Evaluation of Antioxidant and Prooxidant Activities of Bamboo *Phyllostachys nigra* Var. *Henonis* Leaf Extract in Vitro

Chun Hu,[†] Ying Zhang,[‡] and David D. Kitts*[,†]

Food, Nutrition and Health, Faculty of Agricultural Science, University of British Columbia, 6650 N.W. Marine Drive, Vancouver, BC, Canada V6T 1Z4, and Department of Food Science and Nutrition, School of Agricultural Engineering and Food Science, Zhejiang University, Hangzhou, Zhejiang, People's Republic of China

Solvent-extracted bamboo leaf extract (BLE) containing chlorogenic acid, caffeic acid, and luteolin 7-glucoside was evaluated in vitro for free radical scavenging and antioxidant activities using a battery of test methods. BLE exhibited a concentration-dependent scavenging activity of DPPH radical. BLE prolonged the lag phase and suppressed the rate of propagation of liposome peroxidation initiated by peroxyl radical induced by 2,2′-azobis(2-amidinopropane dihydrochloride (AAPH) at 37 °C. BLE also prevented human low-density lipoprotein oxidation, mediated by Cu^{2+}, which was monitored by the lower formation of conjugated diene and fluorescence and a reduced negative charge of apo-B protein. Finally, BLE protected supercoiled DNA strand against scission induced by AAPH-mediated peroxyl radical. Prooxidant activity of BLE was seen in a Cu^{2+}-induced peroxidation of structured phosphatidylcholine liposome, indicating catalytic peroxidation due to a relatively high reducing power of BLE. It was concluded that the BLE has both antioxidant activity and prooxidant activity; the antioxidant activity was attributed to free radical scavenging activity, and the prooxidant activity, albeit minor, resulted from the reducing power of plant phenolics in the presence of transitional metal ions.

Keywords: *Antioxidant; bamboo leaf extract; free radical scavenging; peroxyl radical; prooxidant; reducing power; cupric ion*

INTRODUCTION

Free radical chain breaking, metal chelating, and singlet oxygen quenching represent three major characteristics of antioxidant activity (Hall and Cuppett, 1997). Natural antioxidants derived from plant products have been proposed as replacements for synthetic food antioxidants, to prevent edible oils from rancidity. In addition, antioxidant activities of different agents have been claimed to have potential health functions for reducing aging and possible prevention of cancer and heart disease (Halliwell, 1997). Antioxidant activities from components of food ingredients and food products, such as wine (Tesissere et al., 1996), vegetables (Cao et al., 1996), and tea (Lin et al., 1996), have been reported. Similar studies are available from herbs, ginseng (Kitts et al., 2000), *Ginkgo biloba* (Yan et al., 1995), artichoke (Brown and Rice-Evans, 1998), and pine bark (Virgili et al., 1998). The French paradox was explained, at least partially, by the consumption of red wine, which contributes antioxidant phenolic components in the diet (Lynch and Frei, 1994), with anthocyanins derived from grape juice (Frankel et al., 1998), wine (Tesissere et al., 1996), and berries (Abuja et al., 1998) being the major components contributing to the in vitro antioxidant activity preventing low-density lipoprotein (LDL) oxidation. Similarly, consumption of green tea in the Far East has been associated with low incidences of coronary artery disease mortality rates, as a result of antioxidant activities of four major catechin derivatives, which include (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate (Ishikawa et al., 1997).

Bamboo grows particularly in Southeast Asia and represents a traditionally important commodity used for building materials, in traditional medicine, and as a source of food. To date, the potential health benefits of bamboo leaf extract have not been studied. The objective of this study was to standardize an extract derived from bamboo leaf and to evaluate the antioxidant/prooxidant activities of the bamboo leaf extract (BLE). Our study places a special focus on the efficacy of BLE to inhibit transition metal ion and free radical induced deterioration of macromolecules in vitro.

MATERIALS AND METHODS

Bamboo leaves (*Phyllostachys nigra* var. *henonis*) were collected during the autumn season in Anji County, Zhejiang Province, People's Republic of China. Phosphatidycholine (from soybean), 1,1-diphenyl-2-picylhydrzyl (DPPH), 2-deoxy-D-ribose, 2-thiobarbituric acid (TBA), cupric chloride, potassium ferricyanide, barbital buffer (50 mM, pH8.6), Chelex 100 chelating resin, pBR322 plasmid DNA (from *Escherichia coli*, strain RRI), and human LDL (in PBS, pH 7.4, containing 0.01% of EDTA) were purchased from Sigma Chemical Co. (St. Louis, MO). Electrophoretic grade agarose was purchased from Bio-Rad Laboratories (Richmond, CA). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA Inc. (Richmond, VA). Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) was purchased

* Author to whom correspondence should be addressed [telephone (604) 822-5560; fax (604) 822-3959; e-mail ddkitts@interchange.ubc.ca].

[†] University of British Columbia.

[‡] Zhejiang University.

10.1021/jf0001637 CCC: $19.00
Published on Web 07/14/2000

from Aldrich Chemical Co. Inc. (Milwaukee, WI). Trichloroacetic acid (TCA) and hydrogen peroxide (30%) were purchased from Fisher Scientific (Fari Lawn, NJ). Phosphate buffers made with distilled deionized water were eluted through a Chelex 100 column to eliminate transition metal concentration.

Preparation of BLE. One hundred grams (20−40 mesh) of freeze-dried bamboo leaf powder was refluxed with 1.5 L of 30% (v/v) ethanol in a boiling water bath for 1.5 h, followed by filtration (Whatman No. 4) and rotary vaporization (<40 °C). The concentrated extract was further extracted by an equal volume of *n*-butanol three times. A yellow-brown powder referred to as BLE was recovered (yield percentage = 6%) after *n*-butanol was removed.

Reducing Power of BLE. Reducing power was determined according to the method of Yen and Chen (1995), using L-ascorbic acid as the reference standard. Reducing power of BLE was expressed as ascorbic acid equivalent (AAE) (milligrams of ascorbic acid per milligram of BLE) from a standard curve made from ascorbic acid.

