Comparative Study of *Puerariae lobatae* and *Puerariae thomsonii* by HPLC-Diode Array Detection-Flow Injection-Chemiluminescence Coupled with HPLC-Electrospray Ionization-MS

Cui-Ling Zhang,[a,b,#] Xiao-Ping Ding,[a,#] Zheng-Fang Hu,[a] Xin-Tang Wang,[c] Lin-Lin Chen,[a] Jin Qi,[a] and Bo-Yang Yu*,[a]

[a] *Department of Complex Prescription of Traditional Chinese Medicine, China Pharmaceutical University;* [c] *Department of Pharmacognosy, China Pharmaceutical University; Nanjing 211198, China: and* [b] *Department of Traditional Chinese Medicine, Beng Bu Medical College; Beng Bu 233030, China.*

Received September 9, 2010; accepted December 28, 2010; published online February 24, 2011

An on-line HPLC-diode array detection-flow injection chemiluminescence (HPLC-DAD-FICL) method was applied to estimate the difference of *Puerariae lobatae* and *Puerariae thomsonii*. Their chemical and active profiles could be obtained by HPLC-DAD-FICL in one run. Seventeen compounds in two species were tentatively identified by HPLC-electrospray ionization-MS (HPLC-ESI-MS) method. The main antioxidants were rapidly screened by active fingerprints coupled with MS data. Similarity and Hierarchical clustering analysis (HCA) were used to distinguish different samples. The results suggested that the chemical fingerprints of 16 batches of samples were similar by similarity evaluation, while HCA could discriminate the two species. The active fingerprints of *Puerariae lobatae* and *Puerariae thomsonii* were significantly different. More antioxidants were found in *Puerariae lobatae* than in *Puerariae thomsonii*. Main antioxidants, including 3′-hydroxypuerarin, genistein 8-*C*-glycoside-xyloside, puerarin, 6″-*O*-xylosylpuerarin, mirificin and daidzein in two species, may be reasonable markers for the discrimination of the two species. The integrated fingerprint based on the chemical and active characteristics may provide an objective quality evaluation for *Puerariae lobatae* and *Puerariae thomsonii*.

Key words integrated fingerprint; *Puerariae lobatae*; *Puerariae thomsonii*; HPLC-diode array detection-flow injection chemiluminescence; HPLC-electrospray ionization-MS; antioxidant activity

Puerariae lobatae and *Puerariae thomsonii* distributed in American and Southeast Asia, and have been widely used as herbal medicines in China. The two herbs are simultaneously listed in *Radix Puerariae* of Chinese Pharmacopoeia till 2000 edition. However, *Puerariae lobatae* and *Puerariae thomsonii* are displayed as two herbal medicines in 2005 edition due to different content of puerarin. *Puerariae lobatae* is a main plant resource for clinical applications, while *Puerariae thomsonii* is widely used as a soup material in south of China. It is valuable to study the significant differences of the two plants based on their active ingredients. Although the comparative study on antioxidant activity and HPLC fingerprint of aqueous root extracts of the two herbs have been reported,[1] the differences of their antioxidant activity characteristics and chemical compounds did not be comprehensively evaluated due to the impossibility of separating each compound from the two herbs.

Modern theory of free radical biology and medicine demonstrates that free radicals are concerned with the course of cancers, ageing and cardiovascular diseases, and supplementation of plant-derived antioxidants is considered to supply a good protection against such disorders.[2—4] Therefore, plant-derived antioxidant activity has gained more and more attention and been internationally accepted. At present, antioxidant activity study is mainly determined by a single component or extracts of plant by off-line analysis. In order to avoid the complex separation of a single component and loss of active components in the separation process, on-line chromatographic separation combining activity determination methods have been developed to screen the active compounds in complex matrixes. On-line analysis is chiefly achieved by HPLC-diode array detection (DAD)-1,1-diphenyl-2-picrylhydrazyl (DPPH) method for the determination of DPPH bleaching, and HPLC-DAD-chemiluminescence (CL) method for the detection of H_2O_2 and $O_2^{-}\cdot$ scavenging activities.[5,6] In our previous study, the on-line HPLC-DAD-CL method was applied to evaluate the quality of herbal medicines based on the chemical and active fingerprints.[7] However, the validity of this strategy still needed to be further verified.

