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Food Chemistry 94 (2006) 520–528
Food Chemistry
www.elsevier.com/locate/foodchem

Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models

Sindhu Mathew, T. Emilia Abraham *

Bioactive Polymer Engineering Section, CSIR Regional Research Laboratory, Pappanamcode, Trivandrum 695 019, Kerala, India

Received 16 August 2004; received in revised form 8 November 2004; accepted 8 November 2004

Abstract

The antioxidant activities of the methanolic extract of *Cinnamomum verum* barks (CBE) were evaluated with reference to antioxidant compounds like butylated hydroxyl anisole, trolox and ascorbic acid. By virtue of their hydrogen donating ability, all of the tested compounds and CBE exhibited reducing power. They were found to be potent in free radical scavenging activity especially against DPPH$^{\cdot}$ radicals and ABTS radical cations. The hydroxyl ($^{\cdot}$OH) and superoxide radicals ($O_2^{\cdot -}$) were also scavenged by the tested compounds. CBE also exhibited metal chelating activity. The peroxidation inhibiting activity of CBE recorded using a linoleic acid emulsion system, showed very good antioxidant activity.

Keywords: Free radical scavenging capacity; Antioxidant activity; Plant extract; Phenolic compounds; Lipid peroxidation

1. Introduction

Free radicals and other reactive oxygen species, collectively known as ROS are generated continuously via normal physiological processes, more so in pathological conditions. Reactive oxygen intermediates (ROIs) are partially reduced forms of atmospheric oxygen (O_2). They typically result from the excitation of O_2 to form singlet oxygen (O_2^1) or from the transfer of one, two or three electrons to form a superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) or a hydroxy radical ($HO^{\cdot}$), respectively. They are simultaneously degraded to non-reactive forms by enzymatic and non-enzymatic antioxidant defence mechanisms.

Autoxidation of polyunsaturated fatty acids not only lowers the nutritional value of food (Farag, Badei, & El Baroty, 1989), but is also associated with membrane damage, aging, heart disease and cancer in living organisms (Cosgrove, Church, & Pryor, 1987). Oxidative damage in the human body plays an important causative role in disease initiation and progression (Jacob & Burri, 1996). Damage from free radicals and reactive oxygen species has been linked to some neuro-degenerative disorders (Floyd, 1999) and cancers (Goodwin & Brodwick, 1995), and oxidation of low-density lipoprotein is a major factor in the promotion of coronary heart disease (CHD) and atherosclerosis (Frankel, Kanner, German, Parks, & Kinsella, 1993).

The addition of antioxidants to food products has therefore become popular as a means of increasing shelf life and to reduce wastage and nutritional losses by inhibiting and delaying oxidation (Tsuda, Ohshima, Kawakishi, & Osawa, 1994). Synthetic antioxidants such as 2,3-*tert*-butyl-4-methoxy phenol (BHA) and 2,6-di-*tert*-butyl-4-methyl phenol (BHT) are widely used in the food industry. However there are serious concerns about the carcinogenic potential of these substances (Branen, 1975), and there has been a general desire to

* Corresponding author. Tel.: +91 471 2515253; fax: +91 471 2490186/491172.
E-mail address: emiliatea@yahoo.com (T.E. Abraham).

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doi:10.1016/j.foodchem.2004.11.043

replace synthetic food additives with natural alternatives (Howell, 1986). Therefore, intensive research is being carried out on the extraction, characterization and utilization of natural antioxidants, that may serve as potent candidates in combating carcinogenesis and aging processes.

Sources of natural antioxidants are primarily plant phenolics, that may occur in all parts of the plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt & Hudson, 1990). Plant phenolics are multifunctional and can act as reducing agents (free radical terminators), metal chelators and singlet oxygen quenchers. Studies have shown that consumption of foods and beverages rich in phenolic content is correlated with reduced incidence of heart disease (Verhagen, 1989). The most common plant phenolic antioxidants include flavonoid compounds, cinnamic acid derivatives, coumarins, tocopherols and polyfunctional organic acids (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993).

