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Full Length Research Paper

Antioxidant activities of seed extracts from *Dalbergia odorifera* T. Chen

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The heartwood or root of *Dalbergia odorifera* T. Chen is an important traditional Chinese medicine. Antioxidant activities of seed extracts from *D. odorifera* T. Chen were first investigated in this study. Ethanolic extracts were suspended in distilled water and partitioned successively with petroleum ether, ethyl acetate, n-butanol (n-BuOH) and water, yielding four extracts named as PE, EE, BE and WE, respectively. The EE exhibited the highest total phenolic, total flavonoid, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, reducing power, linoleic acid and lard peroxidation inhibition, but lowest chelating ability. Liquid chromatography mass spectrometry (LC/MS) analysis of EE revealed that there was a predominant component with negative molecular ion $[M-H]^-$ at m/z 373.2, a fragment at m/z 343.2 and UV λ_{max} at 263 and 297nm. The mechanisms of antioxidant activities of seed extracts were exploited. Positive linear correlations were observed between reducing power and DPPH radical scavenging activity (R^2 = 0.836), and linoleic acid peroxidation inhibition (R^2 = 0.920), respectively. Similarly, high positive linear correlations of the total phenolic and total flavonoid with DPPH radical scavenging activity, reducing power and linoleic acid peroxidation inhibition were observed. This study therefore suggests that seeds of *D. odorifera* T. Chen have the potential to be used as natural antioxidants in food or pharmaceutical industry.

Key words: Antioxidant activity, *Dalbergia odorifera* T. Chen, seed, liquid chromatography mass spectrometry (LC/MS).

INTRODUCTION

In recent years, people pay more attention to the safety of their food and the potential effect of synthetic additives on their health. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) are widely used in the food industry. However, BHA and BHT have begun to be restricted because of their toxicity and carcinogenicity (Ito et al., 1985; Sasaki et al., 2002). Therefore, many researchers try to find natural antioxidants from medicinal plants which have been used for many years.One of such medicinal plants is *Dalbergia odorifera* T. Chen, which is indigenous in China and belongs to the *Leguminosae*. This plant is not only used as spices and materials for luxurious furniture and crafts, but also used in the pharmaceutical industry. The dried heartwood or root of the plant is an important traditional Chinese medicine named Jiangxiang in Chinese. Dissipating blood stasis, regulating the flow of vital energy and relieving pain are the main actions in traditional Chinese medicine (Liu et al., 2005a). Modern pharmacological studies show that Jiangxiang possesses various biological activities, such

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Abbreviations: PE, Petroleum ether extract; **EE,** ethyl acetate extract; **BE,** n-butanol extract; **WE,** water extract; **DPPH,** 1, 1-diphenyl-2-picrylhydrazyl; **Vit. C,** ascorbic acid.

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as anti-inflammatory (Lee et al., 2009), anti-platelet (Tao and Wang, 2009), anti-coagulant, anti-tumor, antihyperlipidemic, vasodilative effects (Zhao et al., 2000), stimulating the activity of tyrosine (Wu and Wang, 2003), inhibitory effects on central nervous system (Zhao et al., 2000) and α-glucosidase (Gao et al., 2008). It has been used as the main ingredient in many formulae such as Xiangdan injection (Zhao et al., 2000), Guan-Xin-Er-Hao (Huang et al., 2009), Qi-Shen-Yi-Qi dropping pill (Li et al., 2008) to treat cardiovascular and coronary heart diseases and Tongxinluo capsule (Chen et al., 2009) to treat angina pectoris and ischemic stroke. According to previous reports, volatile oil and flavonoids are major components in Jiangxiang (Zhao et al., 2000; Liu et al., 2005b; Tao and Wang, 2009).

In addition to the aforementioned activities, antioxidant activity of the heartwood or root of *D. odorifera* T. Chen was also reported. Several flavonoid compounds were isolated and reported to have high antioxidant activities on lard or human low density lipoprotein peroxidation inhibition (Cheng et al., 1998; Wang et al., 2000; Jiang and Sun, 2004; Chen et al., 2006; Yu et al., 2007). Study also showed that Guan-Xin-Er-Hao exerts significant cardioprotective effects against acute ischemic myocardial injury in rats, likely through its antioxidation and antilipid peroxidative properties (Qin et al., 2009). However, the antioxidant properties of extracts from *D. odorifera* T. Chen have not been fully addressed because the antioxidant activity may be due to different mechanisms.

