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Antioxidant Properties of Du-zhong (*Eucommia ulmoides* Oliv.) Extracts and Their Effects on Color Stability and Lipid Oxidation of Raw Pork Patties

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The ethanol extracts from leaf, roasted cortex, and seed of Du-zhong (*Eucommia ulmoides* Oliv.) were examined for total phenolics content (TPC) and total flavonoids content (TFC). The antioxidant activity of the extracts was evaluated by measuring the radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and lipid peroxidation inhibition capacity in a β-carotene/linoleic acid system. Du-zhong leaf extract was found to have the highest TPC content (94.46 $\pm$ 1.17 mg of gallic acid equiv/g of solid extract) and TFC content (61.36 $\pm$ 0.59 mg of catechin equiv/g of solid extract). In the above three antioxidant assay systems, Du-zhong leaf extract also exhibited the strongest antioxidant capacities, followed by roasted cortex extract and seed extract. The effects of Du-zhong extracts (leaf, roasted cortex, and seed) on lipid oxidation, meat color, and metmyoglobin (MetMb) formation in raw pork patties were investigated and compared with that of butylated hydroxytoluene (BHT) during refrigerated storage at 4 °C for 8 days. The results indicated that the addition of leaf extract at 0.1% (w/w), roasted cortex extract at 0.1% (w/w), and BHT at 0.01% (w/w) decreased day 8 TBARS values by 35, 20, and 37%, respectively. Du-zhong leaf extract at 0.1% (w/w) also exhibited a certain stabilizing effect on meat redness *a** value and retarded the formation of MetMb. This study suggests that Du-zhong leaf extract may be a potential source of natural antioxidants.

KEYWORDS: Antioxidant; phenolic compounds; *Eucommia ulmoides* Oliv.; lipid oxidation; raw pork patties

INTRODUCTION

Lipid oxidation and discoloration are two major factors that lead to the deterioration of meat and meat products during storage (*1*). Lipid oxidation in muscle foods results in a variety of breakdown products that affect meat flavor, color, texture, and nutritional value (*2*). In fresh meat and meat products, color is an important parameter used to judge the freshness and wholesomeness of meat, thereby influencing the purchase decision of consumers. Myoglobin pigments are responsible for the red color in raw meat. Generally, myoglobin in the ferrous state is oxidized to ferric metmyoglobin, resulting in a consumer-disliked brownish-red meat (*3*). Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG), are commonly employed as food antioxidants and can effectively inhibit lipid oxidation and discoloration in meat and meat products. However, their use in food has been restricted because of their potential toxicity and carcinogenicity (*4*, *5*). Under this situation, naturally occurring antioxidants have recently attracted special interest and are widely regarded as safe alternatives to synthetic antioxidants (*6*).

Since ancient times, herbs and spices have been added to foods to improve sensory properties and prolong the storage life. In addition to imparting characteristic flavors, certain herbs and spices have been recognized to have potential medicinal properties and possible health benefits. In the past few years, the antioxidant effects of many natural antioxidants obtained from medicinal plant materials were evaluated in meat and meat products (*2*, *7*, *8*). These herb extracts have been demonstrated to be effective in inhibiting lipid oxidation and stabilizing meat color, thus extending the shelf life of meat and meat products. The antioxidant properties of plant extracts are closely associated with their phenolic compounds such as phenolic acids, flavonoids, and aromatic compounds (*9*). The antioxidant effect of phenolic compounds is mainly due to their redox properties and chemical structures such as hydroxyl groups, which can scavenge free radicals, chelate metal ions, quench singlet and triplet oxygen, or decompose peroxides (*10*, *11*).

Eucommia ulmoides Oliv., also called Du-zhong, is one of the oldest tonic herbs in traditional Chinese medicine. The roasted cortex of Du-zhong, the dry heated outer portion of the stem, is a folk medicine traditionally used for reinforcing the muscles and lungs, lowering blood pressure, preventing miscarriages, improving the tone of the liver and kidneys, and increasing longevity (*12*). Its leaves have also been used in foods. Du-zhong tea, the aqueous extract of Du-zhong leaves, a popular drink in China and Japan, has been known as a functional health food and commonly used for the treatment of hypertension (*13*). Modern pharmacological

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Table 1. Extraction Yield, Total Phenolics Content (TPC), and Total Flavonoids Content (TFC) of Ethanolic Extracts from Leaf, Roasted Cortex, and Seed of Du-zhong[a]

extract	extraction batch (*n*)	extraction yield[b] (%, w/w)	TPC[c] (mg of GAE/g of solid extract)	TFC[d] (mg of CE/g of solid extract)
leaf	3	16.77 ± 0.35a	94.46 ± 1.17a	61.36 ± 0.59a
roasted cortex	3	9.83 ± 0.21c	40.07 ± 0.45b	16.84 ± 0.20b
seed	3	11.62 ± 0.47b	19.11 ± 0.12c	7.97 ± 0.11c

[a] The data are presented as mean ± standard error of three replications. Values in the same column followed by different letters (a−c) are significantly different ($P < 0.05$). [b] The extraction yield is presented as % weight (gram of solid extract for each 100 g of dry material). [c] Total phenolics content is expressed as mg of gallic acid equivalents (GAE)/g of solid extract. [d] Total flavonoids content is expressed as mg of catechin equivalents (CE)/g of solid extract.

research has shown that the cortex and leaves of Du-zhong have a series of pharmacological actions such as antioxidation, antifungal, anti-inflammatory, and antihypertensive (*14*). This plant, including the leaf or bark, is rich in polyphenolic compounds such as lignans, phenolic acids, and flavonoids (*15*), which are typical antioxidant components possessing multiple biological functions. The pharmacological properties of *E. ulmoides* Oliv. are closely related to these polyphenolic compounds. Yen and Hsieh (*16*, *17*) reported that the water extracts of Du-zhong, including leaves, raw cortex, and roasted cortex, have significant antioxidant activity against various lipid peroxidation systems in vitro and also can scavenge reactive oxygen species (ROS) such as hydroxyl radical, superoxide anion, and hydrogen peroxide. The antioxidant activity was correlated to the polyphenolic compounds in the water extracts of Du-zhong. The research of Ohmori et al. (*18*) showed that the aqueous extracts of *E. ulmoides* leaves have remarkable scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and an inhibitory effect against low-density lipoprotein (LDL) oxidation.

