Advance Journal of Food Science and Technology 13(1): 11-17, 2017
DOI:10.19026/ajfst.13.3411
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: November 27, 2015 Accepted: February 10, 2016 Published: January 25, 2017

Research Article

Preliminary Enrichment and Separation of Total Flavonoids from *Cryptotaenia japonica* Hassk. Extract by Macroporous Resins

[1, 2]Jun Lu, [1]Ting Liu, [1]Jiaqi Zhang, [1]Perui Zhao and [1, 2]Zhonghai Li
[1]National Engineering Laboratory of Rice and By-product Deep Processing, College of Food Science and Engineering,
[2]National Engineering Laboratory of Rice and By-product Deep Processing, Central South University of Forestry and Technology, Changsha, 41004, P.R. China

Abstract: In this study, the protocol for enrichment and separation of flavonoids from *Cryptotaenia japonica* Hassk. was developed using macroporous resins. After comparison of six kinds of macroporous resins for their adsorption and desorption properties of total flavonoids, LS-46D was found to be the most powerful resin for total flavonoids isolation from the plant. Under optimal conditions, after one round enrichment with LS-46D, the total flavonoids content in the product increased by 3.5 times, with a recovery yield of 94.84%. The contents of the main active components such as luteolin and pigenin increased 8.35 and 6.29 times, respectively. These results demonstrated that the method has great potential for production of total flavonoids from *Cryptotaenia japonica* Hassk.

Keywords: *Cryptotaenia japonica* Hassk., enrichment, flavonoids, macroporous resins, separation

INTRODUCTION

Cryptotaenia japonica Hassk. (Chinese name: Yajiaoban) is a widespread perennial plant and a popular forest vegetable in China. It is also used as a herbal medicine to treat various ailments such as detumescence and inflammation (Yang and Xia, 2010). The pharmacological efficacy and application potential of *C. japonica* were attributed to its essential oils and flavonoids (Okude and Hayashi, 1970; Cheng *et al*., 2010; Li and Niu, 2012; Yao and Ren, 2011). During the past decades, flavonoids have received a great deal of attention because of their obvious pharmacological activities such as antioxidant, anti-inflammation, anticancerogenic and other beneficial properties for human health (Kang *et al*., 2012; Kamiyama and Shibamoto, 2012; Ahmad *et al*., 2015; Cheng *et al*., 2015).

Traditionally, flavonoids were separated from the crude extracts by solvent extraction, solid–liquid extraction, polyamide chromatography and gel chromatography. But the drawbacks of these methods are the consumption of large amount of reagents and energy. In recent years, high-speed counter-current chromatography has been used for efficient separation of flavonoids from a variety of plants (Jiang *et al*., 2015), but the expensive cost and low yields limit its application. A new approach based on macroporous resins has been widely applied in separation of bioactive compounds such phenolics and flavonoids (Wan *et al*., 2014; Yang *et al*., 2016; Xiong *et al*., 2014). These macroporous resins have special characteristics such as diversity in types of resins, large surface area, easy regeneration and relatively low cost. Based on their polarity, macroporous resins were classified as non-polar, middle polar and polar types with different adsorption capacities. These resins could be used for selectively adsorb or desorb components from extraction solutions of natural products by size sieving, bonding interaction, electrostatic force, etc. However, to the best of our knowledge, there has been no report on the use of macroporous resin in separating and purifying flavonoids from *C. japonica* extracts. So in this study, the major objective was to develop the optimum conditions for enrichment and separation of total flavonoids from *C. japonica* using macroporous resins.

MATERIALS AND METHODS

Plant material: *Cryptotaenia japonica* Hassk. was collected from the western region of Hunan Province, China. The plant was authenticated by Professor Meiqun Zhang and voucher specimen (YY.201105) was deposited at our laboratory.