At present, some analytical methods, including HPLC, UV, TLC and capillary electrophoresis (CE), have been applied to determine the active ingredients in *Puerariae lobatae* and *Puerariae thomsonii*.[8—10] These methods mainly based on the quantitive analysis of several compounds in the two herbs, did not display their activity characteristics. The investigations indicated that the two herbs possessed better antioxidant activity. Isoflavonoids, such as puerarin, daidzin and daidzein, were confirmed to be the major efficient ingredients in the two medicinal materials,[11—13] and had superior free radicals scavenging capacity.[14] Although the comparative study on antioxidant activity and HPLC fingerprint of aqueous root extracts of the two plants was reported in previous study and two kinds of medicinal materials were distinguish by several isoflavonoids,[15—17] the active properties of the two herbs were yet unclear. So the establishment of a rapid separation coupled with on-line activity analysis method for integrity evaluation of the two species will be necessary.

In this study, the combination analysis of HPLC-DAD-flow injection-chemiluminescence (FICL) and HPLC-electrospray ionization (ESI)-MS methods was applied for the detection of radical scavenging characteristics and identification of main active ingredients in *Puerariae lobatae* and *Puerariae thomsonii*. The main differences of the two species were displayed by the proposed method. The chromatog-

* To whom correspondence should be addressed. e-mail: boyangyu59@163.com

# These authors contributed equally to this work.

raphic fingerprints and active profiles of *Puerariae lobatae* and *Puerariae thomsonii* from different habitats were utilized to evaluate the quality of *Puerariae lobatae* and *Puerariae thomsonii*.

Experimental

Plant Materials and Chemicals *Puerariae lobatae* and *Puerariae thomsonii* were collected in different natural growth sites in China. All of them were identified by Professor Bo-yang Yu, Department of Complex Prescription of Traditional Chinese Medicine, China Pharmaceutical University. Their habitats and codes were listed as follows: Guangxi (S01); Guangxi (S02); Fujian (S03); Anhui (S04); Anhui (S05); Guangzhou (S06); Guangzhou (S07); Guangxi (S08); Anhui (S09); Guangxi (S10); Guangxi (S11); Anhui(S12); Hunan (S13); Hunan (S14); Hubei(S15); Dabieshan (S16). S01 to S11 were *Puerariae thomsonii*, while S12 to S16 were *Puerariae lobatae*.

Acetonitrile (Dikma, U.S.A.) and analytical grade phosphoric acid (Nanjing Chemical Plant, Jiangsu, China) were used for chromatographic preparation. Luminol (Sigma corporation, U.S.A.), Hydrogen peroxide (30% H_2O_2 in water), Na_2CO_3 and $NaHCO_3$ (Nanjing Chemical Reagent Corp., Jiangsu, China), ethylenediaminetetraacdetic acid (EDTA) (Shanghai Chemical Reagent Corporation, Shanghai, China), were applied for CL detection. The purified water was prepared using a Millipore water purification system (Millipore, Bedford, MA, U.S.A.).

Sample Preparation *Puerariae lobatae* and *Puerariae thomsonii* from different habitats were milled to 60 mesh powder. Each dry sample powder (2.5 g) was accurately weighted into a conical flask, and was refluxed with 60 ml methanol–water (95 : 5, v/v) for 1 h. The process was repeated twice. The extract solution was filtered, and then the residues were rinsed with 20 ml methanol–water (95 : 5, v/v) for two times. The extracts and washings were mixed and evaporated to dryness under vacuum. The dry residue was dissolved and diluted with methanol–water (95 : 5, v/v) into a 25 ml volumetric flask. After filtration through a 0.45 μm filter, an aliquot of 5 μl solution was injected to HPLC.

The Preparation of CL Solutions A 1.85×10^{-2} mol/l stock solution of luminol was prepared in 0.1 mol/l Na_2CO_3 solution and stored in a refrigerator for at least 3 d before dilution. 0.1 mol/l carbonate buffers (pH 10.0) were prepared by mixing appropriate volumes of 0.1 mol/l Na_2CO_3 and 0.1 M $NaHCO_3$ solutions. 9.1×10^{-6} mol/l luminol solution was prepared by mixing 1.85×10^{-2} mol/l luminol, 0.1 mol/l carbonate buffer and 6.3×10^{-3} mol/l EDTA. 4.4×10^{-4} mol/l H_2O_2 solution was prepared from 30% H_2O_2 diluted in water.

HPLC-DAD-FICL Procedures The chromatographic analysis was performed on an Agilent 1100 system equipmented with binary pump, autosampler, thermostated column compartment and a photodiode array detector (DAD). A Lichrospher C18 column (4.6 mm×250 mm, 5 μm) (Hanbon Sci & Tech, Jiangsu, China) was used for all chromatographic separation. BT-200 peristaltic pump (Huxi Analysis Instrument Factory, Shanghai, China) was used to deliver the CL solutions. The CL emission was detected by BPCL system (Academia Sinica Biophysics Institute, Beijing, China).

