# Antioxidant Flavone Glycosides from the Root of *Pteroxygonum giraldii*

**Bao-Lin Li,* Zhan-Jun Yang, Lin-Ling Jiang, Xi-Quan Zhang,‡ Hong-Mei Gu,† Hui-Chun Wang, and Xian-Hua Tian**

*Key Laboratory of the Ministry of Education for Medicinal Resources and Natural Pharmaceutical Chemistry, and School of Chemistry and Materials Science, Shaanxi Normal University, Xi'an 710062, P. R. China*
**E-mail: baolinli@snnu.edu.cn*
*‡Jiangsu Chia Tai Tianqing Pharmaceutical Co., Ltd. Nanjing 210018, P. R. China*
*Received March 28, 2009, Accepted May 1, 2009*

Two new flavone glycosides, giraldiin A and B, together with three known compounds, annulatin, myricetin 3-*O*-*α*-L-rhamnopyranoside and gallic acid, were isolated from the ethanol extract of the root of *Pteroxygonum giraldii* Damm. et Diels. The structures of giraldiin A and B are designated as 3'-(*α*-L-arabinopyranosyloxy)-4',5,5',7-tetrahydroxy-3-methoxyflavone and 4'-(*β*-D-glucopyranosyloxy)-5,5',7-trihydroxy-2',3-dimethoxyflavone, respectively, on the basis of detailed spectroscopic analyses. The free radical scavenging activity of giraldiin A was evaluated by decolouring spectrophotometry of pentamethine cyanine dye (Cy5) with $Fe^{2+}$-$H_2O_2$ Fenton radical generating system. The results indicated the hydroxyl free radical scavenging activity of giraldiin A ($ED_{50}$ = 23.7 nmol/mL) is higher than that of some known antioxidants such as rutin, puerarin, daidzein and 2,6-di-tert-butyl-4-methylphenol.

**Key Words**: *Pteroxygonum giraldii*, Flavone glycosides, Giraldiin A, Giraldiin B, Antioxidant

## Introduction

*Pteroxygonum giraldii* Damm. et Diels is a medicinal plant.[1] Its root is extensively used as herbal medicine for hemostasia, rheumatism and detoxification etc in China.[2] It is found widely in Hebei, Shanxi, Henan, Shaanxi, Gansu, Hubei and Sichuan provinces of China, which usually grows in clefts of fissured rocks, hillside and shrub in valleys at altitudes from 600 to 2000 m.[3] Though the root of *P. giraldii* have a good medicinal utility, so far, there was no report on its chemical constituents. Previous studies on this plant only concentrated on its biological characteristic, geographic distribution[4] and the chemical components of the essential oil from fresh leaves and flowers of *P. giraldii*.[5] Therefore, to explore the substance foundation for the bioactivity of the root of *P. giraldii*, we investigated in the chemical constituents of the root of this plant, and got two new flavone glycosides, giraldiin A (**1**) and B (**2**), together with other three known compounds, gallic acid (**3**), annulatin (**4**)[6] and myricetin 3-*O*-*α*-L-rhamnopyranoside (**5**)[7] (Figure 1). Herein this paper deals with the isolation, structure elucidation of these compounds, and the evaluation of the radical scavenging activity for giraldiin A.

Giraldiin A was obtained as a straw yellow amorphous powder and its molecular formula $C_{21}H_{20}O_{12}$ was established from ESI-MS (*m/z* 463[M-H]$^-$) and HR-FABMS (*m/z* 464.3742 [M]$^-$ (calcd. 464.3763)). The IR spectrum showed functional group absorptions corresponding to at 3539, 3394 (OH), 1659 (conjugated and 5-OH chelated carbonyl) and 1614 cm$^{-1}$ (aromatic ring), respectively. The UV spectrum of **1** showed absorptions $\lambda_{max}$ at 269 and 359 nm. These indicated the presence of a flavone skeleton[8,9] in compound **1**. A hydroxyl attached at 5-postion of flavone skeleton was confirmed by the resonance signal at $\delta_H$ 12.67 ppm in NMR spectrum (see Table 1) resulting from the H of this hydroxyl, which exhibited a significant downfield shift due to the hydrogen bond formed from the oxygen of carbonyl at 4-positon. $^1$H, $^{13}$C NMR of **1**

