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Macroporous Resin Extraction of Chlorogenic Acid From *Eucommia* Leaves

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The most suitable absorption of *Eucommia* chlorogenic acid from *Eucommia* leaves by macroporous resin has been identified. Ultraviolet spectrometer through a qualitative and quantitative determination, In: $\lambda = 330$ nm; the standard curve is $C = 0.67649 + 21.2607 A$; $R = 0.9998$ and the limited scope: 51 μg/mL < C < 255 μg/mL; six different models of macroporous resins were tested through static adsorption and one macroporous resin labeled as D201 was selected. By dynamic adsorption experiment, adsorption reached saturation time was 80 min; 45 % ethanol with 5.5 bed volume (BV) was found to be able to elute most of the chlorogenic acid adsorbed by the resin. Chlorogenic acid with content of 74.14 % was obtained with the chlorogenic acid recovery of 53.4 % in the purification process Therefore, it is concluded that results in this study may provide scientific references for the large-scale chlorogenic acid production from plants extracts.

Key Words: *Eucommia* leaves, Chlorogenic acid, Macroporous resin.

INTRODUCTION

Adsorbent resins are durable, non-polar or slightly hydrophilic polymers having high adsorption capacity with possible recovery of the adsorbed molecules, relative low cost and easy regeneration. They are currently used for adsorption of flavonoids and other components extracted from many plants. Non-polar resins have been used to recover hesperidin from citrus peel or the wastewater flowing from centrifuges of essential oil separation (yellow water)[1,2] and anthocyanins from pulp wash of pigmented oranges[3]. They have also been used to remove naringin and limonin from citrus juices and to recover cold pressed grapefruit oil from wastewater[4]. Resins are effective adsorbent material for anthocyanins from different sources and have been widely used in research and in the production of anthocyanins[5-7].

Chlorogenic acid existed in many plants, had various pharmacological actions. Recent researches indicated that chlorogenic acid had relatively low bioavailability for oral use. It existed in plasma in the form of metabolites which are excreted mainly by rena1. Chlorogenic acid has multiple actions including antioxidant, anticancer, antibiosis, antiviral, immune modulability and hyperglycemic activity[8].

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EXPERIMENTAL

Eucommia leaves were provided by Tianxiakang Industry Co. Ltd. (China); ethanol, hydrochloric acid, sodium hydroxide, acetic acid (all AC grade), methanol (HPLC grade) were purchased from Beijing Chemical Plant. De-ionized water was used in all experiments. Chlorogenic acid ($C_{16}H_{18}O_9$, m.w. 354.30, 99 %) used for the HPLC analysis standard was purchased from the Chinese Institute of Drug and Biological Products Identification (China), chlorogenic acid (35%) used for the adsorption experiment were purchased from the Beijing Jijiyuan Medical Industry Co. Ltd. (China). The resins tested were NKA-9, AB-8, D4020, ADS-8, D201 and D301 (Chemical Industrial Company affiliated to Nankai University, Tianjin, China).

Plant material treatment: 500 g *Eucommia* leaves powder was extracted with 1500 mL of ethanol-water (20:80, v/v) solution in a bath for 1 h, repeated three times. The extracted solutions were combined and centrifuged at 6000 rpm for 10 min using a centrifuge. The supernatant extracts were concentrated to dryness by removing the ethanol solvent in a rotary evaporator at 60 °C and *Eucommia* extracts residue was obtained.

Resins: Pretreatment and activation: The adsorbent was pre-treated to remove the monomers and porogenic agents entering inside the pores during the synthesis process. All resins were cross-linked polystyrene copolymers. Their physical and chemical properties are shown in Table-1.