Previous studies on *E. ulmoides* Oliv. have mainly focused on its physiological actions and have demonstrated its remarkable antioxidant capacity. On the other hand, there are also a few reports that Du-zhong has been used as a feed additive to improve the meat quality of livestock and aquatic products. The work of Lee et al. (*19*) indicated that *E. ulmoides* leaf supplementation improved growth performance, blood, and meat quality parameters in growing and finishing pigs. Wang et al. (*20*) found that incorporating Du-zhong leaf or bark extract into pig diets improved carcass quality and muscle amino acid content. However, few studies have been conducted to investigate the feasibility of using Du-zhong as a potential natural antioxidant in foods and specifically the meat industry.

Therefore, the objectives of this study were (1) to evaluate the total phenolics and flavonoids content and the antioxidant capacities of ethanolic extracts from Du-zhong leaves, roasted cortex, and seeds, (2) to quantify levels of representative individual phenolic constituents present in the Du-zhong extracts by RP-HPLC, and (3) to investigate the effects of Du-zhong extracts on meat color, color stability, and lipid oxidation of raw pork patties.

MATERIALS AND METHODS

Plant Materials. Du-zhong (*E. ulmoides* Oliv.), including leaves, roasted cortex, and seeds, was obtained from 15-year-old Du-zhong trees within a woods in Luoyang County, Henan Province, China. Leaves and cortex of Du-zhong were collected in the middle of June, and the seeds were collected in later autumn when they were fully ripe. Leaves and seeds were air-dried at room temperature (25 °C) in the shade, and the cortex was roasted in a thermoelectric oven at 100 °C for 60 min to destroy the gutta-percha in the cortex. After that, leaves, roasted cortex, and seeds were randomly divided into three lots, respectively, for further use.

Chemicals and Reagents. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), 2,6-di-*tert*-butyl-4-methyphenol (BHT), Folin−Ciocalteu's phenol reagent, 1,1,3,3-tetraethoxypropane (TEP), and β-carotene were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). HPLC-grade methanol and formic acid were purchased from Fisher Scientific (Fair Lawn, NJ). Authentic standards, such as gallic acid, catechin, chlorogenic acid, caffeic acid, protocatechuic acid, rutin, quercetin, and kaempferol, were purchased from Tauto Biotech Co. Ltd. (Shanghai, China). All other chemicals and solvents used were of analytical grade and purchased from Lanyi Chemical Articles Co. Ltd. (Beijing, China).

Preparation of Herbal Extracts. For each lot, dried samples were further air-dried in a ventilated oven at 40 °C for 24 h, then ground into powder, and passed through a sieve (60 mesh, 0.3 mm aperture). Each 10 g of powdered samples (3 × 10 g for each lot) was extracted with 100 mL of 70% (v/v) aqueous ethanol at room temperature (25 °C) for 6 h in a shaking incubator (130 rpm). After extraction, the mixture was filtered, and the residue was re-extracted with 100 mL of 70% (v/v) aqueous ethanol overnight. The combined filtrates were centrifuged at 3600*g* for 10 min at 4 °C using a high-speed freezing centrifuge (GI-20G, Anke, Shanghai, China). The supernatants were collected and evaporated to a final volume of 45 mL under reduced pressure in a rotary evaporator (RE-52AA, Yarong Biochemical Analysis Co., Ltd., Shanghai, China) at 40 °C. The resulting solutions were defatted three times with petroleum ether to remove lipids. The remaining solutions were lyophilized, vacuum packaged, and stored at 4 °C until use. All lot extractions followed the same procedures, and the extraction yield is presented as percent weight (grams of solid extract for each 100 g of dry material), and the results are shown in **Table 1**.

Analysis of Total Phenolic Compound (TPC). The amount of total phenolics in extracts was determined using the Folin−Ciocalteu method as described by Zheng and Wang (*10*) with some modifications. An aliquot (1.0 mL) of appropriately diluted extracts solution was added into test tubes, and then 5.0 mL of diluted (1:10 with distilled water) Folin−Ciocalteu reagent was added with mixing. After 5 min, 4.0 mL of 7.5% (w/v) Na_2CO_3 solution was added. The test tubes were properly shaken and then incubated for 1 h in the dark at room temperature. The absorbance of the mixture was determined at 765 nm using a Unicam UV4 spectrophotometer (Unicam Ltd., China). Quantification was done on the basis of a standard curve prepared using gallic acid, and the results are expressed as milligrams of gallic acid equivalent (GAE) per gram of solid extract.

Determination of Total Flavonoids Content (TFC). TFC was measured by colorimetric assay according to the method of Kim et al. (*21*). The absorbance was determined at 510 nm using a Unicam UV4 spectrophotometer (Unicam Ltd., China). The TFC was calculated from a standard curve prepared with catechin and expressed as milligram of catechin equivalent (CE) per gram of solid of extract.