The objective of the present study was to explore the antioxidant activities of seed extracts from *D. odorifera* T. Chen. Seeds of this plant were selected for the following reasons: (1) the heartwood or root of this plant needs more than 20 years to grow until it can be used while satisfactory yield of seeds can be obtained after 5 years; (2) the collection of seeds does not destroy the parent plant; and (3) some crude extracts and pure compounds from leaves, bark and seeds of other species of the same genus *Dalbergia* were reported to have various bioactivities (Hajare et al., 2001; Phimonphan et al., 2007; Khan et al., 2000), although, very few data on bioactivities and phytochemical composition of other parts of this plant are available.

Hence, for the first time, a detailed study on antioxidant activities of seed extracts of this plant was done. Five different experimental models were used: (1) DPPH radical scavenging activity model; (2) ferric ion reducing power model; (3) ferrous ion chelating activity model; (4) linoleic acid peroxidation inhibition model and (5) lard peroxidation inhibition model. To the best of our knowledge, correlations of the models and correlations of antioxidant activity with the total phenolic and total flavonoid were exploited to estimate the mechanisms of antioxidant activities of seed extracts from this plant.

MATERIALS AND METHODS

Seeds of *D. odorifera* T. Chen were collected from Hainan Province of China. Lard was rendered in the laboratory from fresh pig leaf fat, purchased from local market. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), ferrozine and linoleic acid were purchased from Sigma Co. (St. Louis, MO, USA). Ascorbic acid (Vit. C), BHT, gallic acid, rutin and Folin-Ciocalteau reagent were purchased from the China National Medicines Corporation Ltd. All the other solvents and chemicals used were of analytical grade.

Preparation of seeds extracts

Dried and powdered seeds of *D. odorifera* T. Chen (90 g) were extracted with 70% ethanol at 40°C for 2 h. After filtration, the combined extracts were evaporated to dryness to yield the ethanolic extract. The ethanolic extract was suspended in distilled water at room temperature and partitioned successively with petroleum ether, ethyl acetate, n-butanol (n-BuOH) and water, yielding four extracts named as PE, EE, BE and WE, respectively. The extracts were concentrated to dryness under reduced pressure at 40°C and kept in dark at 4°C until further analyses.

Determination of total phenolic and flavonoid contents

The presence of phenolics, flavonoids, tannins was detected by simple qualitative methods of Adeneye et al. (2006). The total phenolic content of the seed extracts was determined with the Folin-Ciocalteau method with little modification (Liu and Yao, 2007). Briefly, each extract (1.0 mg/ml, 0.1 ml) in 70% ethanol was mixed with 0.5 ml each of Folin-Ciocalteau reagent and distilled water. After the mixture was shaken, 1.5 ml of 15% Na_2CO_3 was added and the mixture was shaken for 0.5 min. Finally, the solution was brought up to 10 ml by adding 70% ethanol. The mixture was then incubated at 70°C. After 10 min, the absorbance at 750 nm was evaluated. The concentrations of total phenolic were calculated according to the following Equation obtained from the standard gallic acid graph:

Absorbance = 0.1004 gallic acid (μg) + 0.0365 (R^2 = 0.9993)

Total flavonoid content of the seed extracts was determined according to the method reported by Jia et al. (1998). Briefly, each extract (2.0 mg/ml, 2.0 ml) in 70% ethanol was mixed with 0.5 ml of 5% $NaNO_2$ solution. The mixture was shaken and allowed to stand at room temperature for 6 min before 0.5 ml of 10% $Al(NO_3)_3$ was added. This mixture was allowed to stand for a further 6 min before further addition of 4.0 ml of 1 M NaOH. The solution was then shaken vigorously before absorbance at 510 nm was measured. The concentrations of total flavonoid were calculated according to the following equation that was obtained from the standard rutin graph:

Absorbance = 0.0119 rutin (μg) - 0.0129 (R^2 = 0.9994)

Free radical scavenging activity

Free radical scavenging capacity of seed extracts was evaluated according to the reported procedure using the stable DPPHradical (Chen et al., 2009). Briefly, each extract (0.1 to 0.8 mg/ml, 1 ml) in ethanol was mixed with 2 ml DPPH radical solution in ethanol (0.1

mM). The reaction mixture was shaken and incubated in the dark for 30 min. The absorbance of the solution was measured at 517 nm by a spectrophotometer (UV-2800, Shanghai Unico Instrument Co.Ltd., China). Vit. C dissolved in ethanol was also analyzed for comparison. Scavenging ability of free radical DPPH was calculated by using the following equation:

Scavenging ability (%) = [(Absorbance$_{517\ nm\ of\ blank}$ - Absorbance$_{517\ nm\ of\ sample}$) / Absorbance $_{517\ nm\ of\ blank}$] × 100.

Reducing power

The reducing power was determined by the method of Oyaizu (1986). Each extract (0.1 to 1.0 mg/ml, 1 ml) in ethanol was mixed with sodium phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was then incubated at 50°C for 20 min. At the end of the incubation, trichloroacetic acid (2.5 ml, 10%) was added to the mixtures, followed by centrifuging at 3000 rpm for 10 min. The upper layer (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Vit. C was used for comparison.

