Chemical Engineering Journal 237 (2014) 138–145

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

A dual-task method for the simultaneous detoxification and enrichment of stilbene glycoside from *Polygonum multiflorum* roots extract by macroporous resin

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HIGHLIGHTS

- A dual-task method for simultaneous detoxification and enrichment was established.
- A modified gradient elution strategy was proposed.
- Accumulative desorption ratio–volume/time curves were plotted.
- The recovered target compound was nearly free of hepatotoxic impurities.

ARTICLE INFO

Article history:
Received 19 July 2013
Received in revised form 5 October 2013
Accepted 7 October 2013
Available online 17 October 2013

Keywords:
Polygonum multiflorum roots
2,3,5,4′-Tetrahydroxystilbene-2-O-β-D-glycoside
Anthraquinones
Detoxification
Macroporous resin

ABSTRACT

In this study, a dual-task method for the simultaneous detoxification and enrichment of the pharmacologically active compound, 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glycoside (TSG) from crude extract of *Polygonum multiflorum* roots was established by macroporous resin. The performances of TSG on six macroporous resins were compared with regard to their adsorption and desorption properties. Kinetics tests indicated that the adsorption process fitted the pseudo-second-order model much better than the pseudo-first-order model. The solute affinity of TSG towards the AB-8 resin at different temperatures was described in terms of Langmuir and Freundlich isotherms. The optimal adsorption conditions were: 2.24 mg/mL of TSG in feed solution, 4.5 bed volume (BV) of loading volume, loading time no less than 120 min. The elution conditions were as follows: 6.0 BV of water, followed by 2.5 BV of 40% ethanol with flow rate of 1.5 BV/h. After the AB-8 resin treatment, the concomitant hepatotoxic anthraquinones were almost completely removed. Meanwhile, the purity of TSG was 8.6-fold increased from 7.51% to 64.76% with a recovery of 68.34%. The process fulfilled the task of detoxification and enrichment of TSG, and proved to be a promising basis for pharmaceutical preparations.

1. Introduction

The root of *Polygonum multiflorum* Thunb. (family Polygonaceae), well known as He-Shou-Wu in Chinese, has been widely used as a tonic and an antiaging remedy for a long time in the history of traditional Chinese medicines. It is officially listed in Chinese Pharmacopoeia [1]. The therapeutic actions to some common diseases, such as dizziness, early graying of hair, hypertension, backache and constipation, make this herb one of the most popular herbal medicines in the pharmaceutical markets. However, associated hepatotoxicity is being increasingly documented in parallel with the rising popularity of those He-Shou-Wu-containing medicines in clinical use [2,3]. The efficacy as well as safety of this herbal remedy has attracted much attention in recent years [4].

Chemical profiling of *P. multiflorum* root has revealed that the herb contains relatively higher content (as much as 40 mg/g) of 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glycoside (TSG, Fig. 1) and lower content of anthraquinones including emodin, physcion, emodin-8-O-β-D-glycoside, and physcion-8-O-β-D-glycoside (abbreviated as ED, PS, EDG, and PSG, respectively, Fig. 1) [5]. Among these compounds, TSG has been reported to possess antioxidative [6], Alzheimer's disease preventive [7], cardiovascular system protective and hyperlipidemia reducing activities [8]. TSG is therefore considered to be responsible for the curative effects of *P. multiflorum* root. By contrast, anthraquinones have been ascribed to the hepatotoxic compounds in He-Shou-Wu-preparations [9,10]. Thus, if a dual-task method can be established to simultaneously remove the anthraquinones and enrich the TSG from

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http://dx.doi.org/10.1016/j.cej.2013.10.020

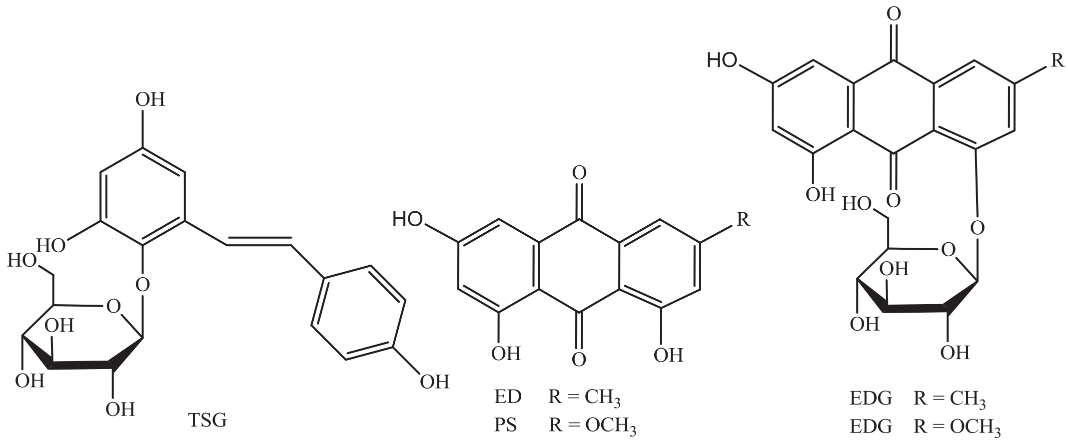

Fig. 1. Chemical structures of TSG, ED, EDG, PS and PSG.