Corresponding Author: Zhonghai Li, National Engineering Laboratory of Rice and By-product Deep Processing, College of Food Science and Engineering, Central South University of Forestry and Technology, Changsha, 41004, P.R. China, Tel./Fax: +86-731-85623074

Table 1: Physical properties of macroporous resins

Resin series	Polarity	Particle size (mm)	Surface area (m^2/g)	Appearance	Average pore diameter (A°)
AB-8	Middle	0.315–1.25	400-500	White particles	30
XDA-8	Weak	0.315–1.25	1100-1300	Brown granules	30
LX-60	Nonpolar	0.315–1.25	500-600	White particles	30
LS-303	Nonpolar	0.315–1.1	600	White particles	25
LS-308	Weak	0.315–1.1	450	White particles	26
LS-46D	Weak	0.315–1.1	700	White particles	26

All plant materials were washed and separated into roots and aerial parts. The aerial parts were dried in an oven at 60°C and powdered to 60 meshes and then stored in sealed polyethylene bags in a dry and dark place until use.

Chemicals: Luteolin and apigenin were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. Rutin was purchased from the National Institutes for Food and Drug Control, China. All the other chemicals and solvents are of analytical grade.

Pretreatment of resins: Six types of macroporous resins were used in this study. LS-303, LS-308 and LS-46D were purchased from Shanxi Lanshen Special Resin Co., Ltd. (Shanxi, China). AB-8, LX-60 and XDA-8 were purchased from Shanxi Lanxiao Technology Co., Ltd. (Shanxi, China). The physical characteristics of these resins were listed in Table 1. These macroporous resins were pretreated by washing with abundant distilled water to remove residue salts or impurities and then dried at 60°C for 12 h and kept at a dry and dark area until use. The dried macroporous resins were immersed for 12 h with 95% ethanol and subsequently washed thoroughly with distilled water prior to the adsorption experiments (at a flow rate of 3 mL/min until the eluent was neutral).

Preparation of crude extracts from *Cryptotaenia japonica* Hassk: The extracts were obtained by refluxing 30 g of sample powder in 360 mL of 70% ethanol in a water bath at 85°C for 60 min. After initial extraction, the extract was removed and 90 mL of 70% ethanol was added and extracted for another 30 min. The extracts were combined, filtered and centrifuged for 10 min at 6000 rpm. The supernatant was collected and evaporated under vacuum to remove the ethanol. Then, deionized water was added to give flavonoids concentration of 5-6 mg/mL.

Determination of total flavonoids: The concentrations of the total flavonoids were measured by a modified colorimetric method (Lu *et al*., 2013). Briefly, one mL of the sample solution and 0.6 mL of NaNO2 (5%) were removed to a volumetric flask (10 mL) and shaken for 6 minutes, then 0.5 mL of 10% $Al(NO_3)_3$(w/v) was added and mixed. The mixture was allowed to stand for another 6 minutes prior to the addition of 3.0 mL of 4.3% NaOH and then, 70% ethanol (v/v) was added to the scale. The mixture was well shaken and incubated for 15 min and then measured at 507 nm using an UV-vis spectrophotometer (Shanghai Precision Instrument Co., Ltd, Shanghai, China). The content of total flavonoids was calculated by using rutin as standard. The regression equation was A = 1.0286C-0.0161 (R^2 = 0.9993), where A is the absorbance of the sample and C is the flavonoids content (mg/mL). The data were reported as mean ±SD for triplicate independent measurements.

Static adsorption and desorption experiments: The static adsorption experiments were conducted as follows: pretreated hydrated resins (equal to 4 g of the dry resins) and 40 mL of crude extract were added to a flask and shake (50 rpm) in a water-bath shaker at 25°C. One mL of the adsorption solution was removed for flavonoids concentration analysis at 0.5, 1, 2, 4, 6 and 8 h, respectively. The adsorption capacity (Q) and adsorption ratio (E) of each resin for the flavonoids were preliminarily determined using the following equations:

$$Q = (Co–Cr)\ Vr/W \quad (1)$$

$$E = (Co–Cr)/Co\times100 \quad (2)$$

where, Co and Cr are the initial and residual concentrations (mg/mL) of the total flavonoids in the solutions, respectively; Vr(mL) and W (g) are the volume of the initial flavonoids solution and the weight of the dried resins, respectively.

When the adsorption reaches the adsorption equilibrium, the residual solution was removed and 40 mL of 70% ethanol was added and then shaken (50 rpm) for 8 h at 25°C in a water-bath shaker. The concentration of the total flavonoids in the desorption solutions was determined then. Meanwhile, the desorption ratio (D) of different resins was calculated using the following equation:

$$D = \frac{CdVd}{(Co-Cr)Vr}\times100 \quad (3)$$

where, Cd is the total flavonoids concentration in the desorption solutions (mg/mL); Vd is the volume of the desorption solution; and Co, Cr and Vr are the same as those defined above.