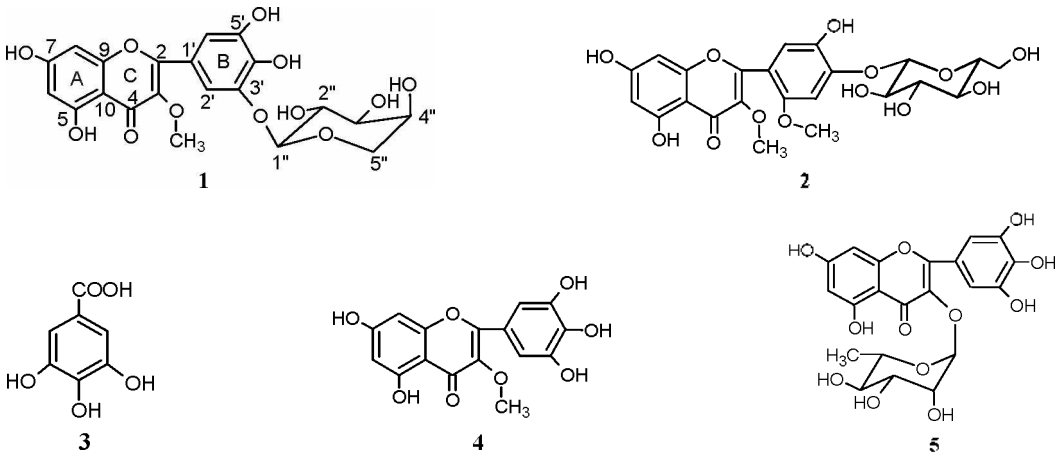


**Figure 1.** Structures of compounds **1-5**.

**Table 1.** $^1$H NMR data and principal HMBC correlations* of **1**, **2** and **4** in DMSO-$d_6$

| Proton | 1 | | 2 | | 4 |
|---|---|---|---|---|---|
| | $\delta_H$, mult, intgr, ($J$, Hz) | HMBC (carbon) | $\delta_H$, mult, intgr, ($J$, Hz) | HMBC (carbon) | $\delta_H$, mult, intgr, ($J$, Hz) |
| 6 | 6.20,d,1H,(1.53) | (5),(7),8,10 | 6.20,s,1H | (5),(7),8,10 | 6.20,br s, 1H |
| 8 | 6.42,d,1H,(1.53) | 6,(7),10 | 6.33,s,1H | 6,(7),(9),10 | 6.40,br s, 1H |
| 2' | 7.33,d,1H,(1.44) | 2,(3'), 4', 6' | | | 7.13,br s, 1H |
| 3' | | | 6.99,s,1H | 1',(2'),(4'),5' | |
| 6' | 7.42,d,1H,(1.44) | 2,2',4',(5') | 6.84,s,1H | 2,2' ,4' ,(5') | 7.13,br s, 1H |
| 1" | 4.76,d,1H,(7.23) | 3' | 4.78,d,1H,(7.62) | 4' | |
| 2" | 3.24,m,1H | | 3.11,m,1H | | |
| 3" | 3.30,m,1H | (2") | 3.17,m,1H | | |
| 4" | 3.32,m,1H | | 3.07,m,1H | | |
| 5"A | 3.87,m,1H | | 3.22,m,1H | | |
| 5"B | 3.28,m,1H | (4") | | | |
| 6"A | | | 3.38,m,1H | | |
| 6"B | | | 3.72,m,1H | | |
| 3-$OCH_3$ | 3.81,s,3H | 3 | 3.70,s,3H | 3 | 3.78,s,3H |
| 2'-$OCH_3$ | | | 3.84,s,3H | 2' | |
| 5-OH | 12.67,s,1H | | 12.71,s,1H | | 12.72,s,1H |
| 7-OH | 10.88,s,1H | | 10.78,s,1H | | |
| 3'-OH | 9.46,s,1H | | | | 9.29,s,1H |
| 5'-OH | 9.01,s,1H | | 8.96,s,1H | | 9.29,s,1H |
| glycosyl-OH | 5.13,d,1H,(4.53); 5.17,d,1H,(3.84); 5.57 br s | | 4.65,br s,1H; 4.98,m,3H | 2",3",4" | |