Separation of samples was achieved using a mobile phase consisting of 0.1% (v/v) aqueous H_3PO_4 (A) and acetonitrile (B) with the following gradient elution program: 0—20 min, 10—12% B; 20—40 min, 12—15% B; 40—60 min, 15—25%; 60—80 min, 25—50% B; 80—100 min, 50—60%. The flow rate was 1.0 ml/min and the detection wavelength was set at 280 nm. Column temperature was maintained at 30 °C. On-line post-column addition of CL reagents was delivered with a peristaltic pump at the flow rate of 1.2 ml/min for luminol and H_2O_2 solutions, respectively. CL detector was equipped with a flat glass coil (100 μl) as detection cell and a photomultiplier operated at −1000 V. HPLC-DAD-FICL detection system was interconnected with PEEK tubes.[7]

HPLC-ESI-MS Analysis The above HPLC system was interfaced with an Agilent 1100 LC/MSD Trap XCT ESI (Agilent Technologies, MA, U.S.A.). The HPLC-MS analysis was performed under the same gradient program with HPLC-DAD-FICL using the 0.1% (v/v) aqueous formic acid (A) and acetonitrile (B). The ESI-MS spectra were acquired in negative ionization modes recorded on a mass range over *m*/*z* 100—1000. Capillary voltage was 3300 V. Drying gas temperature was set at 350 °C with a flow rate of 9.0 l/min and nebulizing pressure was of 40 psi.

Data Analysis Data analysis was performed by professional software named Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004 A), which was edited by Chinese Pharmacopoeia Committee. The similarities of different chromatographic patterns were compared with mean chromatogram between the samples tested.

Hierarchical clustering analysis (HCA) was performed to analyze the data from chemical and active fingerprint by SPSS software (SPSS for Windows 12.0, SPSS Inc., U.S.A.). The nearest neighbor method, a pattern similarity measure, was applied and cosine was selected as measurement for hierarchical cluster analysis. In the present work, similarity evaluation and HCA were used to assess chromatographic and active fingerprints of all samples.

Results and Discussion

The Optimization of HPLC-DAD-FICL Detection Condition Chemiluminescence (CL) as a detection technique of HPLC has been developed since the 1980s. Although it is not as universal as other detection systems, such as DAD and mass spectrometry, CL detection is rapidly growing and very attractive due to its high sensitivity, wide dynamic range and simple instrumentation. HPLC-CL is becoming a powerful analytical tool with widespread application in various fields, including the determination of pesticides,[18] drugs,[19—21] antioxidants[22] and others.[23,24] Considering the factors influencing the CL reaction, the pH of luminol solution and the concentrations of luminol and H_2O_2 solutions in luminol–H_2O_2 reaction are usually studied to obtain maximal enhanced CL intensity. However, to our knowledge, the chromatographic separation conditions of HPLC markedly affect CL intensity in the on-line HPLC-FICL analysis. Therefore, the above factors should be optimized for the stable baseline and high luminescence intensity of CL detection.

In HPLC separation, the mixture of methanol or acetonitrile with water or acids was currently used as the mobile phase in separating herbal medicines. The CL characteristic of the mobile phases was investigated, and the results indicated that the gradient elution using methanol and acids of high percentage could not achieve the HPLC-FICL analysis of the complex sample due to intense CL baseline drift. Therefore, acetonitrile and acids of low percentage were applied to build the chromatographic and CL profiles of herbal medicines. In our previous study, acetonitrile–0.1% phosphoric acid was regarded as the best eluent because it provided the highest CL signal with a good separation.

Furthermore, the factors influencing the CL reaction such as the pH of luminol solution, the concentrations of luminol and H_2O_2, and flow rate of solutions were studied in order to obtain maximal enhanced CL intensity. To find out the optimal concentration of luminol and H_2O_2, a series of concentrations of luminol and H_2O_2 were tested.

The effect of the concentration of luminol solution was evaluated in the range 5.49×10^{-6}—2.2×10^{-5} mol/l. Increasing the luminol concentration (5.49×10^{-6}—1.46×10^{-5} mol/l) resulted in an increase in CL intensity and the luminol concentrations higher than 1.46×10^{-5} mol/l caused a decrease in CL intensity and low CL inhibition. The CL intensity increased while the concentration of luminol increased, but high concentration affected the detection sensitivity. Considering the sensitivity of CL inhibition, 9.15×10^{-6} mol/l of luminol was selected as optimum.