Table 2: Adsorption and desorption properties of six macroporous resins for flavonoids

Resin series	Adsorption capacity (mg/g dry resin)	Adsorption rate (%)	Desorption capacity (mg/g Dry resin)	Desorption rate (%)
AB-8	43.20±0.51	12.75±0.33	25.11±0.35	58.14±0.44
XDA-8	46.70±0.45	13.79±0.61	29.195±0.43	62.52±0.62
LX-60	56.61±0.43	16.71±0.22	33.278±0.38	58.78±0.34
LS-303	49.32±0.48	14.56±0.56	36.19±0.45	73.39±0.61
LS-308	58.36±0.71	17.23±0.86	29.78±0.52	51.02±0.52
LS-46D	60.11±0.62	17.75±0.49	41.15±0.72	68.46±0.56

Dynamic adsorption and desorption experiments: The dynamic adsorption and desorption experiments were performed as follow: First, the pretreated wet resin (equal to 8 g of the dried resins) was packed into the chromatographic column (2.4×40 cm) to give a Bed Volume (BV) of 50 mL and height of 14.4 cm. In the adsorption process, the effects of different adsorption conditions including the initial total flavonoids concentration, feed flow rate and the volume of the sample on the adsorption capacity of the resin were investigated. After the adsorption was completed under the optimized adsorption conditions (total flavonoids concentration in the feed solution: 6 mg/mL; flow rate: 3 mL/min; feed volume: 50 mL), the desorption experiments were performed to study the effects of ethanol concentrations, eluting flow rates and ethanol consumption on desorption. The adsorption capacity and desorption ratio of the six kinds of resins were calculated using Eq. (3). All the dynamic adsorption and desorption tests were performed in triplicates at room temperature.

HPLC analysis: The dried, purified extract (0.0575 g) and crude extract (0.2673 g) were dissolved in 25 mL of methanol and filtered through 0.45 μm membrane filters respectively. The flavonoids and phenols concentrations in samples before and after enrichment were determined by HPLC (Shimadzu, Tokyo, Japan) equipped with Photodiode Array Detector (PDA), A reverse-phase C18 column (symmetry 150 × 3.0 mm, 5 μm particles, Shimadzu, Tokyo, Japan) was used for the chromatographic analysis. The mobile phase consisted of 0.6% (v/v) phosphoric acid (A) and 100% acetonitrile (B) and was used for the gradient elution as follows: 5-20% B from 0 to 20 min, 20% B from 20 to 30 min, 20-25% B from 30 to 40 min, 25-35% B from 40 to 50 min, 35% B from 50 to 60 min, 35-20% B from 60 to 80 min and 20-25% B from 80 to 90 min. The flow rate was 0.2 mL/min. The injection volume was 10 μL. The oven temperature of the column was maintained at 30°C and the wavelength of the detector was 275 nm. The compounds were identified according to their retention times and UV spectra and compared with the standard. The contents of the phenolics and flavonoids were calculated by the external standard method. The regression equations for luteolin and pigenin were $Y = 60907.1X-61891.2$ ($R^2 = 0.9999$, n = 5) and $Y = 84028.2X- 4604.55$ ($R^2 = 0.9998$, n = 5), respectively, where Y and X are the peak area and injection quantity of the analyte, respectively. The calibration curves were obtained by plotting the concentration (μg/mL) of selected flavonoids in the range 10–100 μg/mL against the peak areas.

RESULTS AND DISCUSSION

Preliminary selection of resin: The results of static adsorption/desorption experiments were shown in Table 2. LS-308, LS-46D and LX-60 exhibited higher adsorption capacities for the total flavonoids than other resins and the desorption ratios for the flavonoids on LS-303 and LS-46D resins (73.39 and 68.46%, respectively) were relatively higher than those on the other resins. Clearly, the nonpolar and weak polar resins exhibited higher adsorption capabilities than middle polar resins, probably because of their comparable polarity with the flavonoids and appropriate pore diameters (around 26 nm). The results indicated that among the six resins investigated, LS-46D possessed a comparatively higher adsorption capacity (60.11 mg/g of the dry resin) and desorption ratio (68.46%) than other resins.