Chelating ability on ferrous ions

Chelating ability was determined according to the method of Lee et al. (2008). Each extract (0.5 to 2.0 mg/ml, 1 ml) in ethanol, was mixed with 3.7 ml ethanol and 0.1 ml 2 mM ferrous sulfate. The reaction was initiated by 0.2 ml 5 mM ferrozine. After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. Na_2EDTA was used for comparison. Chelating ability on ferrous ions was calculated by using the following Equation:

Chelating ability (%) = [(Absorbance$_{562\ nm\ of\ blank}$ - Absorbance$_{562\ nm\ of\ sample}$) / Absorbance $_{562\ nm\ of\ blank}$] × 100.

Ferric thiocyanate method (FTC)

The antioxidant activity of extracts on inhibition of linoleic acid peroxidation was assayed using the ferric thiocyanate method (Yen et al, 2003). The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 and 50 ml phosphate buffer (0.2 M, pH 7.0), and then the mixture was homogenized. Each extract (1.0 mg/ml, 1 ml) in ethanol, was mixed with linoleic acid emulsion (2.0 ml, 0.02 M) and phosphate buffer (2 ml, 0.2 M, pH 7.0). The reaction mixture was incubated at 37°C in dark for 60 h to accelerate the oxidation process. Aliquots (0.1 ml) were drawn from the incubation solution and mixed with 75% ethanol (9.7 ml) and 30% ammonium thiocyanate (0.1 ml). Precisely 3 min after addition of 0.1 ml 20 mM ferrous sulfate in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm. The control and standard were prepared as the sample. For the control, there was no addition of sample, while for the standard, extract was replaced by BHT at same concentration. The antioxidant activity was expressed as percentage of inhibition of peroxidation (%) and calculated by using the following Equation:

Inhibition (%) = [(Absorbance$_{500\ nm\ of\ blank}$ - Absorbance$_{500\ nm\ of\ sample}$) / Absorbance $_{500\ nm\ of\ blank}$] × 100

Schaal oven test

The antioxidant activity of extracts on inhibition of lard peroxidation was measured using the method of Schaal oven test. Lard samples (each sample was 100 g) were thoroughly mixed with PE, EE, BE, WE and BHT (each was 20 mg), respectively. Lard (100 g), containing no additive, was run as a control. All samples were kept in conical flask with stopper under dark conditions at 60 ± 2°C. Peroxide values of lard samples were measured every 48 h by the method of The National Standard of PR China (2006).

LC-MS profiling of ethyl acetate extract (EE)

HPLC-UV analysis was performed using an Agilent 1100 Series HPLC system (Palo Alto, CA, USA) with a diode-array detector (DAD); the separation used was a Zorbax SB-C18 column (250 × 4.6 mm, 5 mm) with a Zorbax SB-C18 guard column (20 × 4 mm, 5 mm). The mobile phase consisted of (A) methanol and (B) 0.3% aqueous phosphoric acid (v/v), using a gradient elution of 10 to 80% A at 0 to 15min and then maintained. The flow rate was 0.8 ml/min and the temperature of the column oven was 40°C. Detection wavelength was set at 280 nm and UV spectra from 200 to 400 nm were also recorded for peak characterization. After passing through the flow cell of the DAD, the column eluate was directed to an LCQ ion trap mass spectrometer fitted with an electrospray interface (Thermo Finnigan, San Jose, CA). Experiment was performed in negative ion mode with a scan range was 100 to 2000. The desolvation temperature was 300°C. High spray voltage was set at 5000 V. Nitrogen was used as the dry gas at a flow rate of 75 ml/min. MS was carried out using helium as target gas, and collision energy was set at 30%.

Statistical analysis

Experimental results were mentioned as means ± SD of three parallel measurements. P values < 0.05 were regarded as significant. The statistical analysis was performed with the Excel 2003 and SPSS 16.0.

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents

The seed extracts of *D. odorifera* T. Chen were analyzed using various color and precipitation reactions. Whilst phenolics and flavonoids (ferric chloride test and lead acetate test) could be detected in these extracts, the presence of tannins (bromine water test) was not observed. This conclusion agrees with the results of previous studies (Zhao et al., 2000; Liu et al., 2005b; Tao and Wang, 2009).

Total phenolic and total flavonoid contents of seed extracts from *D. odorifera* T. Chen were examined and are presented in Table 1. The EE exhibited the highest

Table 1. Yield and content of total phenolic and flavonoid in seed extracts from *Dalbergia odorifera* T. Chen.

