Asian Journal of Chemistry; Vol. 23, No. 9 (2011), 3964-3968

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Separation of Flavonoids from Crude Extract of *Gynura divaricata* by Macroporous Resin

MIN JIE[1], CHUNPENG WAN[1], YANYING YU[2,*] and SHUWEN CAO[1,2]

[1]State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, P.R. China
[2]Department of Chemistry, Nanchang University, Nanchang 330031, P.R. China

*Corresponding author: Tel: +86 791 3969610; E-mail: yuyanying@ncu.edu.cn

(*Received*: 20 October 2010; *Accepted*: 14 May 2011) AJC-9945

The flavonoids in *Gynura divaricata* has obvious effect of glucose-lowering and antiatherosclerosis. In this paper, an efficient separation process of the flavonoids from *Gynura divaricata* crude extracts has been developed. During the five kinds of macroporous resins tested preliminarily, HPD-100 offers the best adsorption capacity, so the dynamic adsorption and desorption experiments have been carried out on it to optimize the separation process of the flavonoids from *Gynura divaricata* crude extracts further. After treatment with HPD-100 resin under the optimal conditions, the flavonoids content was increased more than 6-fold from 7 to 43 %, with a recovery yield of 85 % and the content of kaempferol-3-O-glucoside enhanced 7-fold. It is concluded that the results in this study may provide a potential approach for the large-scale flavonoids production from *Gynura divaricata* extracts for its medicinal use.

Key Words: *Gynura divaricata*, Flavonoids, Macroporous resin.

INTRODUCTION

Gynura divaricata (L.) DC (Compositae) belongs to Gynura Cass., is called "Bai Bei San Qi", which has been planted widely from Taiwan to southern part and southwest of China. It is one of the most famous traditional Chinese medicinal herbs and usually used to cure bronchitis, pulmonary tuberculosis, kink cough rheumatism, diabetes and so on[1]. The literatures have reported some alkaloids, flavonoids, terpenoid, steroids, nucleosides, alkanes, alcohols, fatty acid, phytosterols and cerebroside from this species[2-4]. And the flavonoids from this genus have obvious glucose-lowering effect and antiatherosclerosis activities. In Fujian area, the leaf of *G. divaricata* is a commonly tea for reducing blood glucose. It could significantly decrease the blood glucose of diabetic patient[5-7].

The conventional method for enrichment and separation of flavonoids from the crude extracts of *G. divaricata* was performed by solid-liquid extraction or solvent extraction, followed by silica gel chromatography and polyamide chromatography[7,8]. The traditional separation process is not effective regarding reagents, energy consumption and industrial production.

There has been a growing interest in employing macroporous resins to enrich the flavonoids from many medicine plants because of their unique adsorption properties including ideal pore structure and various surface functional groups. They have high adsorption capacity and recovery ratio, relative low cost and easy regeneration[9-11]. Macroporous resins can be used to selectively adsorb constituents from aqueous solution as well as non-aqueous systems through electrostatic force, hydrogen bonding interaction, size sieving action, etc and they are durable non-polar, middle polar and polar macroporous polymers[12]. Few studies have been attempted to separate the flavonoids from *G. divaricata* by macroporous resins.

In order to achieve efficient adsorption-desorption separation process of the flavonoids from *G. divaricata*, a detailed investigation on suitable macroporous resin and its adsorption properties is needed. The aim of the present work is to investigate the adsorption and desorption properties of the flavonoids on different macroporous resins and to develop an efficient method for the preparative separation of the flavonoids from *G. divaricata* crude extracts with the optimal resin.

EXPERIMENTAL

Dry *G. divaricata* leaves were provided by Guangzhou province of China; ethanol and methanol were both AR grade; acetonitrile (HPLC grade) were purchased from JingYan Chemical Technology. De-ionized water was used in all experiments. Kaempferol ($C_{15}H_{10}O_6$, m.w. 286.23, 98 %) was used as standard to evaluate the contents of the flavonoids. The macroporous resins tested were HPD-100, HPD-826, AB-8, D101 (Cangzhou Bon Adsorber Technology Co., Ltd, Hebei,

China) and LX-8 (Xian Lanxiao Technology Co., Ltd, Xian, China).