*Two-bond correlations are indicated in parentheses.

were very similar to that of known compound **4** except for the signals of two secondary H and four tertiary H in $^1$H NMR of **1**, and the signals of a secondary carbon and four tertiary carbons in $^{13}$C NMR of **1** (see Table 1 and 2). This indicated that compound **1** was a derivative bearing a pentose glycosyloxy of annulatin. Acid hydrolysis of the compound afforded arabinose as the sugar residues confirmed by cochromatography with authentic samples. The stereochemistry structure of glycosyl was identified as L-arabinopyranosyl by comparing the spectroscopic data with literature[10] and deducing from the signal at $\delta_C$ 69.9 (C-4").[11] In addition, the doublet at $\delta_H$ 4.76 ($J$ = 7.23 Hz, H-1") and the signal at $\delta_C$ 103.7 (C-1") demonstrated the $\alpha$-configuration of arabinopyranosyl.[11,12] The position of arabinosyloxy was deduced by HMBC experiments (see Table 1). The signal at $\delta_H$ 4.76 assigned to the resonance of H-1" in arabinopyranosyl was correlated with the signal at $\delta_C$ 146.4 (s, C-3') in the HMBC spectrum of **1**, this suggested arabinopyranosyloxy was at 3'-position. Thus, compound **1** was identified as 4',5,5',7-tetrahydroxy-3-methoxy-3'-*O*-$\alpha$-L-arabinopyranosyloxyflavone, a new flavone glycoside named giraldiin A.

Giraldiin B was also obtained as a straw yellow powder. The molecular formula was deduced as $C_{23}H_{24}O_{13}$ from ESI-MS ($m/z$ 507[M-H]$^-$) and HR-FABMS ($m/z$ 508.4276 [M]$^-$ (calcd. 508.4289)). In the IR spectrum of **2**, it revealed hydroxyl, carbonyl at 3364 and 1655 cm$^{-1}$, and aromatic ring at 1600 cm$^{-1}$. The UV spectrum of **2** showed absorptions $\lambda_{max}$ at 256 and 360 nm, which indicated a flavone skeleton exhibited in the molecule of **2**. In the $^{13}$C NMR spectra of **2** (see Table 2), in range of $\delta_C$ 102.4-60.5 ppm presented five tertiary carbons, a secondary carbon and two methoxyls. Compound **2** afforded glucose as the sugar residues on acid hydrolysis. These characters showed a flavone moiety bearing a D-glucopyranosyloxy.[13] two methoxyl groups and three hydroxyls of polyphenol in compound **2**. The $^{13}$C NMR of **2** was similar to that of **1** in the ring A and C, thus a methoxyl and two hydroxyl groups of phenol should attached at C-3, C-5 and C-7 positions, respectively. The signals at $\delta_H$ 6.99 (s, 1H) and 6.84 (s, 1H) were designated to the H of ring B; $\delta_H$ 6.84 presented HMBC correlation (see Table 1) with $\delta_C$ 156.5 (s, C-2) and 141.4 (s); $\delta_H$ 6.99 exhibited HMBC correlation with $\delta_C$ 150.7 (s) and 149.4 (s), these spectra characters indicated the resonance signals at $\delta_H$ 6.99 (s, 1H) and 6.84 (s, 1H) were resulted from H-3' and H-6', and peaks at 150.7, 149.4, and 141.4 (s) were the resonance ones of C-2', C-4' and C-5'. In the HMBC of **2**, $\delta_H$ 3.84 (s, 3H, -$OCH_3$) correlated with $\delta_C$ 150.7 (s), and $\delta_H$ 4.78 (d, 1H, $J$ = 7.62 Hz, H-1") correlated with $\delta_C$ 149.4 (s, C-4'), these suggested the methoxyl and glucopyranosyloxy attached at C-2' and C-4' position, respectively, thus other hydroxyl should be at C-5'. $\beta$-Glucosyl linkage was deduced from the coupling constant of the anomeric proton at $\delta_H$ 4.78 ($J$ = 7.62 Hz).[14] On the basis of above evidences, **2** was determined as 5,5',7'-trihydroxy-2',3-dimethoxy-4'-*O*-$\beta$-D-glucopyranosyloxyflavone, a new flavone glucoside named giraldiin B.

