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Short communication

Application of preparative high-speed counter-current chromatography for separation of methyl gallate from *Acer truncatum* Bunge

Xiaofeng Ma [a], Linhuan Wu [b], Yoichiro Ito [c], Weixi Tian [a,*]

[a] *Department of Biology, The Graduate School of Chinese Academy of Sciences, Beijing 100049, China*
[b] *Faculty of Applied Bioscience and Engineering, Katholieke Universiteit Leuven, Parkstraat 15, Leuven 3000, Belgium*
[c] *Center of Biochemistry and Biophysics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bldg. 50, Room 3334, 50 South Drive MSC 8014, Bethesda, MD 20892-8014, USA*

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Abstract

Preparative separation of methyl gallate in leaves extract of *Acer truncatum* Bunge was conducted using high-speed counter-current chromatography (HSCCC) with a solvent system composed of ethyl acetate–ethanol–water at volume ratios of 5:1:5 (v/v/v). In a single operation, 57.5 mg of methyl gallate was obtained from 120 mg of the extract. HPLC analyses of the counter-current chromatography (CCC) fraction revealed that the methyl gallate was having over 97% purity. Its structure was identified by ^{1}H NMR and ^{13}C NMR.

Keywords: *Acer truncatum* Bunge; Counter-current chromatography; Preparative chromatography; Methyl gallate

1. Introduction

Acer truncatum Bunge is a particular species of Acer in China [1]. It is a prominent species in the hardwood forests of north China. It is horticulturally important and widely planted for the brilliant autumn colours of its leaves. It has been used as a Chinese folk medicine for the treatment of coronary artery cirrhosis, cerebrovascular diseases and angina pectoris [2]. The extract of the leaves of *A. truncatum* Bunge shows high oxidation resistance [3]. Few phytochemical investigations about this plant have been described in literature up to now. It contains tannin [4], chlorogenic acid [5], flavonoids [6]. In this paper, we report a compound with the FAS (fatty acid synthase) inhibition activity from the EtOAc extract of the leaves of the title plant collected from Beijing, China.

High-speed counter-current chromatography (HSCCC), being a support-free liquid–liquid partition chromatographic technique, eliminates irreversible adsorption of the sample onto the solid support [7], and has been widely used in preparative separation of natural products, such as alkaloids [8–10], flavonoids [11–13], coumarins [14,15] and hydroxyanthraquiones [16,17].

Methyl gallate, whose chemical structure is given in Fig. 1, is a potent and highly specific inhibitor of herpes simplex virus [18,19]. It has shown antioxidant activity [20–22], free-radical scavenging activity [22], antimicrobial activity [23], and cancer chemopreventive effects [24]. The present paper describes the successful preparative separation of methyl gallate from the partially purified extract of *A. truncatum* Bunge by HSCCC.

2. Experimental

2.1. Apparatus

The analytical HSCCC instrument employed in the present study is a Model GS 20 analytical high-speed counter-current chromatograph designed and constructed in Beijing Institute of New Technology Application (Beijing, China). The apparatus holds a pair of column holders symmetrically

* Corresponding author. Tel.: +86 10 88256346; fax: +86 10 88256353.
E-mail address: tianweixi@gscas.ac.cn (W. Tian).

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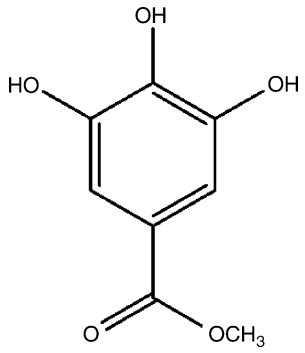

Fig. 1. The chemical structure of methyl gallate.

on the rotary frame at a distance of 5 cm from the central axis of the centrifuge. The multilayer coil separation column was prepared by winding a 50 m long, 0.85 mm I.D. PTFE (polytetrafluoroethylene) tube directly on to the holder hub forming multiple coiled layers with a total capacity of 30 ml. The β-value varied from 0.4 at the internal terminal to 0.7 at the external terminal ($\beta = r/R$, where r is the distance from the coil to the holder shaft, and R, the revolution radius or the distance between the holder axis and central axis of the centrifuge). Although the revolution speed of the apparatus could be regulated with a speed controller in the range between 0 and 2000 rpm, an optimum speed of 1800 rpm was used in the present study.

