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Short communication

Preparative isolation and purification of gastrodin from the Chinese medicinal plant *Gastrodia elata* by high-speed counter-current chromatography

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Abstract

Gastrodia elata Blume is a famous Chinese medicinal plant, which has been widely used for the treatment of rheumatism, epilepsy, paralysis, hemiplegia, lumbago, headache and vertigo. High-speed counter-current chromatography was successfully used for the first time for the preparative isolation and purification of the bioactive component gastrodin from *G. elata* Blume. The crude gastrodin was obtained by extraction with ethanol from the dried roots of *G. elata* Blume under sonication. Preparative high-speed counter-current chromatography with a two-phase solvent system composed of *n*-butanol–ethyl acetate–water (2:3:5, v/v/v) was successfully performed yielding 48 mg gastrodin at 96% purity from 500 mg of the crude extract (10.3% gastrodin) with the recovery of approximately 90% in a one-step separation.

Keywords: *Gastrodia elata*; Counter-current chromatography; Preparative chromatography; Plant materials; Gastrodin

1. Introduction

Traditional Chinese herbal medicines have been used to treat human diseases in China for centuries. People are becoming increasingly interested in traditional Chinese medicines because of their low toxicity and good therapeutic performance. *Gastrodia elata* Blume (Tianma in Chinese) is a notable Chinese medicine. Its roots have been widely used for the treatment of rheumatism, epilepsy, paralysis, hemiplegia, lumbago, headache and vertigo [1,2]. Gastrodin is the bioactive component of *G. elata* Blume, which has sedative and anticonvulsant actions, neuroprotective effect, facilitating memory consolidation and retrieval, and antioxidant and free radical scavenging activities [3–7]. The chemical structure of gastrodin is shown in Fig. 1.

In order to study their pharmacological properties and to control their quality, separation and purification of active components in herbal medicines have become increasingly urgent. Crude extracts from plant sources usually contain a large number of compounds with a broad range of hydrophobicity. Most often only one component needs to be separated from the others. High-speed counter-current chromatography (HSCCC) is a unique liquid–liquid partition chromatography that uses a liquid stationary phase. HSCCC has been applied to the separation of a number of natural products [8–15]. However, no report has been published on the use of HSCCC for the isolation and purification of the highly bioactive component gastrodin from plants. The aim of this study, therefore, was to develop an efficient method for the isolation and purification of gastrodin from the Chinese medicinal plant *G. elata* Blume by HSCCC.

2. Experimental

2.1. Apparatus

Preparative HSCCC was carried out with a Model CCC-1000 high-speed counter-current chromatograph (Pharma-

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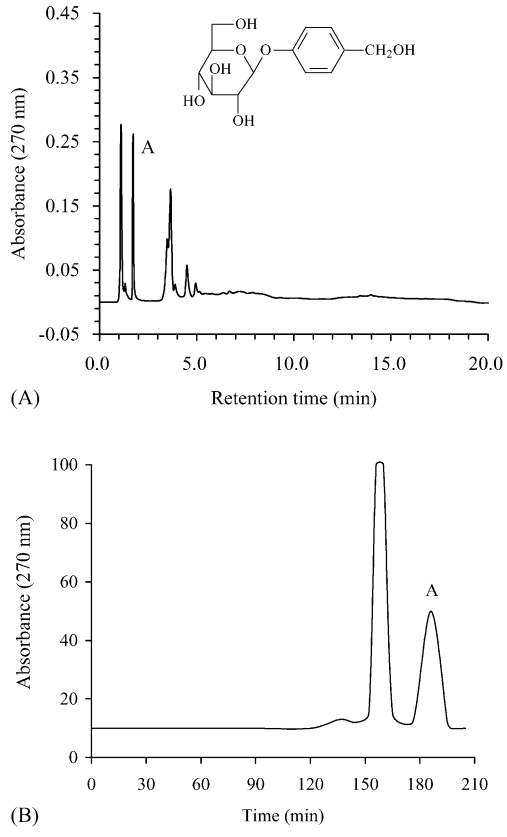