Determination of free radical scavenging activity for giraldiin A was based on the hydroxyl radical (·OH) produced from $H_2O_2$-$FeSO_4$ Fenton system[15] could reduce the color intensity

**Table 2.** $^{13}$C-NMR* data of **1**, **2** and **4** in DMSO-$d_6$

| Carbon | 1 | 2 | 4 |
|---|---|---|---|
| 2 | 155.5(s) | 156.5(s) | 156.2(s) |
| 3 | 138.4(s) | 139.5(s) | 137.3(s) |
| 4 | 178.0(s) | 178.6(s) | 178.3(s) |
| 5 | 161.2(s) | 161.9(s) | 161.7(s) |
| 6 | 99.1(d) | 98.9(d) | 99.01(d) |
| 7 | 164.8(s) | 164.4(s) | 164.6(s) |
| 8 | 94.0(d) | 94.3(d) | 93.9(d) |
| 9 | 156.7(s) | 157.6(s) | 156.7(s) |
| 10 | 104.7(s) | 105.4(s) | 104.6(s) |
| 1' | 120.2(s) | 111.9(s) | 120.1(s) |
| 2' | 111.0(d) | 150.7(s) | 108.1(d) |
| 3' | 146.4(s) | 102.5(d) | 146.3(s) |
| 4' | 139.1(s) | 149.4(s) | 138.3(s) |
| 5' | 146.1(s) | 141.4(s) | 146.3(s) |
| 6' | 109.1(d) | 116.5(d) | 108.1(d) |
| 1'' | 103.7(d) | 102.4(d) | |
| 2'' | 76.2(d) | 73.7(d) | |
| 3'' | 73.6(d) | 77.2(d) | |
| 4'' | 69.9(d) | 70.7(d) | |
| 5'' | 66.3(t) | 77.8(d) | |
| 6'' | | 61.5(t) | |
| 3-$OCH_3$ | 60.1(q) | 60.5(q) | 60.1(q) |
| 2'-$OCH_3$ | | 56.1(q) | |

*The $^{13}$C NMR multiplicities were obtained from the 135° DEPT and HMQC spectra.

of pentamethine cyanine dye (Cy5) aqueous solution.[16] Thus the concentration of ·OH produced from $H_2O_2$-$FeSO_4$ Fenton system can be indirectly evaluated by using the absorbance ($A_0$) of $FeSO_4$-Cy5 solution minus the absorbance ($A$) of the same concentration of $H_2O_2$-$FeSO_4$-Cy5 system determined at 647 nm. With increasing the concentration of antioxidants or the free radical scavenging activities of antioxidants in $H_2O_2$-$FeSO_4$-Cy5 system, the absorbance ($A_s$) of the $H_2O_2$-$FeSO_4$-Cy5 system could be increased. Based on this principle, the hydroxyl radical scavenging ratio ($S$) of tested compound can be calculated according to the equation (1).

