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Purification of Flavone *C*-Glycosides from Bamboo Leaves by Macroporous Adsorption Resin

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A simple and efficient process for purification flavone *C*-glycosides from bamboo leaves was developed in this paper. The adsorptive properties of flavone *C*-glycosides on the resins were evaluated. Among the ten macroporous resin adsorbents tested, the non-polar resin H103 has the best adsorption and desorption performance. Besides, the static equilibrium adsorption data fitted well with the Langmuir equation. The gradient elution program was as follows: 2 BV (bed volume) of deionized water, 2 BV of 20 % ethanol, 5 BV of 80 % ethanol at a flow rate of 1 mL/min. After the H103 resin treatment, the contents of the total 6 flavone *C*-glycosides were increased from 2.49 to 50.40 %, the recovery rate of which was 69.73 %. Therefore, the method can be applied to the separation of flavone *C*-glycosides from bamboo leaves.

Keywords: Bamboo leaves, DesorptBn, Flavone *C*-glycosides, Macroporous resin.

INTRODUCTION

Flavonoids, a large variety of plant polyphenol secondary metabolites, are widely distributed in medicinal plants, fruits, teas and health beverages[1,2]. Flavone *C*-glucosides, an important constituent of the flavonoid family, are found in some plants, such as the tree *Pterocarpus marsupium*[3] and the fruits of *Cucurbitaceous*[4], *etc*. Bamboo is a giant, woody grass widely distributed in tropic and subtropic[5]. Many papers have shown that bamboo leaves are rich in flavonoid *C*-glycosides, such as orientin, isoorientin, vitexin and isovitexin[6], as well as phenolic acids, coumarin lactones, anthraquinones and amino acids[7]. Other two flavone *C*-glycosides, 2"-O-β-L-galactopyranosylorientin, 2"-O-β-arabinopyranosylorientin, (Fig. 1) were also isolated in previous studies[8]. These important active components of bamboo leaf showed a variety of biological activity, including antioxidation and free radical scavenging[9,10], antiaging and preventing aging dementia[11], antiinflammatory[12], antibacterial[13], antihyperglycemia[14,15], cancer prevention[16], inhibition of tumor initiation[17,18] and reduction the risk of cardiovascular diseases[19-22].

A few methods have been developed to separate and purify the flavone *C*-glucosides from plants, such as heat reflux extraction[23], microwave-assisted extraction[24], enzyme-assistant extraction[25], preparative column chromatography[26] and high-speed counter-current chromatography[27], *etc*. However, these methods are only suitable for initial separation or purification of small quantities for analysis purposes. Moreover, the aforementioned separation processes require multiple fractionation

