Original Article

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Preparative separation and purification of geniposide from *Gardenia jasminoides* ellis fruit using Macroporous adsorption resin D101

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ABSTRACT

Geniposide, a main iridoid glycoside component found in *Gardenia jasminoides* Ellis fruit, possesses various precious biological activities including anti-inflammatory, anti-viral, anti-diabetic, and hepatoprotective effects. For thaose reasons, the techniques for extraction and isolation of geniposide from *Gardenia jasminoides* fruit have been widely developed in recent years. In this report, geniposide was successfully purified by combining D101 macroporous resin column chromatography and crystallization method. The optimal separation parameters were as follow: The *Gardenia jasminoides* fruits were extracted with 60% ethanol - water solution (v/v) by percolation method. The extraction solution after partially purified with a geniposide concentration of 6.231 mg/mL was loaded in the column wet - packed with 100 g resin and the bed volume (BV) = 200 mL at the flow rate of 2 BVs/h. The feeding volume was determined as 2.5 BVs. The adsorbate - laden column was washed with 3BVs water at the flow rate of 3 BVs/h to remove water - soluble impurities and further eluted using 6 BVs of 20% ethanol at the flow rate of 2 BVs/h to get geniposide. The desorption solution was concentrated under vacuum, precipitated and recrystallized in acetone to obtain geniposide with the high purity. The structure of product was confirmed by model method, including: UV, IR, MS, and ^{1}H, ^{13}C NMR spectra and compared with geniposide standard as well as data referred from previous studies. The separation process was straightforward, highly efficient, rapid, low cost making it a potential approach for the large - scale production of geniposide in real practice.

1. INTRODUCTION

Gardenia fruit (*Gardenia jasminoides* Ellis), which are also known as the Fructus *Gardenia*, is widely distributed in Southeast Asia. In the Vietnamese and Chinese traditional medicine, *Gardenia* dried ripe fruit was used as an antiphlogistic, choleretic, diuretic, hemostatic and anticancer agent, and externally as a resolutive in treatment of sprains and bruises[1]. Phytochemical studies have been indicated that the principal bioactive compounds group in fruits are iridoid glycoside, in which geniposide is a main component with various biological activities such as anti-inflammatory, anti-depressive, anti-angiogenic, and hepatic protection effects[2, 3].

In the past, the isolation techniques of geniposide from Gardenia fruit has been mainly performed by liquid-liquid extraction, absorption on activated charcoal[4], silica gel column chromatography[4], high-speed counter-current chromatography[5], and centrifugal partition chromatography[6]. However, these methods have several disadvantages such as time - consumption, labor intensiveness, harmful and environmental pollution as well as very difficult to scale-up. Recently, macroporous resins have been applied in the purification and separation

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of natural compounds due to its unique advantages such as high adsorption capacity, easy elution, low expense, less and "green" solvent consumption and easy regeneration[7, 8]. Hence, the aim of this study is established a simple, efficient method for isolating geniposide from *G. jasminoides* using D101 macroporous resin. The effects of different solvent systems on preparing crude extract, volume of loading sample on the dynamic adsorption, concentration and volume of eluent solvent on the desorption were determined.

2. METARIALS AND METHODS

2.1 Instrumentation

A CAMAG TLC-scanning system (Switzerland) equipped with an automatic TLC sampler 4 applicator, a winCATS V 1.4.4 software, a CAMAG twin trough glass chamber (20 x 10 cm) for developing the plate and attached with CAMAG TLC Scanner 3 was used for densitometric scanning of the TLC plate in this study. The chromatography of geniposide was performed on the aluminium TLC plate precoated with silica gel 60F254 (E. Merck). The mobile phase used for developing the plate was ethyl acetate : acetone : formic acid : water (5:5:1:1, v/v). The geniposide spot was detected under UV at 254 nm and visualized by spraying with 10% H_2SO_4 in 96% ethanol - water solution and then heating at 110°C until brown color spot of geniposide appear. Also the plate after derivatized was scanned under white light and geniposide concentration was calculated based on the calibration curve of standard prepared in the same conditions. Infrared (IR) spectra was recorded on a spectrophotometer (Shimadzu, Japan). Ultra-violet absorption analysis was performed using a spectrophotometer (U-1900, Hitachi, Japan) with distilled water as the reference. The melting point was measured using a melting point apparatus (FP62, Toledo, Switzerland). The nuclear magnetic resonance (NMR) spectrometer used in this study was a Bruker 500 Avance NMR system (Bruker, Germany). The ESI-MS spectra was identified on LC-MS/MS equipment (1290/6460, Agilent, USA).