Several methods are used to measure the antioxidant activity of a biological material. The most commonly used for their ease, speed and sensitivity are those involving chromogen compounds of a radical nature to stimulate RONS. The presence of the antioxidant leads to the disappearance of these radical chromogens, the two most widely used being the $ABTS^{+\cdot}$ and the DPPH radicals. DPPH is a free radical that is acquired directly without preparation while $ABTS^{+\cdot}$ must be generated by enzymatic or chemical reactions (Brand-Williams, Cuvelier, & Berset, 1995; Miller & Rice Evans, 1997).

Cinnamomum verum belongs to the family Lauraceae and possesses significant antiallergic, antiulcerogenic, antipyretic and anaesthetic activities (Kurokawa, Kumeda, Yamamura, Kamiyama, & Shiraki, 1998). The bark yields an essential oil containing cinnamaldehyde and eugenol. Several biological activities such as peripheral vasodilatory, antitumor, antifungal, cytotoxic and antimutagenic activities has been attributed to cinnamaldehyde (Bullerman, Liew, & Seier, 1977; Koh et al., 1998; Kwon et al., 1998; Shaughnessy, Setzer, & DeMarini, 2001).

2. Materials and methods

2.1. Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), Phenazine methosulphate (PMS), β-Nicotinamide adenine dinucleotide (NADH) from Sigma (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). 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, nitroblue tetrazolium (NBT), trichloroacetic acid (TCA), potassium persulphate, ascorbic acid, eugenol, gallic acid and linoleic acid from Sisco Research Lab (India). All the other chemicals employed were of standard analytical grade.

2.2. Plant material

The plant, *C. verum* is widely distributed throughout tropical and subtropical India and barks from healthy plants were collected for the preparation of the methanolic extract.

2.3. Preparation of extracts

The barks of *C. verum* were shade dried initially, freeze dried and then ground to a fine powder. Four grams of the powdered sample was then extracted for 5 h with methanol under continuous stirring at room temperature (28 °C) and the extraction process was repeated until the solvent became colorless. The extracts were then concentrated in vacuo at 50 ± 1 °C in a rotavapor (Buchi, Model R-205, Germany), followed by lyophilisation (Hetosicc, Model CD2.5) and the solid mass obtained was resuspended in methanol and stored at 0–4 °C.

2.4. Evaluation of antioxidant activity

2.4.1. Rapid screening of antioxidant activity by dot-blot and DPPH staining

An aliquot (3 μl) of each dilution of the methanolic plant extract and standard compounds like cinnamaldehyde and eugenol were carefully loaded on a 10×20 TLC layer (Silica gel 60 F_{254}; Merck) and allowed to dry. Drops of each sample were loaded in the order of increasing concentration along the row. The staining of the silica plate was based on the procedure of Solver-Rivas, Carlos Espin, and Wichers (2000).

2.4.2. DPPH free radical scavenging assay

The antioxidant activity of CBE and the standard compound BHA was measured in terms of hydrogen donating or radical scavenging ability using the stable $DPPH^{\cdot}$ method (Brand-Williams et al., 1995) as modified by Sanchez-Moreno, Larrauri, and Saura-Calixto (1998). A methanolic solution (0.1 ml) of the sample at various concentrations were added to 2.9 ml of $DPPH^{\cdot}$ (60 μM) solution. When $DPPH^{\cdot}$ reacts with an antioxidant compound that can donate hydrogen it gets reduced and the resulting decrease in absorbance at 517 nm was recorded at 10 min intervals up to 30 min using a UV–Vis Spectrophotometer (Shimadzu UV–Vis 2100).

The mean values were obtained from triplicate experiments.

The remaining concentration of DPPH· in the reaction medium was calculated from a calibration curve, determined by linear regression: $A_{515\ nm} = 0.0209[DPPH^{\cdot}]_T - 0.0078$ and $r = 0.9992$. The percentage of remaining DPPH· ($(DPPH^{\cdot})_R$) was calculated as $(DPPH^{\cdot})_R$ (%) = $(DPPH^{\cdot})_T/(DPPH^{\cdot})_{T=0} \times 100$, where $(DPPH^{\cdot})_T$ is the concentration of DPPH· at 30 min time and $(DPPH^{\cdot})_{T=0}$ is the concentration at zero time (initial concentration). The percentage of remaining DPPH· was plotted against the sample or standard concentration to obtain the amount of antioxidant necessary to decrease the initial concentration of DPPH· to 50% (EC_{50}). A lower EC_{50} value indicates greater antioxidant activity.

