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Short communication

Isolation of quercetin-3-O-L-rhamnoside from *Acer truncatum* Bunge by high-speed counter-current chromatography

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Abstract

Preparative high-speed counter-current chromatography (HSCCC) was successfully used for isolation and purification of quercetin-3-O-L-rhamnoside from the ethyl acetate extract of the leaves of *Acer truncatum* Bunge using a two-phase-system composed of ethyl acetate–ethanol–water at a volume ratio of 5:1:5 (v/v/v). In a single operation, 41.9 mg of quercetin-3-O-L-rhamnoside was obtained from 366 mg of the crude extract. High-performance liquid chromatography (HPLC) analyses of the CCC fraction revealed that the purity of quercetin-3-O-L-rhamnoside was over 96%. Its structure was identified by MS, ^{1}H NMR and ^{13}C NMR. Quercetin-3-O-L-rhamnoside was obtained from this plant for the first time.

Keywords: *Acer truncatum* Bunge; High-speed counter-current chromatography; Preparative chromatography; Quercetin-3-O-L-rhamnoside

1. Introduction

Acer truncatum Bunge, known as Yuanbaofeng in China, is a prominent species in the hardwood forests of north–west China, and widely planted for the brilliant autumn colors of its leaves. It has been used as a Chinese folk medicine for the treatment of coronary arteriosclerosis, cerebrovascular diseases and angina pectoris [1]. No phytochemical investigation about this plant has been described in literature up to now. In this paper, we report a flavonoid with the FAS inhibition activity from the ethyl acetate extract of the leaves of the above plant collected from Beijing China.

The flavonoids were well known as one group of the beneficial components in *A. truncatum* Bunge [2], and have suitable chromophores for UV detection so that it can be chosen as "marker compounds" for the chemical evaluation or standardization of *A. truncatum* Bunge and its products [1,2]. Quercetin-3-O-L-rhamnoside, a well known flavonoid with antidiarrhoeic activity [3], sedative activity [4], anti-inflammatory effect [5–7], antifungal activity [8], was found to be a main compound in the crude extract of *A. truncatum* Bunge.

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 [9], is considered as a suitable alternative for the separation of phenolic compounds such as flavonoids and hydroxyanthraquinones [9–16]. The present paper describes the successful preparative separation of quercetin-3-O-L-rhamnoside (Fig. 1) from the crude 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

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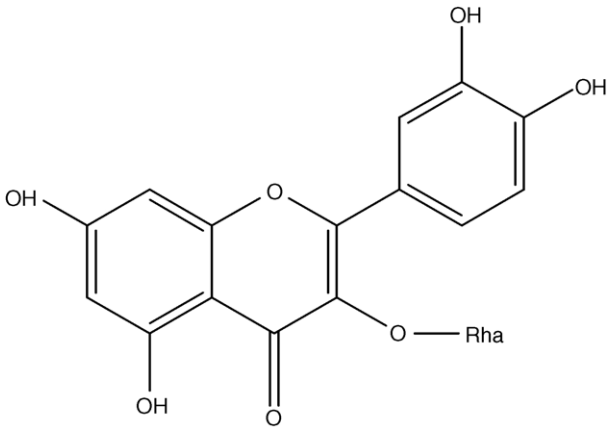

Fig. 1. The chemical structure of quercetin-3-O-L-rhamnoside.

Institute of New Technology Application, Beijing, China. The apparatus holds a pair of column holders symmetrically 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. polytetrafluoroethylene (PTFE) tube directly onto the holder hub forming multiple coiled layers with a total capacity of 40 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 1500 rpm was used in the present studies.

Preparative HSCCC was performed using a Model GS10A2 multilayer coil of 110 m × 1.6 mm i.d. with a total capacity of 300 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). Continuous monitoring of the effluent was achieved with a Model 8823A-UV Monitor (Beijing Institute of New Technology Application) at 254 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 Co., 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 quaternary pump with a degasser, a dual λ absorbance detector, an auto injector, and an Agilent 1100 ChemStation software.

2.2. Reagents

All organic solvents used for HSCCC were of analytical grade and purchased from Beijing Chemical Factory, Beijing, China. Acetonitrile used for HPLC analysis was of chromatographic grade.

2.3. Extraction of crude samples

An amount of 180 g raw leaves of *A. truncatum* Bunge were extracted three times by 50% ethanol (1000 ml for each time) with ultrasonic treatment and yield 40 g of crude extracts. Then, the extract was combined and evaporated to dryness under reduced pressure. 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 subjected to sephadex LH-20 column chromatography to yield crude samples. Portions of the above crude samples of *A. truncatum* Bunge were subjected to HSCCC.

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

For the present study, we selected two-phase solvent systems composed of ethyl acetate–ethanol–water at a (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.

2.5. Separation procedure

The analytical HSCCC separation was performed with a Model GS 20 HSCCC instrument as follows: the multilayer coiled column was first entirely filled with the stationary upper phase. The lower phase was then pumped into the head end of the column at a flow-rate of 1.0 ml/min, while the apparatus was run at a revolution speed of 1500 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 254 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 stationary upper phase followed by injection of the sample solution (366 mg in 20 ml of upper organic phase) 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 254 nm. Peak fractions were manually collected according to the chromatogram.

2.6. HPLC analysis and identification of CCC peak fractions

The crude sample of *A. truncatum* Bunge and each peak fraction from HSCCC were analyzed by HPLC. The analyses were performed with a Diamonsil 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 fractions were carried out by MS, ^{1}H NMR and ^{13}C NMR spectra.

3. Results and discussion

The crude sample of *A. truncatum* Bunge was analyzed by HPLC. The result indicates that the crude sample contains several compounds including quercetin-3-O-L-rhamnoside and some unknown compounds (see Fig. 3A).

