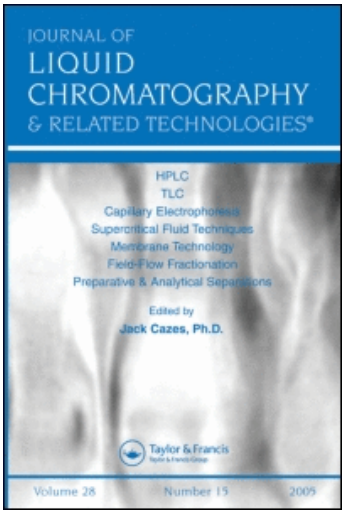

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Preparative Separation of Saponins from the *Luffa Cylindeica* (L.) Roem. by Slow Rotary Countercurrent Chromatography

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Abstract: Slow rotary countercurrent chromatography (SRCCC) was used for the separation of saponins from towel gourd crude extract (*Luffa cyclindria* Roem.) with a biphasic solvent system composed of chloroform-methanol-water (13:7:8, v/v). In each separation of SRCCC, 3 g crude saponins sample yielded 357 mg Lucyoside Q **(1)** and 213 mg Lucyoside H **(2)**. The structures of the two compounds were confirmed by means of ESI-MS, ^{1}H- and ^{13}C-NMR.

Keywords: *Luffa cyclindrica*, Saponins, Slow rotary countercurrent chromatography, Preparative isolation, Lucyoside Q, Lucyoside H

INTRODUCTION

Towel gourds are the fruits of *Luffa cylindrical (L.)* Roem. cultivated in the tropical and subtropical Asian region. The young fruits are a daily vegetable in China, India, and Japan. The fruits are also used in a traditional Chinese herb medicine as an anthelmintic, stomachic, and antipyretic.[1,2] The study of chemical constituents on this plant mainly involved saponins,[3–8] which showed bioactivity of anti-inflammation. The traditional preparative separation of the saponins mainly employed silica gel column chromatography and preparative HPLC, which have disadvantages such as lower recovery of saponins or lower sample load. Slow rotary countercurrent chromatography

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(SRCCC) has been developed for the preparative separation of natural products,[9,10] because it not only possesses the separation effect of high speed countercurrent chromatography (HSCCC) but also can be scaled up for industrial preparative separation. The present paper describes an excellent separation of saponins in *Luffa cylindrical* by using SRCCC.

EXPERIMENTAL

Reagents

The organic solvents, ethanol, methanol, and chloroform used for extraction and HSCCC separation were of analytical grade, and water was distilled water.

Preparation of Crude Saponins

One kg of freeze-dried sample was extracted two times with 5 liters of 90% ethanol at 80°C for 30 minutes. The combined extracting solution was evaporated into syrup. The syrup was defatted with ether, and then freeze-dried to yield 141 g of crude extract. The crude extract, 100 g, was subjected to column chromatography with macroporous resin AB-8 (Nankai Chemical Factory, Tianjin, China). A glass column (8 cm i.d. × 1.5 m length) stuffed with 5 kg of the macroporous resin AB-8 was loaded with the sample, and then orderly eluted with 10, 30, 50, and 70% of ethanol to yield 4 fractions. The effluents of 70% ethanol were evaporated and freeze-dried to yield 7.1 g of F70 that contained saponins components demonstrated by TLC with different color development reactions.

Selection of Solvent System for HSCCC

A partition test was used for selection of the solvent system. TLC was used for checking the partition coefficients of the saponins in a crude sample, in the two phases of a series of solvent systems composed of chloroform, methanol, and water in different proportions. Finally, chloroform-methanol-water (13:7:8, v/v), which provided a suitable partition coefficient were chosen as the solvent system for the separation of the crude sample.

TLC Analysis

TLC of saponins was conducted on GF254 plates (Merck, Germany), which was developed with chloroform-methanol-water (7:3:1) and colorized with 5% H_2SO_4 in ethanol at 110°C.

HPLC-MS Analysis

A system composed of a Waters 2695 and Waters 2420 Evaporative Light Scattering Detector (ELSD) was used for HPLC analysis, and an electronic spray ionization mass spectrometer (ESI-MS) was employed for mass analysis of the compounds. HPLC separation was performed on a Waters Symmetry C18, 5 μ (3.9 × 150 mm) column at 30°C, eluted by a gradient solvent system composed of A (H_2O with 0.05% HCOOH (v/v)) and B (CH_3CN with 0.05% HCCOH (v/v)). The initial condition of the gradient was 95% A and 5% B, a linear gradient in 20 min to 5% A and 95% B, 95% B from 20 to 25 min and back to the initial condition from 25 to 30 min. All ESI-MS experiments were conducted in positive and negative ionization mode, analyzing ions up to m/z 2200.

