Antioxidant Activity of Extracts from Du-zhong (*Eucommia ulmoides*) toward Various Lipid Peroxidation Models in Vitro

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The antioxidant effect of extracts from leaves, raw cortex, and roasted cortex of Du-zhong was evaluated using various lipid peroxidation models. The inhibitory activity of extracts of Du-zhong (200 μg/mL) on the peroxidation of linoleic acid measured by thiocyanate method followed the order leaves (99.9%) > roasted cortex (95.9%) > raw cortex (77.2%) at 60 h of incubation. The IC_{20} for leaves, roasted cortex, and raw cortex on the peroxidation of liposome, induced by Fe^{3+}/H_2O_2/ascorbic acid, was <0.06, 0.24, and 0.81 mg/mL, respectively. The thiobarbituric acid reactive substances values for leaves, roasted cortex, and raw cortex were 0.12, 1.54, and 1.81 μmol of malondialdehyde/mg of protein in enzyme-mediated microsomal peroxidation and 0.08, 0.69, and 0.88 μmol of malondialdehyde/mg of protein in the nonenzyme-mediated microsomal peroxidation, respectively. The antioxidant activity of extracts of Du-zhong correlates to their polyphenol content. The results presented herein indicate that extracts of Du-zhong leaves may be useful in inhibiting membrane lipid peroxidation and preventing free radical-linked disease.

Keywords: *Du-zhong tea; water extracts; antioxidant activity; lipid peroxidation; free radical*

INTRODUCTION

Recent developments in biomedical science point to the involvement of free radicals in many diseases (Ames et al., 1993; Gutteridge and Halliwell, 1994). Free radicals attack the unsaturated fatty acids in the biomembrane, resulting in membrane lipid peroxidation, decrease in membrane fluidity, loss of enzyme and receptor activity, and damage to membrane proteins leading to cell inactivation (Dean et al., 1993). Free radicals also attack DNA and cause mutation leading to cancer (Cerutti, 1994; Diplock et al., 1994). Hence, identifying antioxidants that block the generation of free radical chain reactions is important.

The aqueous extract of *Eucommia ulmoides* leaves, commonly known as Du-zhong tea, is a popular folk drink in Japan, is thought to be a functional health food, and is commonly used for treatment of hypertension (Nakazawa, 1997). Research showed that Du-zhong is mild but long in duration and has antihypertensive and diuretic effects (Huang, 1993). Recently, their recuperative effects in hypercholesterolemia and fatty liver have also been reported (Nakasa et al., 1995). Roasted cortex or its powder is used traditionally as a medicinal food by Chinese women after birth labor; the raw cortex however, is not widely consumed. According to ancient records, the roasting process increases the physiological effect of Du-zhong cortex by promoting the absorption by the kidney. Roasted cortex is recommended as a folk medicine to reinforce muscles and lungs, lower blood pressure, and prevent miscarriages and occasionally to improve the tone of the liver and kidney and also to increase longevity.

Recently, it has been indicated that Du-zhong has an antimutagenic effect besides its physiological effect. Sasaki et al. (1996) reported that drinking Du-zhong (leaf) tea has a suppressing effect on mutagenicity of urine samples collected from individuals after ingestion of raw fish and cooked beef. Nakamura et al. (1997) demonstrated that Chinese hamster ovary (CHO) cells treated with Du-zhong tea crude extracts, following mitomycin C treatment, have reduced frequency of chromosome aberration. There is an important correlation between anticancer, antimutagenic, and antioxidant properties (Slaga, 1995). Consistent with this contention, Yen and Chen (1995) showed that the antioxidant activity of various tea extracts is related to their antimutagenicity. However, the relationship between the antioxidant activity of the leaves, raw cortex, and roasted cortex of Du-zhong has not been determined. The objective of this study was to address this aspect by comparing the inhibition of lipid peroxidation by the extracts from leaves, raw cortex, and roasted cortex of Du-zhong, using various lipid models in vitro.