The adsorption kinetics curves for the total flavonoids on different resins were presented in Fig. 1. The adsorption capacities of LS-303, LS-308 and LX-60 increased rapidly in the first 120 min and then increased slowly thereafter. The adsorption capacities of AB-8 and XDA-8 increased slowly with the time during the experimental procedure. Only LS-46D showed the highest increase rate. In comprehensive consideration of the adsorption capacity and desorption ratio, LS-46D had the highest adsorption and desorption capacity; therefore, LS-46D was chosen as the suitable

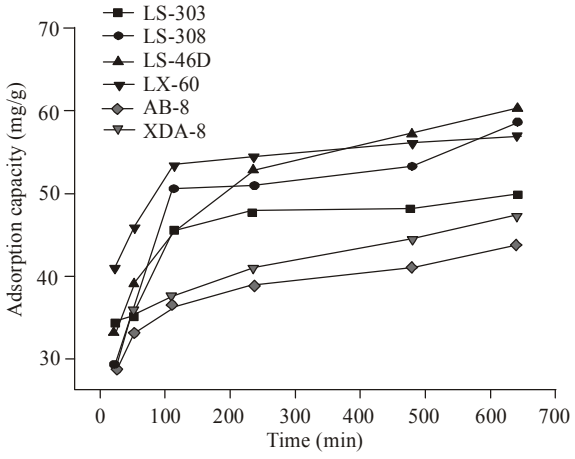

Fig. 1: Adsorption kinetics curves for the total flavonoids on different resins

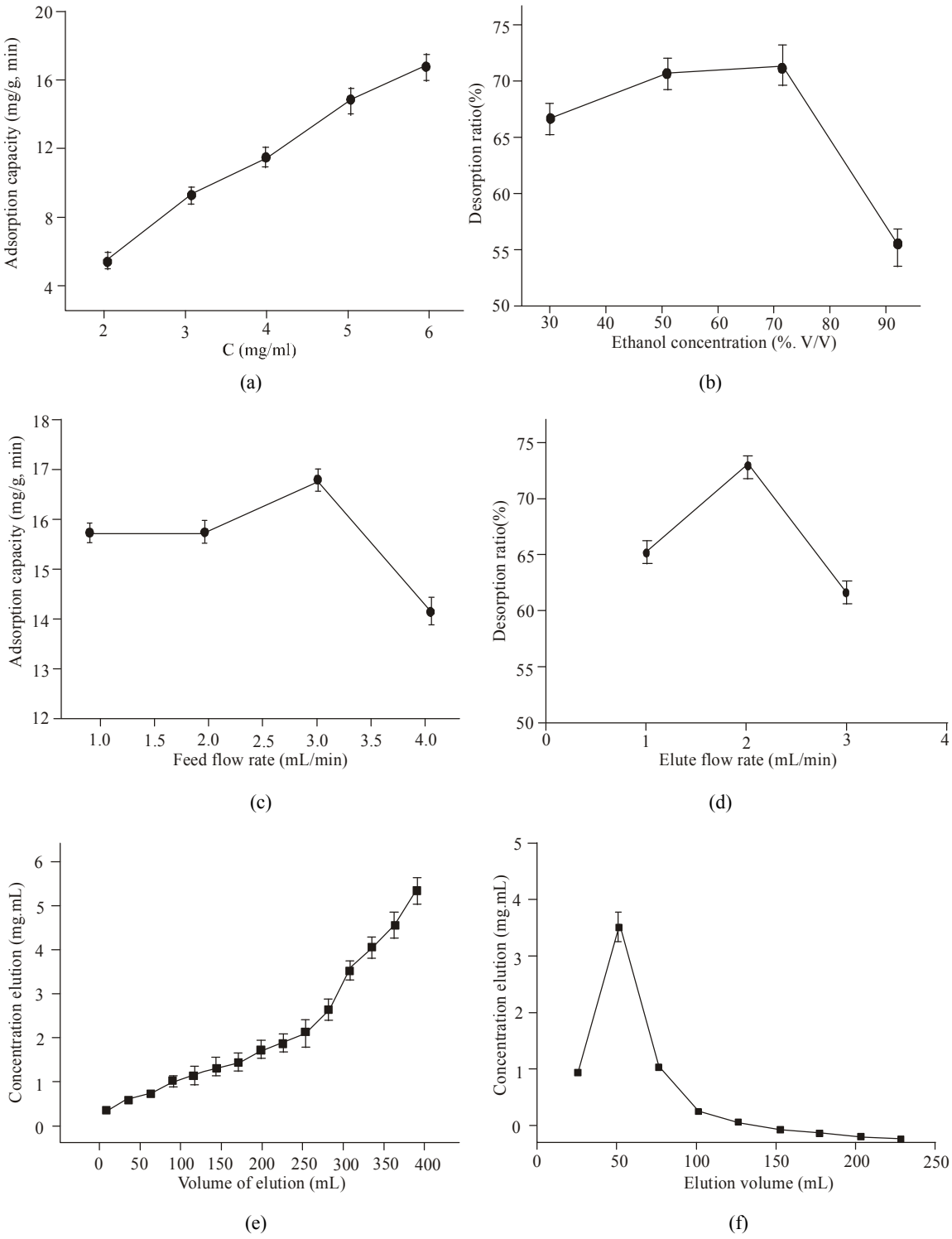

Fig. 2: Effect of initial concentration (A), feed rate (B) on the adsorption capacity of LS-46D and Leakage curve (C); Effect of ethanol concentration (D), elute flow rate (E) on the desorption ratio and dynamic elution curve (F)

resin for separation of the total flavonoids from *Cryptotaenia japonica* Hassk

Effect of initial concentration of sample on adsorption capacity: It is reported that adsorption capacity and ratio of macroporous resins for flavonoids increase with the decrease of pH because of the hydrogen bonding (Fu *et al*., 2005). And the increase in temperature leads to the decrease of adsorption because of exothermic reaction in resin (Peng *et al*., 2004).

Therefore, in this study, the pH and temperature of the crude extract solution were fixed at 6.5 (the natural pH of crude extracts) and room temperature, respectively. To study the effect of the initial flavonoids concentration of sample on adsorption capacity, 40 mL of the crude extract with different flavonoids concentrations (2, 3, 4, 5 and 6 mg/mL) was transferred into the laden at a feed rate of 2.0 mL/min. After the adsorption reached saturation, 120 mL of deionized water was used to wash the resin and then desorbed with 70% ethanol at a flow rate of 2.5 mL/min until the eluent was colorless. In Fig. 2A, the adsorption capacity of LS-46D for the flavonoids increased proportionally as the increase of the initial concentration of the samples. However, in our sample preparation procedure, it became more and more difficult to concentrate the crude extract as the extracts became thicker after removing the