HPLC Analysis of Selected Phenolic Compounds in Extracts. The selected phenolics constituents in Du-zhong extracts, namely, phenolic acids (chlorogenic acid, caffeic acid, protocatechuic acid) and flavonoids (rutin, quercetin, kapempferol), were identified by using a reverse-phase high-performance liquid chromatography (RP-HPLC) method. The preparative HPLC apparatus was a Shimadzu HPLC system (model LC-10ATvp, two pumps and DGU-12A degasser) equipped with a diode array detector (model SPD-M10Avp) (Shimadzu, Kyoto, Japan). The column used was a Diamonsil C18 (*2*) (250 mm × 4.6 mm i.d., 5 μm particle size). The HPLC conditions used were based on the procedure of Luo et al. (*22*) with minor modifications. The mobile phase consisted of solvent (A) water containing 0.1% formic acid and solvent (B) methanol. The gradient elution program was as follows: 0−5 min, 20% B; 5−18 min, 20−30% B; 18−23 min, 30% B; 23−35 min, 30−50% B; 35−50 min, 50−90% B; 50−65 min, 90−20% B; 65−70 min, 20% B. The flow rate of the mobile phase was 0.8 mL/min, and the injection volume was 10 μL. Column temperature was set at 30 °C. Detection was carried out between the wavelengths of 200 and 450 nm. Identification of individual phenolic constituents was based on the comparison of the retention time and UV spectrum of unknown peaks with those of reference authentic standards

analyzed under the same chromatographic conditions. The phenolic compounds were expressed as milligrams per gram of solid extract.

Antioxidant Activity of Scavenging DPPH Radical. The DPPH radical scavenging capacity of each herbal extract was evaluated according to the method described by Zhang et al. (*23*) with minor modifications. DPPH radical was prepared in ethanol to a final concentration of 0.1 mmol/L. An amount of 0.2 mL samples at a concentration of 1 mg/mL (solid extract dissolved in 70% ethanol) was added to 4 mL of freshly prepared DPPH radical solution, and the mixture was kept in the dark for 1 h. The absorbance of the reaction mixture was measured at 517 nm. A control was measured in the same way except that the extract was replaced by 70% ethanol. The scavenging activity was calculated by the equation

$$\text{scavenging activity } (\%) = [1 - (A_1/A_0)] \times 100$$

where A_1 is the absorbance of the sample and A_0 is the absorbance of the control. BHT was used as positive control.

Ferric Reducing Antioxidant Power (FRAP) Assay. The antioxidant capacity of each extract was measured following the procedure described by Tian et al. (*24*), with a little modification. Briefly, 0.12 mL of sample and 0.36 mL of deionized water were added to 3.6 mL of freshly prepared and warmed (37 °C) FRAP reagent. The mixture was shaken well and kept in the water bath at 37 °C for 10 min. Then, the absorbance of the mixture was determined at 593 nm. The FRAP reagent was prepared by mixing 0.3 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ solution (dissolved in 40 mmol/L HCl), and 20 mmol/L $FeCl_3 \cdot 6H_2O$ solution in proportions of 10:1:1 (v/v), respectively. The results were calculated by standard curves prepared with known concentrations of $FeSO_4 \cdot 7H_2O$ and expressed as EC_1 value, which means the concentration of antioxidant in the reactive system having a ferric-TPTZ reducing ability equivalent to that of 1 mmol/L $FeSO_4 \cdot 7H_2O$. BHT was used as positive control.

Antioxidant Activity in β-Carotene/Linoleic Acid System. The antioxidant activities of samples were measured according to the method of Tian et al. (*24*). Half a milliliter of β-carotene in chloroform (2 mg/mL) was added into a flask containing 0.2 mL of linoleic acid in chloroform (100 mg/mL) and 1.0 mL of Tween 80 in chloroform (200 mg/mL). The chloroform was removed by rotary evaporation at 40 °C for 5 min, followed by 100 mL of deionized water that was slowly added to the residue with vigorous agitation to form an emulsion. Forty-five milliliters of the emulsion was mixed with 4.0 mL of phosphate buffer (0.2 M, pH 6.84) to gain the reactive reagent. An 8.0 mL aliquot of this reagent was added to a tube containing 0.2 mL of test sample at a concentration of 1 mg/mL (solid extract dissolved in 70% ethanol), and the absorbance was measured at 470 nm immediately. The reaction mixture was incubated at 50 °C in a water bath for 30 min for thermal autoxidation, and the absorbance was measured again. A control was prepared using 0.2 mL of 70% ethanol instead of the test sample. The antioxidant activity (AA) of sample was evaluated in terms of bleaching of β-carotene using the formula

$$\text{AA } \% = [1 - (S_{A0} - S_{At})/(C_{A0} - C_{At})] \times 100$$

where S_{A0} and C_{A0} are the absorbance values of the sample and the control determined at 0 min; the S_{At} and C_{At} were the absorbance values of test sample and control measured after 30 min. BHT was used as positive control.

Preparation of Pork Meat Samples. A total of 12 pork strip loins (longissimus dorsi muscle at the 8−13th thoracic vertebra) were obtained from porcine carcasses at a local abattoir 24 h post-mortem and then transported to the laboratory under refrigerated conditions (4 °C) within 1 h. After removal of visible fat and connective tissues, four loins were finely minced twice using a conventional mincer (model MM 12, Shaoguan Food Machinery Co., Ltd., Guangdong, China) through a plate with 6 mm holes and then divided into five equal-quantity sections (15 total sections). One of five treatments was assigned randomly to each of the sections by using a completely randomized block design, resulting in three treatment replications. All meat processing was performed at 4 °C. Each section (900 g) was mixed homogeneously with a total of 100 mL of Du-zhong extract solution (prepared by dissolving solid extract in 70% (v/v) aqueous ethanol, with final concentration made up to 10 mg/mL). Treatment groups included (a) leaf extract of Du-zhong at 0.1% (w/w) (percentage of solid extract in the final meat mixture), (b) roasted cortex extract of Du-zhong at 0.1% (w/w), (c) seed extract of Du-zhong at 0.1% (w/w), (d) BHT at 0.01% (w/w), and (e) the control (no extract, only 100 mL of 70% (v/v) aqueous ethanol). Each treated section was formed into 15 patties (60 g portions, 1.2 cm thick, 8.5 cm diameter), which were randomly designated for traits analysis on days 0, 2, 4, 6, and 8 of storage, using a meat former and placed on styrofoam trays. Each tray with the round pork patty was overwrapped with polyvinyl chloride film (23 °C, 60% RH, 350−400 $cm^3\ m^{-2}\ h^{-1}\ atm^{-1}$, Mitsui Chemical, Japan) and stored in a 4 °C refrigerator (dark). Lipid oxidation, metmyoglobin (MetMb, %), and the color of the samples were determined at 0, 2, 4, 6, and 8 days of storage.