Treatment of *Gynura divaricata* material: 50 g dry *Gynura divaricata* leaves were ground into powder and then were extracted with 2000 mL ethanol-water (45:55, v/v) solution in a bath at 90 ºC for 0.5 h, repeated two times. The extracted solutions were collected and centrifuged at 3800 rpm for 10 min by a centrifuge, then the supernatant was concentrated to dryness by removing the ethanol solvent in a rotary evaporator at 50 ºC and *G. divaricata* extracts residue was obtained. It was dissolved in water by ultrasonic, then centrifuged and the aqueous solution of *G. divaricata* was gained finally.

Method of detecting the contents of flavonoids: The contents of flavonoids were determined by the $AlCl_3$ colorimetric method used kaempferol as standard reference[13-15]. The absorbance of the standard reference and the extracts of *G. divaricata* was measured at 350 nm using UV-2450 spectrophotometer (Shimadzu, Japan). Calibration curve was determined using a series of standard solutions in a range of concentration of 2.0-16.0 μg/mL. The calibration equation for was $Y = 0.04177X + 0.01484$, $r^2 = 0.9993$ (Y: absorption value, X: concentration of the μg/mL, r: related coefficient).

Pretreatment and activation of resins: The macroporous resins were cross-linked polystyrene interpolymer. Some residues including monomers, cross-linking agents, filler and impurity might enter inside the pores during the synthesis process. So it was needed to pre-treat the resins to remove them before application. Their properties are shown in Table-1.

TABLE-1
PHYSICAL PROPERTY OF THE TESTED MACROPOROUS RESINS

Name	Particle diameter (mm)	Surface area (m^2/g)	Average pore diameter (Å)	Polarity
AB-8	0.30-1.25	480-520	130-140	Low polar
LX-8	0.30-1.25	400-440	100-105	Polar
HPD-100	0.30-1.20	650-700	85-90	Non-polar
HPD-826	0.30-1.25	500-600	90-100	Hydrogen bond
D101	0.25-0.84	500-550	90-100	Non-polar

The resins were pretreated and activated according to the manufacturer's recommendation and literature[16]. Firstly, they were soaked with 95 % ethanol for 24 h. After soaking, the resins were introduced into a glass column and rinsed with a further 2 BV (bed volume) of 95 % ethanol at the flow rate of 2 BV/h. Subsequently they were rinsed with distilled water to remove the ethanol at the same flow rate. Finally, 1 BV of 4 % (w/v) sodium hydroxide and distilled water rinsed it to neutral, 1 BV 4 % (v/v) hydrochloric acid and distilled water rinsed it until the pH of the fluent became neutral.

Static adsorption and desorption test: In the adsorption experiment, all macroporous resins were tested as follows: 1 g hydrated resin was put into an Erlenmeyer flask and 30 mL aqueous solution of *G. divaricata* extracts was added. The flask was then shaken (100 rpm) in a water-bath shaker at 25 ºC for 24 h, after that, the static desorption was performed in the shaker at 30 ºC for 4 h. Desorption test were conducted using 30 mL of 70 % ethanol solutions (v/v) as eluting phase.

Then the static adsorption kinetics of the flavonoids on all macroporous resins was also studied. A specified mass of hydrated resin was introduced into an Erlenmeyer flask containing 30 mL aqueous solution of *G. divaricata* extracts and the flask was shaken (100 rpm) at 25 ºC. The concentration of the flavonoids in liquid phase was monitored at certain time intervals till equilibration.

The preliminary choice of these resins was assessed by their adsorption ratio, desorption ratio, recovery ratio and adsorption kinetics. Then, HPD-100 resin was selected from the initial experiments, its adsorption property was further investigated at different pH values of sample solution firstly. Secondly, after saturation adsorption of HPD-100 resin on condition of the optimalizing pH value, different concentration of ethanol solution was used as eluent, then the optimal desorption eluent was screened out by calculating their desorption ratio.