Effect of BLE on Scavenging DPPH Radical. The method of Blois (1958) with modification (Kitts et al., 2000) was used to assess BLE scavenging capacity of DPPH radical. A 0.1 mM DPPH solution in ethanol was mixed with various amounts (0, 1, 5, and 20 μg/mL) of BLE. After vortexing, samples were kept at room temperature for 30 min, and an absorbance reading at 519 nm followed. The scavenging percentage was calculated according to

$$\%\ \text{scavenging} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

where $\text{Abs}_{\text{control}}$ is the absorbance at 519 nm of 0.1 mM DPPH and $\text{Abs}_{\text{sample}}$ is the absorbance at 519 nm of 0.1 mM DPPH with BLE.

Determination of the Formation of Conjugated Diene in a Liposome Model. A structured liposome stock solution was made from soybean lecithin in 10 mM PBS (pH 7.4) at an ice−water bath by sonication (Bransonic 220, Branson Cleaning Equipment Co., Shelton, CT). Liposome (0.1 mg of lecithin/mL) was mixed with various amounts (0, 0.5, 1.0, and 2.0 μg/mL) of BLE, and peroxidation was initiated at 37 °C by either 0.1 mM cupric chloride or in separate experiment by 2 mM AAPH. Absorbance at 234 nm was continuously recorded for 100 min at 37 °C (Unicam UV−vis spectrometer UV2, equipped with a Haake constant temperature recirculator). Results from the conjugated diene measurement are expressed as the lag phase duration, which is defined as the amount of time that is required for an intersection point to be identified between two best fit linear regression lines for both the induction period and the propagation period for the plotted conjugated diene−time course data. The rate of propagation was defined as $\Delta A_{234\text{nm}}$/min occurring during the propagation phase.

Effect of BLE on Preventing Human LDL (hLDL) Peroxidation Induced by Cupric Ion. The method of Hu and Kitts (2000) was used to measure hLDL oxidation. hLDL (LDL in PBS, pH 7.4, with 0.01% of EDTA) was dialyzed against 10 mM PBS (pH 7.4) at 4 °C for 24 h under an atmosphere of N_2. The EDTA-free hLDL (0.5 mg of protein/mL) was mixed with BLE in 10 mM PBS (pH 7.4), and oxidation was initiated by adding 10 μM $CuCl_2$ at 37 °C. The reaction mixture was incubated at 37 °C for 20 h, and EDTA (40 μM) was added to stop the reaction. Three approaches were employed to evaluate the extent of hLDL oxidative modification.

The negative charge of LDL apo-B protein was measured using agarose gel electrophoresis. Briefly, samples were processed using a 0.6% (w/v) agarose gel for 2 h at constant voltage of 46 V in a 50 mM barbital buffer (pH 8.6), employing a horizontal sub-marine gel electrophoresis apparatus (EC apparatus Corp., St. Petersburg, FL). Gels were fixed in 5% of acetic acid (75% of ethanol solution) and stained with 0.1% Sudan Black B in 60% ethanol (Noble, 1968). The inhibition percentage of negative charge of oxidative modified hLDL was evaluated by the equation

$$\%\text{Inh}_{\text{NC}} = \frac{D_{\text{oxidative}} - D_{\text{sample}}}{D_{\text{oxidative}} - D_{\text{native}}} \times 100$$

where $D_{\text{oxidative}}$ is the migration distance of hLDL incubated with $CuCl_2$, D_{sample} is the migration distance of hLDL incubated with $CuCl_2$ with test sample, and D_{native} is the migration distance of hLDL incubated without $CuCl_2$.

For the formation of conjugated diene in hLDL, the reaction mixture was assayed in 10 mM PBS (pH 7.4) at an absorbance of 234 nm. The inhibitory effect of BLE samples on the formation of conjugated diene was calculated according to the equation

$$\%\text{Inh}_{\text{CD}} = \frac{\text{Abs}_{\text{oxidative}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{oxidative}} - \text{Abs}_{\text{native}}} \times 100$$

where $\text{Abs}_{\text{sample}}$ is the absorbance at 234 nm of hLDL incubated with $CuCl_2$ and test samples, $\text{Abs}_{\text{native}}$ is the absorbance at 234 nm of hLDL incubated without $CuCl_2$, and $\text{Abs}_{\text{oxidative}}$ is the absorbance at 234 nm of hLDL incubated with $CuCl_2$.

Measurement of hLDL fluorescence was made after samples were diluted with 10 mM PBS (pH 7.4). Fluorescence was measured at an excitation wavelength of 332 nm and an emission wavelength of 381 nm against PBS. The suppression of fluorescence was calculated according to the equation

$$\%\text{Inh}_{\text{F}} = \frac{\text{fluorescence}_{\text{oxidative}} - \text{fluorescence}_{\text{sample}}}{\text{fluorescence}_{\text{oxidative}} - \text{fluorescence}_{\text{native}}} \times 100$$

where $\text{fluorescence}_{\text{sample}}$ is the fluorescence of hLDL incubated with $CuCl_2$ and test samples, $\text{fluorescence}_{\text{native}}$ is the fluorescence of hLDL incubated without $CuCl_2$, and $\text{fluorescence}_{\text{oxidative}}$ is the fluorescence of hLDL incubated with $CuCl_2$.