Measurement of Lipid Oxidation. Lipid oxidation was determined by measuring 2-thiobarbituric acid-reactive substances (TBARS) content using the method described by Mielnik et al. (*25*) with little modification. In brief, 10 g of meat sample was homogenized (15000 rpm, 30 s) in 50 mL of 7.5% trichloroacetic acid solution with a superfine homogenizer (F6-10, Fluko, Shanghai, China). The dispersion was filtered through Whatman no. 1 filter paper. The supernatant (5 mL) was mixed with 5 mL of 0.02 mol/L aqueous 2-thiobarbituric acid (TBA), heated in a boiling water bath for 30 min to develop the rose-pink color, and subsequently cooled for 10 min in cold water. The absorbance was measured at 532 nm against a blank prepared with 5 mL of distilled water and 5 mL of TBA reagent, using a Unicam UV4 spectrometer (Unicam Ltd., China). The TBARS values expressed as milligrams of malondialdehyde per kilogram of meat sample were calculated from a standard curve of TEP standards.

Determination of Metmyoglobin. Pattie samples (5 g) were homogenized together with 25 mL of ice-cold 0.04 M phosphate buffer (pH 6.8) for 10 s using a superfine homogenizer (F6-10, Fluko), which was set at speed setting 2 (10000 rpm). The homogenate was allowed to stand for 1 h at 4 °C and centrifuged at 4500*g* for 20 min at 4 °C using a high-speed freezing centrifuge (GI-20G, Anke, Shanghai, China). The absorbance of the filtered supernatant was read at 572, 565, 545, and 525 nm with a Unicam UV4 spectrometer (Unicam Ltd., China). The percentage of metmyoglobin was determined using the formula of Krzywicki (*26*):

$$\text{MetMb } (\%) = [-2.514(A_{572}/A_{525}) + 0.777(A_{565}/A_{525}) + 0.8(A_{545}/A_{525}) + 1.098] \times 100$$

Color Measurement. The color of pork patties was determined using a reflectance colorimeter (WSC-S, Shanghai, China), which was standardized with a white tile [$L^* = 94.61$, $a^* = -0.52$, and $b^* = 1.88$]. Illuminant A, 10° standard observer, and a 3.18 cm diameter aperture were used when collecting values. Values for CIE L^* (lightness), a^* (redness), and b^* (yellowness) were measured. Each patty was scanned at three different locations, and values were averaged for statistical analyses.

Statistical Analysis. The design for experiments of investigating the effects of Du-zhong extracts on meat color, color stability, and lipid oxidation of raw pork patties was a split plot. In the whole plot (complete randomized block), 12 loins served as blocks, and each of the five sections with 4 loins minced together was an experimental unit to which treatments were applied randomly. This resulted in three replications of the five treatments. In the subplot, each of the 15 patties from a section was considered to be an experimental unit to which storage time (0, 2, 4, 6, and 8 days) was assigned randomly.

The data for meat color, MetMb (%), and lipid oxidation of raw pork patties were subjected to analysis of variance by the General Linear Model (GLM) procedure with repeated measures using the SPSS 16.0 statistical package, and other data were analyzed by one-way analysis of variance (ANOVA). Duncan's multiple-range tests were used to compare the significant differences among the mean values, and differences at $P < 0.05$ were considered to be statistically significant. The results are presented as mean values ± SE (standard error).

RESULTS AND DISCUSSION

Total Phenolics, Total Flavonoids, and Quantitative Analysis of Individual Phenolic Constituents. The contents of total phenolics and total flavonoids in Du-zhong extracts are summarized in **Table 1**. The contents of total phenolics and total flavonoids in the extracts of leaf, roasted cortex, and seed of Du-zhong are 94.46 ± 1.17, 40.07 ± 0.45, and 19.11 ± 0.12 mg of GAE/g of solid extract and 61.36 ± 0.59, 16.84 ± 0.20, and 7.97 ± 0.11 mg of CE/g of solid extract, respectively. There were significant differences at a