Sample	Yield (%)	Total phenolic (mg/g extract)[a]	Total flavonoid (mg/g extract)[b]
PE	1.1	135.5 ± 13.4	103.7 ± 1.6
EE	2.2	563.2 ± 11.3	350.3 ± 3.1
BE	7.0	167.3 ± 10.6	79.9 ± 0.6
WE	15.6	135.0 ± 4.2	115.1 ± 1.6

[a]Gallic acid equivalent; [b]rutin equivalent. PE, Petroleum ether extract; EE, ethyl acetate extract; BE, n-butanol extract; WE, water extract

Table 2. DPPH radical scavenging ability (%) of seed extracts from *D. odorifera* T. Chen and Vit. C at different concentrations.

Sample	Sample concentration (mg/ml)			
	0.1	0.2	0.4	0.8
PE	22.7 ± 0.9	35.8 ± 0.3	53.5 ± 0.8	67.1 ± 0.2
EE	43.1 ± 0.7	53.7 ± 0.7	58.7 ± 0.1	62.8 ± 0.5
BE	19.3 ± 1.7	30.8 ± 0.6	42.8 ± 1.4	57.2 ± 1.1
WE	28.7 ± 0.2	45.8 ± 1.6	56.0 ± 0.7	59.5 ± 1.0
Vit. C	84.9 ± 0.4	95.0 ± 0.1	95.0 ± 0.1	95.2 ± 0.1

total phenolic content at 563.2 ± 11.3 mg gallic acid equivalents/g extract, approximately 3.3, 4.1, 4.1 folds more than the BE, PE and WE, respectively. The total phenolic content was not significantly different between PE, BE and WE ($p > 0.05$). The EE also exhibited the highest total flavonoid content at 350.3 ± 3.1 mg rutin equivalents/g extract, approximately 4.3 , 3.4, 3.0 folds more than the BE, PE and WE, respectively. A significant difference of total flavonoid contents was detected among these extracts ($p < 0.05$). However, the yield of EE was far lower than BE and WE. Variations in the total phenolic and total flavonoid contents of these extracts are attributed to the polarities of different solvents used in the experiment. The results indicate that the polarity of ethyl acetate was more selective to the phenolic and flavonoid compounds present in seeds of *D. odorifera* T. Chen than other three solvents, hence supporting why many researchers isolated bioactive compounds from ethyl acetate extract of the heartwood or root of *D. odorifera* T. Chen (Chan et al., 1997; Wang et al., 2000; Jiang and Sun, 2004; Yu et al., 2007) and other materials (Yen et al., 2003; Duan et al., 2006).

In addition, flavonoids account for a large part of total phenolic in these extracts, especially in the PE (83%) and WE (92%). It is interesting to note that a highly positive linear correlation ($R^2 = 0.967$) between total phenolic and total flavonoid was observed, which predicted that the flavonoids play an important role in seeds extracts of *D. odorifera* T. Chen. All these information suggests the potential of seeds from *D. odorifera* T. Chen to be utilized as a source of nutritional phenolics and flavonoids.

Free radical scavenging activity

The DPPH radical method is one of the most widely employed method to evaluate the ability to prevent oxidative damages, based on the reduction of DPPH radicals in the presence of a proton-donating substance resulting in the formation of diamagnetic molecules (Soares et al., 1997). The radical scavenging activities of seed extracts of *D. odorifera* T. Chen were estimated by comparing the scavenging ability of DPPH radical with Vit. C. Table 2 depicts a steady increase in the DPPH radical scavenging activities of the EE and WE up to the concentration of 0.4 mg extract/ml, after which there was a leveling off with much slower increase. This pattern of DPPH radical scavenging activity is commonly observed with plant extracts (Nurhanani et al., 2008) and certain compounds such as coumaric acid and vanillin which never react with more than 75% of the initial DPPH radical even after 7 h of reaction time and at very high concentrations (Brand et al., 1995).

However, the PE and BE did not show a leveling off with increasing concentration. As a result, the radical scavenging activities of WE, PE and BE were much less than the EE at the low concentration of 0.1 mg extract/ml (28.7 ± 0.2, 22.7 ± 0.9, 19.3 ± 2.7 and 43.1 ± 0.7%, respectively), but were close at the high concentration of 0.8 mg extract/ml (59.5 ± 1.0, 67.1 ± 0.2, 57.2 ± 1.1 and

Table 3. Reducing power of seed extracts from *D. odorifera* T. Chen and Vit. C at different concentrations.

