SEPARATION AND PURIFICATION OF GINKGOLIC ACID FROM GINKGO BILOBAL PEEL BY MACROPOROUS RESIN

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ABSTRACT: Ginkgolic acid (GA) was obtained from Ginkgo bilobal peel by means of ethanol and macroporous resin purifying process. The static adsorption and desorption experiments were performed. A kind of suitable macroporous resin was selected from D101, D1300, AB-8 and DA201 resin. The dynamic adsorption and desorption experiments were also performed to analyze the influence of the concentration and flow velocity of the crude extraction solution on adsorption. Moreover, the influence of the flow velocity and concentration of the eluent on desorption were also analyzed, to optimize the parameters of purifying process of GA by macroporous resin. Results showed that D101 resin had much higher static adsorption and desorption ratio, which was a suitable resin for purifying GA. Results of the dynamic adsorption and the desorption experiments showed that the high concentration and the low flow velocity of the crude solution sample were benefit for the process of adsorption. 100% ethanol was used as the eluent and the reasonable amount of eluent was 10 times the volume of the resin column (BV). The flow velocity of the solution sample and the eluent was 1.0ml/min. By optimizing the macro-porous resin purification method, the purity of GA attained 80.79% which was extracted and purified from the Ginkgo bilobal peel.

KEYWORDS: Dynamic adsorption, Ginkgo bilobal peel, Ginkgolic acid, Macroporous resin, Purification, Static adsorption.

INTRODUCTION
With the development of the industrial utilization of ginkgo biloba, the peels of ginkgo biloba were produced in a large quantity and could not be fully utilized. It was more worse that the toxicants of ginkgo biloba peel may pollute the environment and threaten the health of mankind and animals. So how to reuse the ginkgo biloba peel properly becomes more and more important (Li et al. 2004).

Ginkgolic acid (GA) was assumed the active component of the ginkgo biloba peel. GA can be regarded as a series of compounds with salicylic acid molecules, in the C6 position of the benzene ring having the longer side chain, generally from 13 to 17 carbon atoms. The medicinal properties of GA have been demonstrated scientifically and experimentally. For instance, GA has been shown to have antioxidative, anti-inflammatory, anti-tumor, anti-allergic, antibacterial activities, and can be used as bio-pesticides, cosmetic additive and pharmaceuticals (Chen et al. 1996; Tang et al. 2000; Ni et al. 2001; Ni et al. 2006).

There are reports proposing extraction of GA from ginkgo biloba leafs and nuts (Fuzzati et al. 2003; Hong et al. 2010; Smith et al. 1996), by organic solvent extraction method (Iokawa et al. 1987), supercritical CO2 extraction method (Yin et al. 2003), and column chromatography method (Liu & Rao 2008; Ni & Lin 2002; Qin & Gao 2010). But studies on extraction and purification of GA from ginkgo biloba peel have not been reported. In the present study we used Ginkgo bilobal peel as the raw material to extract and purify GA by the method in combination of ethanol and macroporous resin. The present study was designed to select a kind of suitable macroporous resin to purify GA, and to investigate the adsorptive and desorptive characteristics of the selected macroporous resin. This study would provide the possible method of purifying GA from ginkgo biloba peel for industrial purpose.

MATERIALS AND METHODS
Materials
The ginkgo bilobal peel was obtained from Pizhou city, Jiangsu province of China. Ginkgolic acid (GA) standard was
High Performance Liquid Chromatography (HPLC) Analysis of Ginkgolic Acid

The purity of Ginkgolic acid (GA) was determined by HPLC instrument (Agilent 1260, Infinity Agilent company). The chromatography column was Eclipse Plus C18 3.5μm 4.6×100mm. The mobile phase was methanol and 0.5% aqueous acetic acid (90:10) and pH was 3.15. The flow rate was 1 ml/min, the column temperature was 30℃, and the detection wavelength was 310 nm. The different concentration GA standards were 100, 200, 400, 600, 800, 1000, 1200 μg/ml. The calibration curve of GA standard was drawn and the linear regression equation was established by linear regression method. The purity of GA was then calculated based on the calibration curve, which was analyzed by external standard method.

