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Antioxidant Activity and Isoflavonoid Components in Different Sections of Pueraria lobata Root

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ABSTRACT

The isoflavonoids content and antioxidant activity in different sections of Pueraria lobata roots, including the root outer bark, kudzu root and whole root were investigated in this study. Isoflavonoid concentrations in P. lobata roots were determined by high performance liquid chromatography. The antioxidant activity of P. lobata roots was assessed by total phenol content, DPPH free radical scavenging assay, ABTS free radical scavenging assay and reducing power. The root outer bark of P. lobata root possessed higher isoflavonoids content than whole root or kudzu root ($p < 0.05$). The levels of antioxidant potential of the root outer bark assayed by total phenolic content, DPPH, ABTS and reducing power were significantly higher than those of the whole root or the kudzu root ($p < 0.05$). The main isoflavonoids in all sections of P. lobata roots were puerarin, daidzin, daidzein, genistin and genistein. The major isoflavonoid puerarin in root outer bark exhibited the greatest antioxidant activity in P. lobata roots.

Key words: Pueraria lobata, kudzu, isoflavonoids, antioxidant activity

INTRODUCTION

Pueraria lobata (Willd.) Ohwi, a plant in traditional medicine, is widely distributed in China, Korea and Japan. The dried root of P. lobata have been used as the main ingredient of a traditional prescription for the treatment of early symptoms of common cold and as antipyretic, antidiarrhetic, diaphoretic and antiemetic agents[1,2]. The main active constituents of P. lobata are isoflavonoids, such as puerarin, daidzin, and daidzein[3,4]. P. lobata root is also used for the treatment of hypertension[5] and alcoholism[6] or as an antioxidant[7]. It was also shown to possess anti-dipsotropic activity[1].

Isoflavonoids exhibit a wide range of biological activities; they have anti-inflammatory, antithrombotic, antihypertensive, antiarrhythmic, spasmolytic, and cancer chemopreventive properties[8]. The beneficial health effects of isoflavonoids are due to their antioxidative and phytoestrogenic properties[9]. Isoflavonoids are regarded as protective antioxidants based on their ability to donate hydrogen atom to free radicals. Due to the distance of hydroxyl groups at C-7 to C-4' in isoflavonoids is similar to that of hydroxyls at C-3 and C17 in estradiol[10] thus have estrogenic-like property. Due to these properties, isoflavonoids are believed to have potential in the prevention of cancer, atherosclerosis and diabetes[11].

The concentrations of isoflavonoids in kudzu change with the age and the harvest season[12]. This variability has a significant impact on the therapeutic effects of the plant extract, and it also affects the consistency and efficacy of the resulting medicinal products or decoctions. The aim of this study was to investigate the various isoflavonoids content and antioxidant activity of root outer bark (ROB), kudzu root (KR) and whole root (WR).

MATERIALS AND METHODS

I. *Materials*

Puerarin, daidzin, genistin, daidzein and genistein were purchased from Sigma Chemical Company (St. Louis, MO, USA). Trifluoroacetic acid and liquid chromatography grade acetonitrile, methanol and n-hexane were obtained from Merck (Darmstadt, Germany).

II. *Preparation of P. lobata Root*

The P. lobata (Willd.) Ohwi root used in this study was collected from Taipei Chinese herbal stores in Taiwan.

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The kudzu root (KR) was prepared by removing the root outer bark (ROB) from whole root (WR). All assays were performed using the samples of ROB, KR and WR separately.

III. *Sample Extraction*

Isoflavonoids were extracted from the samples as described by Chen *et al*.[13] Samples were mixed with 80% methanol, stirred at 60°C for 1 h and filtered. The filtrate was dried under a vacuum, dissolved in 80% methanol to a final volume of 10 mL and filtered through a 0.45 μm Millipore PVDF filter membrane (Schleicher and Schuell, GmbH, Dassel, Germany).