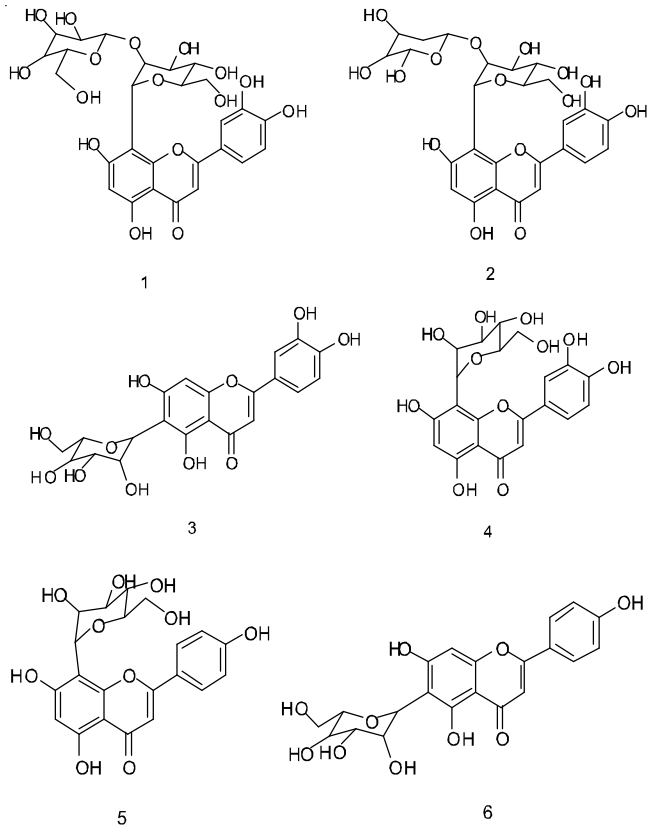

Fig. 1. Chemical structures of flavone C-glycosides of bamboo leaves (1) 2"-O-β-L-galactopyranosylorientin; (2) 2"-O-β-arabinopyranosylorientin; (3) isoorientin; (4) orientin; (5) vitexin; (6) isovitexin

steps, expensive chromatographic matrices and frequent use of poisonous solvents resulting in lower recovery of the products. To cope with this problem, in the present study, various resins with different polarities were used to set up a simple and efficient process for preparative separation of 6 flavone *C*-glycosides from bamboo leaves.

EXPERIMENTAL

2"-O-β-L-galactopyranosylorientinm, 2"-O-β-arabinopyranosylorientin, isoorientin, orientin, vitexin, isovitexin were purchased from Biopurify Phytochemicals Ltd. (Chengdu, China). Acetonitrile and Methanol were obtained from Promptar Co. Ltd. (United States). All the other chemicals (analytical-reagent grade) were obtained from Sinopharm Chemical Reagent Co., Ltd, (Shanghai, China). Water was purified with a Milli-Q system (Millipore, Bed-ford, USA). All solutions prepared for HPLC were passed through a 0.22 μm nylon filter before use.

Crude flavonoids from bamboo leaves: Bamboo leaves dried in the shade were extracted by boiling water for 3 h (solid-liquid, w/v, 1:10, g/mL) and removed the insolubles by filtration, then parted by *n*-butane (liquid-liquid, v/v, 1:3). After solvent recovery by vacuum evaporator, the sample was obtained. The contents of 2"-O-β-L-galactopyranosylorientin, 2"-O-β-arabinopyranosylorientin, isoorientin, orientin, vitexin and isovitexin in the crude extract were 0.15, 0.11, 1.24, 0.41, 0.26 and 0.32 %, respectively detected by HPLC.

Macroporous resins D4020, S-8, XAD-16, AB-8, H103, NKA-9, D101, NKA-II, NKA and X-5 were supplied by Chemical Plant of Nankai University, Haiguang Chemical Ltd. And Nankai Hecheng S&T Co., Ltd. (Tianjin, China). Their chemical and physical properties are listed in Table-1. The resins were dipped in ethanol for 24 h, then, washed by circumfluence until there was no residue after distillation. Before being used, the resins were wet with ethanol and then were washed with deionized water until the ethanol was thoroughly replaced with deionized water.

TABLE-1
PHYSICAL AND CHEMICAL PROPERTIES OF TEN RESINS

Resin series	Polarity	Specific surface area (m^2/g)	Pore size (nm)
AB-8	Weak polar	480-520	13.0-14.0
D101	Nonpolar	500-550	9.0-10.0
D4020	Nonpolar	540-580	10.0-10.5
NKA-9	Polar	250-290	15.0-16.5
NKA-II	Polar	160-200	14.5-15.5
NKA	Nonpolar	570-590	20.0-22.0
X-5	Nonpolar	500-600	29.0-30.0
H103	Nonpolar	1000-1100	8.50-8.50
S-8	Polar	100-120	28.0-30.0
XAD-16	Polar	750-800	14.0-15.0