ethanol. Moreover, with further increase in the feeding concentration, impurities in the crude extract solution increased and the active site-to-absorbate ratio decreased accordingly. The impurities competed with the flavonoids for the active sites on the resins, resulting in reduced adsorption of the flavonoids (Fu *et al*., 2005). Therefore, an initial concentration of 5.0-6.0 mg/mL was chosen for the following experiments.

Effect of feeding speed on adsorption capacity: In order to investigate the effect of feeding speed on adsorption capacity, 40 mL of a 5.7 mg/mL crude extract were run through the column at different feeding speeds (1.0, 2.0, 3.0 and 4.0 mL/min) and then washed with 120 mL of deionized water. The eluent solution was collected for calculation of adsorption capacity. The adsorption ratio increased first with the increase of feeding speed and reached the peak value at feeding speed of 3.0 mg/mL and then decreased rapidly with the further increase of feed rate (Fig. 2B). Too slow or too fast flow rates did not favor the adsorption of flavonoids on LS-46D resin. This was because the contact time between the flavonoids and resin was either too short or long. So the best suitable feeding speed was selected as 3.0 mL/min.

Leakage curve of total flavonoids: The leakage curve was plotted using 8 g of LS-46D under the optimum conditions, i.e., an initial concentration of 5.7 mg/mL and a feeding speed of 3 mL/min. The eluents were collected at 25 mL intervals and monitored by the colorimetric analysis of the flavonoids content. When the flavonoid concentration of the eluent was one tenth of the feed sample, the sample loading was stopped as that was the breakpoint (Jia *et al*., 2013). The leakage curve was shown in Fig. 2C. With the increase in adsorption volume, the flavonoids concentration in the eluent increased gradually. When the adsorption volume reached 75 mL, the concentration of the total flavonoids in the eluent reached the breakpoint (0.613 mg/mL), higher than 10% of the original concentration (5.7 mg/mL). When the loading volume was increased further, the leaking of the total flavonoids in the eluent increased rapidly. This is likely due to the reach of maximum adsorption capacity of the resin. Thus, the maximum treating capacity of 8 g resin was 50 mL of the sample solution.