TABLE-1
PHYSICAL AND CHEMICAL PROPERTIES OF RESIN

Name	Particle diameter (mm)	Surface area (m^2/g)	Pore radius (nm)	Polarity
NKA-9	0.3-1.25	250-290	15.0-16.5	Polar
AB-8	0.3-1.25	480-520	13.0-14.0	Low polar
D4020	0.3-1.25	540-580	10.0-10.5	Non-polar
ADS-8	0.3-1.20	360-420	9.5-10.0	Non-polar
D301	0.3-1.25	530-600	11.5-13.0	Non-polar
D201	0.3-1.25	450-550	10.0-12.0	Non-polar

The resins were pretreated and activated according to the manufacturer's recommendation. Firstly, they were rinsed with distilled water and filtered with nylon filter cloth to retain those with a particle diameter larger than 0.3 mm. They were then soaked overnight in 2 bed volumes (BV) of 95 % ethanol. After soaking, the resins were introduced into a glass column and rinsed with a further 2 BV of 95 % ethanol. Subsequently they were rinsed with 2 BV of distilled water to remove the ethanol, 1 BV of 4% (w/v) sodium hydroxide, 2 BV of distilled water, 1 BV 4 % (v/v) hydrochloric acid and finally by distilled water until the pH of the fluent became neutral.

Static adsorption and desorption: In the static adsorption experiment, 4 g hydrated test resin was put into flask with a lid, 20 mL sample solution of *Eucommia* extracts was added. The flask was then shaken (100 rpm) for 2 h in a water-bath shaker at 25 ºC. The static desorption was also performed in the shaker at 25 ºC for 2 h, using 10 mL 45 of % aqueous ethanol solutions as eluting phase.

Dynamic adsorption and desorption: Dynamic adsorption and desorption experiments were carried out in glass columns (12 mm × 300 mm) wet-packed with the 40 g of selected resin. The feed rate was 4 BV h^{-1} (BV standing for bed volume) and the eluting solvent flow rate was 1 BV h^{-1}. All the dynamic experiments were performed at room temperature. While adsorptive equilibration, the adsorbate-laden column was washed first with de-ionized water and then desorbed with aqueous solution of ethanol-water (45:55, v/v). The fluent was concentrated in the rotary evaporation apparatus and dried under vacuum before further analysis.

Detection and analysis: UV analysis was applied to determine the content of chlorogenic acid by reported method[4]. Chlorogenic acid concentration was calculated using the standard sample as the calibration standard. A good linear relationship was obtained over the range of 51 μg/mL < C < 255 μg/mL and the regression was y = 21.2607x + 0.67649 (R = 0.9998), where y is the absorbance at 330 nm, x is the concentration of chlorogenic acid (mg mL^{-1}) and R is the regression coefficient.

HPLC analysis was performed using a Waters liquid chromatographic systems (Waters Company, USA) HPLC system, A Diamonsil C_{18} column (250 mm × 4.6 mm i.d.,10 μm) was employed for the separation of samples. The detector was set to 326 nm and injection volume was 20 μL. Methanol + water + acetic acid = 24:75:1 was adopted as mobile phase. Flow rate was 0.5 mL min^{-1}. All HPLC were performed at (25 ± 1) ºC. All solutions were prepared in 24 % aqueous methanol and filtered through 0.45 μm membranes (Chromatography Science and Technology Co., Tianjin, China) before HPLC analysis, the retention time of chlorogenic acid was 31.8 min. The chromatographic peak of the chlorogenic acid was confirmed by comparing its retention time and UV spectrum with that of the reference standard. The working calibration curve based on chlorogenic acid standard solutions showed good linearity over the range of 40-200 μg/mL. The regression line was $y = 2\times10^7x + 380379$ ($R^2 = 0.9997$, n = 8), where Y is the peak area of chlorogenic acid and X is the concentration of chlorogenic acid (μg/mL).

RESULTS AND DISCUSSION

Static adsorption and desorption on D201: Six macroporous resins with different properties were tested through static adsorption and the adsorption capacity (Qe) and desorption recovery (D) were used to evaluate their adsorbent efficiency. As shown in Table-2, the adsorption capacity and the desorption ratio of D201 is higher compared with the other resins studied here. Though some other resins also have high adsorption capacities, they are not easy to release the chlorogenic acid. For economical reason, the resin D201 was finally chosen.