MATERIALS AND METHODS

Materials. Du-zhong (*Eucommia ulmoides*) leaves, raw cortex, and roasted cortex were purchased from a local market in Taichung, Taiwan. Gallic acid, dihydronicotinamide adenine dinucleotide phosphate (NADPH), mannitol, adenosine diphosphate (ADP), linoleic acid, and ferrous chloride were purchased from Sigma Chemical Co. (St. Louis, MO). Ammonium thiocyanate, iron(II) chloride tetrahydrate, ascorbic acid, trichloroacetic acid (TCA), lecithin, and thiobarbituric acid (TBA) were purchased from E. Merck Co. (Darmstadt, Germany). Trolox was purchased from Aldrich Chemical Co. (Milwaukee, WI).

Preparation of Water Extracts from Du-zhong. The leaves, raw cortex, and roasted cortex of Du-zhong (20 g) were individually extracted with boiled water (200 mL) for 60 min, respectively. The extracts were filtered through Whatman No. 2 filter paper, and the filtrates were freeze-dried to powder form. The yields of extracts from leaves, raw cortex, and

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roasted cortex of Du-zhong were 1.92, 1.41 and 1.60 g, respectively.

Antioxidant Activity in Linoleic Acid Emulsion. The antioxidant activity of Du-zhong was determined using the thiocyanate method (Mitsuda et al., 1966). Each sample (500 μg) in 0.5 mL of deionized water was mixed with linoleic acid emulsion (2.5 mL, 0.02 M, pH 7.0) and phosphate buffer (2 mL, 0.2 M, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier, and 50 mL phosphate buffer, and then the mixture was homogenized. The reaction mixture was incubated at 37 °C. Aliquots of 0.1 mL were taken at different intervals during incubation. The degree of oxidation was measured according to the thiocyanate method by sequentially adding ethanol (4.7 mL, 75%), an ammonium thiocyanate (0.1 mL, 30%), sample solution (0.1 mL), and ferrous chloride (0.1 mL, 0.02 M in 3.5% HCl). After the mixture had stood for 3 min, the peroxide value was determined by reading the absorbance at 500 nm using a spectrophotometer (Hitachi U-2000). A control was performed with linoleic acid but without the extracts. Trolox and ascorbic acid were used as positive control.

Liposomes Preparation and Oxidation. Liposomes were prepared according to the method of Tsuda et al. (1994). Egg lecithin (5 g) was dispersed in sodium phosphate buffer (500 mL, 20 mM, pH 7.4) and sonicated in a Branson sonicator for 30 min, by which process small vesicles were obtained. All operations were carried out under N_2, and sonication was done in an ice-cold water bath. Various concentrations of the extracts were tested for lipid peroxidation activities as follows. The extract (0.5 mL) was mixed with liposomes (2.0 mL), 25 mM $FeCl_3$ (0.1 mL), 25 mM H_2O_2 (0.1 mL), 25 mM ascorbic acid (0.1 mL), and 0.2 M phosphate buffer (1.2 mL, pH 7.4). The reaction mixture was incubated at 37 °C for 2 h. At the end of incubation, 1 mL of BHT (20 mg/mL in methanol) was added to the mixture to stop the oxidation reaction. The extent of oxidation of liposomes was subsequently determined by the thiobarbituric acid reactive substances (TBARS) measurement. One milliliter each of 1% TBA and 10% HCl was added to the reaction, and then it was heated in a water bath at 100 °C for 30 min. After the mixture had cooled in an ice bath for 15 min, 5 mL of chloroform was added to the mixture, and the mixture was centrifuged at 1600*g* for 20 min. The absorbance of the supernatant was measured spectrophotometrically at 532 nm and quantified as micromoles of TBARS per milligram of protein using $\epsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$ (Tampo and Yonaha, 1995). The IC_{20} value was obtained by regression analysis. Gallic acid and mannitol dissolved in water were assayed for comparison of the results.