Effect of the concentration of ethanol on desorption ratio: After completion the adsorption of flavonoids on LS-46D resin, the separation of the flavonoids in the crude extracts was affected by the desorption process of the adsorbates from the resin. So appropriate desorption solution and elute flow rate are very important. In order to investigate the effect of the concentration of ethanol on desorption ratio, different ethanol concentrations of 30, 50, 70 and 90%, respectively were used to determine the proper desorption solution and the results were shown in Fig. 2D. The desorption ratio of flavonoids increased slowly with the increase of ethanol concentration and reached the highest value at 70% ethanol and then decreased rapidly when the ethanol concentration exceeded 70%. Thus, the 70% ethanol solution was chosen for the subsequent tests.

Effect of eluting flow rate on desorption ratio: The different **eluting rates** (1.0, 2.0, 3.0 and 4.0 mL/min) on desorption results were shown in Fig. 2E. With the increase in flow rate, the desorption rate increased gradually and reached the maximum value (72.11%) at a flow rate of 2.0 mL/min and then decreased rapidly as the flow rate increased. This was perhaps because the flow rate was too fast, resulting in a low recovery of flavonoids. Therefore, 2.0 mL/min flow rate was determined as the optimum flow rate.

Dynamic elution curve: The dynamic **elution** curve was determined under the following conditions: 50 mL of the sample (5.7 mg/mL of the crude flavonoids extracts) was run through LS-46D resin at a flow rate of 3.0 mL/min. Then, the resin was washed with deionized water until the eluent was almost colorless and 70% ethanol was used to elute the flavonoids at a flow rate of 2.0 mL/min. The eluents were collected at 25 mL intervals and monitored the flavonoids content by the colorimetric analysis. The dynamic elution curve was plotted and displayed in Fig. 2F. The concentration of the total flavonoids increased first with the increase in the volume of eluent and reached the maximum value when elute volume was 50 mL and then decreased with the further increase of elute volume. When the elute reached 150 mL, the flavonoids were almost completely desorbed from LS-46D resin. Therefore, 150 mL was selected as the volume of desorption solvent.

Comparison of the characteristics of samples before and after enrichment: The method was validated using a LS-46D resin (8 g, dry weight) under conditions described in previous sections. Under these conditions, the total recovery yield was 94.84% and the content of the total flavonoids in the product reached 11.4%, 3.5