2.2 Reagents and materials

Ethyl acetate, acetone, formic acid, ethanol are of analytical grade from Xilong Chemical Engineering Co. Ltd. (China). D101 macroporous resin (MAR D101) was purchased from Anhui Sanxing Resin Technology Co. Ltd. (Lot. 20150201, China). Geniposide standard (purity ≥ 98%) was gifted from National Institute of Drug Quality Control (Vietnam). *Gardenia jasminoides* Ellis fruits were collected at Hai Hau district, Nam Dinh province (Vietnam) in October 2015 and authenticated. A voucher specimen was deposited at the laboratory of Depatment of Pharmaceutical Industry, Hanoi University of Pharmacy. The samples were dried, ground to coarse powder (1-2 mm) and stored at room temperature.

2.3. Preparation of *Gardenia* crude extracts

The dried *Gardenia* fruit was ground up and extracted by percolation method. Briefly, the coarse powder of *Gardenia* was mixed thoroughly with a portion of the investigated extraction solvent (30, 60 or 96% ethanol - water solution) and allowed to stand for 3 h. After that, the moist mixture was transferred to a percolator and allowed the percolate to flow at room temperature slowly making sure that the herbs were always covered with the remaining extraction solvent. The extraction solution were collected in fractions.

2.4 Dynamic adsorption/desorption experiments

Several important parameters, including the feeding volume, the percentage of ethanol in solutions and the volume of the eluents for desorption process were systematically investigated and optimized at room temperature. Dynamic adsorption/desorption tests were performed on the glass columns (40 x 3.0 cm i.d) wet - packed with 100 g (wet MAR D101). The bed volume (BV) of resin was 200 mL. After the sample loading, the adsorbate - laden column was washed first by distilled water, and then eluted with ethanol - water solution at different ratios.

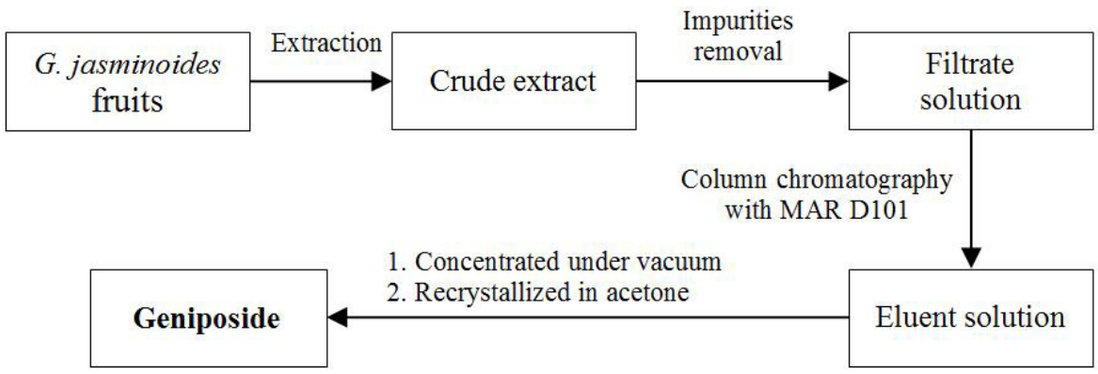

Figure 1. The extraction and separation of geniposide from *G. jasminoides* fruits

2.4.1 Effect of feeding volume

The crude extract solution was adsorbed onto the MAR D101 column chromatography at a flow rate of 2 BVs/h. The effluent was monitored and analyzed by TLC-scanning for geniposide detection.

2.4.2 Effect of the eluent concentration and volume on desorption

To choose the best ethanol concentration for the dynamic desorption of geniposide, after adsorption equilibrium, the adsorbate - laden column was first washed with deionized water, discarded the water fractions and then desorbed using various ethanol concentration ranging from 10 to 40% as eluent. The desorption process ended upon there was no geniposide spot detected by TLC analysis.