The effect of H_2O_2 concentration on the enhanced CL intensity was investigated in the range 4.41×10^{-4}—1.73×10^{-3} mol/l. With the increase of H_2O_2 concentration of in the range 4.41×10^{-4}—8.82×10^{-4} mol/l, the enhanced CL intensity increased sharply, but the inhibited CL intensity decreased beyond 8.82×10^{-4} mol/l. Furthermore, H_2O_2 con-

centration markedly affected the sensitivity of CL inhibition. So 8.82×10^{-4} mol/l of H_2O_2 was selected as optimum for the maximum CL intensity.

The pH of the solution appeared to be the main variable responsible for the CL reaction. The effect of the pH on the CL intensity was studied ranging from 10.0 to 11.0, and the maximum enhanced CL intensity and strong CL inhibition could be reached at pH 10.0. Thus, a Na_2CO_3–$NaHCO_3$ buffer solution with pH 10.0 value was considered to be optimal.

The optimal conditions described above were applied for the further study because they were compatible with the CL detection system.

On-Line Analysis for Chemical and Active Fingerprints of *Puerariae lobatae* and *Puerariae thomsonii* by HPLC-DAD-FICL HPLC-DAD-FICL analysis at 280 nm revealed a serious of chromatographic and corresponding CL inhibited peaks of *Puerariae lobatae* (S14) and *Puerariae thomsonii* (S01) extract (Fig. 1). The antioxidant activities of compounds were proportional to the intensity of negative peaks. The peaks were detected with good baseline separation, many of which exhibited substantial antioxidant activity. More and stronger active compounds could be found in *Puerariae lobatae* than in *Puerariae thomsonii*. Some minor peaks in chromatographic fingerprints showed strong CL inhibition, such as a few inhibiting peaks marked using asterisk. As shown in Figs. 1(A) and (B), peak 2, 3, 4, 5, 6, 8, 16 and several unknown compounds showed stronger antioxidant activities, many of which existed in *Puerariae lobatae*. The unknown active ingredients still needed to be further identified.

The active profiles of 16 batches of samples were shown in Fig. 2. There were much more inhibited peaks in *Puerariae lobatae* except for S11 and S15 than in *Puerariae thomsonii*. The active profiles of 5 batches of *Puerariae lobatae* were different. Although chemical fingerprints of *Puerariae thomsonii* from different habitats were similar, different active characteristics of S01 to S11 could be observed. Some minor peaks of *Puerariae thomsonii* in 70 to 80 min could inhibit CL except for S03, S04 and S05. HPLC fingerprint evaluation usually based on the main peaks, while CL profiles could reveal the potential activity of some trace components due to its high sensitivity. Some smaller peaks might be ignored in HPLC fingerprint analysis. Therefore, the on-line analysis of different detectors could compensate for some shortages of simple analysis method.

The Identification and Comparison of Main Radical Scavengers in *Puerariae lobatae* and *Puerariae thomsonii* In the fingerprints of *Puerariae lobatae* and *Puerariae thomsonii*, 19 peaks were identified by HPLC-ESI-MS and compared with the reported MS data,[25,26] and the compounds listed in Table 1. The results suggested that the compounds were flavonoids except for Sophoraside A (peak 15). The results of MS information combining antioxidant activity detection indicated that the flavonoids in two herbs played an important role in the treatment of diseases.

17 compounds in *Puerariae lobatae* and 15 compounds in *Puerariae thomsonii* were identified, respectively. According to the chemical and active fingerprints of the samples (Figs. 2, 3), peak 2 (3′-hydroxypuerarin), peak 3 (genistein 8-*C*-glycoside-xyloside), peak 4, peak 5 (puerarin), peak 6 (6″-*O*-xylosylpuerarin), peak 7 (mirificin), peak 8 (isomer of daidzin) and several unknown compounds exhibited high contributions to antioxidant activity of *Puerariae lobatae*. However, antioxidant activity of *Puerariae thomsonii* mainly attributed to peak 2, 5, 8, 16 (daidzein) and two unknown components marked using asterisk, and peak 3, 6 and 7 showed minor contributions to antioxidant activity. Peak 4,

