Antioxidant Activity of Cinnamtannin B1 from *Cinnamomum zeylanicum* BLUME

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Abstract

Antioxidative activity of cinnamtannin B1 from *Cinnamomum zeylanicum* Blume was investigated. Antioxidants assay were carried out using lipid peroxidation by ferric thiocyanate (FTC) and DPPH radical scavenging analyzed with ultraviolet spectroscopy and electron spin resonance spectroscopic methods. Cinnamtannin B1 exhibited antioxidant activity in a series of *in vitro* test. It was found to be active inhibit lipid peroxidation in ferric thiocyanate method. It was also active scavenged the radical 1,1-diphenyl-2-picrylhyrazyl which was analyzed with ultraviolet and electron spin resonance spectrometer with IC_{50} of 36 mM and 0.2 μM respectively.

Key words: *Cinnamomum zeylanicum*; proanthocyanidin; cinnamtannin B1; antioxidants activity

1. Introduction

Flavonoids occur in the most plant species, concentrating in seeds, fruit skin or peel, bark and flowers. Flavonoids have been found to have antioxidative, antimicrobial, enzyme-inhibiting, antitumor and antimutagenicity, antiinflamatory activity [1, 2]. Flavonoids are group of compounds characteristic by a C6-C3-C6 configuration and can participate in hydrogen donating, radical scavenging and metal chelating mechanisms [3].

Flavonoids may acts as antioxidants by scavenging radicals that include superoxide anion, lipid peroxyl radicals, and hydroxyl radical [2,3,4].

Organisms are constantly exposed to many different forms of reactive oxygen species and reactive nitrogen species that damage proteins, nucleic acids, and lipids, leading to loss of biological function. The possibility that reactive oxygen/nitrogen-mediated protein damage contributes to the aging process is supported by results of many studies showing that aging is associated with the accumulation of such protein damage [5].

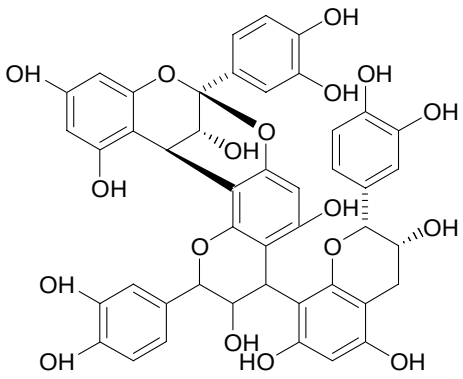

Figure 1. Chemical structure of cinnamtannin B1

Polyphenolic compounds are widely distributed in higher plants and are an integral part of the human diet. Recent interest in these substances has been stimulated by their potential health benefits, which are believed to arise mainly from their antioxidant activity. The antioxidant activity of flavonoids has been studied in detail. An important but often overlooked group of polyphenols is that of the proanthocyanidins [6]. Proanthocyanidins are oligomeric flavonoids, ussually dimers and trimers, based on the flavan-3-ol or catechin molecule [2].

In this paper, we reported the antioxidant activity of a proanthocyanidin, cinnamtannin B1 (Fig.1) isolated from the bark of *Cinnamomum zeylanicum*. Our previous study demonstrated that cinnamtannin B1 has been found to be active in stimulating differentiation of 3T3-L1 adipocytes [7].

2. Experimental

2.1 General

1,1-diphenyl-2-picrylhyrazyl (DPPH), ferric chloride and ammonium thiocyanate were bought from Sigma-Aldrich (St. Louis, MO).UV was recorded on Shimadzu UV-160 spectrophotometer. ESR spectra on a JEOL JES-FA100 instrument using manganese oxide (MnO) as an internal standard.

2.2 Antioxidant assay

2.2.1 Ferric Thiocyanate (FTC) Methods. The detection of lipid peroxidation and preparation of solution were carried out according to method described by Masuda *et al.* [8]. A mixture of sample (2 mg) in 4 ml 99.5% EtOH, 4.1 ml of 2.53% linoleic acid in 99.5% EtOH, 8 ml of 0.05 M phosphate buffer (pH 7) and 3.9 ml of distilled water was placed in a dark vial with screw cap in dark oven at 40 ^{o}C. Oxidation of linoleic acid was monitored by following method. To 0.1 ml of this sample solution was added 9.7 ml of 75% EtOH and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture. Absorbance of the red colour was measured at 500 nm.