Sample	Sample concentration (mg/ml)				
	0.1	0.2	0.4	0.8	1.0
PE	0.031 ± 0.002	0.069 ± 0.004	0.138 ± 0.005	0.288 ± 0.008	0.351 ± 0.017
EE	0.174 ± 0.010	0.308 ± 0.009	0.541 ± 0.031	1.020 ± 0.060	1.230 ± 0.034
BE	0.037 ± 0.011	0.099 ± 0.005	0.193 ± 0.002	0.375 ± 0.006	0.444 ± 0.014
WE	0.103 ± 0.016	0.187 ± 0.011	0.336 ± 0.014	0.638 ± 0.008	0.818 ± 0.006
Vit. C	0.779 ± 0.032	1.295 ± 0.029	2.166 ± 0.033	2.382 ± 0.006	2.408 ± 0.012

Table 4. Chelating ability on ferrous ions of seed extracts from *D. odorifera* T. Chen and Na_2EDTA at different concentrations.

Sample	Sample concentration (mg/ml)			
	0.5	1.0	1.5	2.0
PE	18.6 ± 2.2	35.0 ± 1.9	50.4 ± 0.5	68.4 ± 2.5
EE	6.8 ± 0.4	13.8 ± 1.5	18.4 ± 1.6	21.1 ± 1.5
BE	9.9 ± 1.9	13.9 ± 1.5	20.6 ± 3.4	32.0 ± 2.4
WE	64.4 ± 0.5	79.1 ± 0.7	82.8 ± 1.7	88.1 ± 0.5
Na_2EDTA	96.4 ± 1.5	-	-	-

62.8 ± 0.5%, respectively). The difference between radical scavenging activities of seed extracts was statistically significant ($p < 0.05$). However, the DPPH radical scavenging activities of these extracts were less than Vit. C at 0.1 mg /ml.

Reducing power

The reducing power of a compound is used to evaluate its ability to donate electrons (Dorman et al., 2003) and may serve as a significant indictor of its potential antioxidant activity (Meir et al., 1995). Reducing powers of PE, EE, BE and WE were 0.351 ± 0.017, 1.230 ± 0.034, 0.444 ± 0.014 and 0.818 ± 0.006 at 1 mg/ml, respectively (Table 3). However, Vit. C showed an excellent reducing power of 0.779 ± 0.032 at 0.1 mg/ml. The reducing power of seed extracts increased with increasing concentration and a significant difference in reducing power ($p < 0.05$) was observed between 0.1 to 1.0 mg extract/ml. Reducing powers of different seed extracts exhibited the following order: EE > WE > BE > PE.

Chelating ability on ferrous ions

Ferrous ions are an effective prooxidant in food systems because they can stimulate lipid peroxidation by Fenton reaction (Yamaguchi et al., 1988). The results of the ferrous ion-chelation of seed extracts are shown in Table 4. Chelating abilities of all extracts increased with increasing concentration, especially the PE (from 18.6 ± 2.2 to 68.4 ± 2.5%). Significant differences ($p < 0.05$) were observed among concentrations and seed extracts. Chelating abilities of PE, EE, BE and WE were18.6 ± 2.2, 6.8 ± 0.4, 9.9 ± 3.9 and 64.4 ± 0.5% at 0.5 mg/ml, respectively. However, Na_2EDTA showed excellent chelating ability of 96.4 ± 1.5 at 0.5 mg/ml. Chelating abilities of different seed extracts exhibited the following order: WE> PE >BE > EE.

Ferric thiocyanate method (FTC)

The FTC method was used to measure the peroxide level during the initial stage of lipid oxidation (Liu and Yao, 2007). The results of antioxidant activities of extracts and BHT on inhibition of linoleic acid peroxidation are shown in Table 5. At 1.0 mg/ml, EE exhibited 64.4 ± 2.1% inhibition in the linoleic acid peroxidation system, which was significantly ($p <0.05$) higher than that of PE (14.0 ± 1.0%), BE (30.1 ± 1.4%) and WE (48.5 ± 1.7%) but lower than that of BHT (97.9 ± 2.5%). It is interesting to find that antioxidant activities of different seed extracts in this system exhibited the same order as reducing power.

Schaal oven test

Schaal oven test, the simplest accelerated method was

Table 5. Linoleic acid peroxidation inhibition (%) and IC_{50} value and R^2 of regression equation of DPPH radical scavenging activity, reducing power and chelating ability on ferrous ions of seed extracts from *D. odorifera* T. Chen.

