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Short communication

Preparative isolation and purification of syringin and edgeworoside C from *Edgeworthia chrysantha Lindl* by high-speed counter-current chromatography

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Abstract

The bioactive compound syringin along with edgeworoside C were separated from the *n*-butanol extract of the stems and barks of *Edgeworthia chrysantha Lindl* (*E. papyrifera*) by high-speed counter-current chromatography (HSCCC) while it was difficult to purify each compound by silica gel column chromatography. Syringin was isolated from this plant for the first time. The two-phase solvent system used was composed of ethyl acetate–ethanol–water at an optimized volume ratio of 15:1:15 (v/v/v). Preparative HSCCC yielded, from 110 mg of the partially purified extract, 28 mg of syringin and 45 mg edgeworoside C each at over 96% purity by high-performance liquid chromatography analysis. Their structures were identified by electron impact ionization MS, ^{1}H NMR and ^{13}C NMR.

Keywords: Counter-current chromatography; *Edgeworthia chrysantha*; Preparative chromatography; Plant materials; Pharmaceutical analysis; Syringin; Edgeworoside C

1. Introduction

Edgeworthia chrysantha Lindl (E. papyrifera S. et Z., Thymelaeaceae) is distributed in eastern Asia. It is used to make paper in Korea and Japan while the flowers and the roots are used as the crude drugs ‘meng hua’ and ‘meng hua gen’ in China [1–3]. In the course of our studies of the glycoside components of this plant, we investigated the constituents of the stems and barks of *Edgeworthia chrysantha Lindl*. Several glycoside components were isolated and syringin was isolated from *Edgeworthia chrysantha Lindl* for the first time. Syringin and its aglycone have various pharmacological effects and little toxicity. Various pharmaceutical actions of syringin have been reported. According to two Japanese patents, syringin is effective for the treatment of psychogenic behavior disorder [4] and has a hypnosis inducing action [5]. Pharmaceutical compositions containing syringin have hepato-protective activities [6]. Latest studies found that syringin and its aglycone have TNF a-secretion inhibiting effect, an anti-hypersensitivity effect as well as anti-inflammatory effects on auto-immune diseases like uveitis [7].

We found that the separation and purification of the syringin and edgeworoside C from *Edgeworthia chrysantha Lindl* using conventional methods such as silica gel column chromatography required several steps resulting in time-consuming. However, it is quite easy to purify each compound by high-speed counter-current chromatography (HSCCC).

High-speed counter-current chromatography is considered as a suitable alternative for the separation of glycosides due to its unique advantages, especially some glycosides difficult to isolate by other chromatography methods [8]. The present paper introduces a method for the separation of syringin and edgeworoside C, whose chemical structures are given in Fig. 1, from the extract of *Edgeworthia chrysantha Lindl*, by HSCCC.

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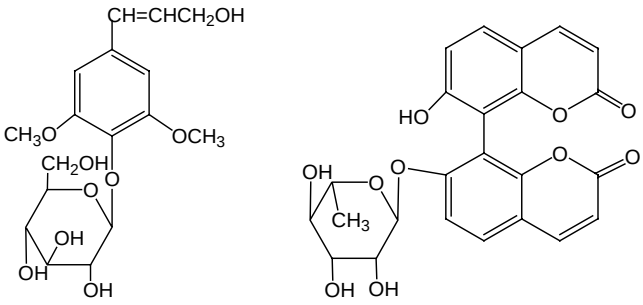

Fig. 1. Chemical structures of syringin (left) and edgeworoside C (right).

2. Experimental

2.1. Apparatus

A Model TBE-300A high-speed counter-current chromatograph (Shanghai Tauto Biotech, Shanghai, China) equipped with three preparative multiplayer coils (290 ml, wound with 2.0 mm i.d. PTFE tubing) was used for the separation and purification of syringin and edgeworoside C. The β values of this preparative column range from 0.46 to 0.73 ($\beta = r/R$, $R = 9.5$ cm, 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). The columns of HSCCC were installed in a vessel which was retained at 20 °C by a4 Model HX-1050 constant-temperature controller (Beijing Detianyou Technology, Beijing, China). The two-phase 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 UV-II detector Monitor (Shanghai Institute of Biochemistry of Academy of Science, Shanghai, China) at 254 nm. A manual sample injection valve with a 20 ml loop (Shanghai Tauto Biotech, Shanghai, China) was used to introduce the sample into the column. Sepu3000 workstation (Hangzhou PuHui Technology, Hangzhou, China) was employed to record the chromatogram. Eluate was collected with a Model BSZ-100 fraction collector (Shanghai Huxi Tech, Shanghai, China), 8 ml for each fraction.