Fig. 1. (A) Chromatogram of crude gastrodin from *G. elata* Blume by HPLC analysis as well as the chemical structure of gastrodin; A = gastrodin. *Conditions*: column, reversed-phase Symmetry C_{18} column (150 mm × 3.9 mm i.d., 5 μm); mobile phase, methanol (solvent A)–water (solvent B) in the gradient mode as follows: 0–9 min, 5–44% A; 9–12 min, 44–65% A; 12–15 min, 65% A; 15–16 min, 65–5% A; flow rate, 1.0 ml min^{-1}; detection, 270 nm. (B) Chromatogram of crude gastrodin from *G. elata* Blume by HSCCC separation; A = gastrodin. *Conditions*: column, multilayer coil of 2.6 mm i.d. PTFE tube with a total capacity of 325 ml; rotary speed, 1000 rpm; solvent system, *n*-butanol–ethyl acetate–water (2:3:5, v/v/v); mobile phase, lower phase (water phase); flow rate, 1 ml min^{-1}; detection, 270 nm; sample size, 500 mg; retention of the stationary phase, 45%.

Tech Research, Baltimore, MD, USA). The apparatus consisted of three preparative coils, connected in series (inner diameter of tube, 2.6 mm; total volume, 325 ml). The revolution radius or the distance between the holder axis and central axis of the centrifuge (*R*) was 7.5 cm, and the β-value varied from 0.47 at the internal terminal to 0.73 at the external terminal ($\beta = r/R$, where *r* is the distance from the coil to the holder shaft). The HSCCC system was equipped with a Model Series II HPLC pump (Pharma-Tech Research), a Model SPD-10Avp UV–vis detector (Shimadzu, Japan), a Model L 120 E flat-bed recorder (Linseis, Germany), and a sample injection valve with a 10-ml sample loop.

2.2. Reagents

All solutions were prepared using analytical grade compounds. Reverse osmosis Milli-Q water (18 MΩ; Millipore, USA) was used for all solutions and dilutions. Ethyl acetate, *n*-hexane, *n*-butanol, ethanol and methanol were obtained from BDH (Poole, UK). The standard gastrodin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products, Ministry of Health, Beijing, China. The gastrodin stock solution was 1.00 mg ml^{-1} which was prepared by dissolving 10.0 mg of the gastrodin in 10.00 ml methanol and then stored in a refrigerator. The working solutions were prepared by suitable dilution of the stock solutions with methanol.

The dried roots of *G. elata* Blume was obtained from Beijing Tong-Ren-Tang drug retail outlet in Hong Kong.

2.3. Preparation of crude gastrodin from the G. elata Blume

Preparation of crude gastrodin was carried out according to the literature [16]. In brief, the dried roots of *G. elata* Blume were ground to powder. The powder (300 g) was extracted with 700 ml ethanol under sonication for 1 h. The mixture was filtered with 0.22 μm film of Type GV (Millipore, USA), and then the residue was repeatedly extracted twice (700 ml each time). The filtrate was combined, and the extract was evaporated to dryness by rotary vaporization at 45 °C. The residue (30.91 g) was stored in a refrigerator for the subsequent HSCCC separation.

2.4. Preparation of two-phase solvent system and sample solution

In the present study, we selected a two-phase solvent system composed of *n*-butanol–ethyl acetate–water at various volume ratios. Each solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated shortly before use.

The sample solution was prepared by dissolving the crude sample in the solvent mixture of lower phase and upper phase (1:1, v/v) of the solvent system because the sample was not easily dissolved in either phase alone.

2.5. HSCCC separation procedure

In each separation, the coiled column was first entirely filled with the upper phase (stationary phase), and then the apparatus was rotated at 1000 rpm, while the lower phase (mobile phase) was pumped into the column at a flow rate of 1.0 ml min^{-1}. After the mobile phase front emerged and hydrodynamic equilibrium was established in the column, about 10 ml of the sample solution containing 500 mg of the crude gastrodin was injected through the injection valve. The effluent of the column was continuously monitored with UV–vis detection at 270 nm. Peak fractions were collected according to the elution profile.