TABLE-2
ADSORPTION CAPACITIES AND DESORPTION RATIOS OF 6 KINDS OF RESINS TOWARDS CHLOROGENIC ACID

Name	Qe (mg/g)	D (%)
NKA-9	13.29	85.3
AB-8	4.54	93.4
D4020	7.38	87.9
ADS-8	12.60	77.6
D301	13.77	84.7
D201	14.31	91.6

The following formulae are used to quantify the adsorption and desorption capacities:

Adsorption capacity:

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

where Q_e is the equilibrium adsorption capacity (mg per g resin); C_0 and C_e are the initial and equilibrium concentrations of solute in the solution, respectively (mg mL^{-1}); V_i is the volume of the initial feed solution (mL) and W is the mass of the dry adsorbent (g).

Desorption recovery:

$$D = \frac{C_d V_d}{(C_0 - C_e)V_i} \times 100\ \% \quad (2)$$

where D is the desorption recovery (%); C_d is the concentration of the solute in the desorption solution (mg mL^{-1}); V_d is the volume of the solution; C_0, C_e and V_i are the same defined as above.

Adsorption kinetics on D201 resin: Adsorption kinetics curve was obtained for chlorogenic acid on D201 resin. As can be seen from Fig. 1, the adsorption capacities towards chlorogenic acid increased with the extension of adsorption time. In 80 min the adsorption capacities increased rapidly, after 80 min it reached equilibrium. The fast initial rate is likely due to the occurrence of adsorption in the easily accessible mesopores of the particles. The later slower uptake, on the other hand, is indicative of process with high mass transfer resistance inside the particle.

Dynamic desorption curve on D201 resin: The dynamic desorption curve using D201 resin was obtained based on the volume of desorption solution and the chlorogenic acid concentration in the desorption solution (Fig. 2).

It is important to choose a proper flow rate to desorb chlorogenic acid from resin effectively. As can be seen in Fig. 2, during the dynamic desorption test, the desorption performance was better with lower desorption flow, the desorption performance at 0.5 BV/h was the best. However, at this desorption flow rate, the working time was too long. Therefore, 1 BV/h was selected as the proper desorption flow rate in consideration of the short working time and lower volume consumption. Under this condition, a desorption solution of approximate 5.5 BV completely desorbed chlorogenic acid from D201 resin.

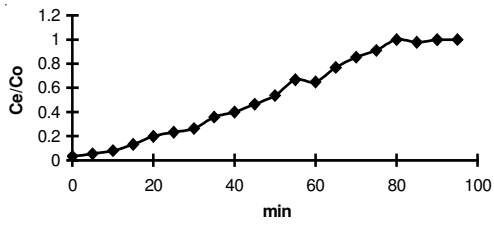

Fig. 1. Adsorption kinetics curve for chlorogenic acid on D201 resin

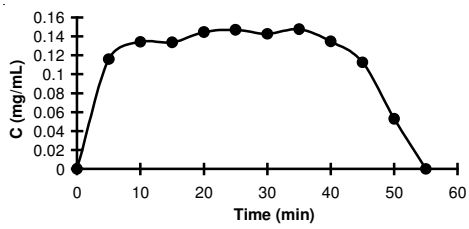

Fig. 2. Dynamic desorption curve for chlorogenic acid using D201 resin

Comparison of chlorogenic acid before and after purification with resin: *Eucommia* leaves were rich in chlorogenic acid, but the concentration of chlorogenic acid in such *Eucommia* extracts residue was low. These products have a relatively short shelf life. Contrary to the crude extracts residue, the eluent of chlorogenic acid purified with resins can be easily concentrated to obtain. During the purification with resins, most of the impurities are removed. When tested by HPLC under the same conditions, the HPLC profiles of the purified (Fig. 4) and crude (Fig. 3) chlorogenic acid extracts were identical. And chlorogenic acid with content of 74.14 % was obtained with the chlorogenic acid recovery of 53.4 % in the purification process.