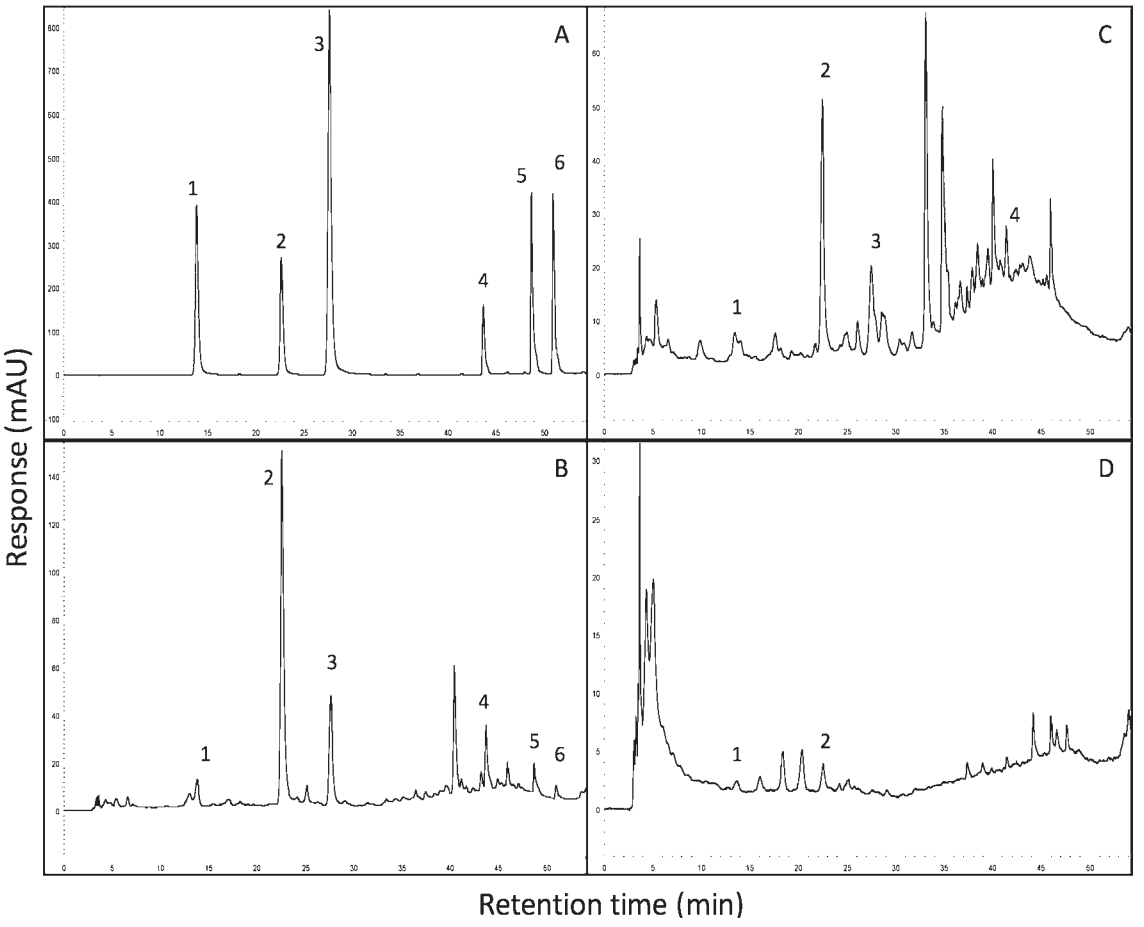

Figure 1. HPLC chromatograms of authentic standards and ethanolic extracts from Du-zhong: (**A**) authentic standards (1, protocatechuic acid; 2, chlorogenic acid; 3, caffeic acid; 4, rutin; 5, quercetin; 6, kaempferol); (**B**) Du-zhong leaf extract; (**C**) Du-zhong roasted cortex extract; (**D**) Du-zhong seed extract. Detection was carried out at 280 nm.

Table 2. Contents of Chlorogenic Acid, Caffeic Acid, Protocatechuic Acid, Rutin, Quercetin, and Kaempferol in Ethanolic Extracts from Leaf, Roasted Cortex, and Seed of Du-zhong[a]

sample	chlorogenic acid	caffeic acid	protocatechuic acid	rutin	quercetin	kaempferol
leaf	18.39 ± 0.09a	2.55 ± 0.08a	1.60 ± 0.03a	9.99 ± 0.07a	3.51 ± 0.05	1.10 ± 0.04
roasted cortex	7.38 ± 0.12b	1.54 ± 0.05b	1.02 ± 0.06b	2.46 ± 0.05b	nd[b]	nd
seed	0.67 ± 0.01c	nd	0.26 ± 0.03c	nd	nd	nd

[a] Each value is the mean ± standard error of three replicate analysis. Values (mg/g of solid extract) in the same column followed by different letters (a−c) are significantly different ($P < 0.05$). [b] Not detectable.

level of $P < 0.05$ in the mass amounts of total phenolics and total flavonoids among the three extracts. Leaf extract was found to have the highest phenolic and flavonoid contents, whereas seed extract had the lowest. This result was in agreement with previous research by Zhang et al. (*27*), who determined the TPC of water extracts from leaf, male flower, raw cortex, and fruit of *E. ulmoides* Oliv. They also found the leaf extract contained much more phenolic content than other extracts.

The phenolic chromatograms from ethanolic extracts of leaf, roasted cortex, and seed of Du-zhong are shown in **Figure 1**. **Figure 1A** shows the chromatogram of authentic standard. The chromatograms of extracts of leaves, roasted cortex, and seed of Du-zhong are given in panels **B**−**D**, respectively. Each phenolic constituent was identified by comparison to the retention time of authentic standards (**Figure 1A**), whereas the quantitative data were calculated from their calibration curves. **Table 2** shows quantitative analysis results of individual phenolic constituents in three Du-zhong extracts. Considerable variation was found in phenolic compounds of different parts extracts of Du-zhong. Du-zhong leaf extract had significantly higher ($P < 0.05$) phenolic acid contents as compared to roasted cortex and seed. Chlorogenic acid was the predominant phenolic constituent in the extracts of leaf and roasted cortex. For Du-zhong seed extract, all kinds of analyzed phenolic compounds were trace constituents. Only minute amounts of chlorogenic acid (0.67 ± 0.01 mg/g of solid extract) and protocatechuic acid (0.26 ± 0.03 mg/g of solid extract) were detected.