IV. *HPLC Aanalysis*

HPLC analysis was performed on a Hitachi Model L-6200 HPLC equipped with an ODS-AM-303 column (250 mm × 4.6 mm i.d., 5 μm; YMC Inc., Kyoto, Japan) and an ultraviolet spectrophotometer L-2000 (Hitachi Ltd.). The gradient mobile phase consisted of glacial acetic acid in water and glacial acetic acid in acetonitrile as described by Chen *et al*.[13]. The flow rate was 1.0 mL/min. The isoflavonoid components were detected at 254 nm. Quantitative data for each isoflavonoid were obtained by comparison to known standards.

V. *Preparation of Methanolic Extracted of Sample*

The dried sample was extracted with 2 mL 80% methanol at 60°C for 1 h. The extraction process was repeated thrice, and the extracts were pooled together and filtered through Whatman no.1 paper. The filtrates were concentration under vacuum and freeze-dried. Prior to use, the lyophilized extract was dissolved in methanol.

VI. *Total Phenol Content*

The total polyphenol concentrations in the P. lobata roots were determined as previously described by Waterhouse[14]. The methanolic extract (0.5 μL) was mixed with 0.5 μL Folin-Ciocalteau phenol reagent, then 200 μL of 5% Na_2CO_3 was added and allowed to react for 1 h at room temperature. The absorbance was measured at 750 nm on an automated microplate reader and then compared to a gallic acid standard.

VII. *DPPH and ABTS Free Radicals Scavenging Activity Assay*

The DPPH radical scavenging effect was estimated as previously described by Katsube *et al.*,[15]. A 0.5 mM DPPH solution in methanol was prepared and 200 μL of the DPPH solution was then added to 25 μL of each test sample. After 90 min incubation at ambient temperature, the absorbance at 517 nm was measured. The inhibitory percentage of DPPH was calculated according to the following equation: Scavenging effect (%) = [1 - absorbance of sample / absorbance of control] × 100%.

The total antioxidant activity of P. lobata root extracts was measured by the $ABTS^+$ radical cation decolorization assay[16]. ABTS was dissolved in 0.01 M sodium phosphate buffer to make a final concentration of 7 mM. $ABTS^+$ was produced by adding potassium peroxodisulfate (2.45 mM final concentration) to the ABTS stock solution and allowing the mixture to stand in the dark at room temperature for 12 h to 16 h before use. The sample of methanolic extractions was serially diluted and a 2 μL aliquot of each dilution was used in the assay. After the addition of 200 μL of the diluted $ABTS^+$ solution to the antioxidant compound or the Trolox standards in methanol, samples were taken at 37°C exactly 7 min after initial mixing. The absorbance of the resulting solution was measured at 734 nm.

The inhibitory percentage of $ABTS^+$ free radical scavenging ability was calculated according to the following equation:

Scavenging effect (%) = [1 - absorbance of sample / absorbance of control] × 100%.

VIII. *Reducing Power Assay*

The Fe^{3+} reducing power of the extracts was determined by the method reported by Oyaizu[17] with slight modifications. The extract (0.75 mL), at various concentrations, was mixed with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mL of potassium hexacyanoferrate [$K_3Fe(CN)_6$] (1% w/v), followed by incubation at 50°C in water bath for 20 min. The reaction was stopped by adding 0.75 mL of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 3000 ×g for 10 min. One point five milliliter of the supernatant was mixed with 1.5 mL distilled water and 0.1 mL ferric chloride (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured to determine the reducing power, with higher absorbance indicating higher reducing power.

VIIII. *Statistic Analysis*

Each test was performed in triplicate. Data were analyzed using the ANOVA procedure in SAS software (version 6.03, SAS Institute Inc., Cary, NC, USA). Duncan's multiple range tests were utilized to analyze differences between treatments (the significance level was $P < 0.05$). Pearson's correlation tests were performed to reveal possible associations between isoflavonoid content and the antioxidant activity.