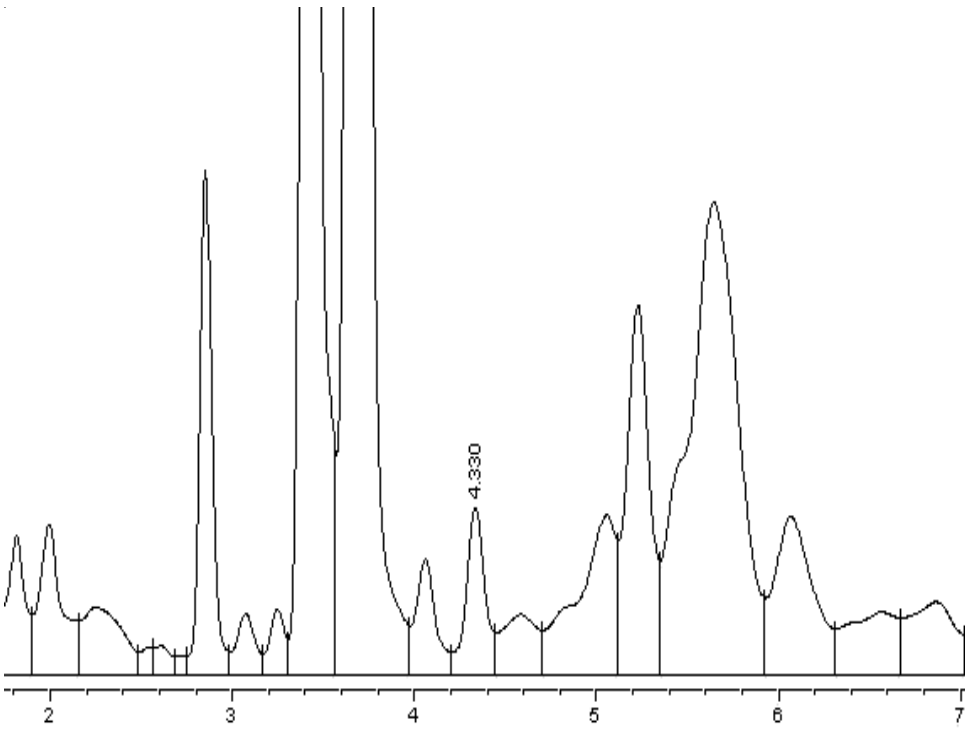

Fig. 3. Chromatograms of sample solution before treatment with resin

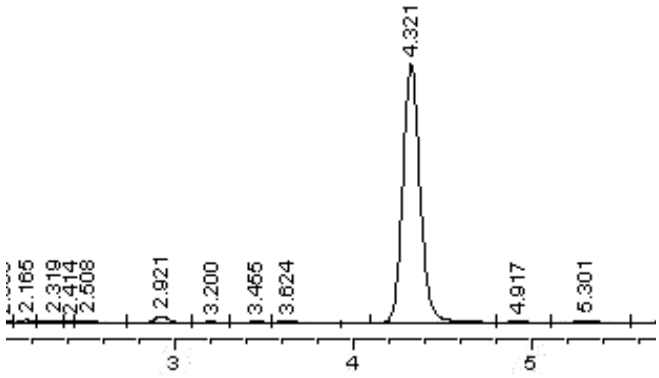

Fig. 4. Chromatograms of sample solution after treatment with D201 resin

Conclusion

In this study, the adsorption and separation characteristics of six widely used macroporous resins were evaluated and the separation process of chlorogenic acid from *Eucommia* leaves extracts with the selected resin was optimized. Among the six resins investigated, D201 resin offers the best separation power for chlorogenic acid. Using D201 resin at optimal conditions, chlorogenic acid with content of 74.14 % was obtained with the chlorogenic acid recovery of 53.4 % in the purification process. Compared to conventional separation methods of chlorogenic acid, this adsorption method is superior because of its procedural simplicity, lower cost, high efficiency and it may provide scientific references for the large-scale production.

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