Preparation of Crude Ethanol Extraction

The *ginkgo biloba* peels were crushed with the mechanical crusher, and then the powder was washed, dried and pulverized. The powder was refluxing extracted with 85% ethanol (the mass and volume ratio was 1:8 g/ml) at 60℃ for 5 h. Then the filtrate was collected and the residue was refluxed with 85% ethanol again in the same above condition. The primo-secondary filtrates were collected and merged. GA content of the crude ethanol extraction solution was assayed by HPLC, and the crude solution was purified by macroporous resins.

Purification of Ginkgolic Acid

a) Pretreatment of Macroporous Resins

Pretreatment of the resins is essential as the monomers and porogenic agents often trap in the pores of macro-porous resins. The resins (D101, D1300, AB-8 and DA201) were soaked in 6 folds volume of 4% HCl as the leaching solution for 3 h. Then the resins were washed twice with distilled water. The resins were then soaked in 6 folds volume of 5% NaOH for 3 h and were washed by distilled water thoroughly. Then resins were pretreated by soaking in ethanol for 24 h. After removal of ethanol, the resins were washed by distilled water thoroughly. Finally, the well-pretreated resins were dried at 30℃ to the constant weight.

b) Static Adsorption and Desorption Experiments

The well-pretreated dry resin 3g was put into the flask with the stopper. Ethanol 20ml was poured in the flask and the soaking procedure lasted 24 h. After removal of the ethanol, the resin was carefully washed by distilled water thoroughly.

Then the distilled water was carefully removed by an injector. The crude ethanol extraction solution 30 ml was added to each flask. The flasks were continually shaken at the velocity of 50 rpm in a thermostatic oscillator at 30℃ for 24 h.

When the equilibrium of the adsorption reached, the supernatant was discarded and the adsorb-laden resin was washed by distilled water twice. Then the resin was desorbed with 90% ethanol. The desorption ratio was expressed as q (mg/g dry resin) = (C_1-C_2)×V/M, in which C_0 (mg/ml) was the initial GA concentration of the extraction solution, C_1 (mg/ml) was the GA concentration of extraction solution at different adsorption stage, V (ml) was the volume of the extraction solution, M (g) was the weight of the resin used. The desorption ratio was expressed as D (%)=C_1×V_1/(C_0-C_2)×V×100%, in which C_1 (mg/ml) was the GA concentration of the desorption solution and V_1 (ml) was the volume of the desorption solution.

c) Dynamic Adsorption Experiment

Dynamic adsorption experiment was carried out using the glass column wet-packed with resin particles. First, wet resin 10g was placed in the column (2.0 cm×60 cm) and the bed volume of the wet-packed resin was 25 ml. The crude ethanol extraction solution (the concentrations of GA was C) and the diluted solution (the concentration of GA was 4/5C, 3/5C, 2/5C, and 1/5C, respectively) was added on the resin. The outflow liquid was collected and was analyzed by HPLC. The dynamic adsorption capacity of resin was calculated to analyze the influence of the concentration of extraction solution sample on adsorption. Moreover, the influence of flow velocity (0.5, 1, 2, 3, and 4ml/min) of the extraction solution sample on adsorption was also investigated.

d) Dynamic Desorption Experiment

The crude ethanol exaction solution was carefully applied on the resin at room temperature. After reaching the adsorption equilibrium, the column was sequentially washed with 5 BV (the volume of the column) of the deionized water and 5 BV of 20% ethanol. The column was then eluted with 20BV of different concentrations of ethanol (85%, 90%, 95%, 100% (v/v)) at room temperature with the constant flow velocity, respectively. Each 1 BV eluent was collected and GA content (W in mg) of each 1 BV eluent was analyzed by HPLC. The elution curve was draw and the influence of the concentration of eluent on desorption was analyzed. Moreover, the influence of the flow velocity of the eluent (1, 2, 3, and 4ml/min, respectively) on desorption was also analyzed.