Effect of BLE on Preventing Peroxyl Radical Induced pBR322 Plasmid DNA Breakage. The method of peroxyl radical-induced pBR322 DNA breakage was modified from our previous method (Wijewickreme and Kitts, 1998). Briefly, 0.2 μg of DNA, with and without test samples, was incubated with 5 mM AAPH at a total volume of 12 μL in a 10 mM PBS buffer (pH 7.4) for 2 h at 37 °C, followed by mixing with gel loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, and 40% sucrose in H_2O). Electrophoresis was performed using a 0.7% (w/v) agarose gel in a Tris−acetic acid−EDTA buffer (40 mM Tris−acetate, 2 mM EDTA, pH 8.5) employing a horizontal sub-marine gel electrophoresis apparatus (EC Apparatus Corp.). DNA was stained with 0.5 μg/mL ethidium bromides and visualized with a UV transilluminator (Bio-Rad Laboratories). Photograph images (Polaroid) were scanned with image densitometer (GS-670, Bio-Rad Laboratories). The amount of supercoiled and nicked DNA bands was analyzed (Molecule Analyst, version 1.3, Bio-Rad Laboratories), and retention percentages of supercoiled DNA strands were calculated from the scan according to

$$\%\ \text{protection} = \text{amount}_{\text{sample}}/\text{amount}_{\text{native}} \times 100$$

where $\text{amount}_{\text{sample}}$ is the amount of supercoiled DNA strand incubated with peroxyl radical and sample and $\text{amount}_{\text{native}}$ is the amount of supercoiled DNA strand incubated in PBS.

High-Performance Liquid Chromatography (HPLC) Profile of BLE. HPLC was used to analyze the phenolic components of the BLE according to the method previously used (Hu and Kitts, 2000). Caffeic acid, chlorogenic acid, and luteolin 7-glucoside were used as standards.

Statistics. All results are expressed as mean ± SD. A *t*-test (SPSS for Windows 10.0, SPSS Inc., Chicago, IL) was used to test the differences among treatments. The level of confidence required for significance was selected at $p \leq 0.05$.

RESULTS

Several phenolic compounds were identified in the BLE by HPLC. These included caffeic acid, chlorogenic

Table 1. Phenolic Components Identified in Bamboo Leaf Extract Quantified by HPLC

phenolic component identified in BLE	retention time (min)	concentration[a] (%)
chlorogenic acid	7.58	1.6 ± 0.1
caffeic acid	9.91	0.1 ± 0.0
luteolin 7-glucoside	21.19	2.8 ± 0.1

[a] Concentration (% of dry weight of BLE) of phenolic component was calculated according to individual standard curves.

Table 2. Effect of BLE on Scavenging Stable DPPH Radical (Expressed as Percentage of Scavenging)

	% scavenging[a]
control, 0 μg/mL	0
BLE, 1 μg/mL	8.9 ± 0.0**
BLE, 5 μg/mL	19.3 ± 0.6**
BLE, 20 μg/mL	40.9 ± 0.5**

[a] Mean ± SD, $n = 3$; **, $p < 0.01$ sample versus control without antioxidant addition.

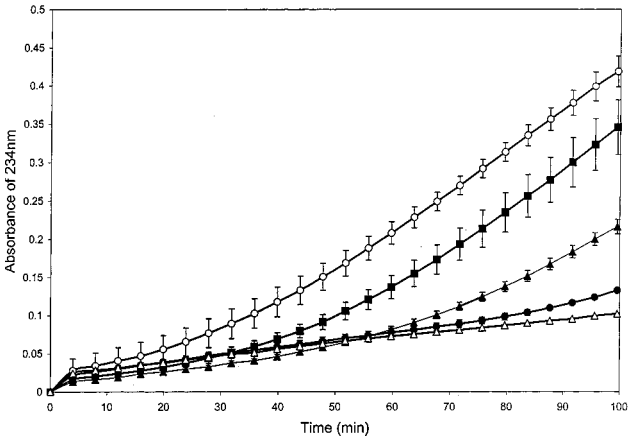

Figure 1. Time course of formation of conjugated diene in the oxidation of structured liposome mediated by peroxyl radical generated by thermal decomposition of AAPH at 37 °C: (○) control incubation; concentration of BLE (■) 0.5 μg/mL, (▲) 1 μg/mL, and (●) 2 μg/mL; concentration of Trolox (△) 1 μg/mL; mean ± SD, $n = 3$.

acid, and luteolin 7-glucoside (Table 1). The reducing power of BLE was equivalent to 0.266 ± 0.022 mg of ascorbic acid/mg BLE. The concentration-dependent scavenging activity of BLE against stable DPPH radical is shown in Table 2. BLE also exhibited an antioxidant property in a peroxyl radical induced liposome peroxidation at 37 °C (Figure 1 and Table 3), which was characterized by both a concentration-dependent prolongation of lag phase duration and a reduction of the rate of propagation. Compared to the control incubation, BLE increased the lag phase by 24, 55 ($p < 0.01$), and 135% ($p < 0.01$) and decreased the rate of propagation by 2, 36 ($p < 0.001$), and 71% ($p < 0.001$) at concentrations varying from 0.5 to 2.0 μg/mL, respectively. The inclusion of Trolox under the same condition resulted in higher ($p < 0.01$) antioxidant activity than BLE in both lag phase and rate of propagation parameters.

In the liposome model system, the presence of 0.1 mM cupric ion resulted in a lag phase of 62.5 ± 2.9 min and a propagation rate of 0.0042 ± 0.0006/min at 37 °C. BLE, under the same condition, produced a significantly shorter ($p < 0.01$) lag phase and a higher ($p < 0.01$) rate of propagation at 2 μg/mL, suggesting that the BLE produced a prooxidant activity, rather than an antioxidant activity, in a cupric ion induced liposome peroxidation model system. Similar findings were obtained for both Trolox and ascorbic acid (Figure 2 and Table 3).