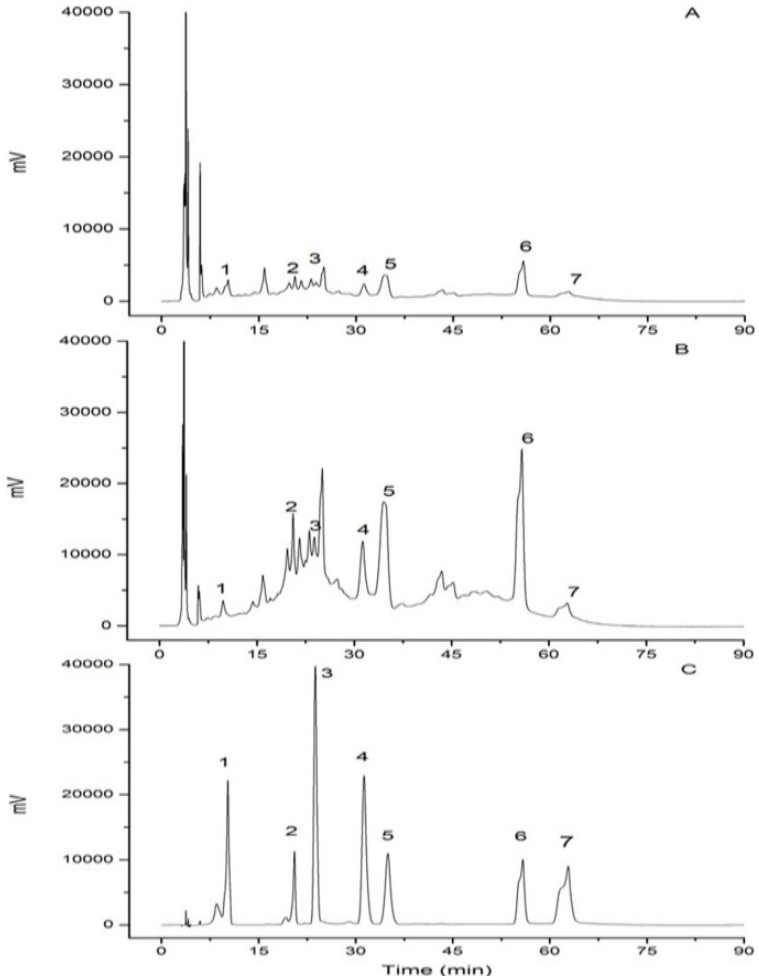

Fig. 3: HPLC chromatograms of the analysis of samples before (A) and after (B) enrichment and the standard reference materials (C). Identified compounds of peaks 1–7 are gallic acid, chlorogenic acid, vanillic acid, coumaric acid, ferulic acid, luteolin and pigenin, respectively

Table 3: Flavonoid and polyphenol contents in the crude and the purified product of *Cryptotaenia japonica* Hassk

Compounds	Content before the treatment (mg/g)	Weight (g)*	Content after the treatment (mg/g)	Weight (g)**	Purification fold
Gallic acid	0.452	8.56	0.915	0.499	2.024
Chlorogenic acid	0.447	8.56	5.264	0.499	11.776
Vanillic acid	0.323	8.56	1.392	0.499	4.310
Coumaric acid	0.770	8.56	4.261	0.499	5.534
Ferulic acid	1.936	8.56	17.367	0.499	8.971
Luteolin	2.554	8.56	21.327	0.499	8.350
Pigenin	0.369	8.56	2.321	0.499	6.290

*: The weight of 50 mL extracts after lyophilization; **: The weight of eluents from 50 mL extracts after lyophilization

times higher than that of the crude extracts. The HPLC chromatograms of samples before and after enrichment were shown in Fig. 3 and the contents of active ingredients were shown in Table 3. The results showed that the main active components (luteolin and pigenin) in *C. japonica* (Li and Niu, 2012; Yao and Ren, 2011) increased 8.35 and 6.29 times after the enrichment (Table 3). Simultaneously, the content of other polyphenols increased 2.024–11.776 times (Table 3). The flavonoids and polyphenol compositions of the samples before and after separation were consistent with those reported by Li and Niu (2012). Thus, our method has great potential for the enrichment and separation of the total flavonoids from *C. japonica* in industry.

CONCLUSION

The enrichment and separation of the total flavonoids in *C. japonica* were successfully achieved using macroporous resins. The separation characteristics of six kinds of resins were evaluated systematically through the static adsorption and desorption experiments. The LS-46D was found to be

the most suitable resin because of its best separation efficiency for the total flavonoids. The optimum conditions for adsorption were as follows: the initial sample concentration of 5-6 mg/mL, load sample volume of 50 mL and feed flow rate of 3.0 mL/min. The conditions for desorption were 150 mL of 70% ethanol and 2.0 mL/min of flow rate. After the treatment with LS-46D resin under these optimal conditions, the weight percentage of the total flavonoids in *C. japonica* increased by 3.5 times from 3.25 to 11.4% with a recovery yield of 94.84%. The contents of luteolin and pigenin increased 8.35 and 6.29 fold, respectively. Simultaneously, the contents of phenols were 2.024-11.776 times higher than that before treatment. The results may provide basis for large-scale enrichment and separation of the flavonoids from *C. japonica.*

ACKNOWLEDGMENT

The project was supported by the Hunan Province Science-technology Support Plan Projects (2015NK3022), the Grain-oil Process and Quality Control 2011 Collaborative and Innovative Grant from Hunan Province (Xiang Jiao Tong [2013] 448), the National Science-technology Support Plan Projects (2012BAC01B07, 2012BAD29B05), Construction Program of the Key Discipline in Hunan Province (2011-76) and the Scientific Research Project of the Education Department of Hunan Province (13C1158).