Liver Microsome Preparation and Assaying the Oxidation Induced by Enzymatic and Nonenzymatic Systems. Microsomes were prepared according to the method of Hanna et al. (1994). Microsomes were isolated from male Sprague−Dawley rats weighing ~200−220 g as follows: the liver was sliced and homogenized in 4 volumes of homogenization buffer (1.15% KCl/10 mM potassium phosphate buffer, pH 7.4). The homogenate was centrifuged at 600*g* for 10 min at room temperature, the resulting supernatant was recentrifuged at 4 °C and 9000*g* for 10 min, and the final supernatant was ultracentrifuged at 4 °C and 100000*g* for 45 min. The resulting pellet was resuspended in homogenization buffer. The protein concentration in microsome was determined according to the method of Lowry et al. (1951) using bovine serum albumin as a standard. Liver microsomal oxidation was performed according to a modified method of Okuda et al. (1983). The extract (0.2 mL) was mixed with liver microsomes (0.5 mg/mL protein). The mixtures were incubated in the presence or absence of either a reduced NADP (NADPH)-generating system (0.2 mL of 1 mM NADPH; 0.2 mL of 1 mM ADP, and 0.2 mL of 0.25 mM $FeCl_3$) or $FeCl_2$ system (0.2 mL of 2.5 mM $FeCl_2$; 0.2 mL of 2.5 mM H_2O_2) in a phosphate buffer (0.2 M, pH 7.4). Total final volume was 1 mL. The reaction mixtures were incubated at 37 °C for 1 h. At the end of incubation, 1 mL of BHT (20 mg/mL) was added to the mixtures to stop the oxidation reaction. The extent of oxidation of microsomes was measured by the TBARS measurement as described earlier, except that 35% TCA was used instead of 10% HCl.

Mitochondrial Preparation and Oxidation Challenge. Mitochondria were prepared according to the method of Ham and Liebler (1995). Mitochondria were isolated from male Sprague−Dawley rats weighing ~200−220 g as follows: rat livers were minced and then homogenized in 9 volumes of homogenization buffer (0.25 M sucrose in 5 mM Tris-HCl buffer, pH 7.4). The homogenate was centrifuged at 600*g* for 10 min, and the supernatant was centrifuged at 90000*g* for 15 min at 4 °C. The resulting pellet was washed three times with homogenization buffer, and the final pellet was resuspended in 0.15 M KCl in 20 mM Tris-HCl buffer (pH 7.4). The protein concentration in mitochondria was determined according to the method of Lowry et al. (1951) using bovine serum albumin as a standard. Oxidation of rat liver mitochondria was carried out in a reaction mixture containing 0.2 mL of mitochondria (equivalent to 0.5 mg of protein), 0.2 mL of extracts (0−0.20 mg/mL), 0.2 mL of 2.5 mM $FeCl_2$, 0.2 mL of 2.5 mM H_2O_2, and 0.2 mL of sodium phosphate buffer (20 mM, pH 7.4). The reaction mixtures were incubated at 37 °C for 1 h. At the end of incubation, 1 mL of BHT (20 mg/mL) was added to the mixture to stop the oxidation reaction. Finally, the oxidation of mitochondria was assessed by TBARS measurement, as described earlier for liver microsomes.

Determination of Total Polyphenolic Compounds. The concentration of phenolic compounds was measured according to the method of Taga et al. (1984) and calculated using gallic acid as standard. The Du-zhong powder (0.1 g) was dissolved in 5 mL of 0.3% HCl in methanol/water (60:40, v/v). The resulting solution (100 μL) was added to 2 mL of 2% Na_2CO_3. After 2 min, 50% Folin−Ciocalteu reagent (100 μL) was added to the mixture, which was then left for 30 min. Absorbance was measured at 750 nm using a spectrophotometer. Results were expressed as milligrams per gram of gallic acid equivalents (GAE).

Statistical Analysis. All results were obtained from three independent experiments and averaged. Data were analyzed using the Statistical Analysis System software package (SAS, 1985). Analyses of variance were performed by ANOVA procedures. Significant differences ($P < 0.05$) between means were determined by Duncan's multiple range test.