The high-performance liquid chromatography (HPLC) (Shimadzu, Japan) equipment used was a CLASS-VP Ver.6.1 system comprised a Shimadzu SPD10Avp UV detector, a Shimadzu LC-10ATvp Multisolvent Delivery System, a Shimadzu SCL-10Avp controller, a Shimadzu LC pump, and a CLASS-VP Ver.6.1 workstation (Shimadzu, Japan).

2.2. Reagents and materials

All organic solvents used for HSCCC were of analytical grade and purchased from Hangzhou HuiPu Chemical Factory, Hangzhou, China. Methanol used for HPLC analysis was of chromatographic grade. Raw stems and barks of *Edgeworthia chrysantha Lindl* were cultivated and collected in the botanical garden of the university.

2.3. Extraction of crude samples

Air-dried stems and barks of *Edgeworthia chrysantha Lindl* (3 kg) were chopped into small pieces and extracted with methanol (10 l × 5) under reflux. The combined methanol extracts were concentrated under vacuum. 210 g of the residue obtained from the combined extract was dissolved with 2 l water and, after filtration, the aqueous solution was extracted three times with 2 l of water-saturated petroleum, ethyl acetate and *n*-butanol successively to yield 30 g of petroleum extract, 55 g of ethyl acetate extract and 46 g of *n*-butanol extract after being combined and evaporated to dryness under reduced pressure. The *n*-butanol extract was further subjected to column chromatography on silica gel (1200 g of silica gel H, 100–200 mesh, Qingdao Haiyang Chemica, Qingdao, China) eluted successively with chloroform–methanol solvent mixture of increasingly polarity to obtain six fractions. On concentration, the 5% methanol eluates gave 110 mg mixture mainly comprised of syringin and edgeworoside C (see Fig. 3A). This partially purified sample of *Edgeworthia chrysantha Lindl* was 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 (15:1:15, v/v/v), which was selected by a partition experiment of the crude extract in a series of solvent systems composed of ethyl acetate–ethanol–water at different volume ratios. The solvent mixture was thoroughly equilibrated in a separation funnel at the same temperature as in the vessel of HSCCC and the two phases separated shortly before use.

The sample solutions was prepared by dissolving the partially purified extract in the mixture solution of lower phase and upper phase (1:1, v/v) of the solvent system used for HSCCC separation.

2.5. Separation procedure

HSCCC was performed as follows. The multilayer coiled column was first entirely filled with the upper phase as a stationary phase. The lower aqueous mobile phase was then pumped into the head end of the column inlet at a flow-rate of 2.0 ml/min, while the apparatus was run at a revolution speed of 850 rpm. After hydrodynamic equilibrium was reached, as indicated by a clear mobile phase eluting at the tail outlet, the sample solution (110 mg dissolved in 20 ml mixture solution of lower phase and upper phase (1:1, v/v) of the solvent system) 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 automatically collected according to the elution profile and determined by HPLC. After the separation was completed, retention of the stationary phase was measured by collecting the column contents by forcing them out of the column with pressurized nitrogen gas.

2.6. HPLC analysis and identification of HSCCC peak fractions

The partially purified extract of *Edgeworthia chrysantha Lindl* and each peak fraction from HSCCC were analyzed by HPLC. The analyses were performed with a Shim-Pack CLC-ODS C_{18} column (250 mm × 6 mm i.d.). The mobile phase composed of methanol–water (50:50, v/v) was eluted at a flow-rate of 0.5 ml/min, and the effluent monitored by a Shimadzu SPD10Avp UV detector at 254 nm.

Identification of HSCCC peak fractions was carried out by electron impact ionization (EI) MS, ^{1}H NMR and ^{13}C NMR spectra. NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer with TMS (tetramethylsilane) as internal standard. EI-MS was obtained on a HP5989B mass spectrometer.

3. Results and discussion

In using HSCCC, successful separation depends upon the selection of a suitable two-phase solvent system, which requires the following considerations: (1) retention of the stationary phase should be satisfactory; (2) the settling time of the solvent system should be short (i.e. <30 s) (2 l); and (3) the partition coefficient (K) of the target compound should fall within a suitable range (i.e. usually between 0.2 and 5) [9]. A series of experiments were performed to determine the optimal solvent two-phase system for the HSCCC separation. The following systems at different volume ratios were tested: *n*-butanol–ethyl acetate–water (4:1:5), (3:2:5), (1:1:2) and (2:3:5); choroform-methanol-water (5:6:4), and ethyl acetate–ethanol–water (10:1:10), (15:1:15) and (50:1:50). Among those ethyl acetate–ethanol–water (15:1:15, v/v/v) gave the best separation of the target compounds by HSCCC.