2.2.2 DPPH Radical Scavenging Method. The detection of DPPH radical scavenging was carried using ultraviolet spectroscopy (UV) and electron spin resonance (ESR). Preparation of solution for UV analysis was carried out according to method described by Tagashira and Ohtake [9] with slightly modified. A test sample solution in methanol 200 μL (1mg/mL) was added to 3.8 mL of 50 μM DPPH methanol solution. After vortexing, the mixture was incubated for 30 minutes in the dark at room temperature and the absorbance at 517 nm was measured. The difference in absorbance between a test sample and a control (methanol) was taken as the activity. DPPH radical scavenging on ESR was carried out according to method described by Ohtani et al. [10] with slightly modified. A test sample solution ethanol 100 μL (1 mg/ mL) and diluting in several concentration with ethanol was added to 100 μL of 39.43 M DPPH ethanolic solution in test tube by shaking 10 seconds.

The mixture was transferred to a flat cell for analysis of the amount of DPPH radical. ESR spectra were recorded after 40 s of mixing the solution. The signal intensity was evaluated by dividing the peak high of the third of the five line signals of DPPH radical. The condition of ESR spectrometer were set at room temperature, power 1 mW, magnetic field 336.000 ± 5mT, field modulation width 0.5 mT, sweep time 30 sec and time constant 0.03 sec.

$$\% \text{ scavenging} = \left(1 - \frac{PHsample}{PHDPPH}\right) \text{x } 100\%$$

2.2.3 Statistical Analysis

Statistical analyses were performed using Sigma Plot 8.0. Data is presented as means standard error of at least triplicate sample.