$$S = \frac{A_s - A}{A_0 - A} \times 100\% \quad (1)$$

This system can be used as a convenient method for the evaluation of the hydroxyl radical scavenging activity of compound, and the screen of antioxidant.[16] The hydroxyl radical scavenging ratios ($S$) for a series of concentrations of giraldiin A in $H_2O_2$-$FeSO_4$-Cy5 system were determined, meanwhile some natural antioxidants such as rutin, puerarin, daidzein and synthetical antioxidant 2,6-di-tert-butyl-4-methylphenol (BHT) were also determined as positive control, the results are presented in Figure 2. The median effective doses ($ED_{50}$) of tested compounds to hydroxyl radical are listed in Table 3. Rutin, puerarin and daidzein are well known for many years as health protection reagent for coronary heart and cardiovascular diseases because of their high free radical

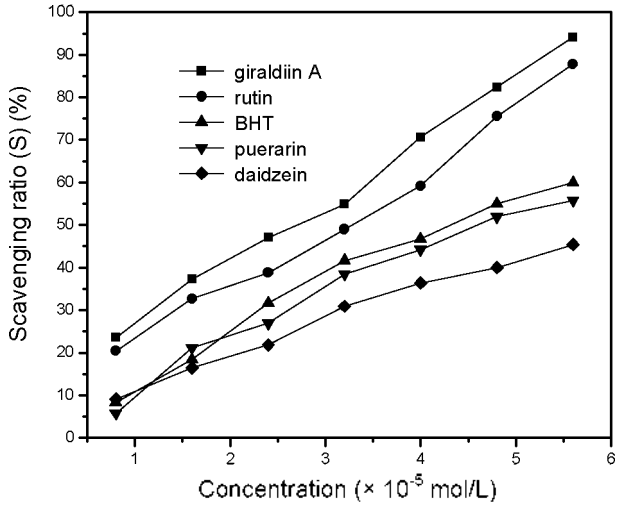


**Figure 2.** The hydroxyl radical scavenging activities of compounds.

**Table 3.** $ED_{50}$ to hydroxyl free radical for giraldiin A and some antioxidants

| Sample | giraldiin A | rutin | BHT | puerarin | daidzein |
|---|---|---|---|---|---|
| $ED_{50}$ (nmol/mL) | 23.7 | 32.1 | 42.2 | 43.8 | 70.4 |

scavenging capacity and antioxidation activities.[17] Herein, giraldiin A exhibited a higher hydroxyl free radical scavenging capability ($ED_{50}$ = 23.7 nmol/mL) than rutin etc natural or synthetical antioxidants. Therefore, it is necessary to further explore the bioactivity of giraldiin A for cardiovascular diseases *etc.*

## Experimental Section

**General experimental procedures.** The IR spectra were recorded with a Nicolet 170SX FT-IR spectrometer using KBr pellets. Melting points were determined with a X-6 micro-melting point apparatus and uncorrected. NMR spectra were preformed on a Bruker AVANCE300 instrument, the chemical shift values are given in ppm using TMS as the internal standard. ESI-MS data were obtained on a Bruker Esquire 3000plus mass instrument; HRFAB mass spectrum was recorded on an Autospec 3000 instrument. pH of Solutions were determined with pHS-3C-meter. The absorbances of solutions were determined with a TU-1901 UV-Visible Spectrophotometer. Silica gel GF254 and 200-300 mesh silica gel (from Qingdao Marine Chemical Co., Qingdao, P. R. China) were used for TLC and column chromatography, respectively.

**Plant material.** The roots of *P. giraldii* Damm. et Diels were collected from Taibai Mountain in altitudes from 800-1500 m, Shaanxi province of China. The specimens were identified by professor Tian Xian-Hua (College of Life Science, Shaanxi Normal University, China) and the voucher specimen was deposited in the Herbarium of Department of Plant Taxonomy, College of Life Sciences, Shaanxi Normal University.