Dynamic adsorption and desorption: Dynamic adsorption and desorption experiments were carried out on glass columns (11 mm × 200 mm) wet-packed with HPD-100 resin and the volume of the resin was 8 mL. During this part, the concentrations of loading sample solution and flow rate were very important influence factor, which need to be investigated further[17].

The same volume of aqueous solutions (pH = 3.5) flowed through the glass column at different flow rate and then the adsorption capacity and ratio were evaluated. Sequentially, different concentrations of aqueous solution with the same content flavonoids were loaded into the glass column at a selected flow rate and the adsorption capacity and ratio were calculated to screen out the satisfying concentration of sample solution.

The same content of the flavonoids were adsorbed by the macroporous resin on optimal adsorption condition screened before. Meanwhile, effluent was collected at 8 mL intervals and the content of flavonoids was detected to draw dynamic adsorption leak curve. While adsorption equilibration, the adsorbate-laden column was washed first with de-ionized water and then desorbed with ethanol-water (70:30, v/v) solution at different flow rate, evaluated the desorption ratio and selected the best flow rate of elution. Then detected the concentration of the flavonoids in eluent which was desorbed at the optimal flow rate and drew the dynamic desorption curve. The eluent was concentrated in the rotary evaporation apparatus before further analysis.

HPLC detection: A definite mass of samples before and after treatment with HPD-100 resin were dissolved in ethanol by ultrasonic, centrifuged, introduced into the 25 mL volumetric flask and filtered through 0.45 μm membranes before HPLC analysis (Hitachi HPLC system, Hitachi High-Technologies Corporation, Tokyo Japan). The condition of chromatographic analysis was as follows: chromatographic column: AlltimaHP C_{18}, 250 mm × 4.6 mm 5 μm; mobile phase: acetonitrile-water system; flow rate: 0.8 mL/min; UV detection wavelength: 347 nm; column temperature: 30 ºC; injection volume: 30 μL.

Calculation of adsorption capacity, adsorption, desorption and recovery ratios: The capacity of adsorption, adsorption

ratio, desorption ratio and recovery ratio were calculated as following equations:

Adsorption capacity:

$$Q_e = \frac{(C_0 - C_e)V_0}{m} \quad (1)$$

Adsorption ratio:

$$E(\%) = \frac{(C_0 - C_e)}{C_0} \times 100 \quad (2)$$

Desorption ratio:

$$D(\%) = \frac{C_d V_d}{(C_0 - C_e)V_0} \times 100 \quad (3)$$

Recovery ratio:

$$P(\%) = \frac{C_d V_d}{C_0 V_0} \times 100 \quad (4)$$

where Q_e (mg/g resin), E, D and P represented the adsorption capacity, adsorption ratio (%) desorption ratio and recovery ratio respectively. C_0 and C_e were the initial and equilibrium concentrations of the flavonoids in solution, respectively (mg/L). C_d was the concentration of the flavonoids in the desorption solution (mg/L). V_0 and V_d were the volume of the initial feed solution and the desorption solution, respectively. m represented the mass of the hydrated resin.

Statistical analysis: Results were given as mean ± standard deviation of three replicates. Experimental results were analyzed by SPSS version 16.0 (SPSS Inc. Chicago, IL). Differences between means were determined using one-way ANOVA and Duncan's test.

RESULTS AND DISCUSSION

Selection of macroporous resins suitable for separation of the flavonoids: Five macroporous resins with different properties were performed through static adsorption and desorption experiment to test their adsorbent efficiency of the flavonoids in *G. divaricata* extracts. The results were listed in Table-2. The adsorption ratio of the flavonoids on AB-8 resin was the highest among these studied resins, but it's desorption ratio was the lowest one, while HPD-100 resin had relatively higher adsorption ratio, desorption ratio and recovery ratio, compared with the other resins.