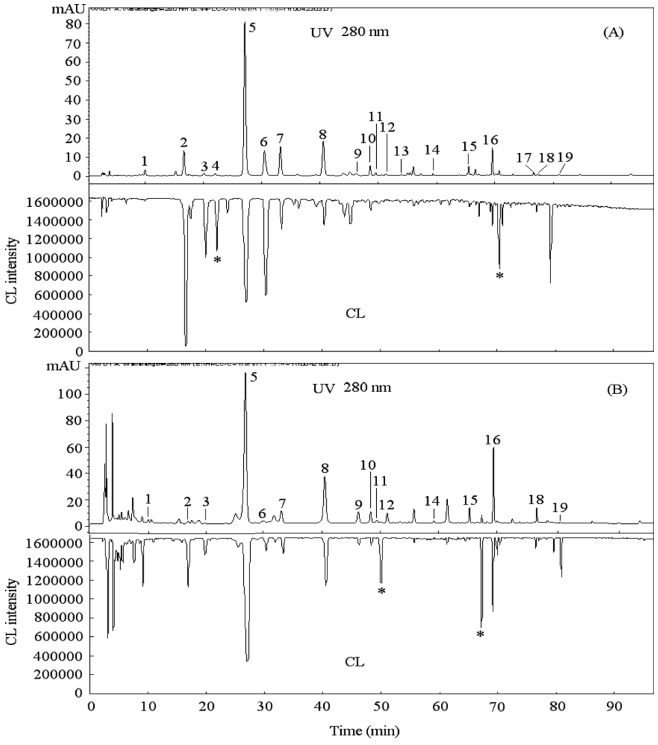

Fig. 1. Chromatograms and CL Inhibited Profiles of *Puerariae lobatae* and *Puerariae thomsonii* Samples by On-Line HPLC-DAD-FICL Detection

(A) *Puerariae lobatae* (S14), (B) *Puerariae thomsonii* (S01).

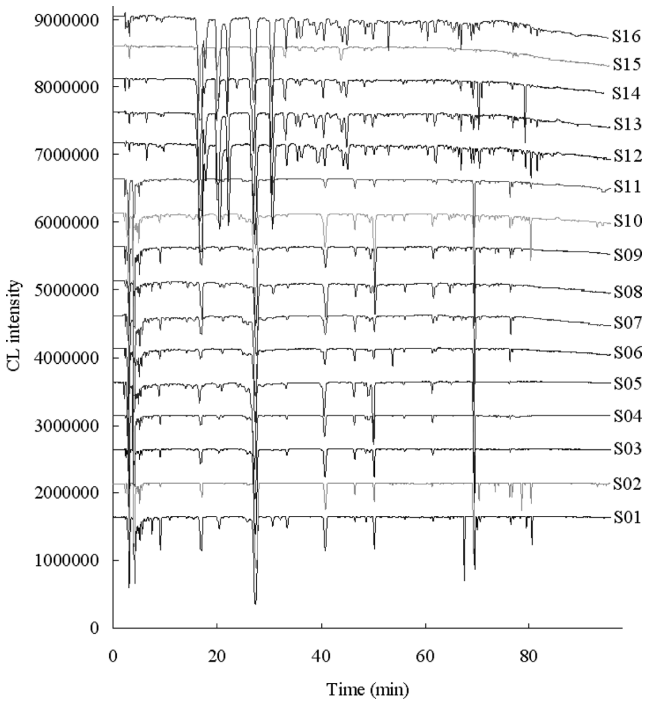

Fig. 2. CL Inhibited Profiles of 16 Batches of Samples from Various Habitats

Table 1. Identification of Main Active Compounds in *Puerariae lobatae* and *Puerariae thomsonii*

Peak No.	t_R (min)	$[M-H]^-/[M+HCOOH-H]^-$	Compounds	HPLC-ESI-MS^n *m/z*
1	9.9	577	Puerarin-4′-*O*-glucoside	MS^2 [577]: 458, 295, 267
2	16.4	431	3′-Hydroxypuerarin	MS^2 [431]: 311, 283
3	19.4	563	Genistein 8-*C*-glycoside-xyloside	MS^2 [563]: 311, 283, 255
4	21.4	563	Unknown compound	MS^2 [563]: 341, 311, 283, 255
5	26.4	415	Puerarin	MS^2 [415]: 295, 267
6	29.9	547	6″-*O*-Xylosylpuerarin	MS^2 [547]: 295, 267
7	32.6	547	Mirificin	MS^2 [547]: 295, 267
8	39.9	461	Isomer of daidzin	MS^2 [461]: 415, 295, 253
9	45.6	431	Genistin	MS^2 [431]: 311, 283
10	48.1	563	6″-*O*-Apiosylgenistin	MS^2 [563]: 311, 283
11	49.1	605	An isoflavone with one rhamnose, one *C*-glycoside	MS^2 [605]: 461, 417, 297, 253, 211
12	50.9	415	Daidzin	MS^2 [415]: 295, 253, 252
13	53.8	445	3′-Methoxypuerarin	MS^2 [445]: 283, 268, 253
14	59.0	253	Isodaidzein	MS^2 [253]: 223, 195, 183
15	65.4	473	Sophoraside A	MS^2 [473]: 311, 267, 252
16	69.4	253	Daidzein	MS^2 [253]: 223, 195, 169, 149
17	76.0	283	Biochanin A	MS^2 [283]: 268, 239, 211
18	76.5	269	Genistein	MS^2 [269]: 253, 223, 195
19	80.9	267	Formononetin	MS^2 [267]: 252, 223