2.4.3. ABTS radical cation decolorisation assay

Generation of ABTS radical cation (Wolfenden & Willson, 1982) forms the basis of one of the spectrophotometric methods that have been applied to the measurement of the total antioxidant activity of solutions of pure substances, aqueous mixtures and beverages widely used for the assessment of antioxidant activity of various substances. The experiments were carried out using an improved ABTS decolorisation assay (Re et al., 1999), which involves the generation of the $ABTS^{+\cdot}$ chromophore by the oxidation of ABTS with potassium persulphate. It is applicable for both hydrophilic and lipophilic compounds.

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 bark extracts with 1 ml of $ABTS^{+\cdot}$ solution. 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 the same antioxidant capacity as the extract. The TEAC values were determined as follows:

$$\Delta A_{trolox} = (A_{t=0\ Trolox} - A_{t=6\ min\ Trolox}) - \Delta A_{solvent\ (0-6\ min)}, \quad (1)$$

$$\Delta A_{trolox} = m \cdot [Trolox], \quad (2)$$

$$TEAC_{extract} = (\Delta A_{extract}/m) \cdot d, \quad (3)$$

where ΔA is the reduction of absorbance; A, the absorbance at a given time; m, slope of the standard curve; [Trolox], the concentration of trolox; d, the dilution factor.

All determinations were carried out in triplicate.

2.4.4. Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of extracts was based on the method described by Liu, Ooi, and Chang (1997) with slight modification of Oktay, Gülcin, and Küfrevioglu (2003). Superoxide radicals are generated non-enzymatically in PMS–NADH systems by the oxidation of NADH and assayed by the reduction of NBT. In this experiment, the superoxide radicals were generated in 1 ml of Tris–HCl buffer (16 mM, pH 8.0) containing NBT (50 μM) solution and NADH (78 μM) solution. The reaction was started by adding PMS solution (10 μM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance at 560 nm in a spectrophotometer was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\%Inhibition = [(A_0 - A_1)/A_0 \times 100],$$

where A_0 was the absorbance of the control and A_1 was the absorbance of extract and the standard compound.

2.4.5. Reductive potential

The reductive potential of the extract was determined according to the method of Oyaizu (1986). The different concentrations of extracts and standard (125, 250, 500, 750 and 1000 μg ml^{-1}) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1% w/v). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10% w/v) was added to the mixture, which was then centrifuged for 10 min at 1000*g*. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1% w/v), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reductive potential.

2.4.6. Metal chelating activity

The chelation of ferrous ions by the extracts and standard was estimated by the method of Dinis, Madeira, and Almeida (1994). Extracts were added to a solution of 1 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 1 mM ferrozine (0.1 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm. All test and analyses were done in triplicate and averaged. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was calculated using the formula given below

$$\%Inhibition = [(A_0 - A_1)/A_0 \times 100],$$

where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample of CB extract. The control contains $FeCl_2$ and ferrozine complex formation molecules.

2.4.7. Antioxidant activity in linoleic acid emulsion system

The antioxidant activity of the CBE and BHA was determined by the thiocyanate method (Duh, Yen, Du, & Yen, 1997). Different concentrations of the sample in methanol were mixed with linoleic acid emulsion in potassium phosphate buffer (0.02 M, pH 7.0). Fifty millilitre linoleic acid emulsion was prepared by mixing and homogenising 155 μl linoleic acid, 175 μg Tween-20 as emulsifier and 0.02 M phosphate buffer. The reaction mixture was incubated at 37 ± 0.5 °C. Aliquots of 0.1 ml were taken at various intervals during incubation. The degree of oxidation was measured by sequentially adding ethanol (5 ml, 75% v/v), ammonium thiocyanate (0.1 ml, 30% w/v), and ferrous chloride (0.1 ml, 0.02 M in 3.5% HCl w/v) to sample solution (0.1 ml) and then reading the absorbance at 500 nm. Solutions without added extracts were used as blank samples. The degree of oxidation was measured every 24 h and the data are the average of triplicate analyses. The inhibition of lipid peroxidation in percent was calculated by the following equation:

$$\mathrm{LPI}(\%) = 100 - [(A_1/A_0) \times 100],$$

where A_1 was the absorbance at 500 nm in the presence of sample and A_0 was the absorbance of the control at 500 nm.

2.4.8. Hydroxyl ($OH^{\cdot}$) radical scavenging activity

The sugar deoxyribose on exposure to hydroxyl radicals, generated by the Fenton reaction model system degrades in to fragments and generates a pink chromogen on heating with TBA at low pH (Halliwell, Gutteridge, & Aruoma, 1987).

The hydroxyl radical scavenging activity was measured by the deoxyribose method (Halliwell et al., 1987). The reaction mixture, which contained CBE, deoxyribose (3.75 mM), H_2O_2 (1 mM), potassium phosphate buffer (20 mM, pH 7.4), $FeCl_3$ (0.1 mM), EDTA (0.1 mM) and ascorbic acid (0.1 mM), was incubated in a water bath at 37 ± 0.5 °C for 1 h. The extent of deoxyribose degradation was measured by the TBA method (Ohkawa, Ohishi, & Yagi, 1979). 1 ml of TBA (1% w/v) and 1 ml of TCA (2.8% w/v) were added to the mixture and heated in a water bath at 100 °C for 20 min. The absorbance of the resulting solution was measured spectrophotometrically at 532 nm. All the analyses were done in triplicates and average values were taken. Inhibition (I) of deoxyribose degradation in percent was calculated according to the equation

$$I = (A_0 - A_1/A_0) \times 100,$$

where A_0 is the absorbance of the control reaction and A_1 is the absorbance of the test compound.

2.5. Total phenolics

The extracts were diluted with the same solvent used for extraction, to a suitable concentration for analysis. Total phenolic content of extracts was assessed approximately by using the Folin–Ciocalteau phenol reagent method (Singleton & Rossi, 1965). To 200 ml of the sample extracts were added 1.0 ml of Folin–Ciocalteau reagent and 0.8 ml of sodium carbonate (7.5% w/v), and the contents were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured in a UV–Vis Spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of sample, using a standard curve generated with gallic acid.

2.6. Statistical analysis

The experimental results are expressed as means ± SD of three parallel measurements. The results were processed using Microsoft Excel 2000 and the data were subjected to one way analysis of variance (ANOVA) and the significance of differences between sample means were calculated by Duncan's multiple range test using SPSS for Windows, Standard Version 7.5.1, SPSS. Inc., Chicago, IL. P values $\leqslant 0.05$ were regarded as significant and P values $\leqslant 0.01$ as very significant.

3. Results and discussion

3.1. DPPH radical scavenging activity

Cinnamon bark extract showed good free radical scavenging capacity at all the concentrations studied. $DPPH^{\cdot}$ is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares, Dinis, Cunha, & Ameida, 1997). Stained silica layers revealed a purple background with yellow spots at the location of the drops, which showed radical scavenging activity in all three cases. The intensity of the yellow color depends on the amount and nature of radical scavenger present in the sample and standard compounds. Eugenol exhibited a faster reaction rate and stronger intensities of white-yellow spots compared to cinnamaldehyde and CBE (Fig. 1). There was a significant decrease in the concentration of $DPPH^{\cdot}$ due to the scavenging ability of the methanolic extracts of CB (Fig. 2). Significant radical scavenging activity was evident at all the tested concentrations of CB. The scavenging activity increased with increasing concentration of CBE and BHA up to 12.5 $\mu g\,ml^{-1}$ and then

