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Application of high-speed counter-current chromatography combined with macroporous resin for rapid enrichment and separation of three anthraquinone glycosides and one stilbene glycoside from *Rheum tanguticum*

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ABSTRACT

In this paper, an efficient method was successfully established by the combination of macroporous resin (MR) and high-speed counter-current chromatography (HSCCC) for rapid enrichment and separation of aloe-emodin 8-*O*-β-D-glucoside, emodin 1-*O*-β-D-glucoside, emodin 8-*O*-β-D-glucoside and piceatannol 4′-*O*-β-D-(6″-O-gallate)-glucoside. Six kinds of macroporous resins were investigated in the first step and X-5 macroporous resin was selected for the enrichment of the target compounds. The recoveries of the target compounds reached 89.0, 85.9, 82.3 and 84.9% respectively after 40% ethanol elution. In the second step, the target compounds were separated by HSCCC with a two-phase solvent system composed of chloroform/ethyl acetate/methanol/water (8:1:6:5, v/v). The established method will be helpful for further characterization and utilization of *Rheum tanguticum*. The results demonstrate that MR coupled with HSCCC is a powerful technique for separation of bioactive compounds from natural products.

1. Introduction

Rheum tanguticum is one of the three genuine rhubarb species reported in the Chinese Pharmacopoeia [1], whose roots and rhizomes have been traditionally used as a folk medicine because of their strong antibacterial, antipyretic and antispasmodic activities [2]. Previous phytochemical studies have demonstrated that *R. tanguticum* contains various constituents, including anthraquinones, dianthrones, stilbenes, anthocyanins, flavonoids, anthraglycosides, polyphenols, essential oils, organic acids, chromenes, chromanone, chromone glycosides and vitamins [3–6]. Pharmacological studies have revealed that the anthraquinone glycosides and stilbene glycosides are the bioactive components because of their pharmacological properties. For example, anthraquinone glycosides have been reported to exhibit antifungal, anti-microbial, cytotoxic and antioxidant activities [7–9], whereas stilbene glycosides have shown anti-HIV, antioxidant, antiplasmodial, antimalarial and antiallergy activities [10–14]. Among the anthraquinone glycosides and stilbene glycosides, aloe-emodin 8-*O*-β-D-glucoside (C1), emodin 1-*O*-β-D-glucoside (C3), emodin 8-*O*-β-D-glucoside (C4) and piceatannol 4′-*O*-β-D-(6″-*O*-gallate)-glucoside (C2) (Fig. 1) are some of the important constituents.

In view of their wide pharmacological activities, large quantities of pure compounds are urgently needed as chemical reference standards and for further pharmacological studies. With regard to the methods available for the separation of organic compounds, silica and gel chromatography are two classical methods due to their different separation principles. Silica chromatography relies on the principles of adsorption and desorption, whereas gel chromatography relies on the principle of molecular sieve. In terms of the above compounds, the similar polarity presents a number of practical difficulties for the separation by silica chromatography. Furthermore, silica has several disadvantages, such as low yields, time-consuming, bulk amount of solvent wastage, poisonous residual solvents (chloroform, ethylacetate, etc.), and is not suitable for large-scale production. The similar polarity and structures of C3 and C4 meant that these two compounds could not be separated using gel chromatography. Based on these, we came to the conclusion that the above compounds could not be well separated by the conventional liquid–solid separation methods. Thus, an efficient

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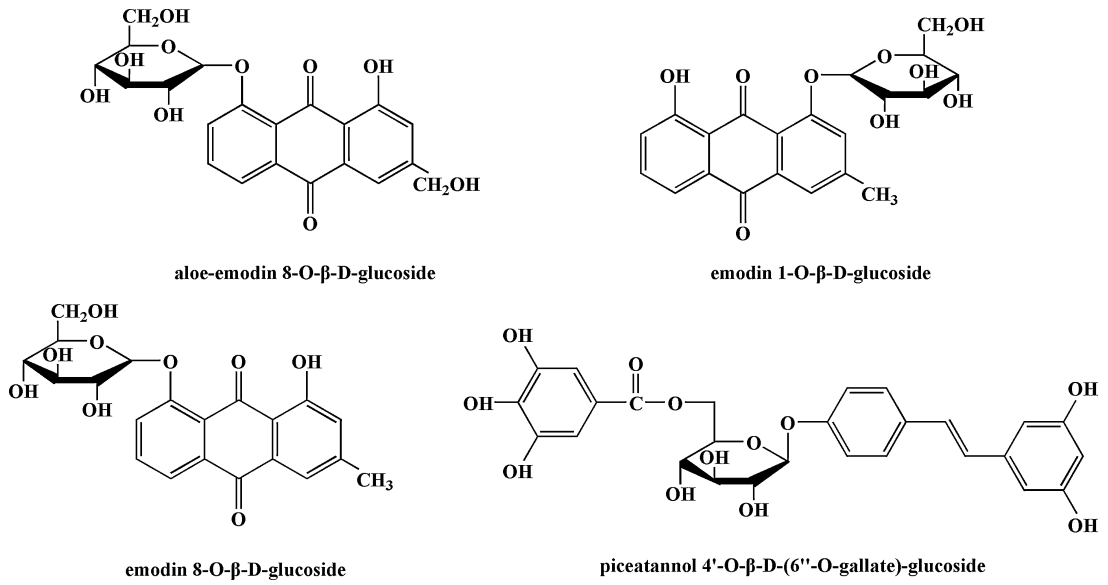