Sample	Inhibition (%)[a]	IC_{50} (mg/ml)					
		b	R_b^2	c	R_c^2	d	R_d^2
PE	14.0 ± 1.0	0.36	0.996	1.41	0.999	1.46	0.999
EE	64.4 ± 2.1	0.17	0.946	0.36	0.999	4.93	0.960
BE	30.1 ± 1.4	0.56	0.997	1.09	0.994	3.36	0.950
WE	48.5 ± 1.7	0.34	0.919	0.60	0.998	< 0.5	0.903
BHT	97.9 ± 2.5	-	-	-	-	-	-

[a]Linoleic acid peroxidation inhibition (%) of seed extracts and BHT. b, c, d IC_{50} of DPPH radical scavenging activity represents reducing power and chelating ability on ferrous ions of seed extracts, respectively, while R_b^2, R_c^2 and R_d^2 of regression equation of DPPH radical scavenging activity represents reducing power and chelating ability on ferrous ions of seed extracts, respectively.

often used to evaluate antioxidant effectiveness in fats, oils and fat-containing foods. The effects of seed extracts and BHT in preventing the peroxidation of lard are shown in Figure 1. The oxidation of lard was slow in the presence of seed extracts. The results show that all seed extracts exhibited effective antioxidant activities ($p < 0.05$). It is interesting to find that the EE exhibited higher activity than other extracts, which is similar to the patterns for DPPH free radical scavenging at low concentration, reducing power and linoleic acid peroxidation inhibition. The antioxidant activity of BHT was similar with that of the EE. There was however, no significant difference between antioxidant activities of the EE and BHT during the storage ($p > 0.05$). Similar result was reported by Jiang and Sun (2004). Some flavonoids and other compounds were isolated from the ethyl acetate extract of heartwood or root of *D. odorifera* T. Chen and their antioxidant activities were tested by an oxidative stability instrument. Results show that strong antioxidant activities was due to their structures, especially the number and position of hydroxy and methoxy groups (Wang et al., 2000; Jiang and Sun, 2004; Yu et al., 2007). This may be one of the reasons for the seed extracts exhibiting different antioxidant activities.

IC_{50} values of seeds extracts from *D. odorifera* T. Chen

IC_{50} value the concentration at which the DPPH radicals were scavenged by 50%; the absorbance was 0.5 for reducing power and ferrous ions were chelated by 50%, respectively. IC_{50} is inversely related to the antioxidant capacity of a compound. The lower IC_{50}, the higher the antioxidant activity of a compound is. IC_{50} is independent of the sample concentration and typically employed to express the antioxidant activity and to compare the antioxidant capacities of various samples (Monica et al., 2009). IC_{50} values of reducing power and chelating ability were obtained by interpolation or extrapolation from linear regression analysis. However, IC_{50} of DPPH radical scavenging activity was obtained by the method of Monica et al. (2009) because of the linear regression analysis was not very suitable for EE and WE. IC_{50} values are exhibited in Table 5.

Correlations of the assays determining antioxidant activity of the extracts

Since the antioxidant activity may be due to different mechanisms, mutual correlations among the methods were therefore determined by linear regression analysis. As shown in Table 6, positive linear correlations were observed between the reducing power and the DPPH radical scavenging activity ($R^2 = 0.836$), FTC ($R^2 = 0.920$), respectively. The results suggest that the components present in the extracts capable of scavenging DPPH radicals and inhibiting linoleic acid peroxidation are also able to reduce ferric ions, and that the reducing power may be an important factor dictating antioxidant capacities of seed extracts from *D. odorifera* T. Chen. However, there was no positive linear correlation between chelating ability and other methods. The reason might be there were no or little ferrous ions in other reaction systems.

Correlations of antioxidant activity with the total phenolic and total flavonoid

As shown in Table 6, positive linear correlations were observed between total phenolic and the DPPH radical scavenging activity ($R^2 = 0.872$), reducing power ($R^2 = 0.784$), FTC ($R^2 = 0.579$), respectively. Similarly, positive linear correlations were observed between total flavonoid