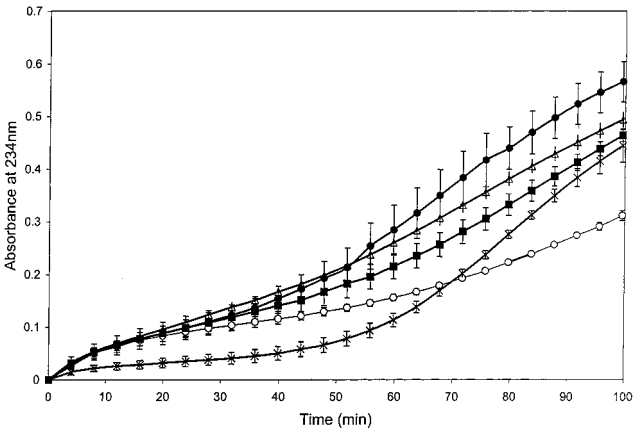

Figure 2. Time course of formation of conjugated diene in the oxidation of structured liposome mediated by Cu^{2+} at 37 °C: (○) control incubation; concentration of BLE (■) 0.5 μg/mL and (●) 2 μg/mL; concentration of Trolox (△) 1 μg/mL; concentration of ascorbic acid (∗) 1 μg/mL; mean ± SD, $n = 3$.

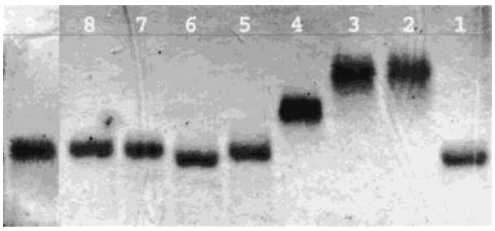

Figure 3. Agarose gel electrophoresis of human LDL incubated with cupric ion: (lane 1) LDL incubated without Cu^{2+} (native); (lane 2) LDL incubated with Cu^{2+} (oxidative); (lane 3) LDL incubated with Cu^{2+} and 10 μg/mL BLE; (lane 4) LDL incubated with Cu^{2+} and 25 μg/mL BLE; (lane 5) LDL incubated with Cu^{2+} and 50 μg/mL BLE; (lane 6) LDL incubated with Cu^{2+} and 100 μg/mL BLE; (lane 7) LDL incubated with Cu^{2+} and 10 μg/mL BHT; (lane 8) LDL incubated with Cu^{2+} and 20 μg/mL ascorbic acid; (lane 9) LDL incubated with Cu^{2+} and 10 μM EDTA.

A concentration-dependent protection of BLE was observed using the human LDL oxidation model (Figure 3 and Table 4). hLDL incubated in the presence of cupric ion resulted in the increased formation of fluorescent products, greater generation of conjugated diene, and increased gel electrophoretic mobility ($p < 0.01$). The latter event reflected the increase of negative charge on apo-B protein due to the oxidation. BLE suppressed hLDL oxidation by lowering all three indices of peroxidation. Reference antioxidants including BHT, ascorbic acid, and EDTA also showed similar protection against hLDL oxidative modification induced by cupric ion.

The presence of peroxyl radical resulted in a dramatic scission of pBR322 supercoiled DNA (Figure 4, lane 2). The addition of BLE provided a protective effect against peroxyl radical induced DNA breakage (lanes 3 and 4 of Figure 4). In the aqueous medium, Trolox concentrations of 0.5 and 2 μg/mL added in vitro significantly ($p < 0.001$) reduced supercoiled DNA strand damage induced by peroxyl radical. High protection against DNA strand breakage observed with 2 μg/mL of Trolox was also achieved by BLE at 20 μg/mL (Table 5).

DISCUSSION

Data on the phytochemical content of bamboo leaf is limited, although Estuko et al. (1998) reported the

Table 3. Lag Phase and Rate of Propagation of Liposome Peroxidation Induced by either Peroxyl Radical (AAPH) or Transition Metal Ion (Cu^{2+})[a]

	AAPH		Cu^{2+}	
μg/mL	lag phase (min)	rate of propagation (ΔA_{234nm}/min)	lag phase (min)	rate of propagation (ΔA_{234nm}/min)
control	35.8 ± 4.5	0.0055 ± 0.000	62.5 ± 2.9	0.0042 ± 0.0006
BLE, 0.5	44.4 ± 0.4	0.0054 ± 0.0002	53.5 ± 6.1	0.0064 ± 0.0004
BLE, 1.0	55.5 ± 1.2**	0.0035 ± 0.0000***		
BLE, 2.0	84.1 ± 10.4**	0.0016 ± 0.0002***	35.1 ± 8.2*	0.0070 ± 0.0004*
Trolox, 1.0	>100***	0.0009 ± 0.0001***	49.0 ± 2.0**	0.0059 ± 0.000*
ascorbic acid, 1.0			56.1 ± 1.6	0.0090 ± 0.0002***

[a] Results expressed as mean ± SD, $n = 3$ individual experiments. *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$, sample versus control within the same column. Reaction conditions and calculation of lag phase and rate of propagation were described under Materials and Methods.

Table 4. Effect of Bamboo Leaf Extract on Suppressing Oxidative Indices of Human LDL Oxidation Induced by Cupric Ion at 37 °C[a]

	$\%Inh_F$	$\%Inh_{CD}$	$\%Inh_{NC}$
control	0	0	0
BLE, 10 μg/mL	13.4 ± 6.7	3.3 ± 3.6	11.2 ± 1.6***
BLE, 25 μg/mL	46.1 ± 3.1**	10.4 ± 0.5**	47.9 ± 3.0***
BLE, 50 μg/mL	67.5 ± 1.6*	80.7 ± 3.1***	93.4 ± 6.5***
BLE, 100 μg/mL	86.7 ± 1.6*	76.1 ± 6.1***	97.4 ± 3.6***
BHT, 10 μg/mL	78.9 ± 1.8**	96.4 ± 3.1***	100 ± 0
ascorbic acid, 20 μg/mL	72.7 ± 2.7**	88.9 ± 4.0***	94.7 ± 4.8***
EDTA, 10 μM	100 ± 0.8*	96.9 ± 2.4***	92.1 ± 8.2***

[a] See Materials and Methods for detail of calculation of inhibition of fluorescence ($\%Inh_F$), inhibition of the formation of conjugated diene ($\%Inh_{CD}$), and inhibition of apo-B negative charge ($\%Inh_{NC}$). *, $p < 0.05$); **, $p < 0.01$; and ***, $p < 0.001$, sample versus control within same column.