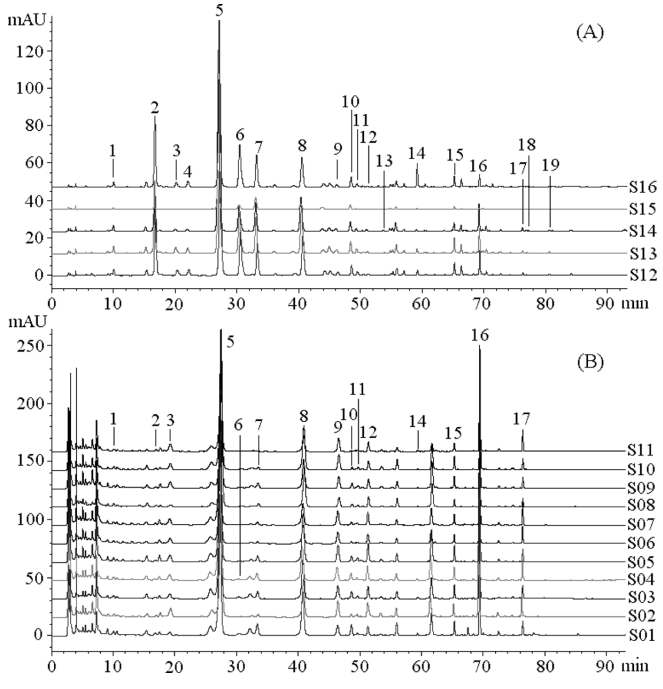

Fig. 3. Chemical Fingerprints of *Puerariae lobatae* and *Puerariae thomsonii* Samples

(A) *Puerariae lobatae*, (B) *Puerariae thomsonii*.

13 and 17 were not detected in *Puerariae thomsonii*.

Similarity Evaluation and Hierarchical Cluster Analysis of *Puerariae lobatae* and *Puerariae thomsonii* Figures 3(A) and (B) showed the chemical fingerprints of *Puerariae lobatae* and *Puerariae thomsonii* from various habitats, respectively. Different fingerprint characteristics between *Puerariae lobatae* and *Puerariae thomsonii* could be observed. Eleven batches of *Puerariae lobatae* displayed similar profiles, while the chemical fingerprints of 5 batches of *Puerariae thomsonii* were different.

In order to investigate the differences of *Puerariae lobatae* and *Puerariae thomsonii* from various habitats, data analysis of 16 batches of the samples were performed by professional software. The similarity values of the samples were obtained and listed in Table 2. Similarity values of 16 batches of samples exceeded 0.9 except for S16. However, the obvious differences between *Puerariae lobatae* and *Puerariae thomsonii* could be observed according to Figs. 3(A) and (B). These results were coincident with those of the previous study, so a quantitative fingerprint evaluation of the two herbs was studied and could reveal satisfactory results.[27] This showed the limitation of the chromatographic fingerprint evaluation. In this study, active fingerprints could effectively reveal the differences among samples and avoid the limitation of chromatographic fingerprint evaluation of *Puerariae lobatae* and *Puerariae thomsonii*. This active fingerprint would be an efficient quality evaluation tool of herbal medicines.

In addition, hierarchical cluster analysis was performed base on the peak areas of 13 compounds in chemical fingerprints and their scavenge rates (%) from active detection. Nearest neighbor method, one of effective methods for the analysis of variance between clusters was applied and Cosine was selected as measurement. Figure 4 showed two dendrograms, which were divided into two main clusters. In Fig. 4(A), cluster I was *Puerariae thomsonii* samples, while cluster II was *Puerariae lobatae* samples. In Fig. 4(B), *Puerariae lobatae* and *Puerariae thomsonii* samples were divided into cluster I and cluster II, respectively. In chemical fingerprints, peak 2, 5, 6, 7, 8 and 16 might be the reasonable markers to distinguish the two species, while peak 2, 3, 4, 5, 6, 7, 8 and 16 exhibiting substantial antioxidant activity were main characteristics for the discrimination of two species based on active fingerprints. The integrated evaluation results of chemical and active characteristics indicated that peak 2 (3′-hydroxypuerarin), peak 3 (genistein 8-*C*-glycoside-xyloside), peak 5 (puerarin), peak 6 (6″-*O*-xylosylpuerarin), peak 7 (mirificin) and peak 16 (daidzein) as common active ingredients in the two species were the main markers to discriminate two species. The combination of chromatographic fingerprint analysis and sensitive activity detection method would be a powerful tool for objective quality evaluation of *Puerariae lobatae* and *Puerariae thomsonii*.

