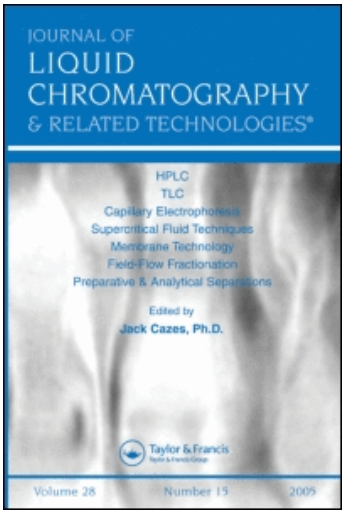

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Preparative Separation of Phenolic Constituents in the Fruits of *Luffa cylindrica* (L.) Roem using Slow Rotary Countercurrent Chromatography

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Abstract: In the present study we performed preparative separations of the phenolic compounds in two pre-purified sponge gourd extracts Fr30 and Fr50 from macroporous resin AB-8, using slow rotary countercurrent chromatography (SRCCC) with two solvent systems, respectively. The separation of 3 g of Fr30 using solvent system $CHCl_3$-MeOH-2-propanol -H_2O (5:6:1:4, v/v) yielded five compounds: *p*-coumaric acid (**1**, 65 mg), 1-*O*-feruloyl-*β*-D-glucose (**2**, 189 mg), 1-*O*-*p*-coumaroyl-*β*-D-glucose (**3**, 96 mg), 1-*O*-caffeoyl-*β*-D-glucose (**4**, 130 mg), and 1-*O*-(4-hydroxybenzoyl)-glucose (**5**, 90 mg), and the separation of 3 g of Fr50 using solvent system $CHCl_3$-MeOH-H_2O (13:7:8, v/v) afforded three compounds: diosmetin-7-*O*-*β*-D-glucuronide methyl ester (**6**, 125 mg), apigenin-7-*O*-*β*-D-glucuronide methyl ester (**7**, 164 mg), and luteolin-7-*O*-*β*-D-glucuronide methyl ester (**8**, 98 mg). The excellent results demonstrate that SRCCC is an efficient method to prepare large amounts of natural products.

Keywords: *Luffa cylindrica*, Sponge gourd, Phenolic compounds, Slow rotary countercurrent chromatography

INTRODUCTION

Sponge gourds are the fruits of *Luffa cylindrica* (L.) Roem cultivated in the tropical and subtropical Asian region. Sponge gourds are used as a routine vegetable in several Asian countries. It is also covered in the traditional

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Chinese medicine (TCM) as an anthelmintic, stomachic, and antipyretic phytomedicinal drug.[1] By employing high speed countercurrent chromatography (HSCCC), phenolic compounds including flavonoid glycosides were separated from sponge gourd extract pre-purified by using macro-porous resin AB-8[2] for structure identification of the compounds. A larger amount of the compounds are expected for advanced bioactive studies.

Slow rotary countercurrent chromatography (SRCCC)[3,4] employs a non-planetary low rotation column (20–150 rpm), which permits the system to be left unattended during the long time separation. The advantage of SRCCC is that the separation can be scaled up to an industrial level.[5] The present study described preparative separations of the phenolic compounds in the sponge gourd extracts to establish a method, which can be scaled up for preparative separation.

EXPERIMENTAL

Materials

One kg of a freeze dried sample was prepared from 30 kg of skin peeled sponge gourds, which were purchased in a local store (Hangzhou, China). All solvents for extraction and separation were of analytical grade (Hangzhou Chemicals Inc., China), and macro-porous gel resin AB-8 was provided by Chemical Factory of Nankai University, Tianjin, China).

One kg of a freeze dried sponge gourd sample was marinated two times with 5 L of 90% ethanol. Each marinate was kept at 50°C for 2 h. The extracts were combined and evaporated to a syrup, defatted with ether, and then lyophilized to yield 141 g of crude extract. Of the crude extract, 100 g was dissolved in 400 mL of water, and then loaded onto a chromatographic column (6 cm i.d. × 1.0 m length) filled with 1 kg of macro-porous gel resin AB-8. Elution was done with increasing solvent strength by using 10, 30, 50, 70, and 90% ethanolic solutions to yield 5 main fractions: Fr10 (22 g), Fr30 (11 g), Fr50 (17 g), Fr70 (15 g), and Fr90 (21 g). Fractions Fr30 and Fr50 were used for SRCCC separation of phenolic compounds.

Slow Rotary Countercurrent Chromatographic Separation

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. It was a seal free instrument equipped with a one layer coil column made of 60 m convoluted polytetrafluoroethylene (PTEF) tubing with an 8.5 mm average inner diameter. The coil hub holder was of 2.2 m length and 17 cm outer diameter. The column capacity was 3.4 L. For the establishment of the SRCCC system, a K-1800

Wellchrom preparative HPLC pump (Knauer, Germany), a 300 mL sample loop made of 5 mm i.d. PTEF tubing, and a B-684 collector (Büchi, Switzerland) with 50 mL tube racks were used. The solvent systems were the same as those for HSCCC separation.[2] The solvent systems of chloroform-methanol-isopropanol-water (5:6:1:4. v/v) and chloroform- methanol-water (13:7:8, v/v) were used for the separation of Fr30 and Fr50, respectively, with aqueous upper phase as the stationary phase. The sample solutions were prepared by dissolving 3.0 g each of Fr30 and Fr50 with 300 mL of corresponding mobile phases, respectively.