RESULTS

I. *Isoflavonoids Content in P. lobata Roots*

The HPLC profile showed that the major isoflavonoid components in ROB and KR of the P. lobata include puerarin, daidzin, daidzein, genistin and genistein (Figure 1). The total isoflavonoid content of the ROB (17.07 mg/g) was significantly ($p < 0.05$) higher than that of WR (6.32 mg/g) and KR

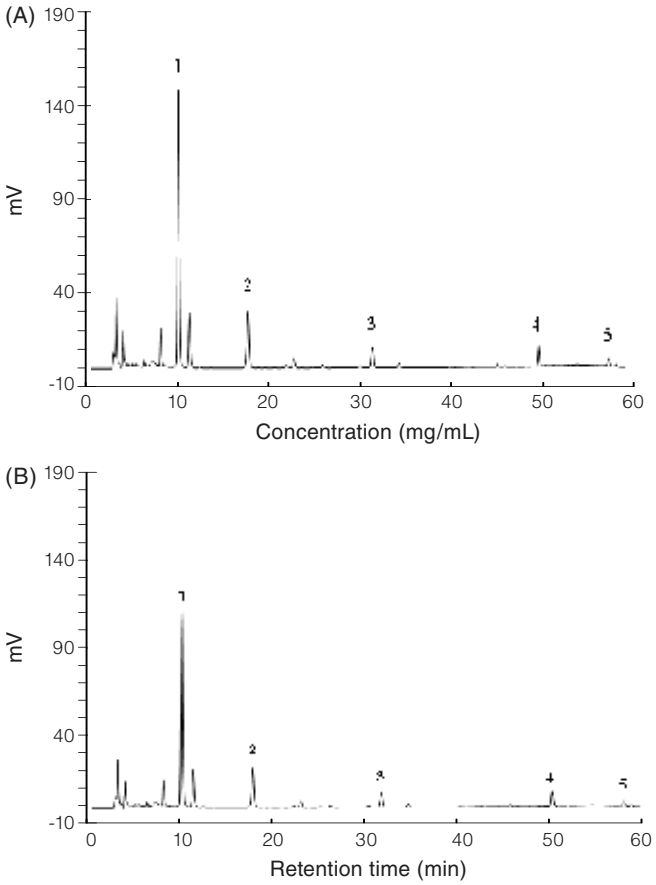

Figure 1. HPLC chromatograms of isoflavonoids in root outer bark (A) and kudzu root (B); 1: Puerarin (9.6 min); 2: Daidzin (17.0 min); 3: Genistin (31.7 min); 4: Daidzein (50.7 min); 5: Genistein (58.3 min).

Table 1. Isoflavonoids content in different sections of P. lobata

Isoflavonoids	Section of P. lobata roots* (mg/g)		
	Whole root (WR)	Kudzu root (KR)	Root outer bark (ROB)
Puerarin	5.21 ± 0.08^{b}** (82.44)***	2.46 ± 0.57^{c} (74.32)	15.81 ± 0.00^{a} (92.61)
Daidzin	0.62 ± 0.03^{b} (9.81)	0.64 ± 0.00^{b} (19.14)	0.82 ± 0.02^{a} (4.80)
Genistin	0.41 ± 0.01^{a} (6.49)	0.16 ± 0.00^{b} (4.83)	0.34 ± 0.13^{a} (1.99)
Daidzein	0.07 ± 0.00^{a} (1.10)	0.03 ± 0.00^{b} (0.91)	0.07 ± 0.00^{a} (0.41)
Genistein	0.01 ± 0.00^{b} (0.16)	0.02 ± 0.00^{b} (0.60)	0.03 ± 0.00^{a} (0.18)
Total	6.32 ± 0.12^{b}	3.31 ± 0.02^{c}	17.07 ± 0.15^{a}

*Values in the same row with different letters are significantly different by Duncan's multiple range tests ($p < 0.05$).
**Values are means ± SD of three replicate analyses (n = 3).
***The percentage of isoflavonoids.

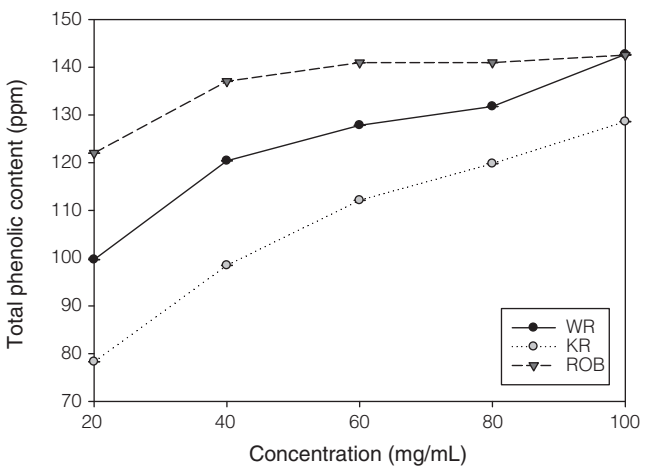

Figure 2. Total phenol in methanolic extract of different sections of P. lobata root. Root outer bark (ROB); Kudzu root (KR); Whole root (WR).