Yen and Hsieh (*17*) reported that protocatechuic acid was the main compound in the water extract of Du-zhong leaf and its content was up to 17.17 mg/g of solid extract, in contrast to our findings, where only a small amount of protocatechuic acid (1.60 mg/g of solid extract) was found in Du-zhong leaf extract. This difference may be explained by the fact that different extraction methods and solutions were used. Apart from that, many studies have shown that various factors, such as plant origin, growing season, and growing location, could influence the contents of bioactive compounds. Luo et al. (*22*) also quantified some bioactive compounds of 50% methanol extracts from Du-zhong leaf

Table 3. DPPH Radical Scavenging Activity, Ferric Reducing Antioxidant Power (FRAP), and Antioxidant Activity against β-Carotene/Linoleic Acid System of Ethanolic Extracts from Leaf, Roasted Cortex, and Seed of Du-zhong[a]

sample	DPPH scavenging activity (%)	ferric reducing power EC_1[b](mg/mL)	antioxidant activity[c] (%)
leaf	81.40 ± 3.63a	0.72 ± 0.02c	43.58 ± 3.29b
roasted cortex	16.72 ± 0.25b	2.81 ± 0.09b	26.71 ± 2.21c
seed	7.65 ± 0.20c	8.43 ± 0.75a	25.10 ± 1.37c
BHT	76.60 ± 2.60a	0.42 ± 0.01c	97.46 ± 0.3 1a

[a] The data are presented as mean ± standard error of three replications. Values in the same column followed by different letters (a−c) are significantly different ($P < 0.05$). [b] Ferric reducing power was expressed as EC_1 value; the unit of EC_1 was mg of extract dry weight/mL in ethanolic solution. [c] The antioxidant activity against the β-carotene/linoleic acid system and DPPH radical scavenging acitivity were measured at a concentration of 1 mg of dry extract/mL in ethanolic solution.

and cortex by HPLC analysis. Their results showed that chlorogenic acid content was remarkably higher as compared to other compounds in the leaves. Roasted cortex did not contain the compounds rutin, quercetin, and kaempferol, whereas the leaves had higher contents of rutin and a small amount of quercetin and kaempferol. Our data are consistent with these results.

In Vitro Antioxidant Capacities of Du-zhong Extracts. In this study, three methods have been used to measure the antioxidant properties of Du-zhong extracts: DPPH radical scavenging assay, FRAP assay, and β-carotene/linoleic acid system.

The scavenging effects of three Du-zhong extracts and BHT on DPPH radical are presented in **Table 3**. The results showed that leaf extract exhibited the highest DPPH radical scavenging activity with an inhibition rate of 81.40%, followed by BHT (76.60%) and roasted cortex extract (16.72%), whereas seed extract had the lowest activity at 7.65%. The DPPH radical scavenging activity of leaf extract was ~10 times higher than that of seed extract. However, there was no significant difference ($P < 0.05$) in the DPPH radical scavenging effect between leaf extract and BHT.

The FRAP of three Du-zhong extracts was expressed in EC_1 values (mg/mL), which are also shown in **Table 3**. The lower the EC_1 value, the higher the ferric reducing activity of a sample. In this assay, the order of ferric reducing activities of three Du-zhong extracts, compared with positive control, was BHT > leaf > roasted cortex > seed. BHT and leaf extract have significantly higher ferric reducing activity than roasted cortex and seed, but there was no statistically remarkable difference ($P < 0.05$) between BHT and leaf extract, which was similar to the result of the scavenging effect on DPPH radicals.

As can be seen in **Table 3**, where the values of antioxidant activity (%) for Du-zhong extracts in the β-carotene/linoleic acid emulsion system at an antioxidant concentration of 1 mg/mL are presented, all of the extracts of Du-zhong showed a certain extent of antioxidant activity. Similar to the results of DPPH radical scavenging effect and ferric reducing effect, leaf extract also possessed better antioxidant capacity (43.58%) than roasted cortex (26.71%) and seed (25.10%), but it was not as good as BHT. In this assay, the antioxidant activity of BHT was found to be significantly more pronounced (97.46%) than that of all three Du-zhong extracts.

In our results, the extract from the leaf of Du-zhong shows a stronger antioxidant activity than those from roasted cortex and seed of Du-zhong in all three assay systems. The reason is probably due to its remarkably higher phenolic and flavonoid contents, which possess a large number of aromatic hydroxyl groups. As is well-known, the phenolic and flavonoid compounds in natural substances have excellent antioxidant qualities. The TPC and TFC have been shown to be positively correlated with antioxidant ability (*28*). Yen and Hsieh (*16*) concluded that the

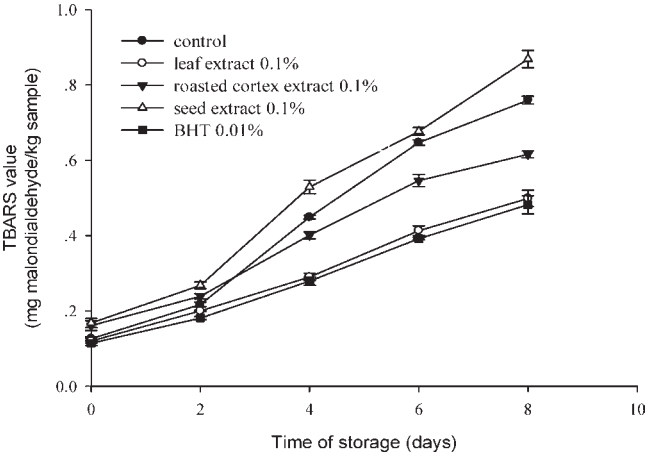

Figure 2. Effects of three Du-zhong extracts added at 0.10% (w/w) and BHT added at 0.01% (w/w) on TBARS values (mg of malondialdehyde equivalent/kg of sample) of raw pork patties during 8 days of refrigerated storage at 4 °C. Results are given as mean ± standard error.