REFERENCES

Ahmad, A., M. Kaleem, Z. Ahmed and H. Shafiq, 2015. Therapeutic potential of flavonoids and their mechanism of action against microbial and viral infections—a review. Food Res. Int., 77(Part 2): 221-235.

Cheng, L.C., V. Murugaiyah and K.L. Chan, 2015. Flavonoids and phenylethanoid glycosides from *Lippia nodiflora* as promising antihyperuricemic agents and elucidation of their mechanism of action. J. Ethnopharmacol., 176: 485-493.

Cheng, M.C., L.Y. Lin, Y.H. Yen and T.H. Yu, 2010. Chemical Composition and antioxidant activity of the essential oils from the stems and leaves of mountain celery (*Cryptotaenia japonica* Hassk). Taiwan. J. Agric. Chem. Food Sci., 48(1): 33-45.

Fu, B.Q., J. Liu, H. Li, L. Li, F.S.C. Lee and X.R. Wang, 2005. The application of macroporous resins in the separation of licorice flavonoids and glycyrrhizic acid. J. Chromatogr. A, 1089(1-2): 18-24.

Jia, D., J. Zhang, R. Lan, H. Yang and Y. Sun, 2013. A simple preparative method for isolation and purification of polysaccharides from mulberry (*Morus alba* L.) leaves. Int. J. Food Sci. Tech., 48(6): 1275-1281.

Jiang, S., Q. Liu, Y. Xie, H. Zeng, L. Zhang, X. Jiang and X. Chen, 2015. Separation of five flavonoids from tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn) grains via off-line two dimensional high-speed counter-current chromatography. Food Chem., 186: 153-159.

Kamiyama, M. and T. Shibamoto, 2012. Flavonoids with potent antioxidant activity found in young green barley leaves. J. Agr. Food Chem., 60(25): 6260-6267.

Kang, S.Y., J.Y. Kang and M.J. Oh, 2012. Antiviral activities of flavonoids isolated from the bark of Rhus verniciflua stokes against fish pathogenic viruses *in vitro*. J. Microbiol., 50(2): 293-300.

Li, S.H. and Y.Y. Niu, 2012. Study on chemical constituents in *Cryptotaenia japonica*. Chinese Tradit. Herbal Drug., 43(12): 2365-2368.

Lu, J., C. Zhou, O. Rong, Y.Y. Xu, B. Zhou and Z.H. Li, 2013. Optimization of microwave-assisted extraction of flavonoids from *Cryptotaenia japonica* hassk using response surface methodology. Adv. J. Food Sci. Technol., 5(3): 310-317.

Okude, T. and S. Hayashi, 1970. Sesquiterpene constituents of the essential oil of mitsuba (*Cryptotaenia japonica* Hassk). B. Chem. Soc. Jpn., 43(9): 2984-2985.

Peng, M.J., S.A. Zhong, C.S. Zhou, C.Q. Dong and Q.F. Lei, 2004. Separation and purification of active components of eucomia on macroporous adsorption resin. Ion Exch. Adsorpt., 20(1): 13-22.

Wan, P., Z. Sheng, Q. Han, Y. Zhao, G. Cheng and Y. Li, 2014. Enrichment and purification of total flavonoids from *Flos Populi* extracts with macroporous resins and evaluation of antioxidant activities *in vitro*. J. Chromatogr. B, 945-946: 68-74.

Xiong, Q., Q. Zhang, D. Zhang, Y. Shi, C. Jiang and X. Shi, 2014. Preliminary separation and purification of resveratrol from extract of peanut (*Arachis hypogaea*) sprouts by macroporous adsorption resins. Food Chem., 145: 1-7.

Yang, Q., M. Zhao and L. Lin, 2016. Adsorption and desorption characteristics of adlay bran free phenolics on macroporous resins. Food Chem., 194: 900-907.

Yang, W.P. and T.H. Xia, 2010. New Chinese Herbal Medicine Map and the Commonly Used Formula. Guizhou Science and Technology Press, Guiyang.

Yao, Y. and G. Ren, 2011. Effect of thermal treatment on phenolic composition and antioxidant activities of two celery cultivars. LWT-Food Sci. Technol., 44(1): 181-185.