e) Verification Experiment

The crude ethanol extraction solution sample was prepared using the method stated above. The suitable kind of macroporous resin was selected and pretreated. The wet packed column was balanced. The dynamic adsorption and desorption experiment was performed under the optimized condition. The desorption solution was collected, in which...
the ethanol was removed using the rotary evaporator at 55°C under the negative pressure, and the extraction was obtained and was desiccated using the electrical vacuum dryer. The GA content and purity of the resultant desiccant powder was determined by HPLC method.

RESULTS AND DISCUSSION

HPLC Analysis of Ginkgolic Acid (GA)

Figure 1a shows the HPLC diagram of GA standard sample (600 μg/ml) which has a relatively symmetric peak at the retention time of 7.408, 8.331, 9.806, 12.526, 13.935 min, representing the peak of five types of GA, which are C13:0, C15:1, C17:2, C15:0 and C17:1 respectively. The linear regression equation is Y = 4.936 X - 49.67 (R²=0.991), in which X represents the concentration of GA standard (μg/ml) and Y represents the absorption peak area.

Preparation of Crude Ethanol Extraction Solution Sample

The HPLC diagram of the crude ethanol extraction solution sample was shown in figure 1b. The retention time was 7.707 min, 8.597 min, 10.176 min, 12.983 and 14.361 min, representing the peak of five types of GA C13:0, C15:1, C17:2, C15:0 and C17:1. The total peak area is 4635.12779 mAU. The concentration of GA of the crude ethanol extraction solution sample was 33.55 mg/ml, which was calculated according to the linear regression equation.

Static Adsorption and Desorption Experiments

It had been reported that the four kinds of macroporous resins D101, D1300, AB-8, DA201 had good adsorptive effects, with large saturated adsorption capacity. The adsorption and desorption properties of the above four kinds of macroporous resins on GA are listed in Table 1. Results showed that the adsorption capacity of the four kind of macroporous resins
was in the order of D101 > DA201 > AB-8 > D1300. The adsorption capacity of D101 was 205.38mg/g, which was much higher than those of the other resins. Moreover, the recovery of GA on D101 was 84.26%, which was also much higher than those of the other three resins.

**TABLE 1. Adsorption and desorption properties of Ginkgolic acid (GA) on four kinds of resin.**

<table>
<thead>
<tr>
<th>Resin</th>
<th>D101</th>
<th>D1300</th>
<th>AB-8</th>
<th>DA201</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorption capacity (mg/g)</td>
<td>205.38</td>
<td>183.04</td>
<td>184.63</td>
<td>204.22</td>
</tr>
<tr>
<td>Desorption recovery (%)</td>
<td>84.26</td>
<td>80.16</td>
<td>83.76</td>
<td>74.51</td>
</tr>
</tbody>
</table>

The resin D101 is a kind of nonpolar macroporous adsorbent resin. On the basis of the theory of similarity and inter-miscibility, D101 had litter adsorption capacity for the substances having strong polarity such as inorganic salts. However, GA was weak in polarity, and thus, the resin D101 had high adsorption capacity for GA. Moreover, the present study demonstrated that D101 macroporous resin had high adsorption capacity and desorption recovery for GA. Therefore, D101 was a suitable resin for purifying GA.

**Dynamic Adsorption and Desorption Experiments**

The dynamic adsorption curve of D101 resin was shown in Figure 2a. The concentration of GA in the crude ethanol extraction sample was 6.28, 12.55, 18.83, 25.11 and 31.39 mg/ml. Results showed that with the increase of concentration of GA, the adsorption ratio increased. So the crude ethanol extraction sample having high concentration of GA was better selected.