HPLC analysis of flavonoids: The 2"-O-β-L-galactopyranosylorientin, 2"-O-β-arabinopyranosylorientin, isoorientin, orientin, vitexin and isovitexin were quantified by HPLC on an Agilent 1200 Series system equipped with a UV-visible detector. Analyses were performed on an Eclipses XDB-C18 column (5 μm, 250 mm × 4.6 mm i.d.). The column temperature was maintained at 30 °C. A gradient program was used with the mobile phase, combining solvent A (acetonitrile) and solvent B (0.2 %, v/v, acetic acid adjusted to pH 3) as follows: 9.5-10.5 % A (32 min), 10.5 % A (16 min), 10.5-13 % A (4 min), 13-16 % A (33 min), 16-9.5 % A (5 min). The flow rate was 1 mL/min, the injection volume was 20 μL and the column temperature was maintained at 30 °C. Signal was monitored at 330 nm with UV-DAD.

The working calibration curves showed good linearity in the ranges of 0.5187-61.01 μg/mL for orientin, 0.4854-63.98 μg/mL for isoorientin, 0.3981-68.80 μg/mL for vitexin, 0.3012-46.23 μg/mL for isovitexin, 0.3990-59.52 μg/mL for 2"-O-β-L-galactopyranosylorientin and 0.4893-57.04 μg/mL for 2"-O-β-arabinopyranosylorientin. The regression curves for orientin, isoorientin, vitexin, isovitexin, 2"-O-β-L-galactopyranosylorientin, 2"-O-β-arabinopyranosylorientin were C = 0.0163A + 0.1112 (R^2 = 0.9998, n = 6), C = 0.0148A + 0.1154 (R^2 = 0.9997, n = 6), C = 0.0162A - 0.0826 (R^2 = 0.9997, n = 6), C = 0.0084A - 0.2818 (R^2 = 0.9998, n = 6), C = 0.015A + 0.1189 (R^2 = 0.9996, n = 6) and C = 0.0152A + 0.1241 (R^2 = 0.9999, n = 6), respectively, where A is the peak area of the analyte and C is the injected concentration (μg/mL) of the orientin, isoorientin, vitexin, isovitexin, 2"-O-β-L-galactopyranosylorientin, 2"-O-β-arabinopyranosylorientin standard. In Fig. 2 the HPLC/DAD chromatograms of the investigated extracts of bamboo at 330 nm are presented.

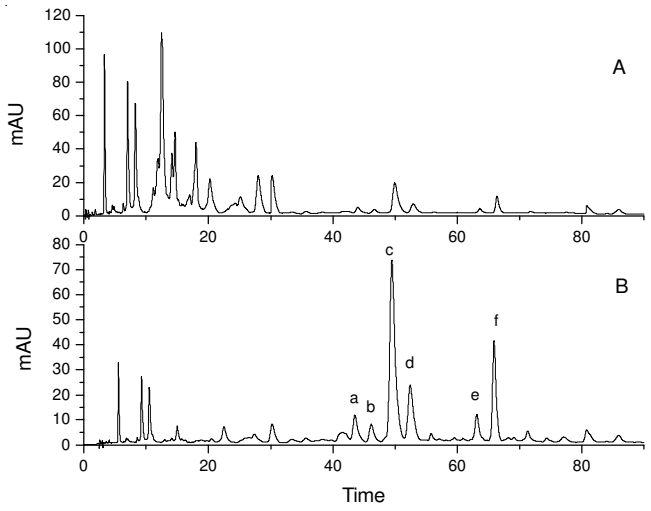

Fig. 2. HPLC chromatogram of flavonoids from bamboo leaves. A: before treated by H103 resin; B: after treated by H103 resin; (a) 2"-O-β-L-galactopyranosylorientin; (b) 2"-O-β-arabinopyranosylorientin; (c) isoorientin; (d) orientin; (e) vitexin; (f) isovitexin

Adsorptive properties of the resin to total flavonoids

Static adsorption resins screening: The flavone 6-*C*-glucosides and 8-*C*-glucosides were the major components in the bamboo leaves. So orientin and isoorientin on behalf of the total flavone *C*-glycosides of bamboo leaves were used in the next adsorption experiments.