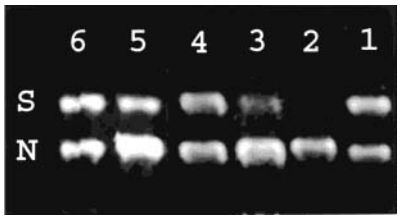

Figure 4. Peroxyl radical leads to supercoiled strand pBR322 DNA scission: (lane 1) DNA incubated without AAPH; (lane 2) DNA incubated with AAPH; (lane 3) DNA incubated with AAPH and 10 μg/mL BLE; (lane 4) DNA incubated with AAPH and 20 μg/mL BLE; (lane 5) DNA incubated with AAPH and 0.5 μg/mL Trolox; (lane 6) DNA incubated with AAPH and 2 μg/mL Trolox; (S) supercoiled strand; (N) nicked strand.

Table 5. Effects of Bamboo Leaf Extract and Trolox on the Retention of Supercoiled Strand of pBR322 DNA under the Peroxyl Radical Induced Scission at 37 °C[a]

	% protection
control	0
BLE, 10 μg/mL	37.5 ± 0.5***
BLE, 20 μg/mL	82.1 ± 5.9***
Trolox, 0.5 μg/mL	46.4 ± 6.1***
Trolox, 2 μg/mL	92.6 ± 2.6***

[a] See Materials and Methods for the calculation of % protection, mean ± SD, $n = 3$; ***, $p < 0.001$ compared with control, which was under stress of peroxyl radical without incubation with antioxidant.

presence of *p*-(hydroxyphenyl)propionic acid, ferulic acid, caffeic acid, and chlorogenic acid in bamboo shoot, an edible vegetable from the Far East. From the viewpoint of antioxidant properties of these phenolic compounds, other workers have reported antioxidant/prooxidant activities of ferulic acid (Graf, 1992; Laranjinha et al., 1996; Bourne and Rice-Evans, 1997) and caffeic acid (Kerry and Rice-Evans, 1999; Laranjinha et al., 1994). Caffeic acid has been shown to not only delay LDL oxidation but also act as a cytoprotective agent by possibly blocking the intracellular signaling triggered by oxidized LDL (Laranjinha et al., 1994). This result produces a sustained rise in calcium required for oxidized LDL induced apoptosis (Vieira et al., 1998). Antioxidant activity of luteolin 7-glucoside has also been reported to lower the rate of propagation and extend the lag phase of Cu^{2+}-induced LDL oxidation in vitro (Brown and Rice-Evans, 1998). As confirmed from our HPLC chromatogram on the BLE, the presence of caffeic acid, chlorogenic acid, and luteolin 7-glucoside could contribute in part to the antioxidant activity of BLE observed in the different model systems tested in this study.

Two mechanisms are basically involved in antioxidant activity, namely, hydrogen donation and chelating of catalytic ion. The propensities of hydrogen donation for exhibiting a redox property are critical factors for characterizing antioxidant activity that involves free radical scavenging. Reducing power of BLE was found to be more than one-fifth that of ascorbic acid on a weight equivalent basis. The hydrogen donating property of BLE was also confirmed from the result of the DPPH scavenging capacity of BLE. As proposed by Blois (1958), the hydrogen donation from an antioxidant involves the decolorization of DPPH (eq 1)

$$(DPPH)^{\bullet} + A - H \rightarrow (DPPH) : H + A^{\bullet} \qquad (1)$$

where A−H is the antioxidant and $A^{\bullet}$ is the antioxidant intermediate radical; thus, the DPPH radical scavenging activity of BLE can be related to a propensity to donate hydrogen.

The hydrogen donating activity of BLE was also seen with protection against peroxyl radical induced liposome peroxidation. Thermolysis of AAPH results in the formation of peroxyl radical ($ROO^{\bullet}$), from which $ROO^{\bullet}$ reacts with nearby substances (eqs 2 and 3). Without

$$ROO^{\bullet} + L - H \rightarrow ROOH + L^{\bullet} \qquad (2)$$

$$ROO^{\bullet} + A - H \rightarrow ROOH + A^{\bullet} \qquad (3)$$

the presence of antioxidant (AH), it can be expected that $ROO^{\bullet}$ will extract one hydrogen atom from nearby substrates (such as lipid or DNA) to generate a new radical ($L^{\bullet}$, eq 2), which in turn invokes a free radical chain reaction resulting in the deterioration of lipids and DNA. However, in the presence of a chain-breaking antioxidant, this chain reaction is terminated by the reaction present in eq 3. Therefore, as shown from Figure 1, the chain-breaking antioxidant activity of BLE can be confirmed by the longer lag phase duration and a lower propagation rate in the peroxyl radical induced liposome peroxidation assay. Several other studies have

reported the relationship between flavonoid structure and antioxidant activity (Salah et al., 1995; Van Acker et al., 1995), showing that flavonoids possessing a di-*o*-hydroxyl group on the B-ring effectively contribute to a chain-breaking antioxidant activity. The presence of B-di-*o*-hydroxyl structure also contributes higher stability to the radical form in electron delocation (Rice-Evans, 1995). Among the phenolic components found in BLE, caffeic acid, chlorogenic acid, and luteolin 7-glucoside all have di-*o*-hydroxyl groups. The fact that reduction potentials of flavonoid radicals are lower than those of alkyl peroxyl radicals makes its possible for flavonoids to prevent alkyl-induced peroxidation reactions (Salah et al., 1995).

As we demonstrate in this study, the reducing power of BLE may contribute to a prooxidant activity when in the presence of free transition metal ions. Cupric ion has been shown to mediate the peroxidation of liposome through a decomposition of a trace amount of lipid peroxide in phospholipid (Coupland and McClements, 1996). The reducing power of the BLE makes it possible to reduce Cu^{2+} to Cu^{+}, which favors the Fenton reaction and in turn promotes the peroxidation reaction of lipids. A similar result is seen in vitro with ascorbic acid, which manifests either antioxidant or prooxidant activity (Otero et al., 1997; Gerster, 1999; Paolini et al., 1999).