TABLE-2
ADSORPTION RATIO (E), DESORPTION RATIO (D) AND RECOVERY RATIO (P) OF DIFFERENT RESINS TOWARDS THE FLAVONOIDS

Name	E (%)	D (%)	P (%)
AB-8	89.3 ± 1.1^{e}	36.8 ± 1.3^{a}	32.9 ± 0.9^{b}
LX-8	28.5 ± 2.1^{a}	78.4 ± 1.7^{d}	22.3 ± 1.4^{a}
HPD-100	69.4 ± 1.3^{c}	81.3 ± 0.8^{e}	56.4 ± 1.0^{d}
HPD-826	66.3 ± 0.7^{b}	75.4 ± 1.1^{c}	50.0 ± 2.4^{c}
D101	72.8 ± 1.0^{d}	68.1 ± 2.4^{b}	49.6 ± 1.6^{c}

Each value is expressed as mean ± SD (n = 3). Means with different small letters within a column are significantly different ($p < 0.05$).

In order to confirm which kind of resins should be selected further, the static adsorption kinetics of the flavonoids on all macroporous resins was also studied. Then the Adsorption kinetic curve was obtained for the flavonoids on all resins. As shown in Fig. 1, the adsorption capacity of all resins increased with the extension of adsorption time, HPD-100 and D101 needed 4 h to reached equilibrium, while HPD-826, AB-8 needed 6 h, LX-8 needed 8 h. Moreover, at the same time, the adsorption capacity of HPD-100 was far higher than the other three kinds of resins, but higher than D101 resin slightly. This may because that the HPD-100 resin with larger surface area showed stronger adsorption capacity. After comprehensive consideration, HPD-100 was picked out for the further study of adsorption process of the flavonoids.

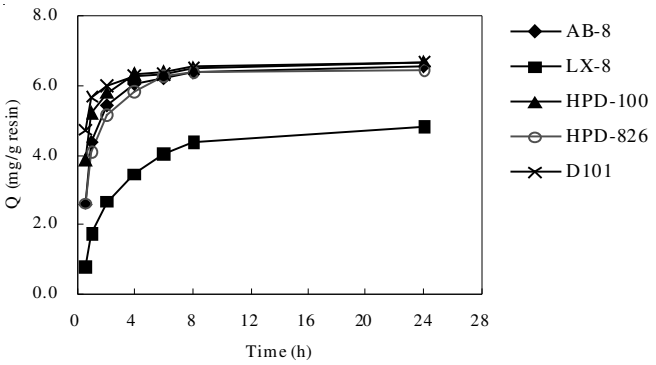

Fig. 1. Adsorption kinetics of the flavonoids on different resins at 25 ºC

Effect of the pH value of sample solution: The most important factor influencing the sorption capacity is the initial pH of adsorption solution[18]. The initial pH of adsorption solution is related to the adsorption mechanisms onto the adsorbent surface from water and reflects the nature of the physicochemical interaction of the flavonoids in solution and the adsorptive sites of adsorbent[12]. As shown in Fig. 2, the adsorption ratio increased remarkably for the flavonoids with the decrease of pH value. There is better adsorption efficiency and no obvious change on adsorption ratio of HPD-100 when pH value was lower than 3.5. Considering the stability of resin and flavonoids in aqueous solution, pH value of 3.5 was selected and would used for all later experiments.

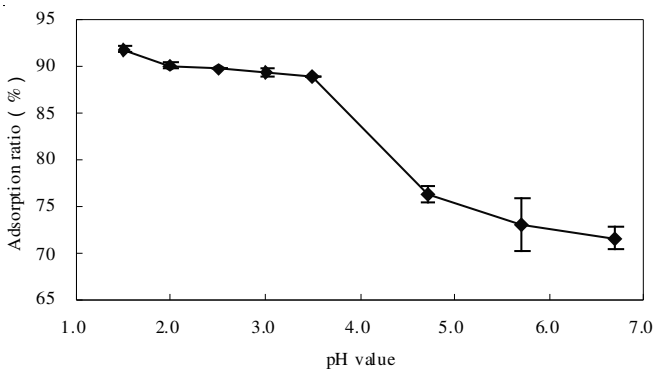

Fig. 2. Effect of pH value on flavonoids adsorption ratio on HPD-100 resin

Static desorption on HPD-100: HPD-100 resin adsorbed the same contents of flavonoids were eluted by various concentrations of ethanol solution from 30 to 90 %, respectively. The results showed in Table-3, the desorption ratio of the flavonoids from HPD-100 resin increased with the increase of ethanol concentration and reached the maximum desorption ratio when