RESULTS AND DISCUSSION

Antioxidant Activity in Linoleic Acid Emulsion. The antioxidant effects of extracts from leaves, raw cortex, and roasted cortex of Du-zhong on the peroxidation of linoleic acid were investigated, and the results are shown in Figure 1. The oxidative activity of linoleic acid was markedly inhibited by all extracts tested. The antioxidant effects of extracts of Du-zhong after incubation for 60 h with linoleic acid are summarized in Table 1. Trolox and ascorbic acid were included as positive controls for the hydrophilic antioxidants. Taken together, the results show that the inhibitory potential follows the order Du-zhong leaves ≅ Trolox > roasted cortex > ascorbic acid > raw cortex. The Du-zhong leaves extracts and Trolox exhibited excellent antioxidant activity with 99.9 and 99.1% inhibition, respectively, of linoleic acid peroxidation. Raw cortex showed the weakest antioxidant activity. The antioxidant activity of raw cortex significantly increased after the roasting process (Table 1 and Figure 1). These results are consistent with those of Nicoli et al. (1997), who found that the coffee brews show strong overall antioxidant properties. This may mainly be attributed to the Maillard reaction products formed during the roasting process. Guillot et al. (1996) also showed that the degree of roasting of the coffee positively correlated with the inhibition of lipid peroxidation in rat liver mem-

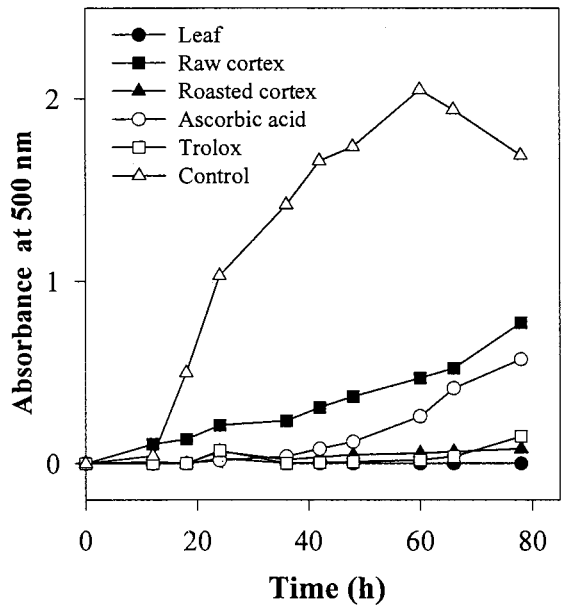

Figure 1. Antioxidant activities of water extracts from Du-zhong (*E. ulmoides*) as measured by the thiocyanate method. A high absorbance at 500 nm indicates a low antioxidant activity.

Table 1. Antioxidant Activity of Water Extracts from Du-zhong As Measured by the Thiocyanate Method after Incubation for 60 h

sample	absorbance at 500 nm	inhibition %[a]
control[b]	2.050 ± 0.026[e c]	0.00[e]
Du-zhong		
leaf	0.002 ± 0.001[a]	99.92 ± 0.02[a]
raw cortex	0.467 ± 0.028[d]	77.20 ± 1.38[d]
roasted cortex	0.085 ± 0.024[b]	95.87 ± 1.18[b]
ascorbic acid	0.257 ± 0.016[c]	87.45 ± 0.76[c]
Trolox	0.019 ± 0.007[a]	99.07 ± 0.35[a]

[a] Inhibition % (capacity to inhibit the peroxide formation in linoleic acid) = [1 − (absorbance of sample at 500 nm)/(absorbance of control at 500 nm)] × 100. A high inhibition % indicates a high antioxidant activity. [b] Control was incubated with linoleic acid but without the samples. [c] Results are presented as means ± standard deviations ($n = 3$). Values in a column with different superscripts are significantly different ($P < 0.05$).

branes and concluded that the heat process is responsible for the generation of novel antioxidant compounds. Ascorbic acid showed slightly higher inhibition of the linoleic acid peroxidation as compared to raw cortex.