The crude ethanol extraction solution sample flowed through the resin layer at the flow rate of 0.5, 1.0, 2.0, 3.0 and 4.0ml/min, respectively. The dynamic adsorption curve was shown in Figure 2b. Results showed that the adsorption capacity decreased as the flow rate of the crude ethanol extraction solution sample increased. The slower the flow rate, the greater was the adsorption capacity of the resin D101. The low flow rate was favorable to the diffusion of the particle of GA molecules in the resin layer. Considering the economic reasons, the flow rate 1.0 ml/min was finally chosen.

The crude ethanol extraction sample flowed through the D101 resin layer at the flow rate of 1.0 ml/min. The column was sequentially washed with 5 BV of deionized water and then 5 BV of 20% ethanol at the flow rate of 1.0 ml/min. Subsequently, the column was eluted with 20BV of different concentrations of ethanol (85%, 90%, 95%, 100% (v/v)) respectively at room temperature with the flow velocity of 1.0 ml/min. Each 1 BV of eluent was collected, and GA content of each 1 BV eluent was analyzed by HPLC. The result was shown in Figure 3a. It was found that 10 BV of eluent was advisable. As the elution solution was 100% ethanol, the elution efficiency was best and the content of GA (W in mg) in the eluent was the highest.

The crude ethanol extraction sample flowed through the
resin layer at the flow rate of 1.0 ml/min. The column was sequentially washed with 5 BV of deionized water, 5 BV of 20% ethanol, and then, the columns was eluted with 10 BV of 100% ethanol at room temperature with the flow velocity 1, 2, 3, and 4 ml/min, respectively. Each 1 BV eluent was collected, and the GA concentration of each 1 BV of eluent was analyzed by HPLC. The result was shown in Figure 3b. Results showed that with the increase of the flow rate of the eluent, the efficiency of GA desorption decreased. The desorption ratio increased as the flow velocity decreased. Combined with economic reasons, the flow velocity of 1 ml/min was finally selected.

**Verification Experiment**

The crude ethanol extraction solution sample was purified by D101 macroporous resin. The flow velocity of the crude extraction solution sample was 1.0 ml/min. The column was sequentially washed with 5 BV of deionized water and then 5 BV of 20% ethanol at the flow rate of 1.0 ml/min. Subsequently, the column was eluted with 10 BV of 100% ethanol at room temperature with the flow velocity of 1.0 ml/min. The eluent was collected, concentrated, dried and the purified sample was obtained. GA content and purity of the purified sample was analyzed by HPLC method.

The HPLC chromatograph of the eluent solution was shown in figure 1c. The retention time was 7.671, 8.552, 10.125, 12.895 and 14.250 min, representing the peak of GA C13:0, C15:1, C17:2, C15:0 and C17:1. The total peak area is 4615.74059 mAU. The content of GA in the purified sample obtained is obviously high in purity, which was 80.79%. This suggested that the sample containing high purity of GA could be obtained by adsorption and desorption with D101 resin as the stationary phase under the optimized conditions.

**CONCLUSION**

Ginkgolic acid (GA) was extracted from Ginkgo biloba peel by ethanol and then purified by macroporous resin. Macroporous resin D101 had much higher static adsorption and desorption recovery for GA, which was a suitable resin for purifying GA. The purifying condition was optimized. The adsorption capacity increased as the concentration of the crude ethanol extraction solution increased, so the high concentration of the crude extraction solution sample was better selected. The adsorption capacity decreased as the flow velocity of the crude extraction solution sample increased and the flow velocity of 1.0 ml/min was chosen. As the elution solution was 100% ethanol, the volume of elution was 10 BV, and the flow velocity of the eluent was 1.0 ml/min, the elution efficiency was best. The content of GA in the purified sample obtained from Ginkgo biloba peel had a high purity of 80.79%. Therefore, the high purity of GA could be obtained from Ginkgo biloba peel by ethanol extraction, combining with adsorption and desorption with the macro-porous resin D101 as the stationary phase under the optimized conditions.

**CONFLICTS OF INTEREST:**

The authors have no potential conflicts of interest relevant to this article to report.

**REFERENCES**


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