Slow Rotary Countercurrent Chromatography

The slow rotary countercurrent chromatograph used in the present study was constructed at the Institute of Food and Biological Engineering, Zhejiang Gongshang University, Hangzhou, China. A free rotary seal apparatus[9] was equipped with a 700 mL column with two layer coils made of 70 m long with 5.7 mm average i.d. convoluted Teflon tubing. The coil holder was 14 cm o.d. and 50 cm long. The apparatus can be operated at a speed up to 150 rpm.

The SRCCC separation procedure was followed as below: Solvent system was prepared by equilibrating chloroform, methanol, and water with a volumetric proportion 13:7:8 in a separatory funnel. The two resulting phases were separated shortly before use. Then, the coil column was entirely filled with the upper phase as the stationary phase and the crude saponins sample (3 g), dissolved in 50 mL of mobile phase, was injected to the SRCCC system through a Teflon sample loop. Next, the SRCCC instrument was operated at a rotary speed of 80 rpm. Afterwards, the mobile phase was pumped into the column at a flow rate of 1.0 mL/min, by a Waters 510 pump (Waters, Milford, MA, USA). The effluent was collected with a fraction collector B-684 (Buchi, Switzerland). The composition in each fraction was analyzed with TLC analysis.

Nuclear Magnetic Resonance (NMR) Analysis

^1H- and ^{13}C-NMR experiments were recorded on a Bruker Advance 400 MHz NMR spectrometer, respectively.

RESULTS AND DISCUSSION

SRCCC Separation

Figure 1 presents the TLC analysis of fractions from SRCCC separation of 3 g of a crude saponins sample with the two-phase solvent system composed of chloroform-methanol-water (13:7:8, v/v). The HSCCC run yielded two components A and B with a single spot on the TLC plate (Figure 1). The combined fractions were evaporated under reduced pressure and subsequent lyophilization to yield 357 mg of component A (tubes 16–34) and 213 mg of component B (tubes 36–70), respectively. Components A and B were very pure, which was demonstrated by analysis of HPLC-ELSD (Figure 2).

Confirmation of Chemical Structures

Components A and B were analyzed by ESI-MS and ^{1}H-NMR and ^{13}C-NMR experiments. The data demonstrated the two components were lucyoside Q **(1)** and lucyoside H **(2)** (Figure 3), respectively, comparing to the data in ref. 7 and 8. Our data were listed as below:

Lucyoside Q: ESI-MS (pos.) m/z: 657.5 $[M + Na]^{+}$. 1H NMR (DMSO-d_6) δppm: 0.91, 1.01, 1.13, 1.16, 1.21, 1.23, 1.25 (s, 3H × 7); 4.50 (1H, br s, olefinic proton); 5.35 (1H, d, J = 7.7 Hz, anomeric proton). ^{13}C-NMR (DEPT, DMSO-d_6) δ ppm: 175.6 (C), 143.3 (C), 123.2 (CH), 78.1(CH), 72.2 (CH), 55.8 (CH), 48.9 (C), 48.1 (CH), 47.0 (CH_2), 42.1 (C), 41.4 (CH), 40.9 (CH_2), 39.8 (C), 39.3 (C), 38.9 (CH_2), 37.3 (C), 36.7 (C), 33.2(CH_2), 29.7 (CH_3), 28.7 (CH_3), 28.3 (CH_2), 25.9 (CH_3), 24.9 (CH_2), 23.8 (CH_2), 18.8 (CH_2), 17.6 (CH_3), 17.5 (CH_3), 16.5 (CH_3), 15.6 (CH_3); Glc: 106.8 (CH), 78.8 (CH), 78.1 (CH), 75.8 (CH), 72.1 (CH), 63.2 (CH_2).

Lucyoside H: ESI-MS (pos.) m/z: 803.2$[M + Na]^{+}$. ^{1}H-NMR(DMSO-d_6) δ_H: 0.86, 0.99, 1.09, 1.16, 1.25, 1.28, 1.30 (s, 3H × 7CH_3); 4.14 (1H, br s, olefinic proton); 5.17, 5.23 (d, 1H × 2, anomeric proton). ^{13}C-NMR (DMSO-d_6) δ_C:177.7 (C), 144.0 (C), 122.2 (CH), 88.4 (CH), 55.5 (CH_2), 48.0 (CH), 47.6 (CH), 43.4 (CH_2), 41.8 (CH), 41.3 (CH), 40.6 (C), 39.6

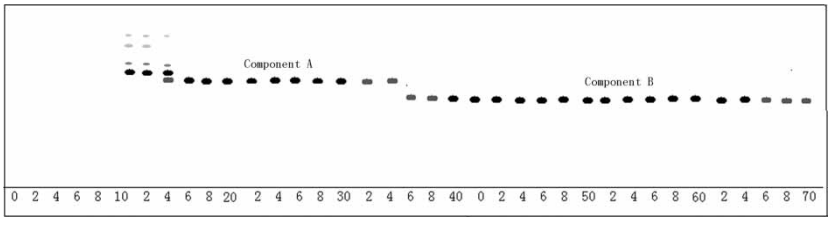

Figure 1. TLC analysis of fractions from SRCCC separation. Spray reagent, 5% H_2SO_4 in ethanol; Fraction volume, 30 mL.