**Extraction and isolation.** The dried powdered root of *P. giraldii* (4.5 kg) were extracted with 95% EtOH (10 L × 4) at

room temperature for 2 days. After removal of the solvent in *vacuo*, the residue was partitioned in $H_2O$ and extracted with petroleum ether (b.p. 60-90 °C, 2 L × 3), EtOAc (2 L × 3), respectively. The EtOAc extracts were combined, and the solvent was removed under reduced pressure to yield extract 71 g.

The EtOAc extract (71 g) was subjected to silica gel column chromatograghy (1.5 kg, 200-300 mesh). The column was eluted with $CHCl_3$ and then with increasing concentrations of MeOH in $CHCl_3$ (10% increments from 0 to 100% MeOH). 250 mL fractions were collected, and fractions were combined by monitoring with TLC. The fraction eluted from 20% of MeOH was further purified by silica gel column chromatograghy to yield 68 mg of gallic acid (**3**, 0.0015%). The fraction eluted from 30% of MeOH was evaporated under reduced pressure to give a yellow solid. After immerse-washing the solid with MeOH (30 mL × 3), the residue of 2.13 g, giraldiin A (**1**, 0.05%), was obtained as straw yellow powder. Immerse-washing solution of MeOH was concentrated and further separated by silica gel column chromatograghy (100 g, 200-300 mesh) eluted with petroleum ether and increasing proportions of acetone (from petroleum ether 100% to acetone 100%), to give annulatin of 54 mg (**4**, 0.0012%), myricetin 3-*O*-$\alpha$-L-rhamnopyranoside of 45 mg (**5**, 0.001%), giraldiin B of 26 mg (**2**, 0.0006%) as straw yellow powder.

**Giraldiin A (1).** 4',5,5',7-Tetrahydroxy-3-methoxy-3'-*O*-$\alpha$-L-arabinopyranosyloxyflavone. Straw yellow powder. Mp 258-259 °C. $^1$H NMR and HMBC are listed in Table 1. $^{13}$C NMR are listed in Table 2. ESI-MS *m/z* (neg.): 463 ([M-H]$^-$). HR-FAB-MS *m/z* (pos.): 464.3742 ([M]$^-$: $C_{21}H_{20}O_{12}$; calc. 464.3763). UV $\lambda_{max}$ (MeOH): 269, 359. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3539, 3394, 2924, 1659, 1614, 1562, 1514, 1447.

**Giraldiin B (2).** 5,5',7'-Trihydroxy-2',3-dimethoxy-4'-*O*-$\beta$-D-glucopyranosyloxyflavone. Straw yellow powder. Mp 250-252 °C. $^1$H NMR and HMBC are listed in Table 1. $^{13}$C NMR are listed in Table 2. ESI-MS *m/z* (neg.): 507 ([M-H]$^-$). HR-FAB-MS *m/z* (pos): 508.4276 ([M]$^+$: $C_{23}H_{24}O_{13}$; calc. 508.4289). UV $\lambda_{max}$ (MeOH): 256, 360. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3364, 2920, 1655, 1600, 1496.

**Acid hydrolysis.** Compounds (**1** and **2**), 5 mg each in a mixture of 8% HCl (1 mL) and MeOH (10 mL) were separately refluxed for 2 h. The reaction mixtures were reduced in vacuo to dryness, dissolved in $H_2O$ (2 mL) and neutralized with NaOH. The neutralized products were subjected to TLC analysis on silica gel (eluent: EtOAc-MeOH-$H_2O$-HOAc, 6:2:1:1). The chromatograms were sprayed with aniline hydrogen phthalate followed by heating at 100 °C. The sugars were identified after comparison with authentic samples.

