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In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies

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Abstract

The free radical scavenging capacity and antioxidant activities of the methanolic extract of *Cinnamomum verum* leaf (CLE) were studied and compared to antioxidant compounds like trolox, butylated hydroxyl anisole, gallic acid and ascorbic acid. The CLE exhibited free radical scavenging activity, especially against DPPH radical and ABTS radical cation. They also exhibited reducing power and metal ion chelating activity, along with hydroxyl radical scavenging activity. The peroxidation inhibiting activity of CLE recorded using the linoleic acid emulsion system, showed very good antioxidant activity.

Keywords: Free radical scavenging capacity; Antioxidant activity; Phenolics; Metal chelator; Lipid peroxidation

1. Introduction

The role of free radicals and active oxygen in the pathogenesis of human diseases including cancer, aging and atherosclerosis has been recognised (Halliwell et al., 1992). Electron acceptors, such as molecular oxygen, react rapidly with free radicals to become radicals themselves, also referred to as reactive oxygen species (ROS). The ROS include superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$) (Grisham and McCord, 1986). Lipid peroxidation, which involves a series of free radical mediated chain reaction processes, is also associated with several types of biological damage. Therefore much attention has been focused on the use of antioxidants, especially natural antioxidants to inhibit lipid peroxidation and to protect from damage due to free radicals.

The medicinal properties of plants have been investigated, in the light of recent scientific developments, through out the world due to their potent pharmacological activities and economic viability. A great number of aromatic, spicy, medicinal and other plants contain chemical compounds, exhibiting antioxidant properties. Sources of natural antioxidants are primarily, plant phenolics that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt and Hudson, 1990). Many of these antioxidant compounds possess antiinflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial or antiviral activities to a greater or lesser extent (Halliwell, 1994; Mitscher et al., 1996; Owen et al., 2000; Sala et al., 2002). Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are increasingly of interest in the food industry, because they retard oxidative degradation of

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lipids and thereby improve the quality and nutritive value of food (Kähkönen et al., 1999; Rice Evans et al., 1995). The extraction, characterization and utilization of natural antioxidants that may serve as potent candidates in combating carcinogenesis and aging process are in progress (Namiki, 1990).

Cinnamomum verum belongs to the family Lauraceae and cinnamaldehyde, one of the component in it has been found to possess significant antiallergic, antiulcerogenic, antipyretic, anaesthetic (Chopra et al., 1980; Kurokawa et al., 1998) and antimutagenic activities (Sharma et al., 2001). *Cinnamomum cassia* has been used traditionally for treating dyspepsia, gastritis, blood circulation disturbances and inflammatory disease in both Eastern and Western countries (Ahn, 1998). The leaves and bark are used as spices and condiments (Joy et al., 1998). The chief constituents of leaf oils were found to be eugenol, benzyl benzoate (Guenther, 1953; Rao et al., 1988), cinnamaldehyde, cinnamyl acetate, cinnamyl alcohol and linalol (Variyar and Bandopadhyaya, 1989).

Several analytical methods have been proposed for determining the total antioxidant activity of biological extracts in order to evaluate the total antioxidant capacity of biological samples (Cano et al., 1998; Cao et al., 1993; Whitehead et al., 1992).

The present paper deals with the free radical scavenging and antioxidant activities of methanolic extract of *C. verum* leaves.

2. Materials and methods

2.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis-3- ethylbenzothiazoline-6-sulfonic acid (ABTS) and 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid (ferrozine) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2- carboxylic acid) and cinnamaldehyde was purchased from Sigma Aldrich Chemical Co. (Milwaukee, WI, USA) butylated hydroxyanisole (BHA) from SD Fine Chemicals (India), thiobarbituric acid (TBA) from CDH (India), ethylene diamine tetraacetic acid (EDTA) and Tween 20 from E-Merck (India) Ltd., Deoxyribose, trichloroacetic acid (TCA), potassium persulphate, ascorbic acid, gallic acid, eugenol, Folin-Ciocalteau reagent and linoleic acid from Sisco Research Lab (India). All the other chemicals used, were of standard analytical grade and solvents were of HPLC grade.

2.2. Plant material

The plant, *C. verum* is distributed through out tropical and subtropical India and leaves from a healthy plant was used for the preparation of the methanolic extract.

2.3. Preparation of extracts

The leaves from a *C. verum* plant, planted in a courtyard were collected during the month of April, average temperature being 28–34 °C. They were shade dried initially, freeze dried and then ground to a fine powder. Four grams each of the powdered leaf sample from a single plant were then extracted separately ($4\,g \times 3$) in methanol at room temperature (27 ± 1 °C) under stirring for 5 h and the extraction process was repeated till the solvent became colorless for calculating the average yield percentage. The total solvent volume employed was 400 ml in each case. The solvent fractions from a single extraction process were pooled and then filtered through Whatman No. 1 filter paper and concentrated in vacuo at 50 ± 1 °C in a rotavapor (Buchi, Model R-205, Germany), followed by lyophilization (Hetosic, Model CD 2.5) to obtain the dry extract which was stored at 0 °C and the lyophilized extract dissolved in methanol were filtered through 0.45 μm syringe nylon filter before analysis.

2.4. Evaluation of antioxidant activity

2.4.1. Determination of ABTS radical cation decolorisation capacity

The experiments were carried out using an improved ABTS decolorisation assay (Re et al., 1999) and it involves the generation of $ABTS^{+\cdot}$ chromophore by the oxidation of ABTS with potassium persulfate. It is applicable for both hydrophilic and lipophilic compounds.

The ABTS radical cation ($ABTS^{+\cdot}$) was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark for at least 6 h at room temperature before use. The $ABTS^{+\cdot}$ solution was diluted to an absorbance of 0.7 ± 0.05 at 734 nm (Shimadzu UV–Vis spectrophotometer, Model 2100). Absorbance was measured 7 min after the initial mixing of different concentrations of the methanolic leaf extracts (final concentration 12.5–150 μg/1.1 ml) with 1 ml of $ABTS^{+\cdot}$ solution. The corresponding concentrations per milliliter of the solution were 11.3, 22.7, 45.4, 68.1, 90.9 and 136 μg respectively. The $ABTS^{+\cdot}$ scavenging capacity of the extract was compared with that of BHA and gallic acid. Trolox, the water soluble analogue of vitamin E was used as a reference standard. A standard curve was prepared by measuring the reduction in absorbance of the $ABTS^{+\cdot}$ solution at different concentrations of trolox over a period of 7 min. The Trolox equivalent antioxidant capacity (TEAC) of an extract represents the concentration of trolox solution that has