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Enrichment and purification of madecassoside and asiaticoside from *Centella asiatica* extracts with macroporous resins

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ABSTRACT

In present study, the performance and separation characteristics of five macroporous resins for the enrichment and purification of asiaticoside and madecassoside from *Centella asiatica* extracts have been evaluated. The adsorption and desorption properties of total triterpene saponins (80% purity) on macroporous resins including HPD100, HPD300, X-5, AB-8 and D101 have been compared. According to our results, HPD100 offered higher adsorption and desorption capacities and higher adsorption speed for asiaticoside and madecassoside than other resins. Column packed with HPD100 resin was used to perform dynamic adsorption and desorption tests to optimize the separation process of asiaticoside and madecassoside from *C. asiatica* extracts. After the treatment with gradient elution on HPD100 resin, the content of madecassoside in the product increased from 3.9 to 39.7%, and the recovery yield was 70.4%; for asiaticoside the content increased from 2.0 to 21.5%, and the recovery yield was 72.0%. The results showed that HPD100 resin revealed a good ability to separate madecassoside and asiaticoside, and the method can be referenced for the separation of other triterpene saponins from herbal raw materials.

1. Introduction

Centella asiatica (L.) urban is a traditional herbal medicine used in Asiatic countries for hundreds of years. It contains pentacyclic triterpenes, mainly asiaticoside, madecassoside, asiatic acid and madecossic acid [1,2], and they were claimed to possess various physiological effects. Their chemical structures are shown in Fig. 1. Reports have revealed that triterpene saponins from *C. asiatica* have been used for the treatment of psoriasis, wound healing, ulceration and eczema [3–8]. They also have the benefits of memory improvement, anti-inflammatory, anticancer, antioxidation, anxiolytic, etc. [9–14]. Thus, an efficient method for the separation of madecassoside and asiaticoside from *C. asiatica* is needed.

The conventional separation method of saponins is normally carried out from the extracts by means of solid–liquid extraction from natural resources, then liquid–liquid extraction is conducted by using different solvents, and then followed by a column chromatography with gradient solvent system [15]. Liquid–liquid separation method has been investigated to get mixture of asiaticoside and madecassoside from extracts of *C. asiatica* [16]. However, this separation method is an inefficient process, consuming longer time and more solvent, and results in lower recovery of the products. Alternatively, the adsorption–desorption process, in general, is an efficient separation method with a moderate purification effect and can be used economically for recovering and concentrating targeted phytochemicals in industrial practices. Alain et al. reported a method for preparing a *C. asiatica* extract rich in madecassoside and terminoloside using a strong anionic resin with functional groups of the quaternary ammonium type [17].

Recently, there has been a growing interest in employing macroporous resins to separate bioactive components from crude extracts of herbal raw materials because of their unique adsorption properties and advantages including ideal pore structure and various surface functional groups available, low operation expense, less solvent consumption and easy regeneration. For example, macroporous resins have been successfully used in the separation of luteolin from pigeonpea [*Cajanus cajan* (L.) Millsp.] leaf extracts, vitexin and isovitexin from pigenonpea extracts, licorice flavonoids and glycyrrhizic acid from licorice, and scutellarin from crude extracts of *Erigeron breviscapus* (vant.) Hand. Mazz, etc. [18–21]. However, there are no reports on using macroporous resin to separate madecassoside and asiaticoside from *C. asiatica* extracts up till now.

This study aims to develop an efficient method for the enrichment and purifying of madecassoside and asiaticoside with the

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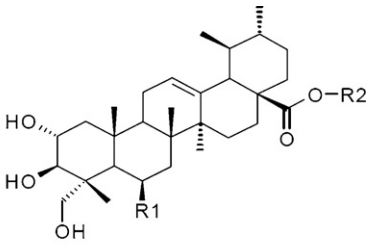

Asiatic acid	R1 = H	R2 = H
Madecassic acid	R1 = OH	R2 = H
Asiaticoside	R1 = H	R2 = Glu-Glu-Rha
Madecassoside	R1 = OH	R2 = Glu-Glu-Rha

Fig. 1. Chemical structures of the studied saponins and sapogenins in *Centella asiatica* extracts.

optimal resin. The information in this study is significant in the selection of adsorption resins for enrichment and purification of triterpene saponins extracts from *C. asiatica* or other herbal materials in general.

