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Antioxidant activity of some leafy vegetables of India: A comparative study

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Abstract

Eleven edible leafy vegetables of India have been analysed for their free radical-scavenging activity in different systems of assay, e.g. DPPH radical-scavenging activity, superoxide radical-scavenging activity in riboflavin/light/NBT system, hydroxyl radical-scavenging activity, and inhibition of lipid peroxidation induced by $FeSO_4$ in egg yolk. Total antioxidant activity was measured, based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of green phosphate/Mo(V) complex at acid pH. The extracts were found to have different levels of antioxidant properties in the systems tested. Considering all the activities, it can be said that *Ipomoea reptans* has good activity amongst the eleven plant materials screened for their antioxidant properties. Lowest activity was found in *Nyctanthes arbortristis*. Many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetables. However, there was no correlation between antioxidant activity and total phenol/flavonoid content.

Keywords: Leafy vegetables; Antioxidant activity; Phenols; Flavonoids

1. Introduction

Generation of oxygen radicals, such as superoxide radical ($O_2^{-\bullet}$), hydroxyl radical ($^{\bullet}OH$) and non-free radical species, such as H_2O_2 and singlet oxygen ($^1O_2$) is associated with cellular and metabolic injury, and accelerating aging, cancer, cardiovascular diseases, neurodegenerative diseases, and inflammation (Ames, 1983; Stadtman, 1992; Sun, 1990). Previous epidemiological studies have consistently shown that consumption of fruits and vegetables has been associated with reduced risk of chronic diseases, such as cardiovascular diseases and cancers (Gerber et al., 2002; Kris-Etherton et al., 2002; Serafini, Bellocco, Wolk, & Ekstrom, 2002) and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases (Di Matteo & Esposito, 2003) as well as inflammation and problems caused by cell and cutaneous aging (Ames, Shigenaga, & Hagen, 1993). Fruits and vegetables contain different antioxidant compounds, such as vitamin C, vitamin E and carotenoids, whose activities have been established in recent years. Flavonoids, tannins and other phenolic constituents present in food of plant origin are also potential antioxidants (Salah et al., 1995; Van Acker, Van den Vijgh, & Bast, 1996). The immune system is vulnerable to oxidative stress. During certain diseased states, as well as during aging, there is a need to boost the antioxidant abilities, thereby potentiating the immune mechanism (Devasagayam & Sainis, 2002). The antioxidants preserve an adequate function of immune cells against homeostatic disturbances (De la Fuente & Victor, 2000). Metabolic activation of carcinogen is a free radical-dependent reaction. DNA damage, mediated by free radicals, plays a critical role in carcinogenesis (Feig, Reid, & Loeb, 1994; Guyton & Kensler, 1993). The potentially cancer-inducing oxidative damage might be prevented or limited by dietary antioxidants found in fruits and vegetables. Studies to date have demonstrated that phytochemicals in common fruits and vegetables can have complementary and overlapping

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mechanisms of action, including scavenging of oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism, and antibacterial and antiviral effects (Waladkhani & Clemens, 1998). During the present study, we have made a comparative study of some leafy vegetables of India for their free radical-scavenging activity and ability to prevent lipid peroxidation.

2. Materials and methods

2.1. Plant materials

Leafy vegetables were collected from Kolkata and surrounding areas. The extracts prepared from the dried leaves were made by boiling in distilled water for 5 min and were used for analyzing antioxidant activity in vitro. Each experiment was repeated five times. Eleven plant materials, which were tested for their antioxidant activity, were *Asteracantha longifolia* Nees (AL), *Bacopa monnieri* (Linn.) Pennell (BM), *Bauhinia racemosa* Lam. (BR), *Centella asiatica* (Linn.) Urban (CAs), *Chenopodium album* Linn. (CAl), *Enhydra fluctuans* Lour. (EF), *Ipomoea reptans* (Linn.) Poir. (IR), *Moringa oleifera* Lam. (MO), *Nyctanthes arbortristis* Linn. (NA), *Paederia foetida* Linn. (PF), and *Trigonella foenum-graecum* Linn. (TF).

2.2. Reagents

Chemicals, such as ethylenediamine tetra acetic acid (EDTA), trichloroacetic acid (TCA), butanol, ammonium molybdate, and sodium dodecyl sulphate, were purchased from E. Merck (India) Limited. 1,1 Diphenyl-2-picrylhydrazyl and catechin were procured from Sigma, USA. Thiobarbituric acid (TBA) was purchased from Spectrochem PVT. Ltd., India. Nitroblue tetrazolium was obtained from Sisco Research Laboratories PVT. Ltd., India. All other reagents were of analytical grade.

2.3. Assay of hydroxyl radical ($^{\cdot}OH$)-scavenging activity

The assay was based on the benzoic acid hydroxylation method (Chung, Osawa, & Kawakishi, 1997). Hydroxyl radicals were generated by direct addition of iron(II) salts to a reaction mixture containing phosphate buffer. In a screw-capped tube, 0.2 ml of sodium benzoate (10 mM) and 0.2 ml of $FeSO_4$, $7H_2O$ (10 mM) and EDTA (10 mM) were added. Then the sample solution and a phosphate buffer (pH 7.4, 0.1 M) were added to give a total volume of 1.8 ml. Finally, 0.2 ml of an H_2O_2 solution (10 mM) was added. The reaction mixture was then incubated at 37 °C for 2 h. After that, the fluorescence was measured at 407 nm emission (Em) and excitation (Ex) at 305 nm. Measurement of spectrofluorometric changes has been used to detect the damage by the hydroxyl radical.

2.4. Assay of superoxide radical ($O_2^{-\bullet}$)-scavenging activity

The method used by Martinez et al. (Martinez, Marcelo, Marco, & Moacyr, 2001) for determination of the superoxide dismutase was followed with modification (Dasgupta & De, 2004) in the riboflavin-light-nitrobluetetrazolium (NBT) system (Beauchamp & Fridovich, 1971). Each 3 ml of reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 μM riboflavin, 100 μM EDTA, NBT (75 μM) and 1 ml of sample solution. The production of blue formazan was followed by monitoring the increase in absorbance at 560 nm after 10 min of illumination from a fluorescent lamp.

2.5. DPPH radical-scavenging activity

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Braca et al., 2001). Aqueous extract was added to a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percent inhibition activity was calculated.

2.6. Lipid peroxidation assay

A modified (Dasgupta & De, 2004) thiobarbituric acid-reactive species (TBARS) assay (Ohkowa, Ohisi, & Yagi, 1979) was used to measure the lipid peroxide formed using egg yolk homogenates as lipid-rich media (Ruberto, Baratta, Deans, & Dorman, 2000). Lipid peroxidation was induced by $FeSO_4$. Malondialdehyde (MDA), produced by the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA) yielding a pinkish red chromogen with an absorbance maximum at 532 nm which was measured. Percentage inhibition of lipid peroxidation by different concentrations of the extract was calculated.

2.7. Determination of total antioxidant capacity

The assay is based on the reduction of Mo (VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH (Prieto, Pineda, & Aguilar, 1999). The tubes containing extract and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE) and gallic acid equivalent (GAE).

2.8. Determination of total phenol content

Phenol was determined by Folin–Ciocalteau reagent in alkaline medium and was expressed as gallic acid equivalents (Sadasivam & Manikam, 1992).