Effect of Extracts of Du-zhong on Fe^{3+}/H_2O_2/Ascorbic Acid Induced Liposomal Lipid Peroxidation. Phospholipid liposomes provide an ideal model membrane system for studying the influence of dietary components and drugs on membrane lipid peroxidation in vitro and are thus used extensively (Chatterjee, 1988). We therefore evaluated the inhibitory effect of extracts of Du-zhong on membrane lipid peroxidation using this system. Lipid peroxidation was stimulated with Fe^{3+}/H_2O_2/ascorbic acid. The antioxidant activity of Du-zhong leaves extract was very high, with IC_{20} values <0.06 mg/mL. The IC_{20} values of roasted cortex and raw cortex were comparatively higher, 0.24 and 0.81 mg/mL, respectively. The roasted cortex extract had a moderate effect on inhibition of lipid peroxidation, whereas raw cortex was almost ineffective. This trend is similar to the antioxidant activity of extracts of Du-zhong toward linoleic acid. However, as shown in Table 2, gallic acid possesses a prooxidant effect and mannitol has a weak antioxidant activity. These results are

Table 2. Comparison of Gallic Acid, Mannitol, and Water Extracts from Du-zhong for 20% Inhibition (IC_{20}) of Liposome Peroxidation

sample	IC_{20}[a] (mg/mL)
Du-zhong	
leaf	<0.0625
raw cortex	0.8143 ± 0.159
roasted cortex	0.2363 ± 0.018
gallic acid	–[b]
mannitol	0.4673 ± 0.018

[a] The concentration of samples that inhibited Fenton-induced liposome peroxidation by 20% (IC_{20}) was determined by linear regression of inhibitory percentages. Results are presented as means ± standard deviations ($n = 3$). [b] Gallic acid represented the prooxidant action.

consistent with the reports of Aruoma (1991) and Aruoma et al. (1993). They reported that gallic acid has dual effects, as an antioxidant or prooxidant, depending on its concentration, in Fe^{2+}- or Cu^{+}-induced lipid peroxidation system. Halliwell and Gutteridge (1989) reported that peroxidation is uninhibited by hydroxyl radical scavengers, such as mannitol, when added to isolated cellular membrane fractions undergoing peroxidation in the presence Fe^{2+}/Fe^{3+} and a reducing agent. From these data, it can be concluded that the raw cortex had no antioxidant effect on lipid peroxidation in the liposomal system.

Effect of Extracts of Du-zhong on Enzyme- and Non-Enzyme-Induced Microsomal Lipid Peroxidation. Membrane lipids are particularly susceptible to oxidation, not only because of their high polyunsaturated fatty acid content but also because of their association in the cell membrane with enzymatic and nonenzymatic systems capable of generating free radical species. Microsomes are a heterogeneous mixture of vesicles derived from both endoplasmic reticulum and plasma membranes and are used as an in vitro test system to assess the lipid peroxidation effect of a wide range of drugs and dietary components. NADPH-cytochrome P-450 reductase is involved in NADPH-induced (i.e., enzymatically induced) microsomal lipid peroxidation, whereas Fe^{2+}/H_2O_2 induces peroxidation nonenzymatically (Buege and Aust, 1978; Halliwell and Gutteridge, 1989).

All extracts of Du-zhong and gallic acid, but not mannitol, dose-dependently inhibited the enzymatic and nonenzymatic lipid peroxidation of microsomal lipids (Figure 2). As shown in Figure 2, the leaves extract possessed significant inhibitory effects. There was approximately 93.9 and 95.8% inhibition of enzymatic and nonenzymatic peroxidation, respectively, at a concentration of 0.2 mg/mL of leaves extract. The leaves extract was significantly more potent than those of raw cortex and roasted cortex. The inhibitory effects of extracts of Du-zhong toward microsomal lipid peroxidation induced by the enzymatic and nonenzymatic systems followed a similar trend. Hanna et al. (1994) reported that the alcoholic and aqueous extracts of MA-631 (a complex herbal mixture) inhibited enzyme- and non-enzyme-induced rat liver microsomal lipid peroxidation in a concentration-dependent manner. Interestingly, the antioxidant potential of extracts of Du-zhong in the nonenzymatic system was more effective than in the enzymatic system. Malterud et al. (1993) studied the activity of anthraquinones and anthrones against nonenzymatic and enzymatic lipid peroxidation in vitro and their ability to scavenge free radical. They concluded that the antioxidant activities in nonenzymatic