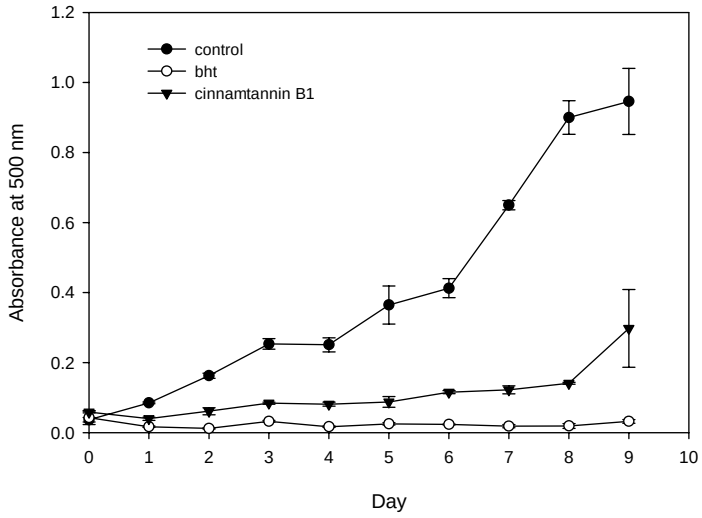

Figure 2. Scavenge activity cinnamtannin B1 by FTC method

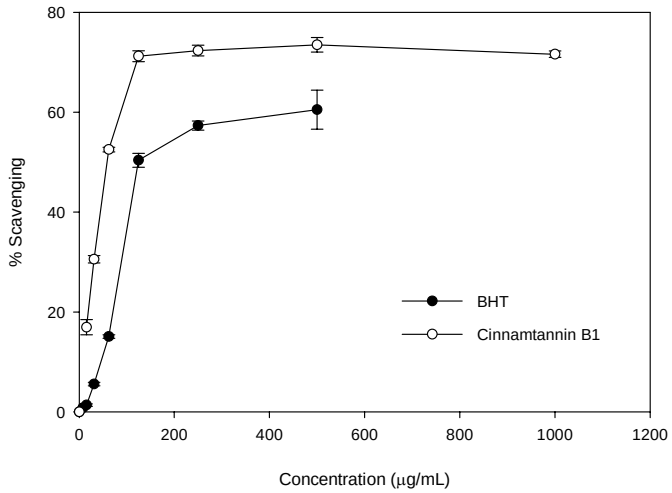

Figure 2. Scavenging activity of cinnamtannin B1 on DPPH radicals

2.4 Results and discussion

Free radicals, reactive oxygen species (ROS) are produced during normal cellular metabolism by oxidation. Other source of free radicals are also inflammation, strenuous exercise, detoxification, exposure to certain chemicals, radiation, ultraviolet light, alcohol, cigarette smoke, air pollutants, excess free calcium, excess stored and unbound iron and high fat diets. ROS are toxic via their effects on cellular components such as denaturing proteins, membrane lipids and DNA [11]. The involvement of these species in the pathogenesis of a large number of diseases, including rheumatoid arthritis, arteriosclerosis, skin aging, nephrites, reperfusion injury, asthma, diabetes, and carcinogenesis [12,13,14,15].

Antioxidants activities were carried out by two different methods of lipid peroxidation and radicals scavenging. The lipid peroxidation was tested using ferric thiocyanate methods (Fig. 2). Free radical scavenging was carried out using DPPH radicals and analysed by the ultraviolet (Fig 3) and electron spin resonance (Fig. 4). Various concentrations of cinnamtannin B1 scavenged the DPPH radicals. Figure 2 shows that cinnamtannin B1 exhibited the activity as inhibitor lipid peroxidation against ferric thiocyanate. However, its activity lowers than BHT that was used as a control antioxidant.

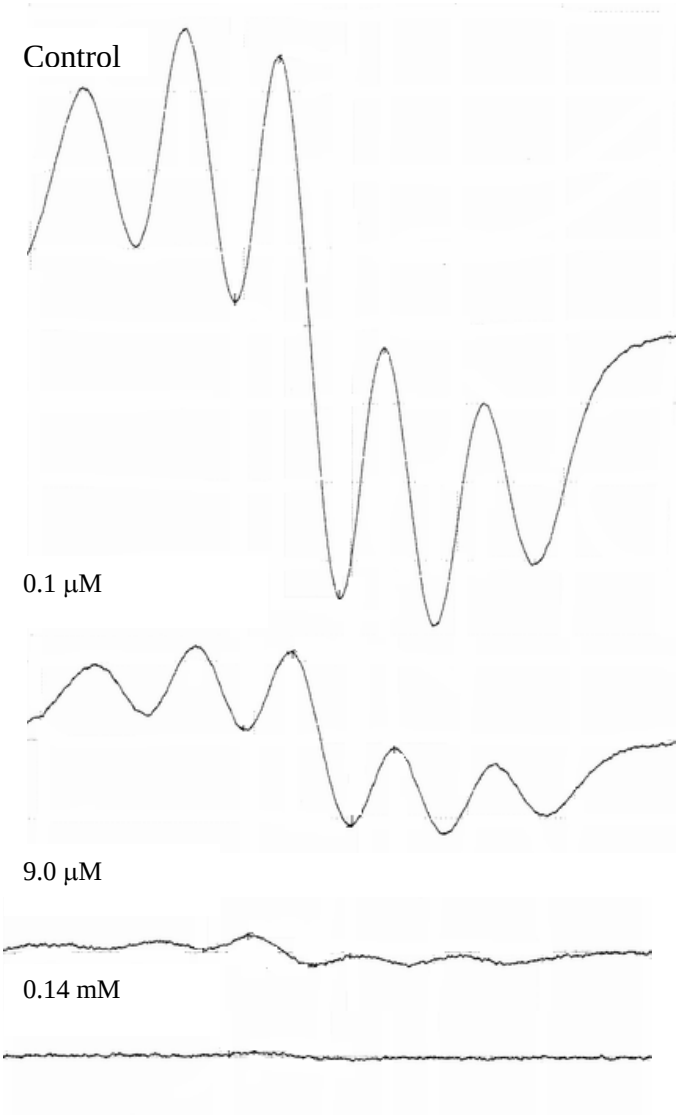

Figure 4. ESR spectra of scavenging activity of different concentration of cinnamtannin B1.

Cinnamtannin B1 exhibited a significant antioxidant activity by scavenging the DPPH radicals. Its activity against DPPH was analyzed using ultraviolet and electron spin resonance with IC_{50} of 36 mM and 0.2 μM respectively.

Acknowledgments

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