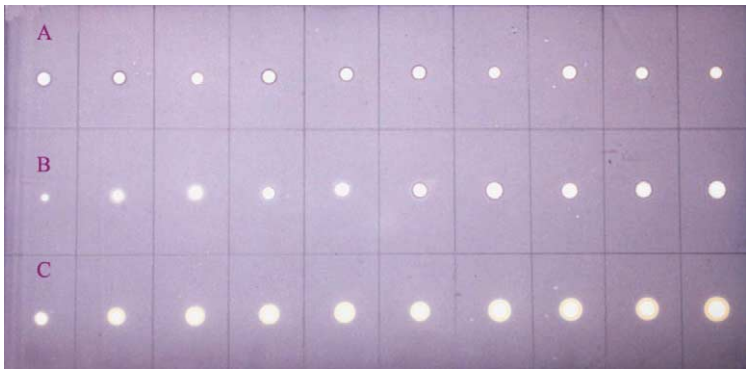

Fig. 1. Dot blot assay on a silica sheet stained with DPPH solution in methanol; First row (A) CBE in the order of increasing concentration (200 μg ml^{-1}–2 mg ml^{-1}); Second row (B) Cinnamaldehyde (10–100 μg ml^{-1}); Third row (C) Eugenol (10–100 μg ml^{-1}).

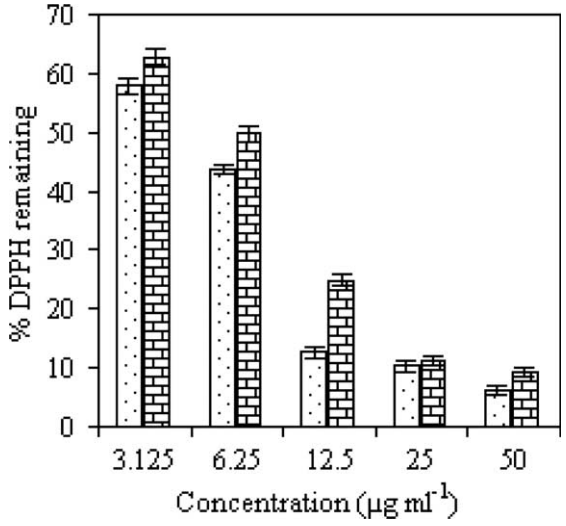

Fig. 2. Free radical scavenging capacity of CBE (white bar) and BHA (checker bar) as determined by the DPPH method. Results are means ± SD of three parallel measurements. P value $\leqslant 0.05$.

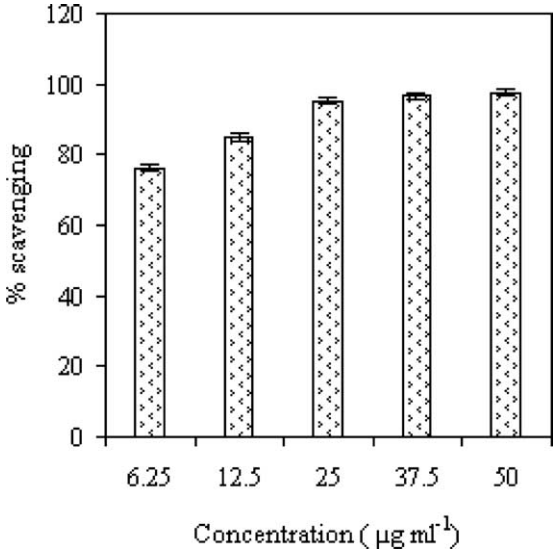

Fig. 3. ABTS radical scavenging capacity of CBE. P value $\leqslant 0.05$.

levelled off with further increase in concentration. The EC_{50} value of CBE was found to be 4.21 μg ml^{-1} and that of BHA 5.79 μg ml^{-1}, which is inversely related to the antioxidant capacity.

3.2. ABTS radical cation scavenging activity

This method measures the relative antioxidant ability to scavenge the radical $ABTS^{+\cdot}$ as compared with a standard amount of Trolox, and is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and of chain breaking antioxidants. Fig. 3 depicts a steady increase in the ABTS radical scavenging capacity of CBE up to a concentration of 25 μg ml^{-1}, followed by a levelling off with further increase in concentration. The TEAC value for CBE at the maximum concentration studied was found to be 18.45 ± 0.6.