The modification of protein by reactive oxygen species can involve direct oxidation of the amino acid side chain residues, the covalent attachment of oxidized metabolites to amino acid side chains on the cleavage of the peptide backbone, or the formation of protein−protein aggregates (Stadtman, 1994). A good example of the significance of oxidative modification of protein in vivo has been reported with LDL, in which modification by oxidation was associated with the pathogenic process of atherogenesis (Steinberg et al., 1989). Metal ion catalyzed oxidation of hLDL associated with the loss of lysine or histidine in apolipoprotein B (Stadtman, 1994; Steinberg et al., 1989) results from either the direct oxidation of protein by metal-catalyzed reaction or the inactivation of these amino acid moieties with lipid peroxidation products. An increased anodic electrophoretic mobility of LDL has been shown to correlate with increased recognition of LDL by the macrophage scavenge receptors (Steinbrencher, 1987), thereby contributing to the formation of lipid-laden foam cells in vivo (Steinberg et al., 1989). In both in vitro and ex vivo LDL models, Fuhrman et al. (1997), using a licorice extract, observed a capacity to prevent LDL from either peroxyl radical or cupric ion induced oxidation due to the polyphenol glabridin content of licorice. This finding suggested that flavonoids prevent free radical damage at the surface of phospholipids (as in the case of AAPH or cupric ion) or, alternatively, in the core of the LDL molecule [as in the case of 2,2-azobis(2,4-dimethylvaleronitrile) (AMVN), which produces lipid-soluble free radical]. Lipophilic antioxidants such as tocopherol are highly efficient antioxidants in the LDL peroxidation model, because tocopherol enters LDL particles. This is in contrast to hydrophilic antioxidants, which act only on the surface of LDL particles (Abuja et al., 1998). Heinonen et al. (1998) evaluated antioxidant activities of fruit berries and found that the anthocyanin content was directly, and the flavonol content indirectly, associated with antioxidant activity using the in vitro Cu^{2+}-induced LDL oxidation model system. Other flavonoid-rich plants, such as *Gingko biloba* extract (as standardized extract, Egb761), have been shown to inhibit in vitro LDL oxidation (Yan et al., 1995). Our current result extends those findings to include similar efficacy for the standardized BLE used in this study, which effectively suppressed the formation of both fluorescence and conjugated diene as well as the negative charge of apo-B protein during exposure of LDL to Cu^{2+}-induced oxidation in vitro. It is noteworthy that within the concentration range tested herein, no prooxidant activity toward LDL by BLE was found, a finding different from that obtained from the Cu^{2+}-induced liposome peroxidation model.

From the fluorescence and conjugated diene data, it is clear that BLE suppressed both indices that characterize the effect of oxidative stress on lipoproteins. Our results showed that both hydrophobic and hydrophilic antioxidants (i.e., 45.5 μM BHT and 114 μM ascorbic acid) also exhibited protection of hLDL, albeit to a comparatively lesser extent than metal chelator EDTA. This result indicated that metal-chelating activity was involved in the protective mechanism against transition metal ion induced LDL oxidation. It was not surprising that EDTA inhibited LDL oxidation because EDTA effectively bound with cupric ion in model systems (Hahnel et al., 1999). EDTA at the same molar concentration as cupric ion will prevent LDL from oxidation induced by Cu^{2+}, suggesting that metal ion catalyzed oxidation of LDL can be prevented by metal-chelating activity. The mechanism of activity of BLE to inhibit cupric ion induced LDL oxidation could also be attributed to the phenolic acid and flavone compositions, which are known transition metal chelators (Markham, 1982).

It is also interesting to note that ascorbic acid, which was found to be a prooxidant in Cu^{2+}-induced liposome peroxidation in this study, exhibited antioxidant activity in the Cu^{2+}-induced LDL oxidation model. Similar results have been reported elsewhere (Retsky et al., 1993; Jialal et al., 1990; Stait and Leake, 1994). The reason of this unusual inhibitory effect of ascorbic acid in the presence of free Cu^{2+} has been explained by the two different Cu^{2+} affinity binding sites of LDL. The redox-active binding site, which primarily consists of histidine residues of the apolipoprotein B-100, is susceptible to undergoing metal-catalyzed oxidation, resulting in the formation of 2-oxohistidine, which has less affinity to bind Cu^{2+} than histidine. Thus, ascorbic acid could protect against Cu^{2+}-induced LDL oxidation by modifying the redox-active binding site of cupric ion (Retsky et al., 1999).

The presence of peroxyl radicals is relevant to biological systems because they are the most abundant free radical (Prior and Cao, 1999). Both phospholipid liposome and DNA treated with peroxyl radical in this study resulted in the formation of conjugated diene and scission of supercoiled DNA strand, respectively. DNA single-strand breakage has also been reported in other free radical involved circumstances, such as γ-radiation (Kumar et al., 1999). Lloyd and Phillips (1999) found that hydroxyl radical generated both from free solution and on the surface of DNA led to the scission of DNA base pairs. In our peroxyl radical induced DNA scission model, BLE prevented breakage of the supercoiled DNA strand to an extent similar to that found with the addition of Trolox, a known water-soluble antioxidant analogue with free radical chain-breaking activity (Castle and Perkins, 1986). Thus, direct free radical scavenging

mechanisms of BLE can be attributed to the prevention of peroxidation reactions.

CONCLUSION

In summary, we have shown that BLE works directly to scavenge free radicals including DPPH radical and peroxyl radical in different model systems. BLE could also act as a prooxidant, under certain conditions, due to its relatively high reducing power. This is particularly plausible when the existence of transitional metal ion (i.e., cupric ion used herein) occurs and a reduction to Cu^{+} favors initiating the peroxidation reaction. However, BLE did not induce pro-oxidation of hLDL oxidation in this study when incubated with Cu^{2+}.