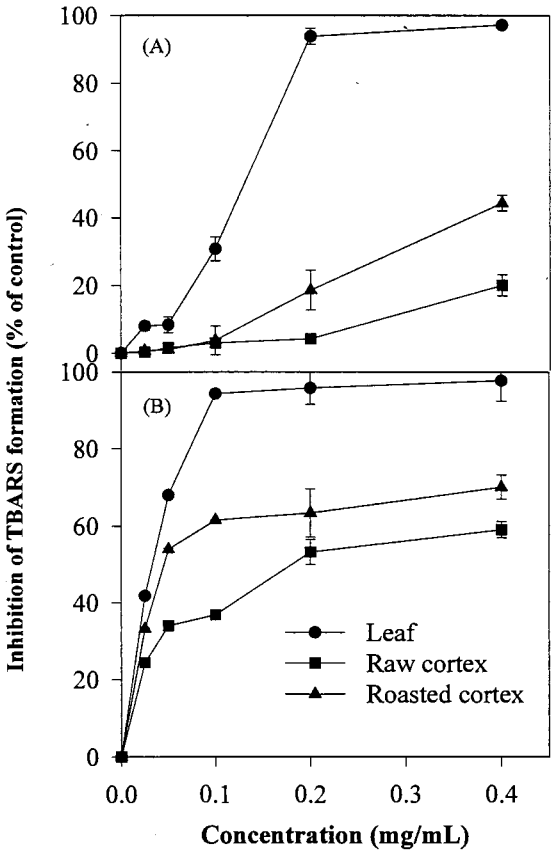

Figure 2. Effect of water extracts from Du-Zhong on the lipid peroxidation of microsomes: (A) enzymatic system induced by NADPH/ADP/Fe^{3+}; (B) nonenzymatic system induced by Fe^{2+}/H_2O_2. One hundred percent inhibition means that the peroxidation of microsomes was completely inhibited by Du-zhong extracts.

peroxidation in rat hepatocytes and the radical scavenging activities were correlated, whereas the inhibition of enzymatic lipid peroxidation showed no correlation with the other two effects. Therefore, we suggest that extracts of Du-zhong are more effective in suppressing the generation of free radicals or scavenging free radicals generated by the Fenton reaction in the nonenzymatic system. In addition, extracts of Du-zhong were less effective in inactivating the enzyme activity, as shown by a weaker inhibition of the lipid peroxidation. This suggests a weaker free radical blocking ability. Typical TBARS formation values of extracts of Du-zhong, gallic acid, and mannitol are shown in Table 3. In the microsomal system, formation of TBARS by the leaves extract and gallic acid was almost equal in enzymatic and nonenzymatic peroxidation. The roasted cortex and raw cortex exhibited around 7- and 9-fold higher TBARS values, respectively, compared to the leaves extract in the nonenzymatic system. These values were about 15- and 18-fold higher than in the enzymatic system.

Effect of Extracts of Du-zhong on Mitochondrial Lipid Peroxidation. The free radical theory of aging proposes that cellular aging results from the oxy-radical attack to the mitochondrial genome. Mitochondria generate high levels of oxygen radicals, and ~2% of all oxygen used by mammalian cells forms oxygen-activated species. The changes occurring in mitochondria upon aging are correlated with mitochondrial transcripts (Cotton et al., 1993). Furthermore, mitochondrial DNA mutations and an impairment of mitochondrial function may contribute to neurodegenerative diseases common