P. multiflorum extract so as to produce detoxified He-Shou-Wu herbal preparations, the He-Shou-Wu-induced hepatotoxicity problem will be solved.

Macroporous resin is widely used in the industrial production due to its high efficiency and low-cost. Moreover, the advantages of macroporous resins, such as ideal pore structure, unique adsorption properties, less solvent consumption and easy regeneration, make them popular in the separation and purification of many pharmacologically important natural products [11–15]. Although some predecessors have concentrated on the purification technologies of stilbene glycoside using macroporous resins [16–18], these enrichment investigations generally focused on how to increase the recovery of the bioactive target, the concomitant hepatotoxic anthraquinones were neglected.

In order to supply safe and economic raw materials for large-scale production of He-Shou-Wu herbal preparations, an efficient separation process capable of simultaneous detoxification and enrichment of TSG is needed. For this purpose, the optimal macroporous resin was screened and the major experimental parameters were optimized. Adsorption kinetics was proposed by pseudo-first and second order models. Meanwhile, the experimental isotherm data were analyzed using the Langmuir and Freundlich equations.

2. Materials and methods

2.1. Chemicals, reagents and adsorbents

Chemical references including TSG, ED, PS, EDG, and PSG were purchased from the Chengdu Must Bio-technology Co. (Chengdu, China). The purity of each compound was determined to be more than 98.5% by the developed HPLC analysis using normalization method.

Acetonitrile (HPLC grade) was purchased from Tedia (Fairfield, OH, USA). Ethanol of analytical grade was purchased from Nanjing Chemical Corporation (Nanjing, China). Deionized water (18 MΩ) was prepared by passing distilled water through a Milli-Q system (Millipore, Milford, MA, USA).

Macroporous resins including D-101, S-8, AB-8, NKA-9, HPD-500 and HPD-600 were provided by Cangzhou Bon Co., Ltd. (Hebei, China). The physical properties of these resins are summarized in Table 1. Prior to use, the resins were pretreated by soaking in 95% ethanol for 24 h and then eluting under reflux until there was no residue after distillation to remove the monomers and porogenic agents trapped inside the pores during the synthesis process. The resins were finally washed with deionized water until the ethanol was thoroughly replaced [19]. The pretreated resins were then placed in the drying oven at 70 °C over 24 h until constant.

2.2. Preparation of sample solution of P. multiflorum roots extract

P. multiflorum roots were purchased from Bozhou, Anhui Province, China. Its botanical origin was authenticated by one of our authors (Prof. Hui-Jun Li). A voucher specimen has been deposited in the Herbarium of Pharmacognosy, China Pharmaceutical University, Nanjing, China.

The dried roots were pulverized into small pieces, then refluxed with 80% ethanol at the ratio of 1:10 (solvent and sample ratio, v/w) for 2 h. The extraction was repeated three times. The extracted solutions were combined, filtered, and then concentrated by using a rotary evaporator under reduced pressure to remove the ethanol solvent. The powdered extract was then dissolved with deionized water at appropriate concentrations for the adsorption and desorption tests. The initial pH value of the sample solution (TSG concentration 2.24 mg/mL) was determined as 5.8. The 0.1 M HCl or NaOH (Nanjing Chemical Corporation, Nanjing, China) was used to adjust the pH values if necessary.

2.3. HPLC analysis

The analysis of TSG, ED, PS, EDG and PSG was performed on an Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a binary pump, an online degasser, an autosampler, a thermostatically controlled column compartment,

Table 1
Physical and chemical properties of the tested resins.

Trade name	Polarity	Structure	Particle diameter (mm)	Surface area (m^2/g)	Average pore diameter (nm)
HPD500	Polar	Styrene	1.10–1.15	500–550	100–120
S-8	Polar	Cross-linked Polystyrene	1.02–1.14	100–120	280–300
D101	Non-polar	Styrene	0.65–0.75	500–550	90–100
AB-8	Weak-polar	Ethylstyrene	1.13–1.17	480–520	130–140
HPD600	Polar	Styrene	0.30–1.25	500–550	75–110
NKA-9	Polar	Cross-linked Polystyrene	1.00–1.17	250–290	155–165