BLE exhibits antioxidant activities in various different model systems that include DNA, lipoprotein, and complex lipid systems. The identification of individual components in the BLE extract responsible for these different antioxidant properties is subject to further investigation. At the present time, we have identified only three primary phenolic components in the BLE, which included chlorogenic acid, caffeic acid, and luteolin 7-glucoside. Additional work is therefore necessary to fractionate the BLE further to elicit a better understanding of how each chemical fraction contributes to the overall antioxidant activity and to determine whether the unique mixture of plant phenolics contributes to a synergistic antioxidant activity.

LITERATURE CITED

Abuja, P. M.; Murkovic, M.; Pfannhauser, W. Antioxidant and prooxidant activities of elderberry (*Sambucus nigra*) extract in low-density lipoprotein oxidation. *J. Agric. Food Chem*. **1998**, *46*, 4091−4096.

Blois, M. S. Antioxidant determinations by the use of a stable free radical. *Nature* **1958**, *181*, 1199−1200.

Bourne, L. C.; Rice-Evans, C. A. The effect of the phenolic antioxidantferulic acid on the oxidation of low-density lipoprotein depends on the pro-oxidant used. *Free Radical Res.* **1997**, *27*, 337−344.

Brown, J. E.; Rice-Evans, C. A. Luteolin-rich artichoke extract protects low-density lipoprotein from oxidation *in vitro*. *Free Radical Res.* **1998**, *29*, 247−255.

Cao, G. H.; Sofic, E.; Prior, R. L. Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem*. **1996**, *44*, 3426−3431.

Castle, L.; Perkins, M. J. Inhibition kinetics of chain-breaking phenolic antioxidants in SDS micelles. Evidence that intermicellar diffusion rates may be rate-limiting for hydrophobic inhibitors such as -tocopherol. *J. Am. Chem. Soc.* **1986**, *108*, 6381−6382.

Coupland, J. N.; McClements, D. J. Lipid peroxidation in food emulsion. *Trends Food Sci. Technol.* **1996**, *7*, 83−90.

Estuko, K.; Nobuyuki, K.; Hironobu, T. Identification and changes of phenolic compounds in bamboo shoots during storage at 20 °C. *J. Jpn. Soc. Hortic. Sci.* **1998**, *67*, 805−81.

Frankel, E. N.; Bosanek, C. A.; Meyer, A. S.; Silliman, K.; Kirk, L. L. Commercial grape juices inhibit the *in vitro* oxidation of human low-density lipoproteins. *J. Agric. Food Chem*. **1998**, *46*, 834−838.

Fuhrman, B.; Buch, S.; Vaya, J.; Belinky, P. A.; Coleman, R.; Hayek, T.; Aviram, M. Licorice extract and its major polyphenols glabridin protect low-density lipoprotein against lipid peroxidation: *in vitro* and ex vivo studies in human and in atherosclerotic apolipoprotein E-deficient mice. *Am. J. Clin. Nutr.* **1997**, *66*, 267−275.

Gerster, H. High-dose vitamin C: a risk for persons with high iron stores? *Int. J. Vitamin Nutr. Res.* **1999**, *69*, 67−82.

Graf, E. Antioxidant potential of ferulic acid. *Free Radical Biol. Med*. **1992**, *13*, 435−48.

Hahnel, D.; Huber, T.; Kurze, V.; Beyer, K.; Engelmann, B. Contribution of copper binding to the inhibition of lipid oxidation by plasmalogen phospholipids. *Biochem. J*. **1999**, *340*, 377−383.

Hall, C. A.; Cuppett, S. L. Structure activities of natural antioxidants. In *Antioxidant Methodology in Vivo and in Vitro Concepts*; Aruoma, O. I., Cuppett, S. L., Eds.; AOCS Press: Champaign, IL, 1997; pp 141−172.

Halliwell, B. Antioxidants and human diseases: a general introduction. *Nutr. Rev*. **1997**, *55*, S44−52.

Heinonen, I. M.; Meyer, A. S.; Frankle, E. N. Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *J. Agric. Food Chem*. **1998**, *46*, 4107−4112.

Hu, C.; Kitts, D. D. Studies on the antioxidant activity of *Echinacea* root extract. *J. Agric. Food Chem*. **2000**, *48*, 1466−1472.

Ishikawa, T.; Suzukawa, M.; Ito, T.; Yoshida, H.; Ayaori, M.; Nishiwaki, M.; Yonemura, A.; Hara, Y.; Nakamura, H. Effect of tea flavonoid supplementation on the susceptibility of low-density lipoprotein to oxidative modification. *Am. J. Clin. Nutr*. **1997**, *66*, 261−266.

Jialal, I.; Vega, G. L.; Grundy, S. M. Physiologic levels of ascorbate inhibit the oxidative modification of low-density lipoprotein. *Atherosclerosis* **1990**, *82*, 185−191.

Kerry, N.; Rice-Evans, C. Inhibition of peroxynitrite-mediated oxidation of dopamine by flavonoid and phenolic antioxidants and their structural relationships. *J. Neurochem*. **1999**, *73*, 247−253.

Kitts, D. D.; Wijewickreme, A. N.; Hu, C. Antioxidant properties of North American ginseng extract. *Mol. Cell. Biol*. **2000**, *203*, 1−10.

Kumar, S. S.; Chaubey, R. C.; Devasagayam, T. P. A.; Priyadarsini, K. I.; Chauhan, P. S. Inhibition of radiation-induced DNA damage in plasmid pBR322 by chlorophyllin and possible mechanism(s) of action. *Mutat. Res.* **1999**, *425*, 71−79.

Laranjinha, J. A.; Almeida, L. M.; Madeira, V. M. Reactivity of dietary phenolic acids with peroxyl radicals: antioxidant activity upon low-density lipoprotein peroxidation. *Biochem. Pharmacol.* **1994**, *48*, 487−494.