Fig. 1. Chemical structures of aloe-emodin 8-*O*-β-D-glucoside, emodin 1-*O*-β-D-glucoside, emodin 8-*O*-β-D-glucoside and piceatannol 4′-*O*-β-D-(6″-*O*-gallate)-glucoside.

method for separation of the above compounds from *R. tanguticum* becomes necessary.

Macroporous resin (MR) is an adsorption material for column chromatography, which can be reused for thousands of times and is friendly to the environment [15]. MR is durable polar, non-polar, or slightly hydrophilic polymers with high adsorption capacity. It can selectively adsorb targeted constituents through electrostatic force, hydrogen bonding interaction, complexation, and size sieving action. However, low purities of the products were often obtained only by using this single method for pure compound separation. Other separation techniques are needed in combination with MAR to obtain final compounds in a pure form. High-speed counter-current chromatography (HSCCC), a solid support-free liquid–liquid partition chromatography, eliminates the irreversible adsorption of sample onto the solid support matrix that is usually encountered in conventional column chromatography [16]. Furthermore, it can be readily scaled up and provide facile access to high quantity of target compounds. It has been widely applied for separation of bioactive compounds from natural products [17–21]. Thus, HSCCC is common used combination with MR to separate bioactive compounds from natural products [22,23].

In the current paper, an X-5 macroporous resin was used for the enrichment of C1, C2, C3 and C4 from *R. tanguticum*, and an efficient method was successfully established by HSCCC for the separation of these compounds.

2. Experiment

2.1. Apparatus

The HSCCC experiment was conducted on a TBE-300B high-speed counter-current chromatography system (Tauto Biotech, Co. Ltd., Shanghai, China), equipped with three polytetrafluoroethylene (PTFE) preparative coils (i.d. of the tubing = 1.6 mm, total volume = 280 mL) and a 20 mL sample loop. The revolution radius of the apparatus was 5 cm, and the β values of the multilayer coil ranged from 0.5 (at the internal terminal) to 0.8 (at the external terminal). For the apparatus, $\beta = r/R$, where r is the distance from the coil to the holder shaft, and R is the revolution radius or the distance between the holder axis and central axis of the centrifuge. Apparatus revolution speed was regulated with a speed controller within the range of 0–1000 rpm. The system was equipped with a model TBP5002 constant-flow pump (Tauto Biotech, Co. Ltd., Shanghai, China), a model UV-500 detector (XUYUKJ Instruments, Hangzhou, China) operating at 254 nm, and a model N2000 workstation (Zhejiang University, Hangzhou, China). A DC-0506 constant temperature-circulating implement (Shanghai Shunyu Hengping Instruments, Shanghai, China) was used to adjust the experiment temperature.

High-performance liquid chromatography (HPLC) analysis was performed on an Agilent 1200 system (Agilent Technologies Co. Ltd., USA). The Agilent 1200 system was equipped with a G1354A solvent delivery unit, a G1315B UV–vis photodiode array detector, a G1316A column thermostat, a G1313A autosampler, an Agilent Eslipse-XDB C18 (250 mm × 4.6 mm, 5 μm) analytical column, and an Agilent HPLC workstation.

The nuclear magnetic resonance (NMR) spectrometer used in this study was a Mercury-400B NMR spectrometer (Varian Co. Ltd., USA).

2.2. Reagents and materials

All of the solvents used in the HSCCC separation were of analytical grade and purchased from the Jinan Reagent Factory (Jinan, China). The methanol used for HPLC analysis was of chromatographic grade, and was purchased from Yuwang Chemical Ltd. (Shandong, China). Six kinds of MRs, including D101, S-8, HPD-600, X-5, AB-8 and D3520, were purchased from Cangzhou Bon Adsorber Technology Co., Ltd (Cangzhou, China). Dimethyl sulfoxide (DMSO-d_6) was used as the solvent for NMR analysis. Deionized water was used throughout the experiment.

R. tanguticum was collected in Huangzhong County, Qinghai Province, China, in October 2010. The species was identified by Professor Jing Sun (Northwest Institute of Plateau Biology, Chinese Academy of Sciences). Voucher specimens were deposited in the archives of the Northwest Institute of Plateau Biology (HNWP), Chinese Academy of Sciences (QZPMB-0277974).

2.3. Preparation of sample solutions

The dried root (0.5 kg) of *R. tanguticum* was powdered and extracted with 70% ethanol (1 L × 3) under reflux for 2 h. All filtrates were combined and concentrated by rotary evaporation at 60 °C under reduced pressure, producing 0.12 kg of crude extract.