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Short communication

Separation of gallic acid from *Cornus officinalis* Sieb. et Zucc by high-speed counter-current chromatography

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Abstract

Gallic acid was separated from a *n*-butanol extract of the fruit of *Cornus officinalis* Sieb. et Zucc by high-speed countercurrent chromatography in two steps using two solvent systems composed of ethyl-acetate–ethanol *n*-butanol–water (5:1.8:6, v/v/v) and ethyl acetate–ethanol–water (5:0.5:6, v/v/v) successively. From 1 g of *n*-butanol extract the method produced 60 mg of gallic acid at a purity of 97%.

Keywords: *Cornus officinalis*; Counter-current chromatography; Gallic acid

1. Introduction

Fruit of *Cornus officinalis* Sieb. et Zucc has been used as a traditional Chinese medicine in China and Japan [1]. It represents one of the eight-component drugs in Hachimi-gan that is one of the most popular traditional Chinese medicines in Japan [2]. A previous study on the constituents of the fruit by Okuda et al. [3] revealed the presence of various organic acids and hydrolyzable tannins. However, separation of these components by conventional chromatographic methods is difficult, because they are strongly adsorbed onto the solid support.

High-speed counter-current chromatography (HSCCC) [4,5], being a support-free partition chromatography, eliminates the above problem and can be an excellent alternative method for separating these compounds. In our present study for developing a new antiager, the *n*-butanol extract of fruit of *Cornus officinalis* Sieb. et Zucc was separated by HSCCC in two steps to purify gallic acid with a high antiaging activity.

2. Experimental

2.1. Apparatus

The present studies were performed with a multilayer coil planet centrifuge constructed at the Beijing Institute of New Technology Application, Beijing, China. The apparatus holds a pair of column holders symmetrically on the rotary frame at a distance of 8 cm from the central axis of the centrifuge. The multilayer coil separation column was prepared by winding a 110 m long, 1.6 mm I.D. PTFE (polytetrafluoroethylene) tube directly onto a holder hub making multiple coiled layers with a total capacity of

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200 ml. The β values varied from 0.5 at the internal terminal to 0.75 at the external terminal. Although revolution speed could be regulated with a speed controller in a range between 0 and 1000 rpm, an optimum speed of 800 rpm was used in the present studies. The solvent was pumped into the column using a Model MS-1007 constant flow pump (Beijing Institute of New Technology Application). The effluent was continuously monitored with a Model 8823A-UV monitor (Beijing Institute of New Technology Application) at 254 nm. A manual sample injection valve with a 20 ml loop (Tianjin High-New Science and Technology, Tianjin, China) was used for sample loading. A portable recorder (Yokogawa Model 3057, Sichuan Instrument Factory, Chongqing, China) was used to draw chromatogram. A model RE-90 rotary evaporator (Beijing Institute of New Technology Application) was also used.

2.2. Reagents

All organic solvents used for HSCCC were of analytical grade and purchased from Beijing Chemical Factory, Beijing, China. Gallic acid standard sample was purchased from national Institute for the Control of Pharmaceutical & Biological Products, Ministry of Health, Beijing, China.

2.3. Preparation of sample solution

About 1.0 kg amount of fruit of *Cornus officinalis* Sieb. et Zucc was extracted in a glass bottle (2500 ml capacity) with 1.3 l of 70% acetone for three times (total volume of 4 l) at room temperature. The extracts were combined, evaporated to dryness under reduced pressure, and redissolved in 500 ml of water. The aqueous solution was again extracted with chloroform (500 ml×3 times) and *n*-butanol (500 ml×10 times) successively. The *n*-butanol extract which showed a high antiaging activity was concentrated to yield 172.8 g, and 1 g of this extract was dissolved in 10 ml of each phase of the solvent system used for separation. The sample solution was sonicated for several min before loading into the column.

2.4. Preparation of two-phase solvent systems

The following two solvent systems were prepared: ethyl acetate–*n*-butanol–water (5:1.8:6, v/v/v) and ethyl acetate–ethanol–water (5:0.5:6, v/v/v). Each solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases separated shortly before use.

2.5. HSCCC procedure

For each separation, the column was first entirely filled with the organic stationary phase. Then the sample solution was injected through the sample port and the aqueous mobile phase was pumped through the column at a flow-rate of 2.0 ml min^{-1} while the column was rotated at 800 rpm. The effluent from the outlet of he column was monitored with a UV detector at 254 nm. Peak fractions were manually collected according to the chromatogram. After the desired peaks were eluted, the rotation and elution were stopped and the column contents were collected in a graduated cylinder by pressured N_2 at approximately 0.5 MPa. The retention of the stationary phase relative to the total column capacity was computed from the volume of the stationary phase collected from the column.

2.6. HPLC analysis

HPLC analysis was performed with a Shimadzu LC-10AVP system including an LC-10AT liquid chromatograph, an SPD-M10AVP photodiode array detector, an SCL-OAVP system controller, a CTO-10SVP column oven and a DGU-12A degasser (Shimadzu, Kyoto, Japan) using an Intersil ODS-3 column (150 mm×4.6 mm I.D.) at a column temperature of 40°C. The mobile phase was composed of acetonitrile–0.1 *M* H_3PO_4 (linear gradient from 0:100 to 40:60, v/v, in 30 min) was eluted at a flow-rate of 1.0 ml min^{-1} and effluent monitored by a photodiode array detector. Identification of HSCCC peak fractions was carried out by comparing the retention time and UV spectra with the standard sample.