Preparative HSCCC was performed using a Model GS10A2 multilayer coil of 110 m × 1.6 mm i.d. with a total capacity of 220 ml. The β values of this preparative column range, from 0.5 to 0.8. The solvent was pumped into the column with a Model NS-1007 constant-flow pump (Beijing Institute of New Technology Application, Beijing, China). Continuous monitoring of the effluent was achieved with a Model 8823A-UV Monitor (Beijing Institute of New Technology Application, Beijing, China) at 280 nm. A manual sample injection valve with a 1.0-ml loop (for the analytical HSCCC) or a 20-ml loop (for the preparative HSCCC) (Tianjin High New Science Technology Company, Tianjin, China) was used to introduce the sample into the column, respectively. A portable recorder (Yokogawa Model 3057, Sichuan Instrument Factory, Chongqing, China) was used to draw the chromatogram.

The high-performance liquid chromatography (HPLC) equipment used was an Agilent 1100 system composed of a G1311A quaternary pump with a G1379A vacuum degasser, a G1315B diode array and multiple wavelength detector, a 1311A micro and preparative autosamplers, and 1100 ChemStation software.

2.2. Reagents

All organic solvents used for HSCCC were of analytical grade and purchased from Beijing Chemical Factory, Beijing, China. Methanol used for HPLC analysis was of chromatographic grade. The leaves of *A. truncatum* Bunge were defoliation or on the point of defoliate which were collected in October 2003 on the Baiwangshan Mountain in Beijing, China.

2.3. Extraction of crude samples

A 180 g amount of raw leaves of *A. truncatum* Bunge were extracted three times by 50% ethanol (1000 ml for each time) with ultrasonic treatment. Then, the extract was combined and evaporated to dryness under reduced pressure which yielded 40 g of crude extract. The residue obtained from the combined extract was dissolved with water. After filtration, the aqueous solution was extracted three times with water-saturated ethyl acetate which yielded ethyl acetate extract after being combined and evaporated to dryness under reduced pressure. The ethyl acetate extract was chromatographed on sephadex LH-20 (600 mm × 25 mm) column. The sample was eluted successively with 900 ml of each of methanol–water (80:20, v/v) and methanol. Thirty-six fractions of 50 ml were collected during the entire chromatography. Fractions 25–30 were subjected to HSCCC.

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

For the present study, we selected a two-phase solvent system composed of ethyl acetate–ethanol–water at volume ratios of 5:1:5 (v/v/v). The solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases separated shortly before use. The sample solutions were prepared by dissolving the crude extract in the upper phase at suitable concentrations according to the analytical or the preparative purpose (for, the injected sample amount see Section 2.5).

2.5. Separation procedure

For each separation, the analytical HSCCC was performed with a Model GS 20 HSCCC instrument, as follows: the multilayer coiled column was first entirely filled with the upper phase. The lower phase was then pumped into the head end of the inlet column at a flow-rate of 1.0 ml/min, while the apparatus was run at a revolution speed of 1800 rpm. After hydrodynamic equilibrium was reached, as indicated by a clear mobile phase eluting at the tail outlet, the sample solution (10 mg in 1 ml of upper organic phase) was injected through the sample port. The effluent from the tail end of the column was continuously monitored with a UV detector at 280 nm. Each peak fraction was collected according to the chromatogram. Preparative HSCCC was similarly performed with a Model GS 10A2 HSCCC instrument as follows: the multilayer coiled column was first entirely filled with the upper phase as stationary phase. Then the sample solution (120 mg in 20 ml of upper organic phase) 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 while the column was rotated at 800 rpm. The effluent from the outlet of the column was monitored with a UV detector at 280 nm. Peak fractions were manually collected according to the chromatogram.

2.6. HPLC analysis and identification of CCC peak fractions

The partially purified sample of *A. truncatum* Bunge and the peak fraction from HSCCC were analyzed by HPLC. The analyses were performed with a Diamonsil^{TM} C_{18} column (4.6 mm × 250 mm, 5 μm). The mobile phase, composed of methanol–acetic acid–water (30:0.7:69.3, v/v/v) was eluted at a flow-rate of 1.0 ml/min, and the effluent monitored by a dual λ absorbance detector.

Identification of HSCCC peak fraction was carried out by ^{1}H NMR and ^{13}C NMR spectra.

3. Results and discussion

The partially purified sample of *A. truncatum* Bunge was analyzed by HPLC. The result indicates that the partially purified sample contains several compounds including methyl gallate (peak 3) and some unknown compounds (peaks 1 and 2) (see Fig. 2A).

In order to achieve an efficient resolution of target compound, the two-phase solvent system of ethyl acetate–ethanol–water was examined using, analytical HSCCC. The result indicated that the volume ratio of 5:1:5 could separate methyl gallate well (Fig. 3).