In order to achieve an efficient resolution of target compound, the two-phase solvent system composed of ethyl acetate-ethanol-water at various ratios was examined using analytical HSCCC. The result indicated that the volume ratio of 5:1:5 could successfully separate quercetin-3-O-L-rhamnoside from impurities.

Fig. 2 shows the result obtained from the crude extract of *A. truncatum* Bunge by preparative HSCCC. The separation produced 4 peaks as labeled in the chromatogram. The analysis of these fractions indicated that the peak 1 and peak 2 fractions were impure. And the peak 4 fraction, which was collected according to the shade part in Fig. 2, contained quercetin-3-O-L-rhamnoside at over 96% purity as measured from HPLC peak areas (Fig. 3B).

The structural identification of quercetin-3-O-L-rhamnoside was carried out by MS, ^{1}H NMR and ^{13}C NMR spectra as follows: FAB-MS *m/z*: 449 (M^+ + 1), 302

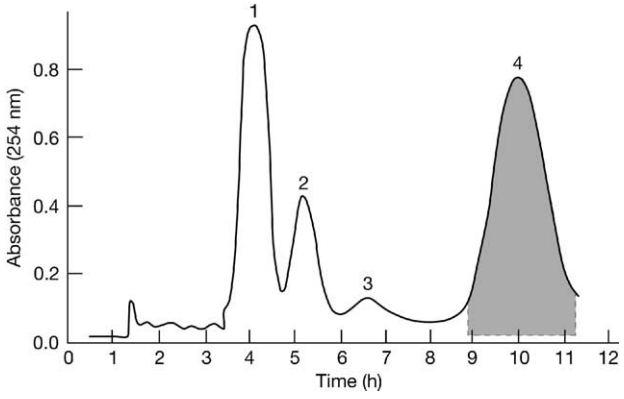

Fig. 2. Preparative HSCCC separation of a crude extract from *A. truncatum* Bunge. Solvent system: ethyl acetate–ethanol–water (5:1:5, v/v/v); stationary phase: upper organic phase; mobile phase: lower aqueous phase; sample: 366 mg dissolved in 20 ml upper phase; flow-rate: 2.0 ml/min; revolution: 800 rpm; retention of stationary phase: 56%.

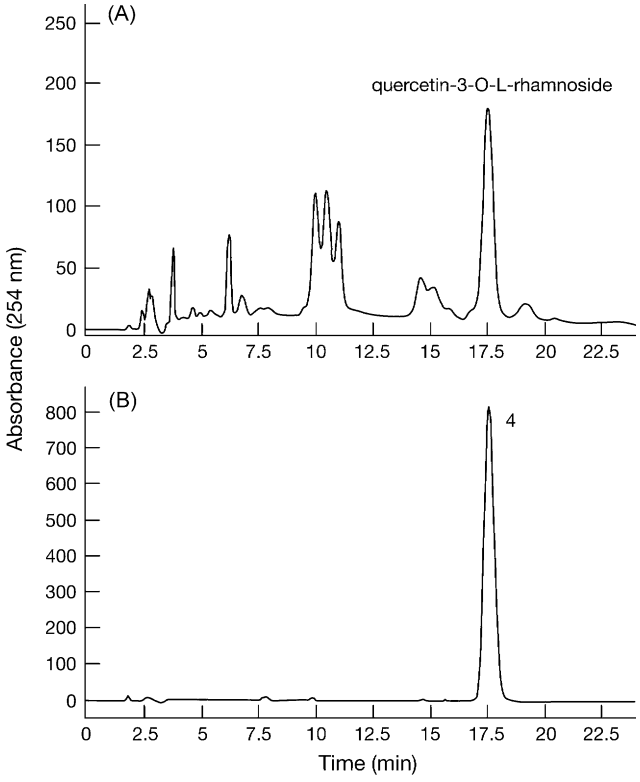

Fig. 3. HPLC analysis of crude sample and the components obtained from HSCCC separation. Experimental conditions: HPLC column: diamonsil C_{18} column (4.6 mm × 250 mm, 5 μm); mobile phase: acetonitrile–acetic acid–water (20:0.8:79.2, v/v/v); flow-rate: 1.0 ml/min; column temperature: 25 °C; detection wavelength: 254 nm.

(M^+ + 1-rhamnose unit); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.60 (1H, s, 5-OH), 7.29 (1H, d, J = 1.5 Hz, H-2′), 7.24 (1H, d, J = 8.4 Hz, H-6′), 6.85 (1H, d, J = 8.4 Hz, H-5′), 6.36 (1H, br.s, H-8), 6.19 (1H, br.s, H-6), 5.25 (1H, s, H-1″), 0.81 (3H, d, J = 6.0Hz, –CH_3). ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 156.9 (C-2), 134.6 (C-3), 178.2 (C-4), 161.8 (C-5), 99.3 (C-6), 165.1 (C-7), 94.1 (C-8), 157.7 (C-9), 104.4 (C-10), 121.6 (C-1′), 115.9 (C-2′), 145.7 (C-3′), 149.0 (C-4′), 116.1 (C-5′), 121.2 (C-6′), 102.3 (C-1″), 70.8 (C-2″), 71.0 (C-3″), 71.7 (C-4″), 70.5 (C-5″), 18.0 (C-6″). Comparing with the reported data, the MS, ^{1}H NMR and ^{13}C NMR data are in agreement with those of quercetin-3-O-L-rhamnoside [17,18].

The above results of our studies clearly demonstrated that HSCCC is very useful in the preparative separation of quercetin-3-O-L-rhamnoside from a crude plant extract. This compound was isolated from *A. truncatum* Bunge for the first time. We believe that the method may be successfully applied for separation of other flavonoids from crude plant extracts by selecting a suitable two-phase solvent system.

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