3.3. Superoxide anion scavenging activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals (Korycka-Dahl & Richardson, 1978).

From the investigations on the superoxide radical scavenging capacities, it was found that the CBE inhibits superoxide radicals in a dose dependent manner. In the PMS/NADH-NBT system, superoxide anions derived from dissolved oxygen by PMS/NADH coupling reaction reduce NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anions in the reaction mixture. The methanol extract of CB exhibited superoxide scavenging activity (Fig. 4) at all the concentrations (12.5–100 μg ml^{-1}). However, the reference compound, ascorbic acid at similar concentrations exhibited a pro-oxidant effect. Ascorbic acid is a potent reducing agent and acts as a free radical scavenger. However, it may act as a

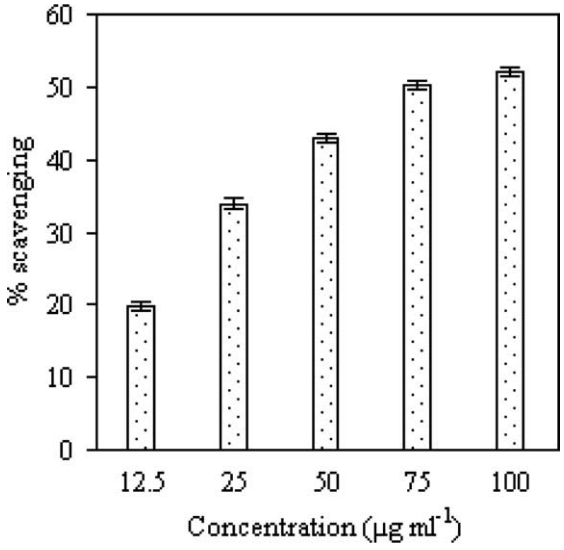

Fig. 4. Superoxide radical scavenging capacity of methanol extracts of CB determined by the PMS/NADH-NBT method.

pro-oxidant in the presence of metals (Bendich, Machlin, Scandurra, Burton, & Wayner, 1986).

3.4. Reducing power

The reducing power of the CBE and the reference compound, ascorbic acid increased steadily with increasing concentration (Fig. 5). The reducing powers (absorbance at 700 nm) of CBE and ascorbic acid were 2.727 and 3.610 at a dose of 1 mg showing that CBE can act as electron donors and can react with free radicals to convert them to more stable products and thereby terminate radical chain reactions. The reducing power of CBE might be due to the di and monohydroxyl substitutions in the aromatic ring, which possess potent hydrogen donating abilities as described by Shimada, Fujikawa, Yahara, and Nakamura (1992).

3.5. Metal chelating activity

The chelating properties of CBE were examined against Fe^{2+}. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. As shown in Fig. 6 the formation of the ferrozine–Fe^{2+} complex is not complete in the presence of CBE, indicating that it can chelate iron. The absorbance of ferrozine–Fe^{2+} complex decreased linearly in a dose dependent manner (750–1750 µg ml^{-1}). However the chelating ability was much lower than that of EDTA.

Metal chelating activity was significant since it reduced the concentration of the catalyzing transition metal in lipid peroxidation (Duh, Tu, & Yen, 1999). It has been reported that chelating agents, which form σ bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gordon, 1990).

3.6. Total antioxidant determination in linoleic acid emulsion system

CBE exhibited effective and powerful antioxidant activity at all the concentrations tested. The effect of various concentrations of CBE (25–200 µg ml^{-1}) on peroxidation of linoleic acid emulsions are represented in Fig. 7. The antioxidant activity of the extract increased with