Table 2. Similarity Values of 11 Batches of *Puerariae thomsonii*

Sample number	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11	S12	S13	S14	S15	S16
HPLC fingerprint	0.985	0.992	0.993	0.991	0.991	0.994	0.992	0.963	0.989	0.988	0.990	0.901	0.921	0.947	0.914	0.886

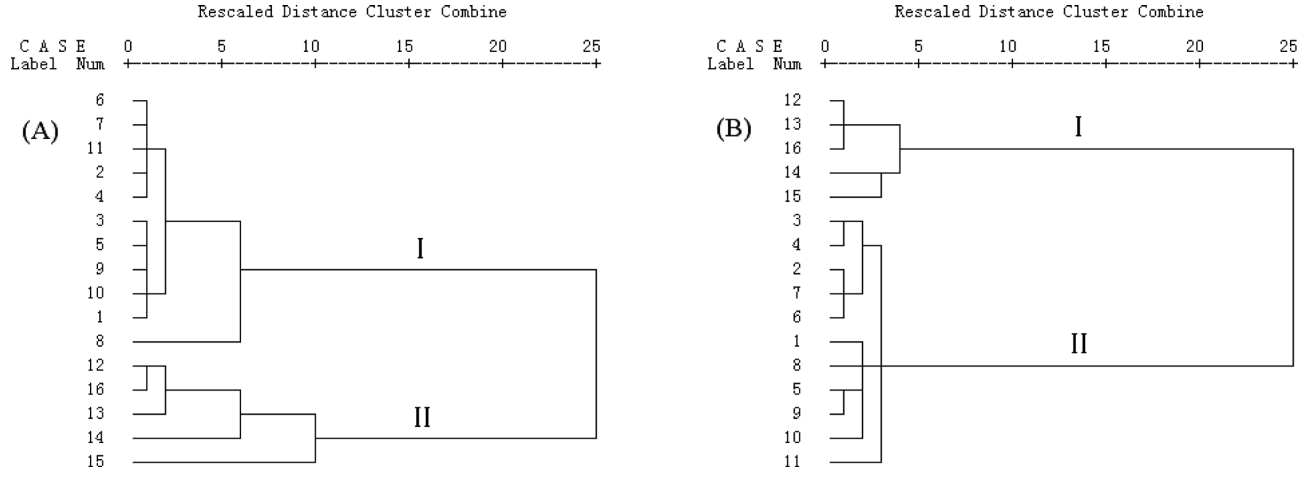

Fig. 4. Dendrogram of Hierarchical Cluster Analysis for the 16 Batches of *Puerariae lobatae* and *Puerariae thomsonii*

The hierarchical clustering was performed by SPSS software. Dendrogram resulting from the areas of 13 fingerprint peaks of the tested samples. (A) Chemical fingerprints. (B) Active profiles.

Conclusion

In this paper, an on-line HPLC-DAD-FICL detection method based on chromatographic separation and CL inhibition was developed for the antioxidant activity determination of *Puerariae lobatae* and *Puerariae thomsonii* from different habitats. The chemical fingerprints coupled with active detection could rapidly screen the main active compounds in two species. Seventeen flavonoids were identified and main active ingredients in two species were compared. The results revealed that more active compounds were found in *Puerariae lobatae* than *Puerariae thomsonii*. Furthermore, the quality evaluation results implied that 3′-hydroxypuerarin, genistein 8-*C*-glycoside-xyloside, puerarin, 6″-*O*-xylosylpuerarin, mirificin and daidzein were main markers to discriminate two species. *Puerariae thomsonii* samples from various habitats displayed similar fingerprints, while the fingerprints of *Puerariae lobatae* samples were obviously different. *Puerariae lobatae* containing more antioxidants showed better quality than *Puerariae thomsonii*. Thereby, on-line HPLC-DAD-FICL analysis is a quick and sensitive antioxidant activity detection method of complex samples, and can provide an objective quality evaluation mode for herbal medicines.

Acknowledgments This work was financially supported by the National Natural Science Foundation of China (Grant No. 30973965), 46th China Postdoctoral Fund (No. 20090461139), 47th China Postdoctoral Science Foundation (No. 20100471480) and Jiangsu Postdoctoral Science Foundation of China (1001077C).