3. Results and discussion

In the present study, 350 g of crude acetone extract from the dried fruit of *Cornus officinalis* Sieb. et Zucc was successively extracted with chloro-

form and *n*-butanol. Pharmacodynamic test on 100 mg each of acetone, chloroform, *n*-butanol, and water extract revealed a high antiaging activity in the *n*-butanol extract. Consequently, the *n*-butanol extract was separated by HSCCC using a solvent system composed of ethyl acetate–*n*-butanol–water (5:1.8:6, v/v/v), yielding six peak fractions (I–V, and the residual in the column) (Fig. 1) among which fraction V showed a high antiaging activity. The HPLC analysis of this fraction indicated that it contained gallic acid at about 70% purity. This partially purified fraction was further subjected to HSCCC using the second solvent system composed of ethyl acetate–ethanol–water (5:0.5:6, v/v/v) (Fig. 2). In the second separation the gallic acid fraction showed a high purity of over 97% by HPLC analysis.

The two-step HSCCC separation described above yielded 60 mg of gallic acid from 1 g of *n*-butanol extract. Gallic acid thus obtained was a white needle-shaped crystal, fast atom bombardment (FAB) MS: *m/z* 171 [M^{+1}]; UV: $\lambda^{EtOH}_{max\ nm}$ 217, 273; [^{1}H NMR ($^{2}H_2O$, TMS) δ ppm: 7.5 (2 H, s, H-2, H-6); ^{13}C NMR 0) δ ppm: 112.6 (C_2, C_6), 124.3 (C_3, C_5), 140.4 (C_4), 147.1 (C_1), 173.4 (COOH).

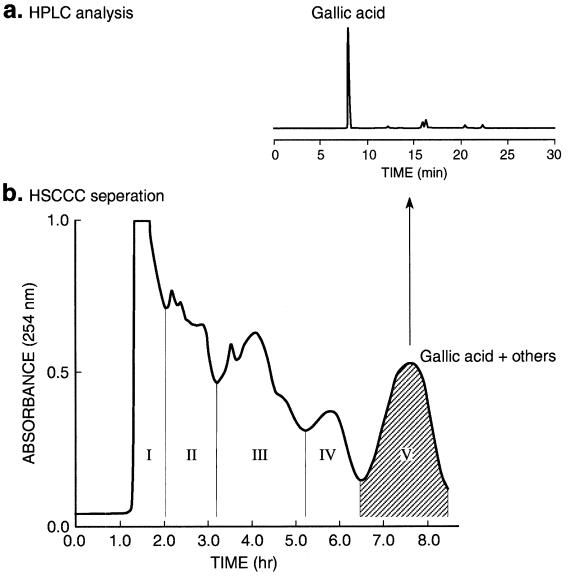

Fig. 1. Preparative HSCCC separation of *n*-butanol extract of *Cornus officinalis* Sieb. et Zucc. (b) Experimental conditions: rotation speed: 800 rpm; solvent system: ethyl acetate–*n*-butanol–water (5:1.8:6, v/v/v); mobile phase: lower aqueous phase; flow-rate: 2 ml min^{-1}; sample size: 1.0 g; retention of the stationary phase: 51%. (a) HPLC analysis: column: Intersil ODS-3 (150 mm×4.6 mm ID); column temperature: 40°C; mobile phase: acetonitrile–0.1 *M* H_3PO_4 (linear gradient, 0:100 to 40:60 in 30 min, v/v); flow-rate: 1.0 ml min^{-1}; detection: 254 nm.

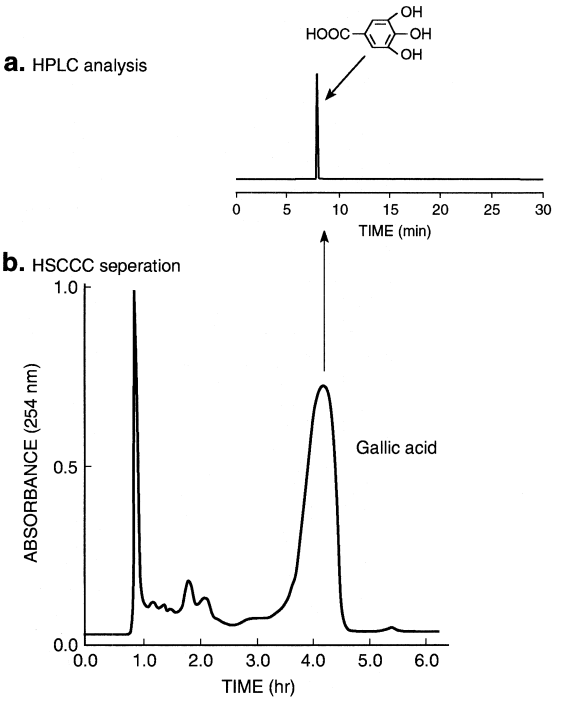

Fig. 2. Preparative HSCCC separation of partially purified extract of *Cornus officinalis* Sieb. et Zucc. (b) Experimental conditions: rotation speed: 800 rpm; solvent system: ethyl acetate–ethanol–water (5:0.6:5, v/v/v); mobile phase lower aqueous phase; flow-rate: 2 ml min^{-1}; detection: 254 nm; retention of stationary phase: 56%. (a) HPLC analysis: see caption to Fig. 1.

4. Conclusion

The results of our studies show that HSCCC is a useful tool to determine the bioactive fraction from a crude extract from *Cornus officinalis* Sieb. et Zucc. The quantity of the sample can be increased several times using a larger multilayer coil separation column suggesting that the method is also ideal for

preparation of the standards of gallic acid and other components.

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