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Separation, purification and quantification of verbascoside from *Penstemon barbatus* (Cav.) Roth

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ABSTRACT

Echinacoside and verbascoside possess a spectrum of significant beneficial bioactivities. A new method of preparative high-speed counter-current chromatography for rapid separation and purification of verbascoside from the leaves of *Penstemon barbatus* (Cav.) Roth was introduced. The solvent system employed here was *n*-butanol–water (1:1, v/v). The structure was confirmed by IR, ^{1}H NMR and ^{13}C NMR. According to the quantification results, echinacoside and verbascoside were found to be as much as 5.25 ± 0.13 mg/g and 11.21 ± 0.16 mg/g, respectively, in the leaves of *P. barbatus*. This indicates that *P. barbatus* might be an ideal alternative resource for large scale preparation of echinacoside and verbascoside.

1. Introduction

The quality of food is increasingly recognised widely for its nutrition and medical benefits. Oxidative stress is the culprit in a wide range of diseases. Natural and synthetic antioxidants are important to control oxidative stress. The dramatic toxic and even carcinogenic effect for synthetic compounds highlighted that natural source antioxidants are highly desirable (Gulcin, 2012).

Echinacoside and verbascoside (Fig. 1), as well-known antioxidants, are extensively studied for their beneficial activities including hepatoprotective, anti-inflammatory, antineoplastic, especially outstanding antioxidant activities (Deepak & Handa, 2000; Funes, Laporta, Calero, & Micol, 2010; He, Fang, & Tu, 2009; Liu, Li, Guo, & Lee, 2003). Verbascoside is a vital precursor of echinacoside and as for pure compounds, compared with other caffeic acid derivatives, such as caftaric acid, chlorogenic acid, caffeic acid, cynarin, cichoric acid, and echinacoside has the highest capacity to quench DPPH· (Pellati, Benvenuti, Magro, Melegari, & Soragni, 2004). Nevertheless, the latest research suggested that they were potential candidates for intervention in neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Kuang, Sun, Yuan, Lei, & Zheng, 2009). However, currently echinacoside and verbascoside are largely extracted from *Cistanche tubulosa* and selected as target compounds to evaluate the quality of the herb material and products of *C. tubulosa* in the 2010 Edition of the Chinese Pharmacopoeia (Pharmacopoeia of the People's Republic of China, 2010). *C. tubulosa* is a slow-growing plant endemic to arid lands and deserts and recalcitrant to domestication (Chen et al., 2007) which limited its applications and development. Increasing demands for the soaring medication value of echinacoside and verbascoside further endangered *C. tubulosa*. Phytochemical analysis of penstemons revealed that dominant presence of various types of iridoids and phenylethanoid glycosides (Dominguez, Marin, Esquivel, & Cespedes, 2007; Ismail, El-Azizi, Khalifa, & Stermitz, 1995; Zajdel, Graikou, Glowniak, & Chinou, 2012) and the most active extracts from the aerial parts of *Penstemon gentianoides* in scavenging DPPH· and inhibiting TBARS formation were the methanol extract and a further ethyl acetate of this methanol extract (Dominguez et al., 2005). Coincidently, this was in accordance with the result that we reported previously. *Penstemon barbatus* (Cav.) Roth, an American and Mexican aborigine, is abound in echinacoside which made it an ideal new alternative resource for the production for commercial demands and environmental protection (Xie, Deng, Tan, & Su, 2010). However, to the best of our knowledge, no work has been published on the separation and purification of verbascoside from *P. barbatus*.

In this paper, the content of echinacoside and verbascoside of *P. barbatus* was quantified by HPLC to justify its in-depth

Abbreviations: HPLC, high performance liquid chromatography; HSCCC, high-speed counter-current chromatography; Glc, glucose; Rha, rhamnose; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TBARS, thiobarbituric acid reactive species; AHUT, Anhui University of Technology; SRTP, student research training program.

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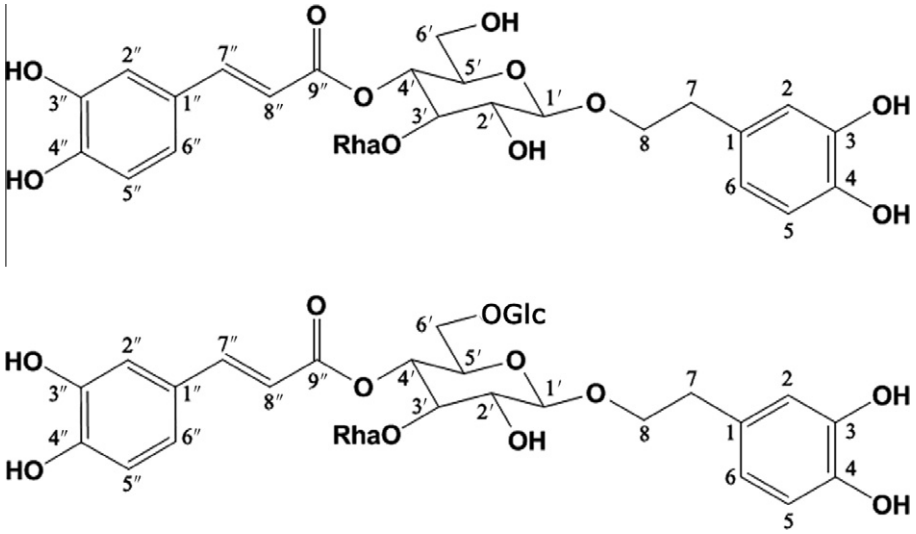