(3.31 mg/g) (Table 1). The most abundant isoflavonoid was puerarin, in concentrations of 15.81, 5.21 and 2.46 mg/g in the ROB, WR and KR, respectively. The concentrations of other four isoflavones, daidzin, daidzein, genistin and genistein, in different sections were lower than 1 mg/g.

II. *Antioxidant Activity in P. lobata Roots*

As shown in Figure 2, total phenolic content increased with methanolic extracts concentration for three test samples at 20-100 mg/mL. At low dose concentration of methanolic extraction, the total phenolic content of the ROB was significantly ($p < 0.05$) higher than those of the WR and KR (Figure 2).

The ROB also possessed significantly ($p < 0.05$) greater DPPH free radical scavenging activity (44.98-87.08%) than the WR (10.87-49.77%) and KR (7.46-38.27%) ($p < 0.05$) (Figure 3). The order of relative scavenging capacity of ABTS radicals was similar to that of DPPH radicals: ROB (27.87-70.62%) > WR (18.15-32.56%) > KR (15.29-30.02%) (Figure 3).

The reducing power of the P. lobata methanolic extracts increased with the increasing concentration and a significant change was observed at 20 to 60 mg/mL concentration (Figure 4). The slope of reducing power with the concentration of ROB (1.32-3.30) methanolic extraction ($p < 0.05$) was significantly stronger than those of KR (0.37-0.99) and WR (0.30-0.81) (Figure 4).

III. *Correlation of Isoflavonoid Content and Antioxidant Activity*

The total isoflavonoid and total phenolic contents in methanolic extracts of ROB, WR and KR exhibited the correlation coefficients between 0.71-0.75 ($p < 0.05$). Total isoflavonoids also had a positive correlation of 0.89-0.97 with DPPH free radical scavenging, ABTS free radicals and reducing power in P. lobata roots ($p < 0.05$) (Table 2). Puerarin

Table 2. Correlation coefficient of isoflavonoid content and antioxidant activity in P. lobata root

	Correlation coefficient			
	Total phenol	DPPH	ABTS	Reducing power
Puerarin	0.714 (0.000)*	0.902 (0.000)	0.945 (0.000)	0.991 (0.000)
Total isoflavonoids	0.753 (0.000)	0.893 (0.000)	0.935 (0.000)	0.976 (0.000)

*Person's correlation coefficient is significant at $p < 0.05$; $n = 45$.