The static adsorption screening of all macroporous resins was performed as follows: 40 mL flavonoid solution was added to 100 mL flasks containing pre-weighed amounts of different hydrated adsorbents (equal to 1 g dry resin). Then the flask was shaken (100 rpm) in a constant temperature shaker at 25 °C for 8 h. After adsorption experiment, the resins were

separated from the solutions by filtration and washed with adequately deionized water and then desorbed with 40 mL of 80 % ethanol.

The macroporous resins were evaluated by their capacities of absorption/desorption and desorption ratios[28,29].

Adsorption evaluation

$$q_e = \frac{(C_0 - C_e) \times V_i}{W} \quad (1)$$

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

where q_e is the adsorption capacity at adsorption equilibrium (mg/g resin); E is the adsorption ratio (%), which is the percentage of the mass of total adsorbate after reaching equilibrium; C_0 and C_e are the initial and equilibrium concentrations in the solutions, respectively (mg/mL); V_i is the volume of the initial feed solution (mL) and W is the weight of the dry adsorbent (g).

Desorption ratio

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

where D is the desorption ratio (%); C_d is the concentration of the solutes in the desorption solutions (mg/mL); V_d is the volume of the desorption solution; and C_0, C_e and V_i are the same as defined above.

Adsorption isotherms on NKA-II and H103: Adsorption isotherms on NKA-II and H103 were performed. 40 mL of crude extraction solutions at different concentrations and resins (equal to 1 g dry resin), respectively were added to 100 mL flasks, which were shaken (100 rpm) for 18 h at 25, 30 and 35 °C separately. The initial and equilibrium concentrations were determined by HPLC.

Two popular theoretical models (Langmuir and Freundlich models) are used to describe the adsorption capacities of resins[30,31].

Langmuir equation:

$$q_e = \frac{q_{max\ KL} C_e}{(1 + {}_{KL} C_e)} \quad (4)$$

The above equation can be rearranged to the following linear form:

$$\frac{1}{q_e} = \frac{1 / q_{max\ KL}\ C_e + 1}{q_{max}} \quad (5)$$

Freundlich equation:

$$q_e = K_F C_e^{1/n} \quad (6)$$

where q_e (mg g^{-1}) and C_e (mg mL^{-1}) represent the same parameters as in formulae 1; q_{max} is the theoretically calculated maximum adsorption capacity (mg g^{-1} resin); K_L is the adsorption equilibrium constant related to the affinity between the adsorbent and adsorbate; K_F is the Freundlich constant, an indicator of adsorption capacity and 1/n is an empirical constant related to the magnitude of the adsorption driving force.

Adsorption kinetics on H103: Adsorption kinetics was carried out to evaluate the effect of contact time on adsorption. 40 mL of crude extraction solutions were added to 100 mL flasks containing pre-weighed amounts of H103 adsorbents (equal to 1 g dry resin). Then the flasks were shaken (100 rpm) in a constant temperature shaker at 25 °C for 8 h. The concentrations of orientin and isoorientin in the adsorption solution were analyzed by HPLC at certain time intervals.

Dynamic adsorption and desorption tests: In view of the above results, the dynamic adsorption experiments were carried out in a glass column (24 × 500 mm) packed with 10 g (dry weight) of H103 adsorbents. The bed volume (BV) of wet packed resin was 45 mL and the length of the bed was 10 cm. The sample solution flowed through the column at the flow of 1 mL/min.

Dynamic leakage curves and dynamic desorption curves experiments were performed as follows. The sample solutions flowed through the column at the rate of 1 mL/min and the concentrations of the orientin and isoorientin and the concentrations of the orientin and isoorientin in the eluents were detected by HPLC analysis. Then the adsorbate-laden column was washed first by deionized water (2 BV) and followed by 20 % aqueous ethanol (2 BV), at last 90 % ethanol (5 BV) at the flow rate of 1 mL/min. The concentrations flavone *C*-glycosides in the desorption solutions were detected by HPLC analysis.