3. RESULT AND DISCUSSION

3.1. Selection of extraction solvent

20 g dried *Gardenia* powder were applied in this experiment in order to obtain four percolated fractions (20 mL one) of each examined ethanol concentration. The geniposide concentration in each fraction was calculated using TLC-scanning tool (See Figure 2 and Table 1).

The results from Table 1 showed that using 30% ethanol - water solution gave the relatively high total peaks area of geniposide (72111 ± 3204 AU). When the crude extract was prepared by 60% ethanol - water solution, the total peaks area of geniposide was the highest with its value of 78,033 ± 2,308 AU. There was no significant difference between the extraction yield obtained from the two solvent. However, regarding to the first fraction of the two solvent systems, the peak area of geniposide in 60% ethanol was higher than 30% ethanol (3,6845 ± 1,203 and 29,396 ± 1093 AU, respectively). These results suggested that geniposide was extracted faster when using 60% ethanol compared to 30% ethanol - water solution. Further increase the concentration of ethanol up to 96%, the geniposide extraction yield decreased. Thus, the 60% ethanol - water solution was selected as the solvent extraction. To continuously, the material powder (1.2 kg) was percolated with 60% ethanol - water solution to obtain ethanol extract solution (4.8 L). The extract was concentrated to paste - like in a rotary evaporator under reduced pressure at 50^{o}C. Then the concentrated extract was suspended in water and placed in a refrigerator at 5^{o}C in 24 h. A large amount of water-insoluble impurities precipitated and was effectively removed by membrane filtration. The concentration of geniposide in the clear filtrate was determined at 6.231 mg/mL and this concentrated solution was stored in fridge at 5^{o}C.

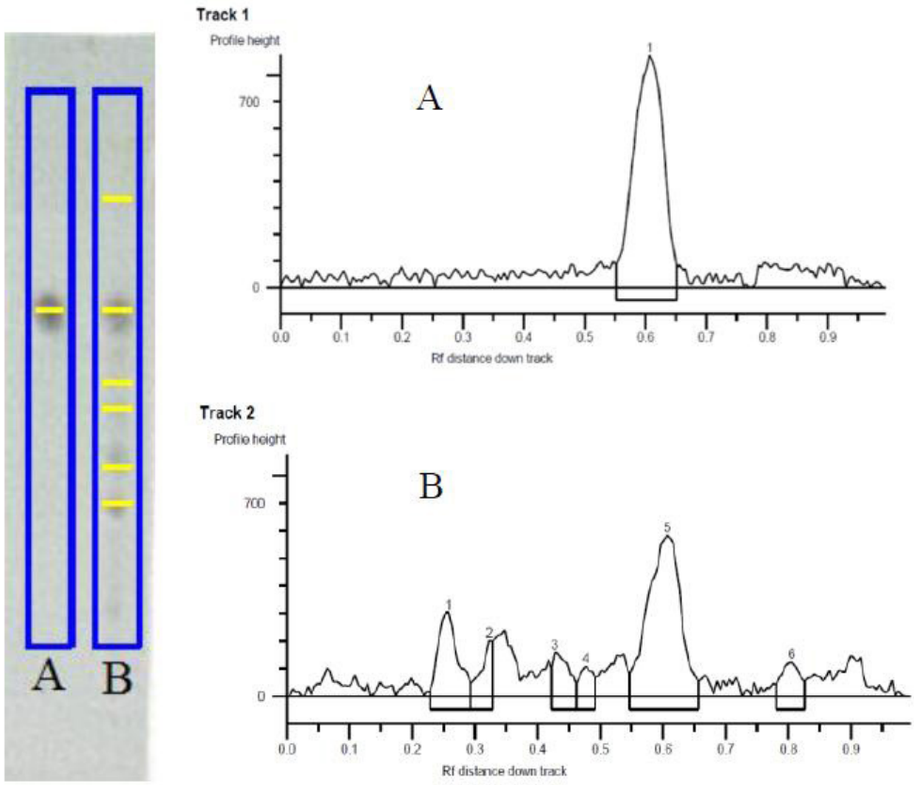

Figure 2. TLC-scanning analysis for the content of geniposide in fractions. TLC was performed by silica gel 60 F_{254} using mixture solvent ethyl acetate : acetone : formic acid:water (5:5:1:1, v/v). (**A**: geniposide standard, **B**: test sample)

Table 1. The peak area of geniposide in the fractions with the different solvent extract.