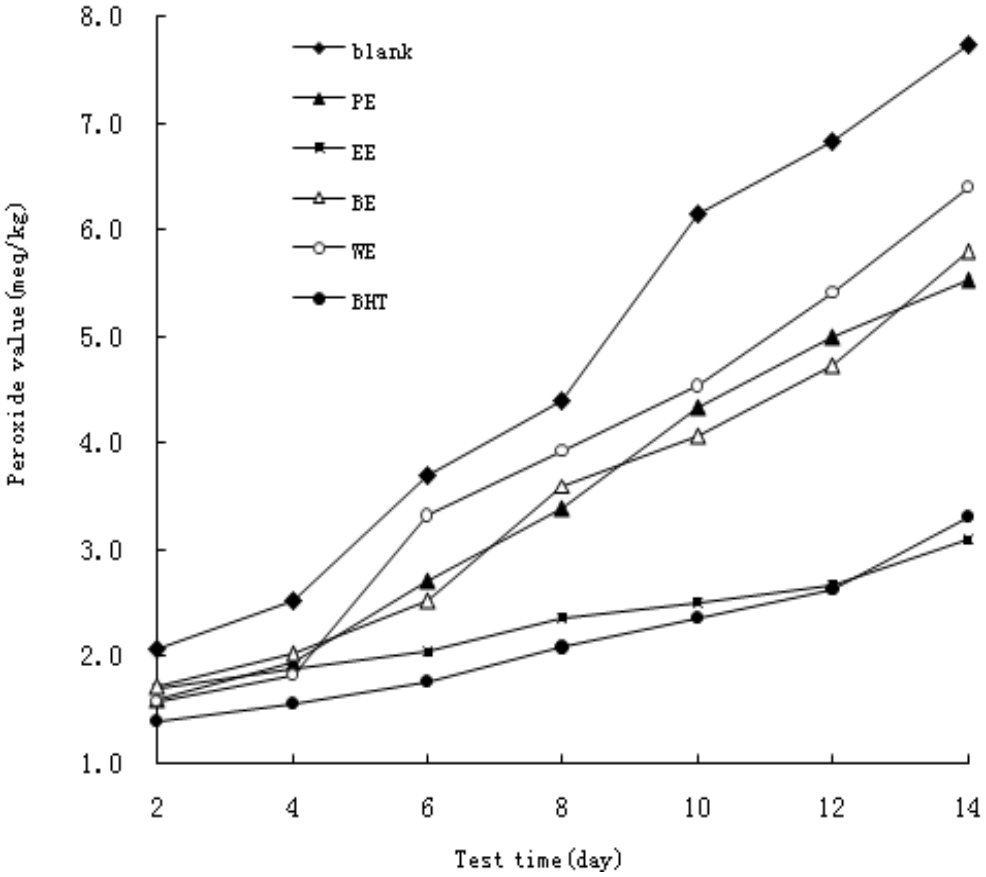

Figure 1. Lard peroxidation inhibition of seeds extracts from *Dalbergia odorifera* T. Chen and BHT. Values are expressed as means of three determinations.

Table 6. Linear correlation coefficients, R^2 for relationships between the assays for the seed extracts from *Dalbergia odorifera* T. Chen.

Parameter	Reducing power (1/ IC50)	FTC (Inhibition)	Total phenolic	Total flavonoid
DPPH (1/ IC50)	0.836	0.583	0.872	0.967
Total flavonoid	0.851	0.618	0.965	
Total phenolic	0.784	0.579		
FTC (Inhibition)	0.920			

and the DPPH radical scavenging activity (R^2 = 0.967), reducing power (R^2 = 0.851) and FTC (R^2 = 0.618), respectively. These results suggest that the total phenolic and total flavonoids were largely responsible for the antioxidant activity of the extracts. Similar conclusion was found in other extracts by Zeng et al. (2011).

In addition, it is interesting to find that there was no positive correlation of chelating ability with the total phenolic and total flavonoid. The EE which had the highest total phenolic and total flavonoid contents exhibited lowest chelating activity. Studies showed that chelating ability was related the structure of flavonoids (Mira et al., 2002). The chelating abilities of flavonoids from different classes, including flavones (apigenin, luteolin, kaempferol, quercetin, myricetin and rutin), isoflavones (daidzein and genistein), flavanones (taxifolin, naringenin and naringin) and a flavanol (catechin) were investigated and results showed that only flavones and the flavanol catechin interact with metal ions (Mira et al., 2002). Other reasons might be the presence