TABLE-3
EFFECT OF ETHANOL CONCENTRATION ON DESORPTION OF FLAVONOIDS ON HPD-100

Concentration of ethanol (%)	D (%)	P (%)
30	64.21 ± 1.64^{a}	56.89 ± 1.41^{a}
50	78.16 ± 2.09^{b}	68.93 ± 1.38^{b}
70	89.89 ± 2.16^{d}	80.90 ± 1.45^{d}
90	84.94 ± 0.54^{c}	74.67 ± 0.48^{c}

D (desorption ratio), P (recovery ratio). Each value is expressed as mean ± SD (n = 3). Means with different small letters within a column are significantly different ($p < 0.05$).

using ethanol at a concentration of 70 %, which was selected as the appropriate desorption solution. Then there was a decline when ethanol concentration was 90 %. This may related to the structure of flavonoids and mechanism of adsorption and desorption, both hydrophobic interaction and hydrogen bonding exist between the resin and flavonoids.

Effect of feeding solution's flow rate and concentration on flavonoids adsorption on HPD-100: Sample solution with fixed volume was loaded into HPD-100 resin at different flow rate, respectively. The adsorption capacity and ratio were shown in Table-4. With the decline of feeding flow rate, the adsorption efficiency increased gradually, the highest adsorption capacity was observed when the flow rate was 0.8 mL/min, but there was not much difference between them. Considering of the solution's volume and time of feeding it, 2 mL/min was selected as the feasible flow rate.

TABLE-4
EFFECT OF FEEDING SOLUTION'S FLOW RATE ON FLAVONOIDS ADSORPTION ON HPD-100

Feeding rate (mL/min)	Q_e (mg/mL)	E (%)
0.8	1.298 ± 0.011^{a}	95.85 ± 0.78^{a}
1.6	1.291 ± 0.011^{a}	95.30 ± 0.85^{a}
2.0	1.271 ± 0.008^{a}	93.90 ± 0.57^{a}

Q_e (adsorption capacity), E (Adsorption ratio). Each value is expressed as mean ± SD (n=3). Means with different small letters within a column are significantly different ($p < 0.01$).

With the same total contents of the flavonoids, different concentration of aqueous solution was introduced into the resin column at rate of 2 mL/min (Table-5). The adsorption capacity and ratio on flavonoids with these tested concentrations were ideal and without obvious difference, its adsorption efficiency was slightly higher than the other's when the initial concentration was 376 mg/L, therefore, 376 mg/L was selected as the appropriate concentration of the sample solution.

TABLE-5
EFFECT OF FEEDING SOLUTION'S CONCENTRATION ON FLAVONOIDS ADSORPTION ON HPD-100

Concentration of solution (mg/L)	Q_e (mg/mL)	E (%)
376	1.293 ± 0.006^{a}	91.8 ± 0.50^{a}
251	1.270 ± 0.010^{a}	89.9 ± 0.86^{a}
125	1.273 ± 0.021^{a}	90.5 ± 1.17^{a}

Q_e (adsorption capacity), E (Adsorption ratio). Each value is expressed as mean ± SD (n=3). Means with different small letters within a column are significantly different ($p < 0.01$).

Dynamic adsorption breakthrough curve, flow rate of desorption and desorption curve: In order to take full use of the resin and choose the fitting contents of sample, dynamic adsorption leak experiment was needed to perform. As shown in Fig. 3, HPA-100 resin reached equilibrium after adsorbed 66 volume of sample solution with the selected concentration of 376 mg/L.