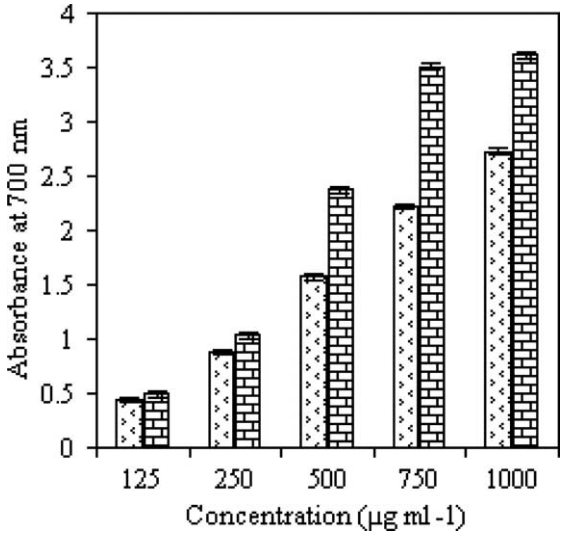

Fig. 5. Reducing power of CBE (white bar) and ascorbic acid (checker bar). Results are means ± SD of triplicate measurements. P value $\leqslant 0.01$.

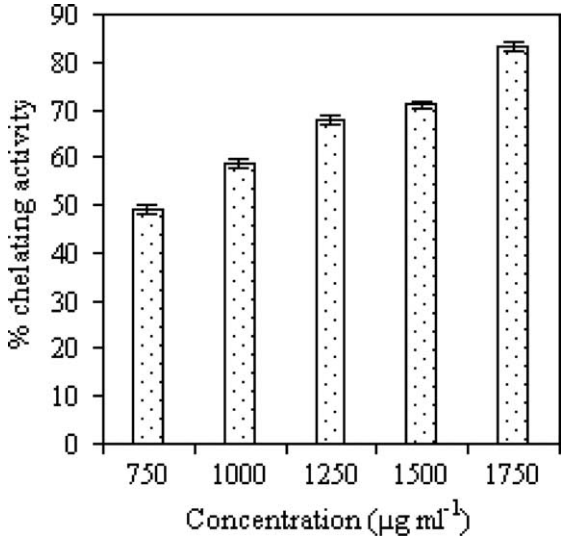

Fig. 6. Metal chelating activity of different concentrations of CBE. Each value is expressed as means ± SD of triplicate measurements. P value $\leqslant 0.01$.