polyphenolic compounds appear to be mostly responsible for the antioxidant activity of water extracts from Du-zhong leaves and cortex. Previous literature has demonstrated that the interaction of a potential antioxidant with DPPH depends on its structural conformation. The number of DPPH molecules that are reduced seems to be correlated with the number of electron-donating hydroxyl groups in the antioxidant molecule (*29*). This structural requirement could be linked to the presence of phenolic compounds, which are known to be widely distributed in natural herb and spice extracts. In the β-carotene/linoleic acid system assay, antioxidants are capable of reducing the rate of chain reaction initiated during lipid peroxidation mainly by scavenging the intermediate peroxyl free radicals formed when linoleic acid is oxidized. This also depends on the hydrogen-donating ability of antioxidants (*24*). Koleva et al. (*30*) reported that the sample polarity was important for the exhibited activity in the β-carotene bleaching test but not for the DPPH method; apolar antioxidants exhibit stronger antioxidative properties in emulsions because they concentrate at the lipid/air surface, thus ensuring high protection of the emulsion itself. Although the extract from the leaf of Du-zhong possess higher TPC, the TPC gives an indication of the levels of both lipophilic and hydrophilic compounds. The β-carotene/linoleic acid system assay, in contrast, gives only an indication of the levels of lipohilic compounds. Probably this is why leaf extract was found to have comparable DPPH radical scavenging effect and ferric reducing effect but was less active in the β-carotene/linoleic acid system assay than BHT. This implies the presence of relatively small amounts of lipophilic antioxidant compounds in the leaf extract.

Effects of Du-zhong Extracts on Lipid Oxidation of Raw Pork Patties. Effects of three Du-zhong extracts and BHT treatment on TBARS value in raw pork patties over 8 days of refrigerated storage are shown in **Figure 2**. All of the treatments, other than seed extract treatment, significantly ($P < 0.05$) decreased the TBARS values after day 2 of storage compared to control. The addition of leaf extract, roasted cortex extract, and BHT decreased day 8 TBARS values by 35, 20, and 37%, respectively, thus indicating high protection of meat against lipid oxidation. The lipid oxidation inhibition effect was highest ($P < 0.05$) in leaf extract compared to roasted cortex and seed at all storage times. However, no difference was observed between leaf extract and BHT treated patties; treatment with 0.1% Du-zhong leaf extract inhibited lipid oxidation to the same extent as 0.01% BHT.

Table 4. Color Changes of Raw Pork Patties Treated with Extracts from Leaf, Roasted Cortex, and Seed of Du-zhong at 4 °C[a]

		treatments[b]				
trait	days	control	leaves	roasted cortex	seeds	BHT
lightness (*L*)	0	61.02 ± 0.29aw	58.35 ± 0.40ax	56.83 ± 0.57bcxy	56.52 ± 0.45ay	60.52 ± 0.84aw
	2	60.77 ± 0.12aw	59.18 ± 0.10ax	58.04 ± 0.12ay	56.69 ± 0.53az	60.55 ± 0.52aw
	4	59.65 ± 0.30bw	56.63 ± 0.15bx	57.43 ± 0.35abx	55.11 ± 0.29by	59.86 ± 0.22abw
	6	58.27 ± 0.01cw	56.40 ± 0.39bx	56.26 ± 0.27cx	54.21 ± 0.34by	58.61 ± 0.55bcw
	8	57.67 ± 0.22cw	55.28 ± 0.29cx	54.03 ± 0.22dy	50.07 ± 0.07cz	56.97 ± 0.33cw
redness (*a**)	0	8.67 ± 0.12aw	8.01 ± 0.07ax	7.48 ± 0.05ay	6.09 ± 0.12az	7.92 ± 0.08ax
	2	7.84 ± 0.06bw	7.27 ± 0.18bx	6.41 ± 0.05by	5.04 ± 0.08bz	7.30 ± 0.04bx
	4	6.62 ± 0.10cw	6.60 ± 0.12cw	5.77 ± 0.08cx	4.36 ± 0.10cy	6.57 ± 0.12cw
	6	5.34 ± 0.23dx	5.98 ± 0.09dw	5.17 ± 0.09dx	3.88 ± 0.07dy	5.79 ± 0.15dw
	8	4.75 ± 0.11ex	5.37 ± 0.05ew	4.82 ± 0.06ex	3.50 ± 0.16ey	5.29 ± 0.08ew
yellowness (*b**)	0	14.79 ± 0.11dz	15.93 ± 0.07dy	17.03 ± 0.17bw	16.19 ± 0.17cxy	16.70 ± 0.27bwx
	2	15.50 ± 0.05cz	16.02 ± 0.14dy	17.26 ± 0.20bw	16.25 ± 0.08cy	16.77 ± 0.17bx
	4	15.99 ± 0.17by	16.51 ± 0.16cxy	17.72 ± 0.42bw	17.27 ± 0.23bwx	17.16 ± 0.21bwx
	6	15.98 ± 0.05bx	16.94 ± 0.18bw	17.09 ± 0.32bw	16.97 ± 0.11bw	16.96 ± 0.20bw
	8	17.43 ± 0.17az	18.53 ± 0.05ax	18.94 ± 0.13aw	19.03 ± 0.05aw	18.17 ± 0.08ay

[a] Values are means ± standard error. Values in the same column not followed by a common letter (a−e) are significantly different ($P < 0.05$). Values in the same row not followed by a common letter (w−z) are significantly different ($P < 0.05$). [b] Treatments with Du-zhong extracts (leaf, roasted cortex, and seed) were added at 0.10% (w/w); treatments with BHT were added at 0.01% (w/w).