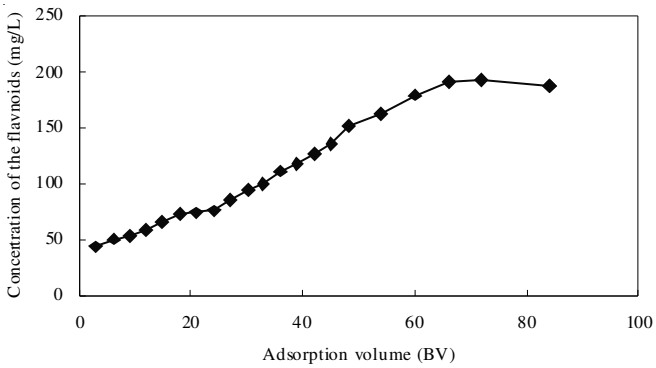

Fig. 3. Dynamic adsorption leak curve of the flavonoids on a column packed with HPD-100

Sequentially, various elution rates was used to desorb the flavonoids on the resin, the effect was shown in Table-6. The desorption performance was better with lower elution flow and it was best at the flow rate of 0.8 mL/min, but without obvious difference compared to 1.5 mL/min. Therefore, 1.5 mL/min was selected as the proper one in consideration of the short working time and lower volume consumption.

TABLE-6
EFFECT OF DESORPTION RATE FOR THE FLAVONOIDS ON HPD-100 RESIN

Desorption rate (mL/min)	D (%)
0.8	91.73 ± 2.25^{b}
1.5	86.77 ± 2.15^{ab}
2.0	80.77 ± 1.55^{a}

D (desorption ratio). Each value is expressed as mean ± SD (n = 3). Means with different small letters within a column are significantly different ($p < 0.01$).

At the proper desorption rate of 1.5 mL/min, approximately 4 BV (Bed volume) of desorption solution could elute the flavonoids completely from HPD-100 resin (Fig. 4).

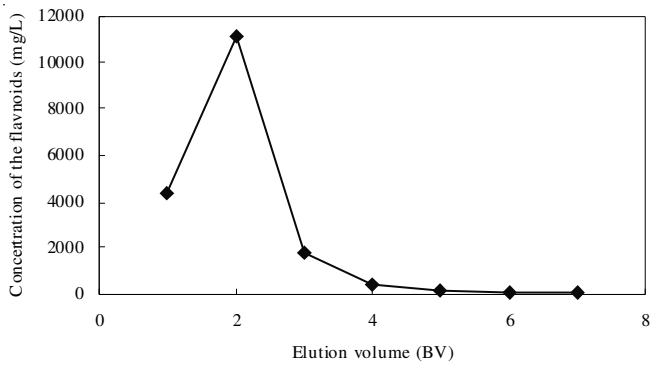

Fig. 4. Dynamic desorption curve of the flavonoids on a column packed with HPD-100

Comparison of the flavonoids before and after purification with resin by HPLC: When tested by HPLC under the same conditions, the chromatograms of samples before and after treatment with HPD-100 resin were shown in Fig. 5. It is observed that the content of components have enhanced with various degree and by compared the peak area of kaempferol-3-O-glucoside, its content increased 7-fold after the separation on HPD-100 resin. The desorption solution was concentrated to dryness by a rotary evaporator. The dried product was weighed and the contents of the flavonoids were calculated. After purification, the flavonoids with content of 43% was obtained, which was 6.14 fold higher than that in *G. divaricata* crude extracts and the recovery yield of flavonoids was 85 %.

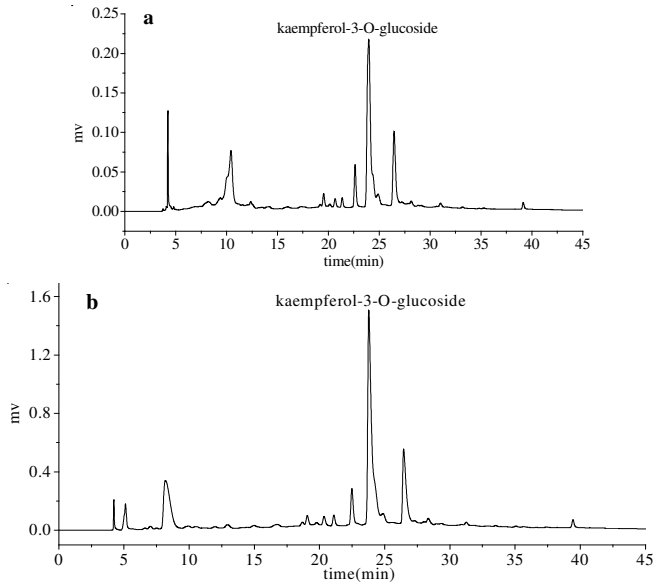

Fig. 5. Chromatograms of sample solution before (a) and after (b) treatment on a column packed with HPD-100 resin