Laranjinha, J. A.; Vierira, O.; Almeida, L.; Madeira, V. Inhibition of metmyoglobin-H_2O_2-dependent low density lipoprotein lipid peroxidation by naturally occurring phenolic acids. *Biochem. Pharmacol.* **1996**, *51*, 395−402.

Lin, Y. L.; Juan, J. M.; Chen, Y. L.; Liang, Y. C.; Lin, J. K. Composition of polyphenols in fresh tea and associated of these oxygen-radicals absorbing capacity with antiproliferative actions in fibroblast cells. *J. Agric. Food Chem*. **1996**, *44*, 1387−1394.

Lloyd, D. R.; Philips, D. Oxidative DNA damage mediated by copper(II), iron(II) and nickel(II) Fenton reactions: evidence for site-specific mechanisms in the formation of double-strand breaks, 8-hydroxyldeoxyguanosine and putative intrastrand cross breaks. *Mutat. Res.* **1999**, *424*, 23−36.

Lynch, S. M.; Frei, B. Mechanisms of copper and iron-dependent oxidative modification of human low-density lipoprotein. *J. Lipid Res.* **1994**, *34*, 1745−1754.

Markham, K. R. *Techniques of Flavonoid Identification*; Academic Press: New York, 1982; pp 36−51.

Noble, R. P. Electrophoresis separation of plasma lipoprotein in agarose gel. *J. Lipid Res.* **1968**, *9*, 693−700.

Otero, P.; Viana, M.; Herrera, E.; Bonet, B. Antioxidant and prooxidant effects of ascorbic acid, dehydroascorbic acid and flavonoids on LDL submitted to different degrees of oxidation. *Free Radical Res.* **1997**, *27*, 619−626.

Paolini, M.; Pozzetti, L.; Pedulli, G. F.; Marchesi, E.; Cantelli-Forti, G. The nature of prooxidant activity of vitamin C. *Life Sci.* **1999**, *64*, 273−278.

Prior, R. L.; Cao, G. Assessing antioxidant capacity in plant foods. *Free Radical Biol. Med*. **1999**, *27* (Suppl. 1), s11.

Retsky, K. L.; Freeman, M. W.; Frei, B. Ascorbic acid oxidation product(s) protect human low-density lipoprotein against

atherogenic modification. Anti-rather prooxidation activity of vitamin C in the presence of transition metal ions. *J. Biol. Chem*. **1993**, *263*, 1304−1309.

Retsky, K. L.; Chen, K.; Zeind, J.; Frei, B. Inhibition of copper-induced LDL oxidation by vitamin C is associated with increased copper-binding to LDL and 2-oxo-histidine formation. *Free Radical Biol. Med*. **1999**, *26*, 90−98.

Rice-Evans, C. A. Plant polyphenols: free radical scavengers or chain-breaking antioxidants? In *Free Radical and Oxidative Stress: Environments, Drugs and Food Additives*; Rice-Evans, C., Halliwell, B., Lunt, G. G., Eds.; Portland Press: 1995; pp 103−116.

Salah, N.; Miller, N. J.; Paganga, G.; Bolwell, G. P.; Rice-Evans, C. Polyphenolic flavonols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Arch. Biochem. Biophys*. **1995**, *322*, 339−346.

Stadtman, R. E. Protein modification in oxidative stress. In *Oxidative Processes and Antioxidants*; Paoletti, R., Ed.; Raven Press: New York, 1994; pp 117−134.

Stait, S. E.; Leake, D. S. Ascorbic acid can either increase or decrease low-density lipoprotein modification. *FEBS Lett*. **1994**, *341*, 263−274.

Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Koo, J. C.; Witzum, J. W. Beyond cholesterol modifications of low-density lipoprotein that increase arthrogenicity. *N. Engl. J. Med*. **1989**, *320*, 915−924.

Steinbrecher, U. P. Oxidation of human low-density lipoprotein results in derivatization of lysine residues of apoprotein B by lipid peroxide decomposition products. *J. Biol. Chem*. **1987**, *262*, 3603−3608.

Tesissere, R. L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of *in vitro* human LDL oxidation by phenolic antioxidants from grapes and wines. *J. Sci. Food Agric*. **1996**, *70*, 55−61.

Van Acker, S. A. B. E.; Van Den Berg, D. J.; Tromp, M. N. J. L.; Griffioen, D. H.; Van Bennekom, W. P.; Van Der Vijgh, W. J. F.; Bast, A. Structural aspects of antioxidant activity of flavonoids. *Free Radical Biol. Med*. **1995**, *20*, 331−342.

Vieira, O.; Escargueil-Blanc, I.; Meilhac, O.; Basile, J. P.; Laranjinha, J.; Almeida, L.; Salvayre, R.; Negre-Salvayre, A. Effect of dietary phenolic compounds on apoptosis of human cultured endothelial cells induced by oxidized LDL. *Br. J. Pharmacol*. **1998**, *123*, 565−573.

Virgili, R.; Kobuchi, H.; Packer, L. Procyanidins extracted from pines maritime (Pycnogenol): scavengers of free radical species and modulators of nitrogen monoxide metabolism in activated murine raw 264.7 macrophages. *Free Radical Biol. Med*. **1998**, *24*, 1120−1129.

Wijewickreme, A. N.; Kitts, D. D. Modulation of metal-induced genotoxicity by Maillard reaction products isolated from coffee. *Food Chem. Toxicol*. **1998**, *36*, 543−553.

Yan, L. J.; Droy-Lefaix, M. T.; Packer, L. *Ginkgo biloba* extract (Egb 761) protects human low-density lipoprotein against oxidative modification mediated by copper. *Biochem. Biophys. Res. Commun*. **1995**, *212*, 360−366.

Yen, G. C.; Chen, H. Y. Antioxidant activity of various tea extracts relation to their antimutagenicity. *J. Agric. Food Chem*. **1995**, *43*, 27−32.

Received for review February 4, 2000. Revised manuscript received May 2, 2000. Accepted May 2, 2000. We thank the National Science and Engineering Research Council for financial support of this research.

JF0001637