Table 3. Comparison of Gallic Acid, Mannitol, and Water Extracts from Du-zhong on TBARS Production in Microsomal Lipid Peroxidation[a]

sample (0.2 mg/mL)	TBARS (μmol/mg of protein) non-enzyme-induced lipid peroxidation	enzyme-induced lipid peroxidation
Du-zhong		
leaf	0.079 ± 0.007	0.115 ± 0.007
raw cortex	0.882 ± 0.007	1.807 ± 0.009
roasted cortex	0.692 ± 0.008	1.535 ± 0.030
gallic acid	0.115 ± 0.004	0.082 ± 0.003
mannitol	1.135 ± 0.004	1.823 ± 0.005

[a] The experiments were conducted essentially as described under Materials and Methods. TBARS production in microsomes exposed to nonenzymatic system induced by Fe^{2+}/H_2O_2 and enzymatic system induced by NADPH/ADP/Fe^{3+}. Results are presented as means ± standard deviation of three independent experiments.

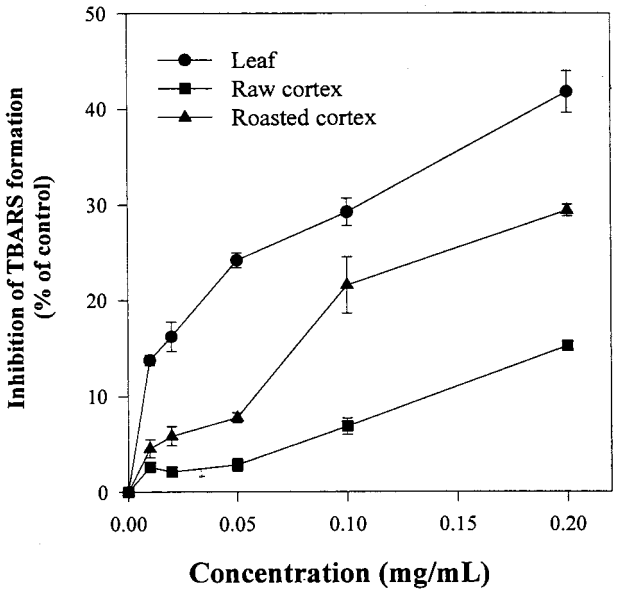

Figure 3. Effect of water extracts from Du-zhong on the lipid peroxidation of mitochondria induced by Fe^{2+}/H_2O_2. Inhibition of TBARS formation (percent of control) indicates the capacity to inhibit the TBARS formation in mitochondria. Each value represents mean ± standard deviation of three independent experiments.

in old age. The phospholipid bilayers of cellular and subcellular membranes are undoubtedly major targets for free radicals. Because redox reactions frequently occur in mitochondria, mitochondria are constantly susceptible to oxidative stress. We found that extracts of Du-zhong also exhibit a concentration-dependent antioxidant effect in the mitochondria. A comparison of the antioxidant activity of extracts of Du-zhong is presented in Figure 3. In this system the leaves extract was found to be the most potent, with an inhibition of 41.8% of the TBARS formation. The potency of the leaves extract was approximately 1.42-fold of the roasted cortex and 2.75-fold of raw cortex at a concentration of 0.2 mg/mL. The use of agents that improve mitochondrial function and neuronal bioenergetics has been proposed for treating neurodegenerative disorders (Sastre et al., 1995). Our results show that the leaves extract is the most potent inhibitor of mitochondrial peroxiadtion among all of the Du-zhong extracts tested. Du-zhong leaves thus have pharmacological potential for preventing the onset of radical-induced neurodegenerative disorders by protecting the function and morphology of mitochondria.