2. Experimental

2.1. Chemicals and reagents

Asiaticoside and madecassoside standards (95% purity) were purchased from Guangxi Changzhou Natural Products Development Co., Ltd. (Guangxi, China). Distilled water was purchased from Hangzhou Wahaha Group Co., Ltd. Methanol and acetonitrile were HPLC grade, and acetic acid was analytical grade. For standard sample solution, the appropriate amounts of standards were dissolved in distilled water to yield the stock solutions at the concentration of 1.1 and 0.2 mg/mL for madecassoside and asiaticoside, respectively. Total triterpene saponins (80%, containing 52% madecassoside and 28% asiaticoside, respectively) used for static adsorption and static desorption tests were purchased from Guangxi Changzhou Natural Products Development Co., Ltd. The whole plants of *C. asiatica* were purchased from China Beijing Tongrentang Group Co., Ltd. (Hangzhou, China) and identified by Prof. Kongrong Chen from College of Medical in Zhejiang Chinese Medical University. Crude extracts prepared for dynamic adsorption, desorption and gradient elution experiments were described in Section 2.4.

2.2. Adsorbents

Macroporous resins including HPD100 and HPD300 were purchased from CangZhou Bonchem Co., Ltd. (Hebei, China), and X-5, D101 and AB-8 from The Chemical Plant of NanKai University (Tianjin, China). Their physical properties are summarized in Table 1. The resins were soaked in 95% ethanol, shaken for 24 h and then washed by distilled water thoroughly before using.

2.3. Determination of moisture content of resins

Three samples of each kind of macroporous resins were weighed, then placed in a drying oven, and dried at 80 °C until the mass did not change. The moisture contents of HPD100, HPD300, X-5, D101 and AB-8 resin are 68.2, 71.5, 65.9, 73.0 and 69.2%, respectively.

2.4. Preparation of C. asiatica extracts

The whole plants (200 g) of *C. asiatica* were extracted by 2000 mL of ethanol–water (70:30, v/v) solution. The extracts were purified by membrane filtration and then evaporated to dryness, and the contents of madecassoside and asiaticoside in extracts were 3.9 and 2.0%, respectively. Distilled water was added to get madecassoside and asiaticoside solutions at the concentration of 1.85 and 0.98 mg/mL.

2.5. Static adsorption and desorption tests

The static adsorption and desorption tests of madecassoside and asiatcoside on macroporous resins were performed as follows: 1 g samples of hydrated test resins together with 50 mL of 0.8 mg/mL total triterpene saponins (80% purity) solutions were added into a flask, shaken (130 rpm) for 24 h at 25 °C. After adsorption, the resins were desorbed with 40 mL ethanol–water (70:30, v/v) solution, shaken (130 rpm) for 12 h at 25 °C. The process was repeated for three times.

The respective concentrations of madecassoside and asiaticoside in the sample solution after adsorption of a certain time were monitored at equal time intervals till equilibration to get adsorption kinetic curves.

The selectivity of resins was based on the capacities of adsorption and desorption, the ratio of desorption and the adsorption speed. The tests for equilibrium adsorption isotherms on the selected resin were conducted by contacting 50 mL sample solutions at different concentrations with pre-weighed 1 g resins, and shaking it for 8 h in a THZ-C constant temperature oscillator (Taicang Laboratorial Equipment Factory, Jiangsu, China) at the temperature of 25, 35 and 45 °C, respectively.

2.6. Dynamic adsorption and desorption

Dynamic adsorption and desorption experiments for asiaticoside and madecassoside were carried out on glass columns (12 mm × 50 mm) wet-packed with 5 g (wet resin) of the selected hydrated resin. The bed volume (BV) of resin was 8 mL. After reaching adsorptive saturation, the column was first washed by distilled water with 4 BV, and then eluted by ethanol–water solution.

The gradient elution tests were taken as follows: after adsorptive saturation, the column was first washed by distilled water with 4 BV, and then eluted by ethanol–water (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, v/v) solutions and the volume of each solution was 1 BV. Each part of desorption solutions was analyzed by HPLC and then concentrated to dryness under vacuum.

Table 1
Physical properties of the test macroporous resins

Trade name	Polarity	Particle diameter (mm)	Surface area (m^2/g)	Average pore diameter (Å)
HPD100	Non-polar	0.3–1.2	650–700	85–90
HPD300	Non-polar	0.3–1.2	800–870	50–55
X-5	Non-polar	0.315–1.25	500–600	290–300
AB-8	Weak-polar	0.3–1.2	480–520	130–140
D101	Weak-polar	0.25–0.84	500–550	90–100