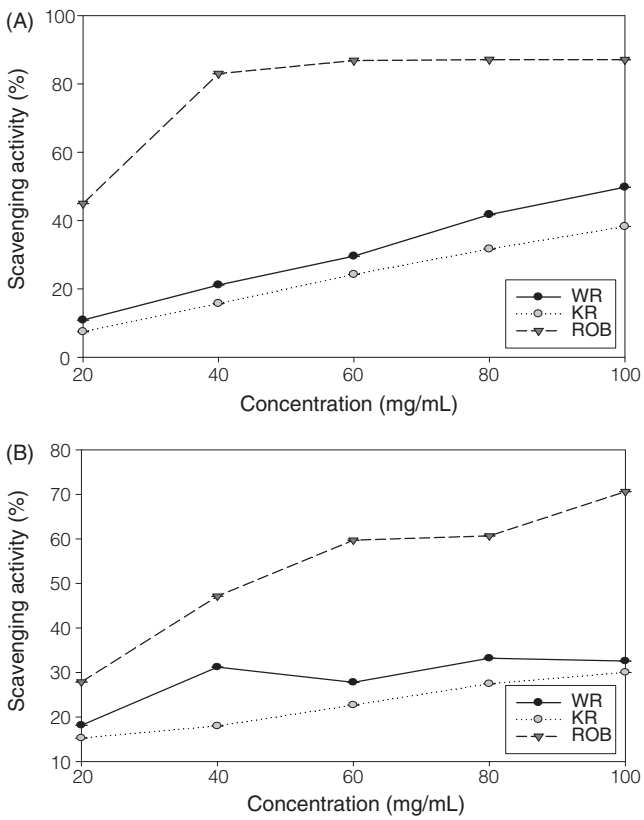

Figure 3. DPPH free radical-scavenging activity (A) and $ABTS^+$ free radical-scavenging activity (B) in methanolic extract of different sections of P. lobata root. Root outer bark (ROB); Kudzu root (KR); Whole root (WR).

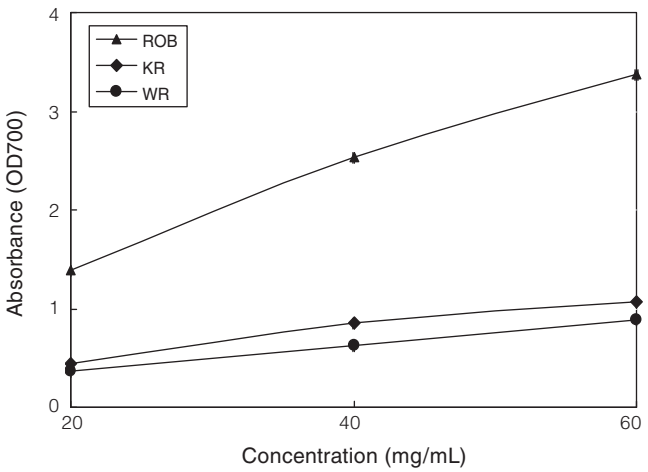

Figure 4. Reducing power in methanolic extract of different sections of P. lobata root. Root outer bark (ROB); Kudzu root (KR); Whole root (WR).

was the major component in all of P. lobata roots samples. The Pearson's correlation coefficient was further applied to assess the correlation of antioxidant activity with puerarin. The results showed a significant positive correlation at 0.90-0.99 between antioxidant activity and puerarin (Table 2).

DISUSSION

The isoflavonoid content as well as total phenol content were higher in ROB over WR or KR. Similar results were found for the distribution of total phenols in olive plants (Olea europaea L.), where the levels in the cortex were higher than that in the pith[18]. The antioxidant activities, including DPPH radical scavenging effect, ABTS radical ability and reducing power, in ROB were also significantly higher than those in WR and KR. The major isoflavonoid components in P. lobata roots include puerarin, daidzin, daidzein, genistin and genistein as previously reported[3,19]. Puerarin was found a significantly greater concentration than the other four isoflavonoids in P. lobata root. Puerarin content as well as antioxidant activity, specific for ABTS radical scavenging ability and reducing power, in ROB was significantly higher than those in WR and KR.

Traditional kudzu root was processed by washing, ROB removing from WR and sliced into pieces. The ROB of P. lobata was thus regarded as a waste in kudzu manufacturing process. In this study we founded the discarded ROB of P. lobata exhibited higher amount of isoflavonoids, especially puerarin, and showed stronger antioxidant activity than KR and WR. Isoflavones are preventive antioxidant substances present especially in leguminous plants in the diet consumed. Some researches applied KR or WR to prepare the health drinks and dietary supplements consumed[20,21]. Kwun, *et al*[20] examined the method of ultrasonication to improve the extracted isoflavones content from the kudzu roots waste. Prasain *et al.*,[21] reported that puerarin was the most abundant component than daidzin in commercially available kudzu

dietary supplements. Thus, ROB should be used to improve the nutritional effect for food industry and Chinese medicine.

CONCLUSIONS

Food and Agriculture Organization (FAO) of the United Nations estimates needs of kudzu to be 50 million tons annually[21]. However, most of ROB of P. lobata was discarded as a waste in Chinese medicine processing. This study showed more isoflavonoids content can be achieved by replacing the kudzu root with the whole root, or to recover abundant isoflavonoids content from the discarding ROB of P. lobata. This result can be readily applied to functional food industry or Chinese medicine production in the future.

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