Statistical data analysis: All the experiments were conducted in triplicate and the data were reported as mean ± SD (standard deviation) of triplicate determinations. Statistical calculations were carried out by SPSS version 16.0 (SPSS Inc., USA). One-way ANOVA was applied to determine the statistic differences. $P < 0.05$ was considered as significantly different.

RESULTS AND DISCUSSION

Static adsorption test of the resins: The results of static adsorption and desorption tests for screening among ten kinds of macroporous adsorption resins were shown in Fig. 3. The optimal choices of the macroporous resin were evaluated by their capacities of adsorption/desorption and desorption ratios. The adsorption capacities of S-8, NKA-II and H103 resins towards isoorientin and orientin were considerably higher than that of other resins, but the desorption ratio of S-8 was very low. D4020, XAD-16, NKA and NKA-9 resins have higher

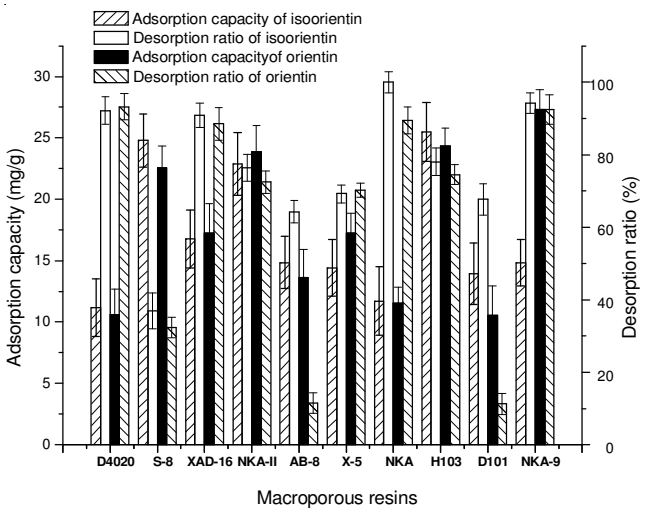

Fig. 3. Adsorption capacities and desorption ratio of orientin and isoorientin on different resins

adsorption but weaker desorption rates than NKA-II and H103 resins. In view of these results, NKA-II and H103 were considered to be further evaluated for their adsorption performance towards flavonoids in the following tests.

Adsorption isotherms on NKA-II and H103: Table-2 lists the two isotherm equations at different temperature and the following parameters: q_m (maximum adsorption capacity) and K_L (adsorption energy) values obtained from the Langmuir isotherm together with K_F and 1/n values obtained from the Freundlich isotherm, which are correlated with adsorption capacity and adsorption intensity, respectively. As can be seen from Table-3, the high correlations 0.927-0.996 for flavone *C*-glycosides showed that both Langmuir and Freundlich models were suitable for describing the tested adsorption system in the concentration range studied. Compared with the fitting results with the Langmuir isotherm model, the Freundlich isotherm model fitting results appeared to be less satisfactory. The R^2 values for the Langmuir model fittings to this two flavone *C*-glycosides were all above 0.97. The q_m decreased with increasing temperature for flavone *C*-glycosides. Thus, the optimum temperature of the adsorption process is 25 °C, the results showed that the adsorption procedure is exothermic process.

It is observed from Freundlich model that the KF of H103 was higher than NKA-II and the q_m of H103 was higher than NKA-II from Langmuir model. In view of these results, H103 resin was considered as the optimum one, to enrich total flavones from bamboo leaves.