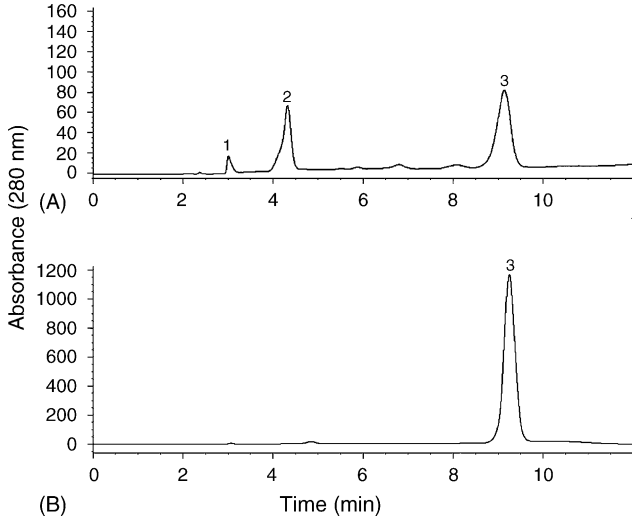

Fig. 2. HPLC analysis of crude sample and the components obtained from HSCCC separation. Experimental conditions: HPLC column: Diamonsil^{TM} C_{18} column (4.6 mm × 250 mm, 5 μm); sample inject: 10 μl; column temperature: 25 °C; mobile phase: methanol–acetic acid–water (30:0.7:69.3, v/v/v); flow-rate: 1.0 ml/min; detection wavelength: 280 nm; peaks 1 and 2: unknown compounds; peak 3: methyl gallate.

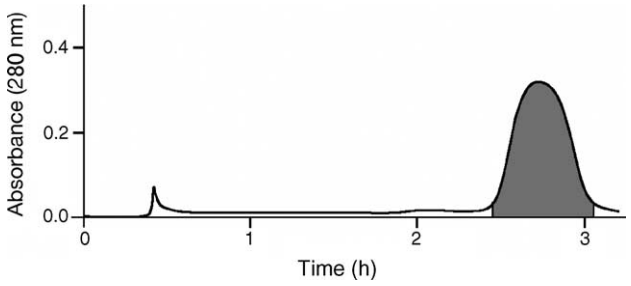

Fig. 3. Analytical HSCCC separation of crude extract from *A. truncatum* Bunge. Solvent system: ethyl acetate–ethanol–water (5:1:5, v/v/v); stationary phase: upper phase; mobile phase: lower phase; flow-rate: 1.0 ml/min; revolution: 1800 rpm; sample: 10 mg dissolved in 1 ml upper phase; retention of stationary phase: 56%; detection wavelength: 280 nm; shade portion: methyl gallate.

Fig. 4 shows the result obtained from the partially purified sample of *A. truncatum* Bunge by preparative HSCCC. After this separation, the fraction containing methyl gallate was collected (the shaded portion). By one step separation, 57.5 mg of methyl gallate was obtained from 120 mg of partially purified extract. The analysis of this fraction indicated that the peak fraction contained methyl gallate at over 97% purity measured from HPLC peak areas (Fig. 2B). The structural identification of the fraction was carried out by ^{1}H NMR and ^{13}C NMR spectra. The result of our studies clearly demonstrated that HSCCC is very successful in the preparative separation of methyl gallate.

The structural identification of methyl gallate was carried out by ^{1}H NMR and ^{13}C NMR spectra as follows: ^{1}H NMR (400 MHz, DMSO-d6) δ6.94(2H, s, H-2, 6), δ3.73(3H, s, –OCH_3). ^{13}C NMR (400 MHz, DMSO-d6): δ166.8(–COOH), δ146.0(C-3, 5), δ138.9(C-4), δ119.8(C-1), δ109.0(C-2, 6), δ52.0(–OCH_3). Comparing with the reported data, the ^{1}H NMR and ^{13}C NMR data are in agreement with those of methyl gallate [25].

The above results demonstrate for the first time that HSCCC can separate methyl gallate in *A. truncatum* Bunge well.

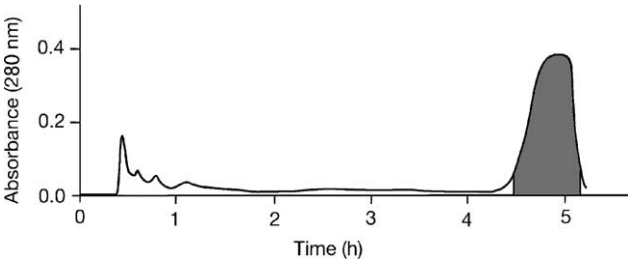

Fig. 4. Preparative HSCCC separation of crude extract from *A. truncatum* Bunge. Solvent system: ethyl acetate–ethanol–water (5:1:5, v/v/v); stationary phase: upper phase; mobile phase: lower phase; flow-rate: 2.0 ml/min; sample: 120 mg dissolved in 20 ml of upper phase; revolution speed: 800 rpm; retention of stationary phase: 60%; detection wavelength: 280 nm; shade portion: methyl gallate.

Acknowledgements

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