**Determination of free radical scavenging activity of giraldiin A.** The evaluation of the hydroxyl free radical scavenging capacity of giraldiin A was carried out according to the protocol described previously using decolouring spectrophotometry of Cy5 with $Fe^{2+}$-$H_2O_2$ Fenton radical generating system.[16] In brief, 0.8 mL of pH 4.0 Tris-HCl buffer, 0.6 mL of $3.3\times10^{-2}$ mmol/L Cy5 aqueous solution and 0.4 mL of 1.0 mmol/L $FeSO_4$ aqueous solution were added to 5 mL color comparison tube. To the result mixture was added 0.4 mL of 0.01% $H_2O_2$, then added $H_2O$ up to 5 mL. After 7 min, the absorbance ($A$) of mixture was determined at 647 nm with a TU-1901 UV-Visible Spectrophotometer. Meanwhile the absorbance ($A_0$) of the system uncontained $H_2O_2$ and the absorbance ($A_s$) of the system contained giraldiin A or other antioxidants were determined at the same wavelength, respectively. The hydroxyl free radical scavenging ratios ($S$) of compounds were calculated according to the equation (1). The results are presented in Figure 2. The median effective doses ($ED_{50}$) of tested compounds to hydroxyl radical are listed in Table 3.

**Acknowledgments.** The authors are thankful for financial support from Science and Technology Key Project of Education Ministry, P. R. China (No: 105153) and Jiangsu Chia Tai Tianqing Pharmaceutical Co., Ltd., P. R. China.

## References

1. Li, Y. L.; Lu, J. X.; Li, X. H.; Han, Q.; Jiang X. T. *Acta Botanica Boreali-occidentalia Sinica* **1994**, *14*, 324-328.
2. Wang, K. J.; Zhang, Y. J.; Yang, C. R. *Chinese Nat. Prod. Res. Dev.* **2006**, *18*, 151-164.
3. Li, A. R.; Gao, C. J.; Mao, Z. F.; Liu, Y. L. *Florae Reipublicae Popularis Sinicae*; Science Press: Beijing, 1998; Vol. 25(1), pp 117-118.
4. Zhang, Q. W. *Journal of Ningxia University (Natural Science Edition)* **2002**, *23*(1), 86-93.
5. Yang, Z. J.; Li, B. L.; Tian, X. H.; Liang, B. *Xibei Zhiwu Xuebao* **2007**, *27*(6), 1261-1264.
6. Yoo, H.; Kim, S. H.; Lee, J.; Kim, H. J.; Seo, S. H.; Chung, B. Y.; Jin, C.; Lee, Y. S. *Bull. Korean Chem. Soc.* **2005**, *26*, 2057-2060.
7. Sun, H. Y.; Long, L. J.; Wu, J. *Chinese Traditional and Herbal Drugs* **2006**, *29*, 671-672.
8. Markham, K. R.; Whitehouse, L. A. *Phytochemistry* **1984**, *23*, 1931-1936.
9. Li, M.; Han, X. W.; Yu, B. *Tetrahedron Letters* **2002**, *43*, 9467-9470.
10. Fu, L.; Li, X. G.; Yang, S. R. *Journal of Jilin Agricultural University* **1998**, *20*(2), 30-34.
11. Chen, J.; Xu, X. F.; Chai, X. Y.; Li, P. *Chinese Journal of Natural Medicines* **2006**, *14*(5), 347-351.
12. Piccinelli, A. L.; Mesa, M. G.; Armenteros, D. M.; Alfonso, M. A.; Arevalo, A. C.; Campone, L.; Rastrelli, L. *J. Agric. Food Chem.* **2008**, *56*, 1574-1581.
13. Marin, P. D.; Grayer, R. J.; Grujic-Jovanovic, S.; Kite, G. C.; Veitch, N. C. *Phytochemistry* **2004**, *65*, 1247-1253.
14. Wang, H. B.; Yao, H.; Bao, G. H.; Zhang, H. P.; Qin, G. W. *Phytochemistry* **2004**, *65*, 969-974.
15. Yang, M. H.; Liu, M. H.; He, L. X.; Cao, Q. E. *Chinese Journal of Analysis Laboratory* **2006**, *25*, 77-80.
16. Jiang, L. L.; Li, B. L.; Wang, L. *Journal of Analytical Science* **2008**, *24*, 212-214.
17. Yang, N.; Ren, G. X. *J. Agric. Food Chem.* **2008**, *56*, 761-764.