Fig. 1. Structure of (1) verbascoside and (2) echinacoside.

exploitation potential. Moreover, a method of preparative HSCCC with the solvent system n-butanol–water (1:1, v/v) was established for rapid separation and purification of verbascoside from *P. barbatus* for the first time. The structure was confirmed by IR, ^{1}H NMR and ^{13}C NMR.

2. Materials and methods

2.1. Reagents and plant materials

All solvents used for preparation of enriched extract and for HSCCC separation were of analytical grade (Kelong Chemical Reagent Factory, Chengdu, China). Methanol and acetonitrile used for HPLC were of chromatographic grade (Honeywell Burdick & Jackson Corporation, Muskegon, MI, USA). Reference standards of echinacoside and verbascoside were purchased from Tauto Biotech, Shanghai, China. The purity of echinacoside and verbascoside determined by HPLC was 99.4% and 99.6%.

P. barbatus has been grown in the Botanical Garden of School of Life Science, Southwest University, China, and was authenticated by Yucheng Liu, a professor in the same school, by morphological analysis. The voucher specimens have been deposited in the Herbarium, Department of Botany, School of Life Science, Southwest University, Chongqing, PR China.

2.2. Apparatus

The HSCCC instrument was TBE-300A high-speed countercurrent chromatography (Tauto Biotechnique, Shanghai, China) with three multilayer coil separation column connected in series (i.d. 1.5 mm, total capacity 260 ml), equipped with a 20 ml sample loop, a S-1007 pump (Beijing Shenyitong Technology Co. Ltd.), a 8823B UV detector (Beijing Bindayingchuang Technology Co. Ltd.), and amodel N2010 workstation (Zhejiang University, Hangzhou, China). HPLC was carried out on a Shimadzu LC-20A system (Shimadzu Co. Ltd., Japan) with a Shimadzu SPD-20A UV detector. Rotational vacuum concentrator RVC 2-33 was used for concentration (Martin Christ Corporation, Germany). The IR experiment was performed on Perkin–Elmer Spectrum One NTS (Perkin–Elmer CO. Ltd., USA) FT-IR Spectrometer. The ^{1}H NMR and ^{13}C NMR experiment was performed on a VARIAN INOVA-600 (Varian Corporation, USA) NMR spectrometer.

2.3. Preparation of crude extracts

The powder of the leaves of *P. barbatus* (10 g) was extracted with 100 ml of 50% aqueous methanol under sonication (40 kHz, 200 W) three times consecutively. The filtrate was combined and evaporated under vacuum to form syrup. Then, the syrup was dissolved with some water, subjected to AB-8 resin (polystyrene resin, 0.3–1.25 mm: NanKai Chemical Factory, Tianjin, China) and eluted in accordance with the sequence of distilled water, 10%, 20% and 50% aqueous methanol. Eluent of 50% aqueous methanol was concentrated to dryness in the rotational vacuum concentrator and the residue (S1) was stored in a refrigerator (4 °C) for further isolation by HSCCC.

2.4. Separation and purification of verbascoside by HSCCC

In the separation process, the entire coiled column has been impregnated with the stationary phase at a flow rate of 9 ml/min, then the apparatus was switched on. The mobile phase was pumped into the head-end of the column at a flow rate of 2 ml/min when the rotate speed reached 800 rpm with the forward mode. The temperature was set at 25 °C. After the mobile phase was eluted from the tail outlet and the hydrodynamic equilibrium of the two phases had been established, samples dissolved in 20 ml mobile phase was injected. The effluent from the outlet of the column was continuously monitored at 280 nm and collected into test tubes with a fraction collector set at 4 min for each tube. The purity of echinacoside and verbascoside within each concentrated fraction was determined by HPLC.

2.5. Preparation of standard solutions and sample solution

Stock standard solutions of echinacoside and verbascoside at a concentration of 10 mg/ml in 50% aqueous methanol were prepared. Finally, they were diluted into five standard concentrations of 10, 25, 50, 125, 250 μg/ml respectively, which were prepared for the construction of the calibration curves.

Leaves of *P. barbatus* were dried at 40 °C in a forced-air oven, ground, and then sifted through a 0.45 mm sieve. One gram of the material was accurately weighed, then extracted with 18 ml 50% aqueous methanol at room temperature under sonication for three times with the interval of 10 min. The supernatant solution was filtered under vacuum and transferred to a 25 ml volumetric