The large amount of phenolic constituents (such as phenolic acids and flavonoids) contained in Du-zhong leaf extract may cause its strong antioxidant ability. Phenolic antioxidants are believed to intercept the free radical chain of oxidation and to give hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product that does not initiate or propagate further oxidation of lipid (*31*). DeJong and Lanari (*32*) reported that the polyphenol extract from the wastewater of olive oil pomace significantly inhibited lipid oxidation in precooked ground beef and pork. They also evaluated the effect of the individual polyphenol in the TBARS values of cooked beef patties. The results suggested that quercetin and rutin as well as caffeic acids exhibited remarkable inhibiting effects on TBARS value. Moreover, they concluded that in polyphenolic compounds, the biggest structural contributors to antioxidant capacity in cooked meat are the *o*-OH groups. In an earlier study by Yen and Hsieh (*16*), it was confirmed that the water extracts of Du-zhong leaves possessed high inhibitory activity against membrane lipid peroxidation by using various lipid peroxidation models and the antioxidant activity correlated to their polyphenol content. In our study, Du-zhong leaf extract was determined to contain a number of phenolic compounds such as chlorogenic acid, caffeic acid, protocatechuic acid, rutin, and quercetin; the antioxidant potential may also due to the additive and synergistic effects of these individual compounds present.

Effects of Du-zhong Extracts on Meat Color and Metmyoglobin Formation. The effects of Du-zhong extracts on meat color changes of raw pork patties during refrigerated storage at 4 °C are shown in **Table 4**. The Du-zhong extracts had an immediate effect on the color parameters of the pork samples after treatments compared with the control. At the initial stage (day 0), the addition of 0.1% Du-zhong extracts (leaf, roasted cortex, and seed) significantly decreased the *L** values (lightness) but increased the *b** values (yellowness) of the pork samples when compared to the control ($P < 0.05$). The redness *a** value followed a pattern similar to the lightness. The redness declined ($P < 0.05$) incrementally with the addition of roasted cortex extract and seed extract, but to a lesser degree in leaf extract and BHT. As storage time progressed, patties treated with Du-zhong leaf extract and BHT had higher *a** values ($P < 0.05$) than control patties at days 6 and 8. In all of the treatments, the *a** values in raw pork patties decreased progressively over the 8 day storage period ($P < 0.05$). However, the Du-zhong leaf extract treatment had a slower rate of *a** decrease than the control. The results indicated that the addition of Du-zhong leaf extract exhibited stabilizing effects on the redness value to a certain extent.

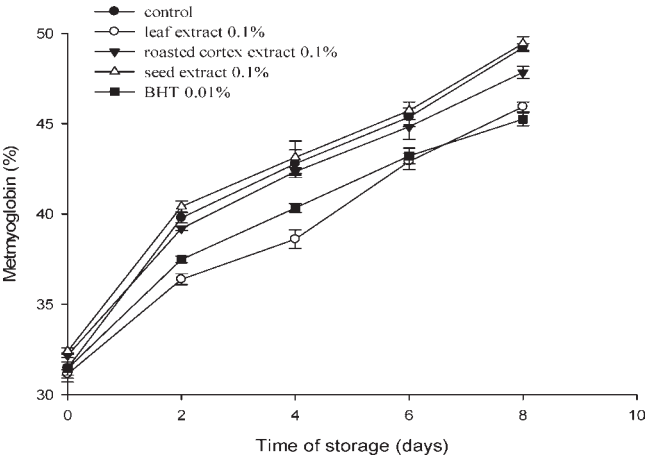

Figure 3. Effects of three Du-zhong extracts added at 0.10% (w/w) and BHT added at 0.01% (w/w) on metmyoglobin changes of raw pork patties during 8 days of refrigerated storage at 4 °C. Results are given as mean ± standard error.

The effect of Du-zhong extracts and BHT on relative MetMb percentage in raw pork patties is presented in **Figure 3**. MetMb (%) generally supports the instrumental color features. The relative MetMb percentage increased as the storage time increased for the 8 days of refrigerated display. The treated groups of leaf extract and BHT had lower ($P < 0.05$) proportions of MetMb compared to the control after day 2, thus exhibiting a certain inhibiting ability against formation of MetMb. However, the addition of roasted cortex extract and seed extract did not exhibit such effects.

Although many factors can influence the color stability of meat and meat products, the susceptibility of myoglobin to autoxidation is a predominant factor. The discoloration of meat from red to brown during storage results from the oxidation of OxyMb to MetMb. Several authors (*33*, *34*) have reported an association between lipid oxidation and myoglobin oxidation or discoloration

in meat products or model systems. The radical species produced during muscle phospholipid oxidation may act to promote OxyMb autoxidation. Conversely, superoxide anion released from oxidized OxyMb can dismutate to hydrogen peroxide and hydroxyl radical, which are potent lipid prooxidants (*35*). The free radical scavenging effects of phenolic compounds occurring in Du-zhong leaf extract are the most likely reason for the retardation of MetMb formation.

The present study has investigated the antioxidant effects of Du-zhong extracts (leaf, roasted cortex, and seed) in raw meat systems. The Du-zhong leaf extract exhibited better antioxidant effects compared to the other two extracts. The data suggested Du-zhong leaf extract at a concentration of 0.1% (w/w) exhibited comparable antioxidant activity to 0.01% (w/w) BHT on raw pork patties. Although the addition of Du-zhong leaf extract changed the color attributes of raw pork patties slightly, Du-zhong leaf extract applied to raw pork patties could inhibit lipid oxidation and stabilize meat color during storage. Because of its rich resources and good antioxidant effects, this kind of herb (Du-zhong leaf) has the potential to be developed as a natural antioxidant source for meat and meat products.

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