Solvents	Peak area (AU)				
	1st fraction	2nd fraction	3rd fraction	4th fraction	Total area
30% ethanol	29,396 ± 1,093	19,981 ± 667	13,985 ± 710	8,749 ± 1,243	72,111 ± 3,204
60% ethanol	36,845 ± 1,203	19,716 ± 610	12,823 ± 1,393	8,649 ± 658	78,033 ± 2,308
96% ethanol	34,616 ± 1,266	18,560 ± 1,001	9,288 ± 515	5,002 ± 371	67,467 ± 2,841

3.2. Dynamic adsorption and desorption

3.2.1 Effect of feeding volume

In the process of dynamic adsorption, the effects of feeding volume on adsorption capacity were investigated, and the results of leakage curve were obtained (Figure 3). The results showed that a geniposide did not appear in the effluent based on TLC analysis until the feeding volume reached 2.5 BVs. Thereafter, geniposide began leaking out of the column, the concentration of geniposide in effluent fractions increased and reached its saturation point at about 1100 mL. Hence, in order to minimize the loss of geniposide, the feeding volume was set at 500 mL for 100 g D101 resin with ratio of corresponding raw material and resin wet mass to be 1.25 g/g.

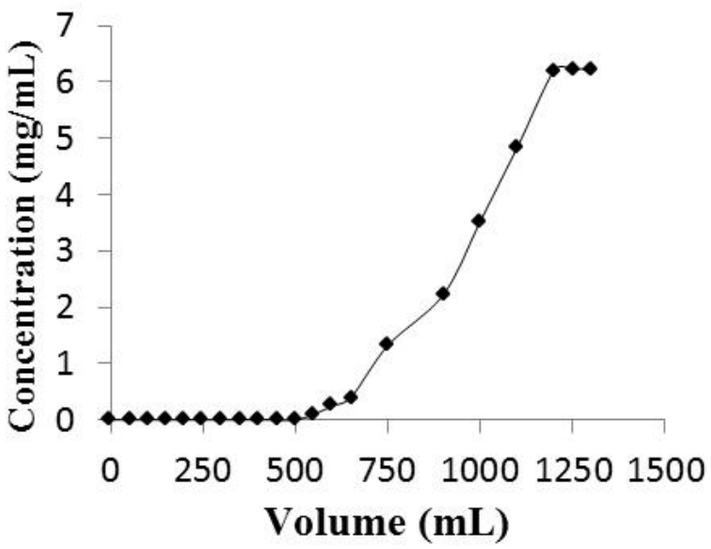

Figure 3. Breakthrough curve of geniposide on D101 macroporous resin chromatography column. The content of geniposide was determined by the TLC-scanning technique using mixture solvent system ethyl acetate : acetone : formic acid : water (5:5:1:1, v/v)

3.2.2 Effect of the eluent concentration and volume on desorption

After the sample loading, the adsorbate - laden column was first washed by distilled water at a flow rate of 3 BVs/h, and then desorbed with several concentrations of ethanol in water with a flow rate of 2 BVs/h to choice the best one. The desorption solutions were then evaporated to dryness, crystallized in acetone to obtain raw geniposide.

The results from Table 2 exhibited that the each of ethanol concentrations gave raw geniposide products with highly different characteristics. The 30 and 40% ethanol - water solution could well desorbed geniposide with the weight values of 2.14 and 1.95 g, respectively, and the eluent volume was both the smallest, at 3 BVs. However, the higher ethanol concentration also dissolved many impurities so that there were many contaminants in the desorption solution which might hindered the geniposide from crystallization. This might be the suitable reason resulting in geniposide with far different melting point compared with that reported in references (163 - 164^{o}C)[4]. By contrast, 10 and 20% ethanol concentration solution did not desorb color impurities, which facilitated the crystallization of geniposide in acetone. However, using 20% ethanol - water solution resulted in geniposide with higher yield and more closely melting point with references. Thus, 20% ethanol concentration was chosen as the desorption solvent.