TABLE-2
(A) LANGMUIR MODEL AND FREUNDLICH MODEL PARAMETERS OF ISOORIENTIN ON NKA-II AND H103 RESINS

	Temp.	Freundlich model			Langmuir model		
	(K)	K_F	N	R^2	q_m	K_L	R^2
NKA-II	298	14.692	1.190	0.966	277.7	0.099	0.985
NKA-II	303	12.914	1.156	0.948	243.9	0.104	0.981
NKA-II	308	10.326	1.122	0.941	200.0	0.139	0.991
H103	298	18.283	1.398	0.927	312.5	0.072	0.978
H103	303	15.957	1.333	0.969	256.4	0.087	0.982
H103	308	13.218	1.185	0.963	212.8	0.095	0.996
(B) LANGMUIR MODEL AND FREUNDLICH MODEL PARAMETERS OF ORIENTIN ON NKA-II AND H103 RESINS							
NKA-II	298	11.261	1.169	0.953	270.3	0.101	0.995
NKA-II	303	9.693	1.153	0.947	227.3	0.110	0.991
NKA-II	308	8.091	1.092	0.937	188.7	0.130	0.985
H103	298	15.753	1.178	0.929	333.3	0.089	0.985
H103	303	13.931	1.124	0.956	263.2	0.098	0.982
H103	308	11.348	1.114	0.951	212.8	0.120	0.996

Adsorption kinetics on H103: After adsorption equilibrium, evaluate the best ratio of ethanol in water, 40 mL of crude extraction solutions and different ratio (50, 70 and 90 %) of ethanol in water were added to 100 mL flasks containing pre-weighed amounts of adsorbents H103 resin (equal to 1 g dry resin). The flasks were then shaken (100 rpm) in a constant temperature shaker at 25 °C for 8 h. The desorption recovery was 90.2, 99.8 and 99.6 % (n = 3), respectively. It is indicated that 70 % ethanol was applied for desorption of total flavones.

The adsorption kinetics of orientin and isoorientin on H103 resin were showed in Fig. 4. Adsorption capacities increased with adsorption time before reaching equilibrium. In the first 60 min, the adsorption capacity increased rapidly and then increased slowly and ultimately reached adsorption equilibrium at 110 min.

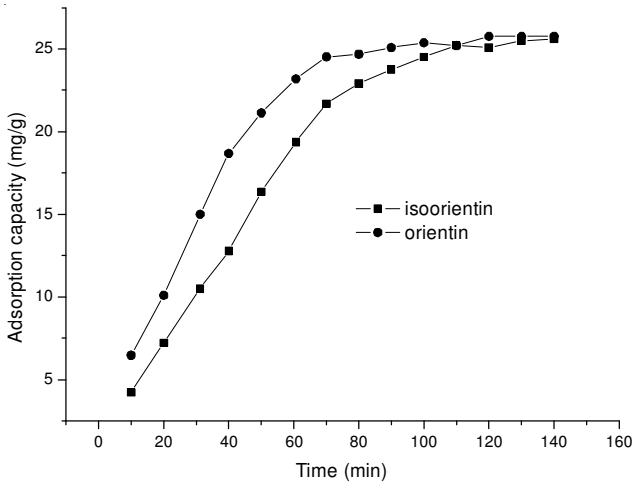

Fig. 4. Adsorption kinetics curve for orientin and isoorientin on H103 resin

Dynamic adsorption and desorption tests

Dynamic leak curves on H103 resin: The leakage curves for orientin and isoorientin on H103 was showed in Fig. 5. Adsorption presumably reached saturation when the concentration in leak solution was 5 % of the original concentration. However, the leak points (5 %) were not the same for these two compounds, the leak points of orientin and isoorientin were 1.8 BV and 2.4 BV at the flow rate of 1 mL/min and 0.8 BV and 1.4 BV at the flow rate of 2 mL/min, respectively. If the volume of feed solution was selected according to orientin, the latter isoorientin would not reach adsorption saturation. Therefore, a feed solution of 2.5 BV was selected for dynamic adsorption experiments.