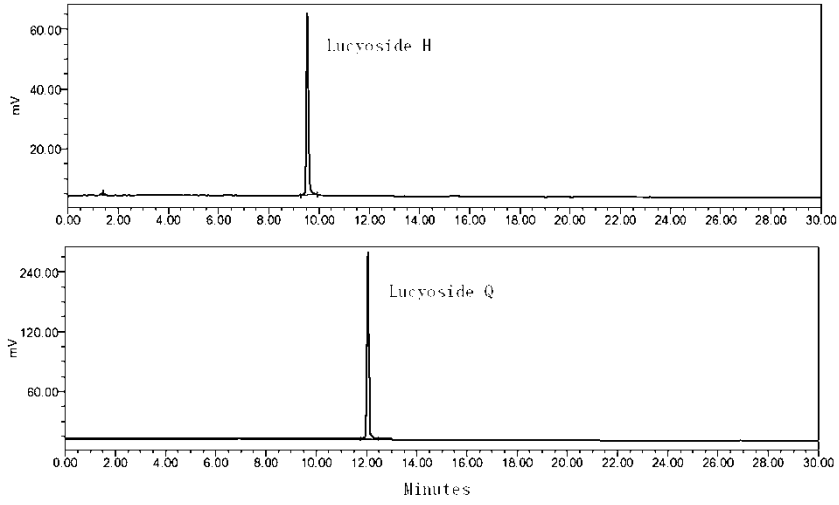

Figure 2. HPLC analysis of the components from SRCCC separation. Component A, lucyoside Q; Component B, lucyoside H.

(C), 39.2 (CH_2), 36.9 (C), 34.2 (CH_2), 33.3 (CH_2), 33.2 (CH_3), 32.7 (CH_2), 30.9 (C), 28.4 (CH_2), 28.4(CH_3), 26.8 (CH_2), 26.3 (CH_3), 25.9 (CH_2), 23.6 (CH2), 17.8 (CH_2), 17.3 (CH_3), 17.0 (CH_3), 15.7 (CH_3), Glu-1: 106.9 (CH), 78.2 (CH), 77.1 (CH), 72.9 (CH), 70.7 (CH), 61.7 (CH_2); Glu-2: 94.6 (CH), 77.4 (CH), 77.2 (CH), 74.6 (CH), 70.0 (CH), 61.2 (CH_2).

The present separation only employed a smaller coil column made of convoluted Teflon tubing with an average I.D. of 5.7 mm. However, it is easy to scale up SRCCC to a industrial level by using a convoluted Teflon tubing with a larger inner diameter, which provides better retention of the stationary phase at a high flow rate of the mobile phase.[11] Thus, the present method will

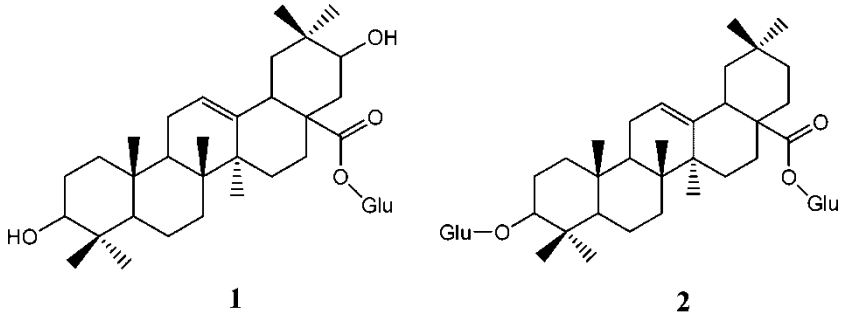

Figure 3. The chemical structures of lucyoside Q (**1**) and lucyoside H (**2**).

provide significant knowledge for the preparation of saponins of *Luffa cyclinderia* with a larger scale SRCCC.

CONCLUSION

SRCCC is an effective methodology for isolation of the saponins in *Luffa cyclinderia*. The method can be accepted for large-scale preparation of the saponins for further biological studies.

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