2.6. HPLC analysis

The crude sample and each peak fraction obtained by HSCCC were analyzed by HPLC according to the literature [17]. The HPLC system used throughout this study consisted of two Waters 510 pumps (Waters, Milford, MA, USA), a sample injector (Rheodyne, Cotati, CA, USA) with a 20-μl loop, and a Waters 996 photodiode array detector. Evaluation and quantification were made on a Millennium chromatography data system (Waters). The column used was a reversed-phase Symmetry C_{18} column (150 mm × 3.9 mm i.d., 5 μm; Waters). The mobile phase was methanol (solvent A)–water (solvent B) in the gradient mode as follows: 0–9 min, 5–44% A; 9–12 min, 44–65% A; 12–15 min, 65% A; 15–16 min, 65–5% A. The flow rate was 1.0 ml min^{-1}, and the effluent was monitored at 270 nm. Routine sample calculations were made by comparison of the peak area with that of the standard.

3. Results and discussion

Fig. 1A shows HPLC analysis of the crude gastrodin from *G. elata* Blume as well as the chemical structure of gastrodin. Peak A corresponds to gastrodin. The content of gastrodin was 10.3%. The crude extract contained several different compounds with a broad range of polarity, of which the major bioactive compound gastrodin was to be separated.

HSCCC is a very useful tool for the separation and purification of natural products. In order to select a suitable two-phase solvent system for the successful separation of a particular compound from a complex mixture, the previous publications on the HSCCC should be consulted, and partition coefficient (*K*) and retention of the stationary phase should be considered. The two-phase solvent systems, *n*-hexane–ethanol–water and *n*-hexane–ethyl acetate–ethanol–water, were previously used for successful separation of bioactive compounds from Chinese herbs and algae in our laboratory [18,19]. Preliminary HSCCC studies were therefore carried out with the two-phase solvent system composed of *n*-hexane–ethanol–water at a volume ratio of 5:2.5:2.5. It was very difficult to separate gastrodin from other components (data not shown). It was also difficult to separate gastrodin from other components with the two-phase solvent system composed of *n*-hexane–ethyl acetate–ethanol–water at a volume ratio of 2.5:2.5:2.5:2.5. In subsequent studies, another two-phase solvent system was thus tested.

Performance of the two-phase solvent system composed of *n*-butanol–ethyl acetate–water at various volume ratios (4:1:5, 3:2:5, 2.5:2.5:5, 2:3:5 and 1:4:5) was evaluated in terms of peak resolution. When the contents of ethyl acetate in the two-phase solvent system were increased from 4:1:5 to 2:3:5, the peak resolution and the retention of the stationary phase were both improved. When it increased to 1:4:5, although the retention of the stationary phase was good (51%), the peak resolution was poor. Fig. 1B shows the preparative HSCCC separation of 500 mg of the crude extract using the solvent system composed of *n*-butanol–ethyl acetate–water (2:3:5, v/v/v). HPLC analysis of each peak fraction of this preparative HSCCC revealed that gastrodin corresponding to peak A was over 96% pure, which was calculated by comparison of the peak area with that of the standard. The yield of gastrodin was 48 mg.

Table 1
The *K*-values (partition coefficient) of gastrodin in several solvent systems

Solvent system	*K*-value
n-Hexane–ethanol–water (5:2.5:2.5)	0.096
n-Hexane–ethyl acetate–ethanol–water (2.5:2.5:2.5:2.5)	0.132
n-Butanol–ethyl acetate–water (4:1:5)	0.651
n-Butanol–ethyl acetate–water (3:2:5)	0.561
n-Butanol–ethyl acetate–water (2.5:2.5:5)	0.534
n-Butanol–ethyl acetate–water (2:3:5)	0.502
n-Butanol–ethyl acetate–water (1:4:5)	0.405

Note: The solvent system 6 (from top to bottom) was selected. The peak resolution was poor with the other solvent systems.

The *K*-values of gastrodin in several solvent systems were measured according to Ito [20], and are given in Table 1.

In conclusion, HSCCC was successfully used for the first time for the efficient separation and purification of gastrodin from the medicinal plant *G. elata* Blume, and yielded 48 mg gastrodin at 96% purity from 500 mg of the crude extract with the recovery of approximately 90% in a one-step separation. The results of our studies clearly demonstrate that HSCCC is a very powerful tool in the preparative separation and purification of bioactive compounds from traditional Chinese medicinal plants including *G. elata* Blume.

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