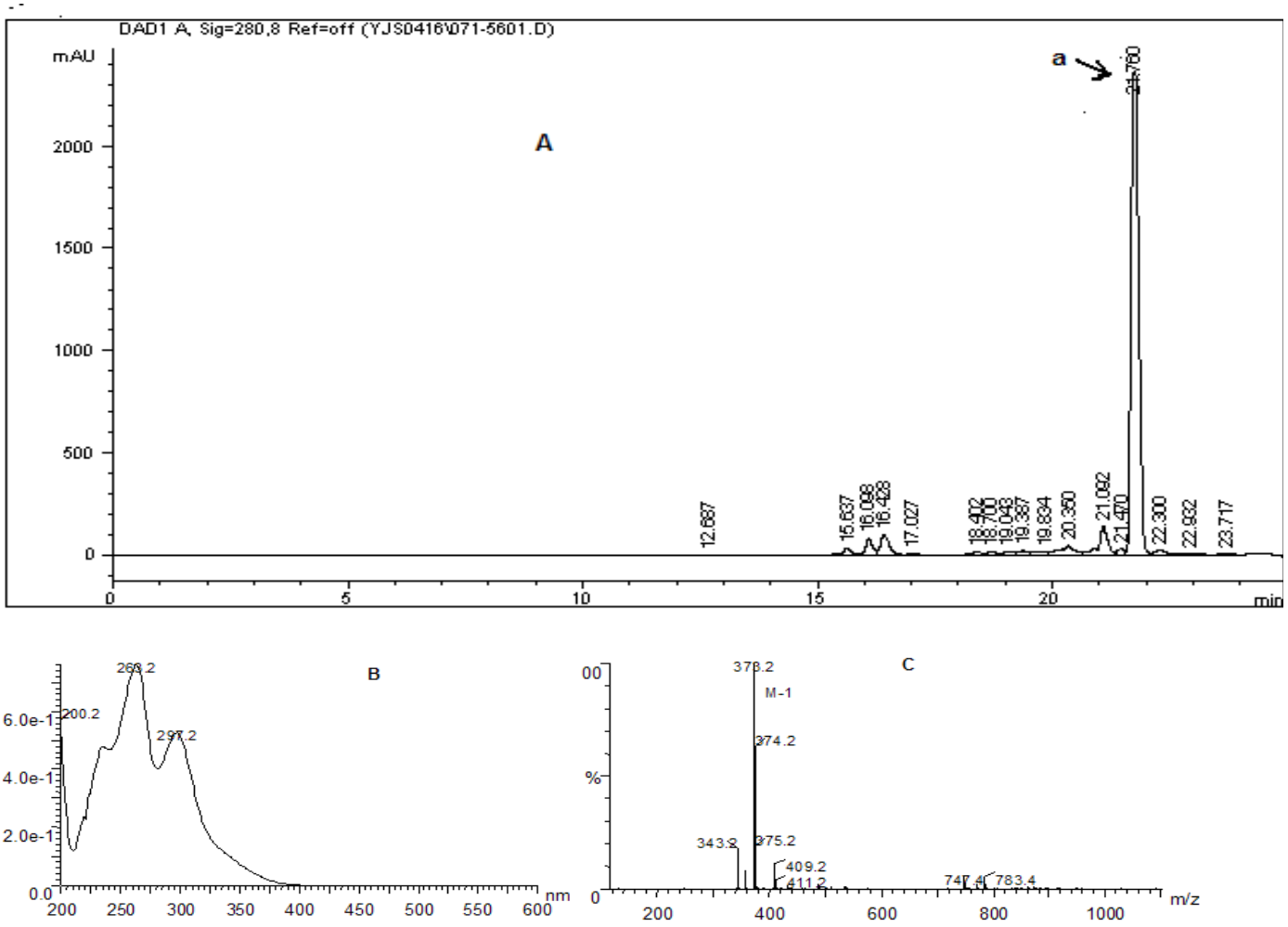

Figure 2. HPLC chromatogram with UV detection at 280 nm of EE (A); UV spectrum of peak a (B); Mass spectrum of peak a (C).

of non-phenolic compounds, antagonistic or synergistic interactions between phenolics and other compounds (Odabasoglu et al., 2005).

Result of LC-MS

Liquid chromatography coupled with mass spectroscopy (LC-MS) has emerged as a powerful tool for the determination of many compounds in crude extracts. The LC/ESI-MS/MS technique in negative ion mode was used to identify 23 flavonoids in the extract of heartwood of *D. odorifera* T. Chen, based on direct comparison with authentic standards (Liu et al., 2005b). To identify major compounds present in the enriched extract, LC-MS profile of EE was taken. The analysis (Figure 2) revealed that EE contained a major component (peak a) at the retention time of 21.76 min. Peak a had a negative molecular ion $[M-H]^-$ at m/z 373.2, a fragment at m/z 343.2 and UV λ_{max} at 263, 297nm. The structure of the component was not identified because of limited information. The component was not reported in the extract of heartwood of *D. odorifera* T. Chen by Liu et al. (2005b). In addition, EE also contained some other undefined compounds.

Therefore, in order to clarify the antioxidant activities of seed extracts of *D. odorifera* T. Chen, further isolation of such bioactive components is needed.

Conclusion

We have so far shown that seed extracts of *D. odorifera* T. Chen had high total phenolic and total flavonoid contents. All seeds extracts exhibited certain antioxidant activities, including radical scavenging activity determined

by DPPH, reducing power, chelating ability on ferrous ions, the linoleic acid and lard peroxidation inhibition. LC-MS analysis of EE also revealed that there was a predominant component with negative molecular ion $[M-H]^-$ at m/z 373.2, a fragment at m/z 343.2 and UV λ_{max} at 263, 297nm. However, the phenolic and flavonoid compounds or other components responsible for the antioxidant of seed extracts from *D. odorifera* T. Chen are still unknown. Therefore, further isolation of such bioactive components could perhaps clarify the antioxidant activities of seeds from *D. odorifera* T. Chen and be further exploited for food or pharmaceutical use.

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