Conclusion

In this study, the adsorption and desorption properties of five macroporous resins were evaluated and the separation process of the flavonoids from *G. divaricata* extracts with the selected resin was optimized. Among these tested resins, HPD-100 resin offers the best separation efficiency for the flavonoids because of its high surface area and appropriate functional non-polarity. Using HPD-100 resin at optimal conditions, the flavonoids with content of 43 % was obtained with a recovery yield of 85 % and the content of kaempferol-3-O-glucoside increased 7-fold. Compared to conventional separation methods of the flavonoids in *G. divaricata*, this purification method is superior because of its operational simplicity, lower cost, high efficiency and it may provide scientific references for the large-scale production.

ACKNOWLEDGEMENTS

This project was supported by the National Natural Sciences Foundation of China (No. 20662008).

REFERENCES

1. Jiangsu New Medial College: The Dictionary of Traditional Chinese Medicines, Shanghai Peoples' Press, Shanghai, p. 1502 (1979).
2. R. Erhard, E. Alexandra and W. Helmut, *Planta Med.*, **62**, 386 (1996).
3. Y. Hu, W.L. Li, H.W. Lin, M. Zhuo and B.R. Ren, *Chin. J. Nat. Med.*, **4**, 156 (2006).
4. L. Chen, H.Q. Li, H.T. Song and G.G. Zhang, *Fitoterapia*, **80**, 517 (2009).
5. G.A. Akowuah, A. Sadikun and A. Mariam, *Pharm. Biol.*, **40**, 405 (2002).
6. X.F. Zhang and B.K. Tan, *J. Singapore Med.*, **41**, 9 (2000).
7. Y. Hu, W.L. Li, H.W. Lin, Y. Hua and L. Feng, *J. Southwest Forestry College*, **27**, 55 (2007).
8. L.M. Li, W.L. Li, Q.S. Guo, B.R. Ren and H.Q. Zhang, *Lishizhen Med. Mater. Med. Res.*, **19**, 118 (2008).
9. B.Q. Fu, J. Liu, H. Li, L. Li, Frank, S.C. Lee and X.R. Wang, *J. Chromatogr. A*, **1089**, 18 (2005).
10. Y. Zhang, J.J. Jiao, C.M. Liu, X.Q. Wu and Y. Zhang, *Food Chem.*, **107**, 1326 (2008).
11. W. Liu, S. Zhang, Y.G. Zu, Y.J. Fu, W. Ma, D.Y. Zhang, Y. Kong and X.J. Li, *Bioresour. Technol.*, **101**, 4667 (2010).
12. B. Zhang, R.Y. Yang, Y. Zhao and C.Z. Liu, *J. Chromatogr. B*, **867**, 253 (2008).
13. M. Bener, M. Özyürek, K. Güçlü and R. Apak, *Ind. Crops Prod.*, **32**, 499 (2010).
14. A. Sharon and J. Gressel, *Pestic. Biochem. Physiol.*, **41**, 142 (1991).
15. S.M. He and J.L. Liu, *Chin. J. Anal. Chem.*, **35**, 1365 (2007).
16. Q. Huo and X.M. Gu, *Asian J. Chem.*, **22**, 6891 (2010).
17. B. Liu, R.B. Shi and C. Yu, *Chin. Tradit. Herbal Drugs*, **33**, 51 (2002).
18. H. Li, B. Chen, L.H. Nie and S.Z. Yao, *Phytochem. Anal.*, **15**, 306 (2004).