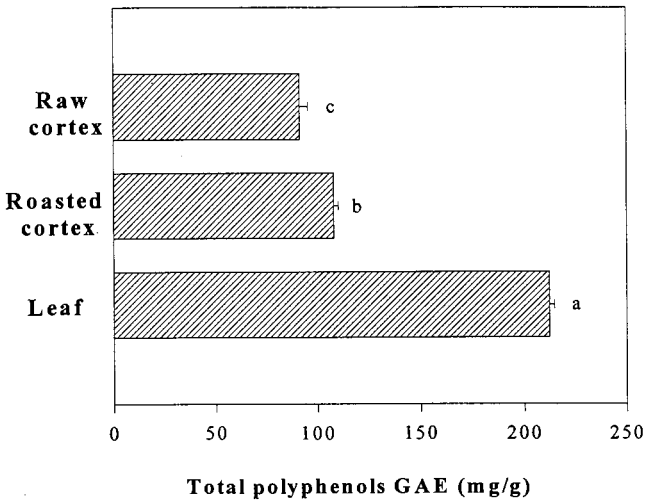

Figure 4. Polyphenol content of water extracts from Du-zhong. Total polyphenols are expressed as GAE. Average values ± standard deviations followed by different letters are significantly different ($P < 0.05$).

Total Polyphenol Content. It is well-known that plant polyphenolic extracts act as free radical scavengers and as antioxidants (Sato et al., 1996). We found that the extracts of Du-zhong leaves contain the highest polyphenol content (Figure 4). The polyphenolic content in roasted cortex is next, followed by that of raw cortex. Total polyphenol contents (GAE) of leaves, roasted cortex, and raw cortex of Du-zhong were 211, 110, and 87 mg/g, respectively. Our results show a good correlation between the polyphenol content of extracts of Du-zhong and the antioxidant activity. Lee et al. (1995) also indicated that the phenolic compounds of fresh pepper correlated well with antioxidant activity. Du-zhong leaves contain unique phytochemicals such as phenolic derivatives, flavonoids, irridoids, and triterpenids (Nakazawa, 1997). The phenolic derivatives—pyrogallol, protocatechuic acid, coumaric acid, and chlorogenic acid—as well as flavonoids—quercetin, kaempherol, and astragarin—have been isolated from Du-zhong tea (Nakamura et al., 1997). The antioxidant activities of phenolic derivatives and flavonoids have been extensively reported. Ueda et al. (1996) reported that protocatechuic acid greatly suppressed the DNA strand scission by •OH which was produced from the reaction of Cu^{2+} with H_2O_2. Chlorogenic acid could react with peroxyl radical (Kono et al., 1994; Laranjinha, 1994) and prevented the formation of the •OH by forming a chelate with the iron ion in the Fenton reaction (Kono et al., 1998). Laranjinha et al. (1995) indicated that *p*-coumaric acid has an antioxidant protection to LDL due to the chain-breaking activity. Also, a phenolic having a pyrogallol nucleus was a potent chelator to act primarily as an antioxidant, decreasing oxidative damage to biomolecules (Moran et al., 1997). Moreover, flavonoids, including quercetin, kaempferol, etc., are strong antioxidants that occur naturally in food and can inhibit carcinogenesis in rodents (Hertog et al., 1993b). The Zutphen elderly study concluded that flavonoids in regularly consumed foods might reduce the risk of death from coronary heart disease in elderly man (Hertog et al., 1993a). Therefore, from the above statement, the polyphenolic compounds appear to be mostly responsible for the antioxidant activity of the Du-zhong extracts, because Du-zhong leaves contain many antioxidant constituents. Furthermore, various antioxidant mechanisms of Du-zhong may be involved in different lipid peroxidation models. Polyphenolic compounds have more than one mechanism of action for free radicals and are able to suppress free radical reactions. Laughton et al. (1991) and Morel et al. (1993) have found that the polyphenols are able to act as antioxidants by virtue of the hydrogen-donating capacity of their phenolic groups. In addition, the metal-chelating potential of polyphenols may also play a role in the protection against iron- and copper-induced free radical reactions.

On the basis of the results of this study, it is clearly indicated that Du-zhong extract has significant antioxidant activity against various lipid peroxidation systems in vitro. Although Du-zhong leaves contain many antioxidants as stated above, further studies are required to reveal whether they contain the other antioxidants or whether the novel antioxidants are formed in the roasted cortex during the roasting process.

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