Regarding the eluent consumption of the separation process as an important factor, the volume of eluent was investigated under the optimal separation conditions. The concentrations of geniposide in the eluent were plotted against eluent volumes, and the elution curves were obtained as shown in Figure 4. For 30 and 40% ethanol concentrations, the concentrations of geniposide in the eluent reached maximum points at the third fraction, then decreased rapidly. In case of 10 and 20% ethanol concentrations, the similar trends were observed at the first stages but geniposide concentration in latter fractions were gradually declined. For 20% ethanol concentration, the concentration of geniposide was less than 2% in the seventh eluent fraction (1.50%). Therefore, eluent volume was selected to be 6 BVs, considering shorter working time with less volume consumption.

Table 2. The effect of eluent solvents on the properties of raw geniposide

Eluent solvents	Ethanol concentration (%)	10	20	30	40
	Volume (BV)	8	6	3	3
Raw geniposide	Appearance	White powder	White powder	Yellow powder	Deep yellow powder
	Weight (g)	1.65	2.16	2.14	1.95
	Melting point (oC)	155.9	163.5	148.0	132.0

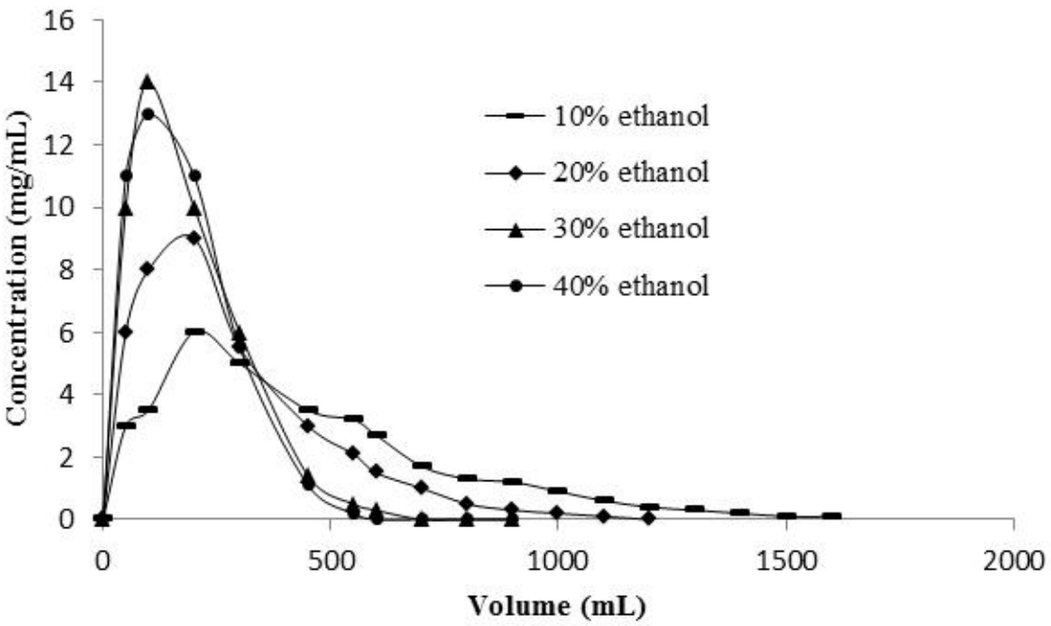

Figure 4. Dynamic desorption curve of geniposide with various ethanol concentrations. The effluent was analyzed by the TLC-scanning using mixture solvent system ethyl acetate : acetone : formic acid : water (5:5:1:1, v/v) for geniposide concentration

3.3. Crystallization and characterization

To obtain geniposide with high purity, the crystallization technique is necessary to be applied in combination with macroporous resin seperation. The raw geniposide obtained after D101 macroporous separation was further recrystallized in acetone to give 1.4 g pure geniposide.