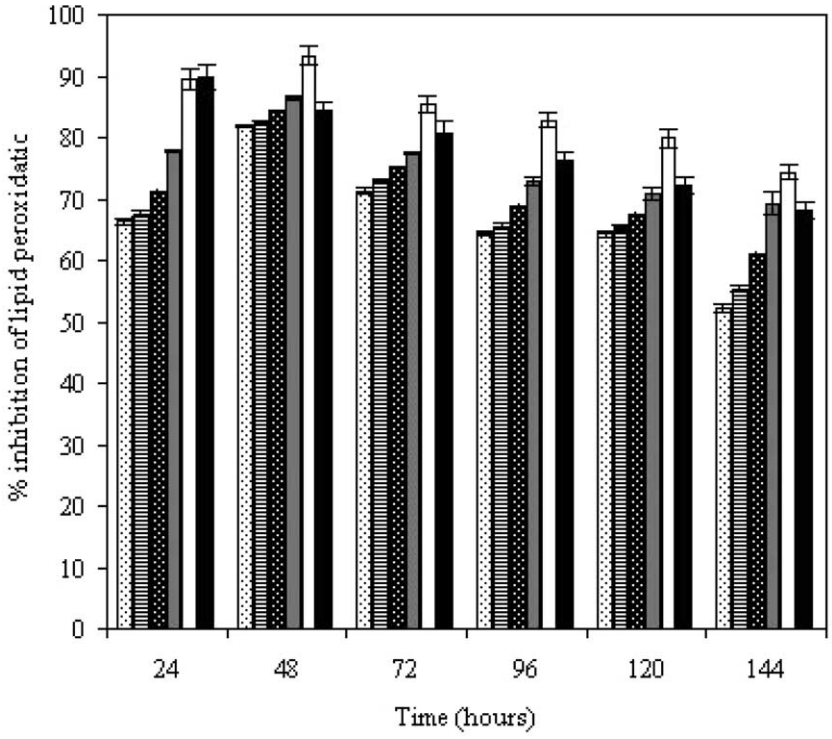

Fig. 7. Antioxidant activity of CBE at different concentrations –25 μg ml^{-1} (white spotted bar); 50 μg ml^{-1} (bar with horizontal lines); 75 μg ml^{-1} (black spotted bar); 100 μg ml^{-1} (grey bar); 200 μg ml^{-1} (white bar) and BHA-200 μg ml^{-1} (black bar) in the linoleic acid emulsion system using the thiocyanate method. Results are expressed as means ± SD of three parallel measurements.

increasing concentration. The percentage inhibition of peroxidation in linoleic acid system by 25, 50, 75, 100 and 200 μg ml^{-1} was found to be 81.8%, 82.4%, 84.5%, 86.5% and 93.3% respectively at 48 h. The percentage inhibition of 200 μg ml^{-1} concentration of BHA was found to be 89.8%.

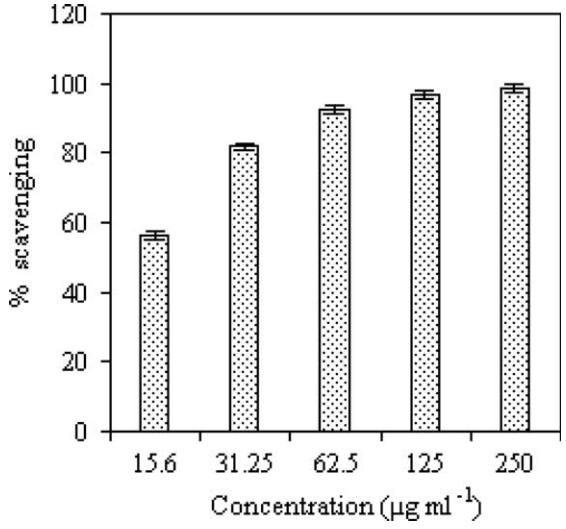

Fig. 8. Hydroxy radical scavenging capacity of CBE. Results are means ± SD of three parallel measurements.

3.7. Hydroxy radical scavenging

The highly reactive ·OH can cause oxidative damage to DNA, lipids and proteins (Spencer et al., 1994). As is the case for many other free radicals, ·OH can be neutralised if it is provided with a hydrogen atom. The sample exhibited hydroxyl radical scavenging activity in a dose dependent manner in the range of 15–250 μg ml^{-1} in the reaction mixture (Fig. 8). Overall, the scavenging activities of phenolic substances might be due to the active hydrogen donating ability of hydroxyl substitutions.

The total phenolic content of the CBE was estimated to be 289.0 ± 2.2 mg Gallic acid equivalents/g of plant extract from triplicate measurements.

4. Conclusion

In conclusion, the results obtained in the present study have shown that the methanolic extract of CB contains a number of antioxidant compounds which can effectively scavenge reactive oxygen species including superoxide anions and hydroxyl radicals as well as other free radicals under in vitro conditions. Moreover the hydrogen donating ability of all the experimental compounds and the extract has been proven through the assessment of reducing power and DPPH· scavenging activity. Even though CBE are weak chelators of

metal ions, their free radical scavenging capacity is comparable to that of synthetic antioxidants such as BHA. CBE possess antioxidant properties, which are concentration dependent.

Antioxidant properties of botanical extracts should be evaluated in a variety of model systems using several different indices because the effectiveness of such antioxidant material is largely dependent upon the chemical and physical properties of the system to which they were added, and a single analytical protocol adopted to monitor lipid oxidation may not be sufficient to make a valid judgement. The ability of the extract to retard lipid oxidation is attributable to the ability of its phenolic constituents to quench reactive oxygen species. Further detailed studies on CBE and its phenolic fractions with regard to antioxidant activity in vivo are needed.

Acknowledgements

The authors are thankful to the Director, Regional Research Laboratory (CSIR), India for providing the facilities for carrying out this research work. The first author acknowledges CSIR, India for the Research Fellowship.

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