A 110 mg quantity of partially purified extract was separated by HSCCC. The retention of the stationary phase was 32.0%, and the separation time was 160 min for a separation run.

The partially purified sample of *Edgeworthia chrysantha Lindl* was analysed by HPLC. The result indicates that it contained several compounds including syringin (about 30%) and edgeworoside C (about 40%) and some unknown compounds (see Fig. 3A).

Fig. 2 shows the result obtained from 110 mg of the partially purified extract of *Edgeworthia chrysantha Lindl* by preparative HSCCC. After this separation, the fractions containing syringin and edgeworoside C were collected, respectively. The analysis of these fractions indicated that the peak 1 fraction contained syringin which weighed 28 mg, at over 96% purity, and the peak 2 fraction contained edgeworoside C which weighed 45 mg, at over 99% purity, as determined by HPLC (Fig. 3B and C).

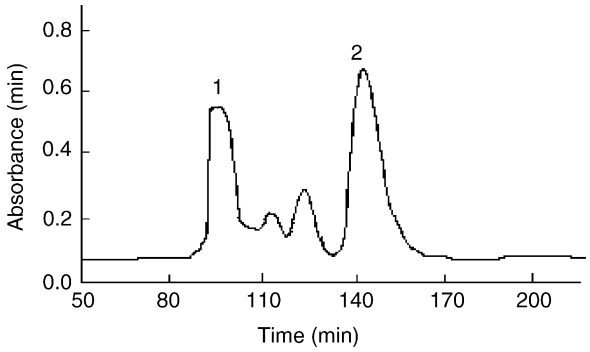

Fig. 2. Chromatogram of the partially purified extract from *Edgeworthia chrysantha Lindl* by preparative HSCCC. Peak 1: syringin; peak 2: edgeworoside C. Solvent system: ethyl acetate–ethanol–water (15:1:15, v/v); stationary phase: upper organic phase; mobile phase: lower aqueous phase; flow-rate: 2.0 ml/min; revolution speed: 850 rpm; sample: 110 mg dissolved in 20 ml mixture solution of lower phase and upper phase (1:1, v/v) of the solvent system; retention of the stationary phase: 32.0%.

The structural identification of the two components was carried out by EI-MS, ^{1}H NMR and ^{13}C NMR spectra. After comparing the data with spectral information from literature [10], the first component was confirmed as syringin. And comparing with the reported data, the spectra data of the second component was in agreement with those of edgeworoside C [3].

The result of our studies described above clearly demonstrated that HSCCC is very successful in the preparative

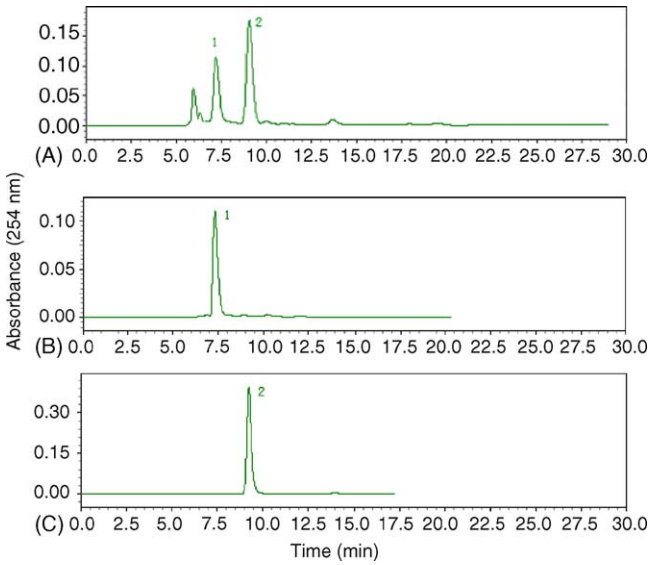

Fig. 3. Results of HPLC analyses of the crude sample of *Edgeworthia chrysantha Lindl* and its HSCCC fractions. Column: Shim-Pack CLC-ODS C_{18} column (250 mm × 6 mm i.d.); mobile phase: methanol–water (50:50, v/v); flow-rate: 0.5 ml/min. (A) The original sample; (B) HSCCC fraction from peak 1 (Fig. 2); (C) HSCCC fraction from peak 2 (Fig. 2).

separation of syringin and edgeworoside C from partially purified extract of *Edgeworthia chrysantha Lindl*.

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