The structural identification of geniposide was carried out by model method and the results as follows:

Geniposide: white crystal. TLC: R_f = 0.60 [mobile phase: acetate ethyl - acetone - formic acid - water (5:5:1:1)] (Figure 5) . Melting point: 162.5°C. UV spectra: λ_{max} = 238 nm. IR spectra (*KBr*): ν_{max} (cm^{-1}) 1064.7 (C-O), 1708.9 (C=O), 1635.6 (C=C aromatic), 3369.6 (OH). ESI-MS spectra: m/z 410.82 $[M + Na]^+$, 386.83 $[M-H]^-$. ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 5.19 (1H, d, J = 7.5 Hz, H-1) 7.53 (1H, s, H-3); 3.22 (1H, overlap, H-5); 2.13 (1H, m, H-6a); 2.84 (1H, dd, J = 8.0, 16.0 Hz, H-6b); 5.82 (1H, s, H-7); 2.74 (1H, t, J = 7.5 Hz, H-9); 4.20 (1H, d, J = 14.5 Hz, H-10a); 4.33 (1H, d, J = 14.5 Hz, H-10b); 3.73 (3H, s, H-12); 4.73 (1H, d, J = 7.5 Hz, H-1′); 3.23-3.41 (4H, m, H-2′, 3′, 4′, 5′); 3.65 (1H, dd, J = 4.0, 9.5 Hz, H-6a′); 3.88 (1H, brd, J = 12.0 Hz, H-6b′).

^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm: 96.8 (C-1); 151.9 (C-3); 111.1 (C-4); 35.2 (C-5); 38.3 (C-6); 143,4 (C-7); 126.9 (C-8); 45.6 (C-9); 60.0 (C-10); 168.1 (C-11); 50.3 (C-12); 98.9 (C-1´); 73.4 (C-2´); 76.4 (C-3´); 70.1 (C-4´); 77.0 (C-5´); 61.2 (C-6´).

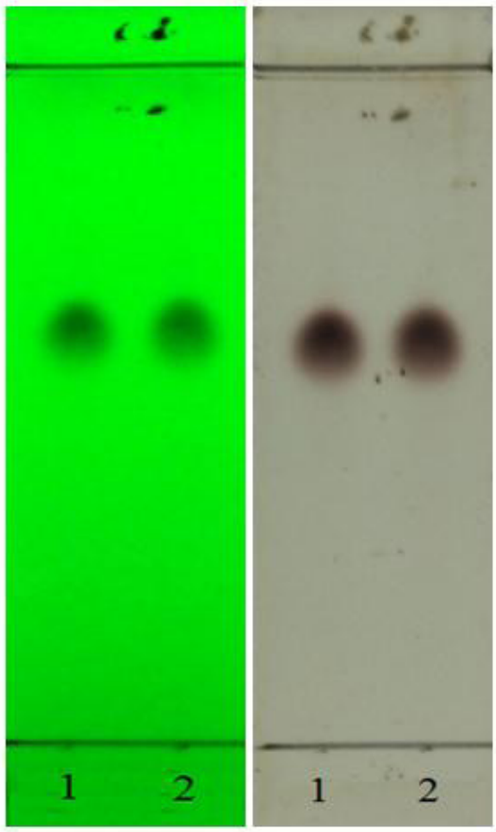

Figure 5. TLC images under UV254 light (left) and white light after derivatization (right) (1: purified geniposide, 2: geniposide standard)

In our study, column chromatography combined with crystallization technique were applied for preparative separation of geniposide from *Gardenia jasminoides* Ellis crude extract. The obtained geniposide exhibited high purity in comparision with geniposide standard (Figure 5) and references[4]. More importantly, this method which is more simple, easier and faster than some previous studies would be a significant contribution to pharmaceutical and phytochemical area, especially to the separation and purification of other natural product from plant materials.

4. CONCLUSION

From *Gardenia jasminoides* fruits, geniposide was isolated and purified using D101 macroporous resin chromatography in combination with crystallization in acetone. The product characteristics was confirmed by spectroscopic methods as well as comparation with that in previous studies. The separation process was highly efficient, rapid, low cost, making it a potential approach for the large-scale production of geniposide. This study would significantly contribute to pharmaceutical industry a simple method to obtain the active compounds from medicinal plant.

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