References

1) Jiang R. W., Lau K. M., Lam H. M., Yam W. S., Leung L. K., Choi K. L., Waye M. M. Y., Mak T. C. W., Woo K. S., Fung K. P., *J. Ethnopharmacol.*, **96**, 133—138 (2005).
2) Valko M., Rhodes C. J., Moncol J., Izakovic M., Mazur M., *Chem.-Biol. Interact.*, **160**, 1—40 (2006).
3) Getoff N., *Radiat. Phys. Chem.*, **76**, 1577—1586 (2007).
4) Valko M., Leibfritz D., Moncol J., Cronin M. T. D., Mazur M., Telser J., *Int. J. Biochem. Cell B*, **39**, 44—84 (2007).
5) Niederländer H. A. G., van Beek T. A., Bartasiute A., Koleva I. I., *J. Chromatogr. A*, **1210**, 121—134 (2008).
6) Shi S. Y., Zhang Y. P., Jiang X. Y., Chen X. Q., Huang K. L., Zhou H. H., Jiang X. Y., *Trac-Trend Anal. Chem.*, **28**, 865—877 (2009).
7) Ding X. P., Qi J., Chang Y. X., Mu L. L., Zhu D. N., Yu B. Y., *J. Chromatogr. A*, **1216**, 2204—2210 (2009).
8) Kirakosyan A., Kaufman P. B., Warber S., Bolling S., Chang S. C., Duke J. A., *Plant Sci.*, **164**, 883—888 (2003).
9) Cao Y., Lou C., Zhang X., Chu Q., Fang Y., Ye J., *Anal. Chim. Acta*, **452**, 123—128 (2002).
10) Wang C. Y., Huang H. Y., Kuo K. L., Hsieh Y. Z., *J. Pharmaceut. Biomed.*, **30**, 843—849 (2002).
11) Zhang H. T., Wang Y., Deng X. L., Dong M. Q., Zhao L. M., Wang Y. W., *Eur. J. Pharmacol.*, **630**, 100—106 (2010).
12) Yeung D. K. Y., Leung S. W. S., Xu Y. C., Vanhoutte P. M., Man R. Y. K., *Eur. J. Pharmacol.*, **552**, 105—111 (2006).
13) Lin Y. J., Hou Y. C., Lin C. H., Hsu Y. A., Sheu J. J. C., Lai C. H., Chen B. H., Chao P. D. L., Wan L., Tsai F. J., *Biochem. Biophys. Res. Commun.*, **378**, 683—688 (2009).
14) Yu W. L., Zhao Y. P., Shu B., *Food Chem.*, **86**, 525—529 (2004).
15) Cherdshewasart W., Sutjit W., *Phytomedicine*, **15**, 38—43 (2008).
16) Bebrevska L., Foubert K., Hermans N., Chatterjee S., Van Marck E., Meyer G. D., Vlietinck A., Pieters L., Apers S., *J. Ethnopharmacol.*, **127**, 112—117 (2010).
17) Chen S. B., Liu H. P., Tian R. T., Yang D. J., Chen S. L., Xu H. X., Chan A. S. C., Xie P. S., *J. Chromatogr. A*, **1121**, 114—119 (2006).
18) Martínez Galera M., Gil García M. D., Santiago Valverde R., *J. Chromatogr. A*, **1113**, 191—197 (2006).
19) Costin J. W., Lewis S. W., Purcell S. D., Waddell L. R., Francis P. S., Barnett N. W., *Anal. Chim. Acta*, **597**, 19—23 (2007).
20) Tsunoda M., Uchino E., Imai K., Hayakawa K., Funatsu T., *J. Chromatogr. A*, **1164**, 162—166 (2007).
21) Wei Y., Zhang Z. J., Zhang Y. T., Sun Y. H., *J. Chromatogr. B*, **854**, 239—244 (2007).
22) Nalewajko E., Wiszowata A., Kojło A., *J. Pharmaceut. Biomed.*, **43**, 1673—1681 (2007).
23) Péter A., Török G., Csomós P., Péter M., Bernáth G., Fülöp F., *J. Chromatogr. A*, **1026**, 57—64 (2004).
24) Kodamatani H., Yamazaki S., Saito K., Amponsaa-Karikari A., Kishikawa N., Kuroda N., Tomiyasu T., Komatsu Y., *J. Chromatogr. A*, **1216**, 92—98 (2009).
25) Dua G., Zhao H. Y., Zhang Q. W., Li G. H., Yang F. Q., Wang Y., Li Y. C., Wang Y. T., *J. Chromatogr. A*, **1217**, 705—714 (2009).
26) Zhang Y., Xu Q., Zhang X., Chen J., Liang X., Kettrup A., *Anal. Bioanal. Chem.*, **383**, 787—796 (2005).
27) Chen J., Song M., Zhang H., Zhang S. Y., Hang T. J., *Chinese J. New Drugs*, **17**, 1240—1246 (2008).