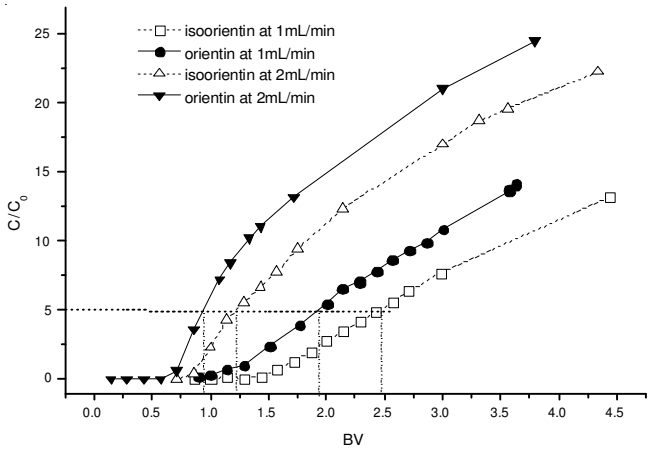

Fig. 5. Leakage curves for orientin and isoorientin on H103

Dynamic desorption curves on H103 resin: The dynamic desorption curves on H103 resin were obtained based on the volume of effluent and the concentration of 2"-O-β-L-galactopyranosylorientin, 2"-O-β-arabinopyranosyl orientin, homoorientin, orientin, vitexin and isovitexin.As shown in Fig. 6, the 6 flavone *C*-glycosides can be completely desorbed from H103 resin by 7 BV solution.

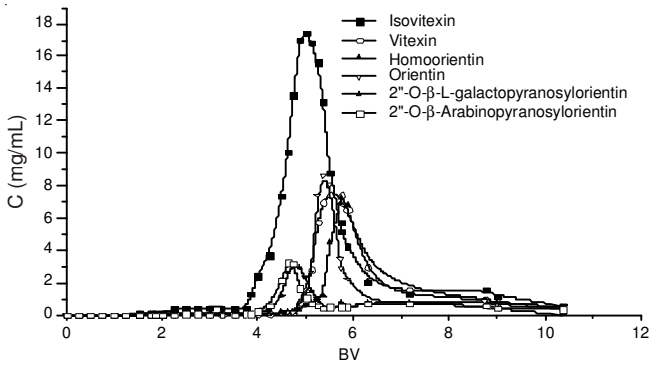

Fig. 6. Dynamic desorption curves of 6 flavone *C*-glycosides on H103 resin

The adsorbate-laden column was washed with deionized water 2 BV first and then desorbed with 20 % ethanol 2 BV, at last desorbed with 80 % ethanol 5 BV at the flow rate of 1 mL/min. Then, 2"-O-β-L-galactopyranosylorientin, 2"-O-β-arabinopyranosyl orientin, isoorientin, orientin, vitexin and isovitexin in the aliquots of 1 mL 90 % ethanol eluents collected at 5 mL intervals were detected by HPLC analysis. After the H103 resin treatment, the contents of 2"-O-β-L-galacto-pyranosylorientin, 2"-O-β-arabinopyranosylorientin, isoorientin, orientin, vitexin and isovitexin were 2.92, 2.16, 25.32, 8.25, 5.30 and 6.45 %, respectively. The contents of total six flavone *C*-glucosides were 50.40 %, the recovery rate of total 6 flavone *C*-glucosides were 69.73 %.

Conclusion

A method for the purification of flavone *C*-glycosides from bamboo leaves was established using macroporous resins. Static adsorption/desorption tests and dynamic separation results shows that H103 had the best desorption ratio and separating selectivity among ten typical macroporous resins investigated. Dynamic absorption/desorption experiments on H103 column were conducted to obtain the optimal parameters.

Flavone C-glycosides, 2"-O-β-arabinopyranosylorientin, orientin, isoorientin, vitexin and isovitexin, 2"-O-β-L-galacto-pyranosylorientin from bamboo leaves were purified by resin, the contents of total six flavone C-glycosides were increased from 2.49 to 50.40 %. These results shows that the established means of flavone *C*-glycosides from bamboo leaves was highly efficient, environmental friendly and economic. The above results suggest that bamboo leaves should be exploited and the extracts as ingredients for diabetes health food.

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