The separation experiment for each sample was conducted with the solvent system described above. The coil column was first entirely filled with stationary phase. Then the apparatus was rotated at 70 rpm and the sample solution was injected into the CCC system through the PTEF sample loop with the mobile phase at a flow rate of 5.0 mL/min. The effluent (mobile phase) was collected into 50 mL tubes for TLC analysis. TLC analysis was performed on GF_{254} (Merck) aluminium plates, using chloroform-methanol-water (7:3:1) as the developing solvent. The visualization was carried out with 10% sulphuric acid in ethanol and heating on a hot plate at 110°C.

HPLC Analysis

A Waters HPLC system (Waters, Milford, USA) was composed of a Alliance 2695, a Symmetry C-18 column (5 mm, 150´ 3.9 mm), a 996 PDA detector, and a Millennium HPLC 2010 processing system. A gradient elution was performed for the separation of fractions with a gradient 95% A and 5% B to 85% A and 15% B from 0 to 10 min, 85% A and 15% B to 40% A and 60% B from 10 to 20 min; A was 0.05% formic acid in water, and B was 0.05% formic acid in methanol. The flow rate of mobile phase was 1.0 mL/min and the detection wavelength was 254 nm.

RESULTS AND DISCUSSION

HSCCC has been used for separations of natural products in worldwide laboratories. But SRCCC is not extensively recognized because the SRCCC instruments are not commercially available up to now, and very few separations have been reported. To demonstrate the availability of SRCCC, we adopted a SRCCC instrument equipped with a 3.4 L column to separate the phenolic compounds in the sponge gourd extracts. The first separation was the SRCCC separation of 3 g of Fr30 using solvent system chloroform-methanol-2-propanol-water (5:6:1:4, v/v). The result was shown in Figure 1. Fractions 40-50, 80-98, 116-142, 148-170, and 176-202 were the single spot fractions. This separation gave a similar resolution compared to

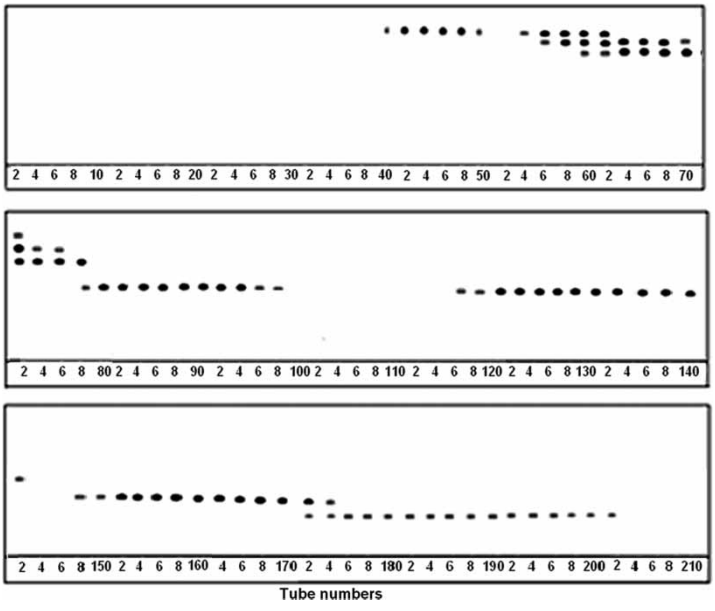

Figure 1. TLC analysis of fractions from SRCCC separation of Fr30. SRCCC conditions: solvent system: chloroform-methanol-2-propanol-water (5:6:1:4, v/v); stationary phase: upper phase; flow rate of mobile phase: 5 mL/min; rotation speed of column: 70 rpm; fraction volume: 50 mL, tubes 40–50: **1**; tubes 80–98: **2**; tubes 116–142: **3**; tubes 148–170: **4**; tubes 176–202: **5**.

the HSCCC separation of 1 g of Fr30, using the same solvent system with a 1200 mL column in ref. 2. The SRCCC fractions exhibiting same TLC behaviors were combined and decolorized with active carbon, then evaporated under vacuum to yield 65 mg of *p*-coumaric acid **(1)**, 189 mg of 1-O-feruloyl-*β*-D-glucose **(2)**, 96 mg of 1-O-*p*-coumaroyl-*β*-D-glucose **(3)**, 130 mg of 1-O-caffeoyl-*β*-D-glucose **(4)**, and 90 mg of 1-O-(4-hydroxybenzoyl)-glucose **(5)**, with purity more than 95% analyzed by HPLC (Figure 2), respectively. The amount of the five compounds was approximately three times of those obtained from the HSCCC separation of 1 g sample in ref. 2. The second separation was the SRCCC separation of 3 g Fr50, which utilized the solvent system chloroform-methanol-water (13:7:8, v/v). The separation gave 3 compounds corresponding to the fractions 46-52, 56-90, and 98-134 (Figure 3). After being decolorized with active carbon, evaporated, and freeze-dried, 125 mg of diosmetin-7-O-*β*-D-glucuronide methyl ester **(6)**, 164 mg of apigenin-7-O-*β*-D-glucuronide methyl ester **(7)**, and 98 mg of luteolin-7-O-*β*-D-glucuronide methyl ester **(8)** were obtained, with purity more than 95% analyzed by HPLC (Figure 4), respectively. Correspondingly, the HSCCC separation with a 1200 mL column allowed loading 1 g of sample and yielded 42 mg of diosmetin-7-O-*β*-D-glucuronide methyl ester, 57 mg of

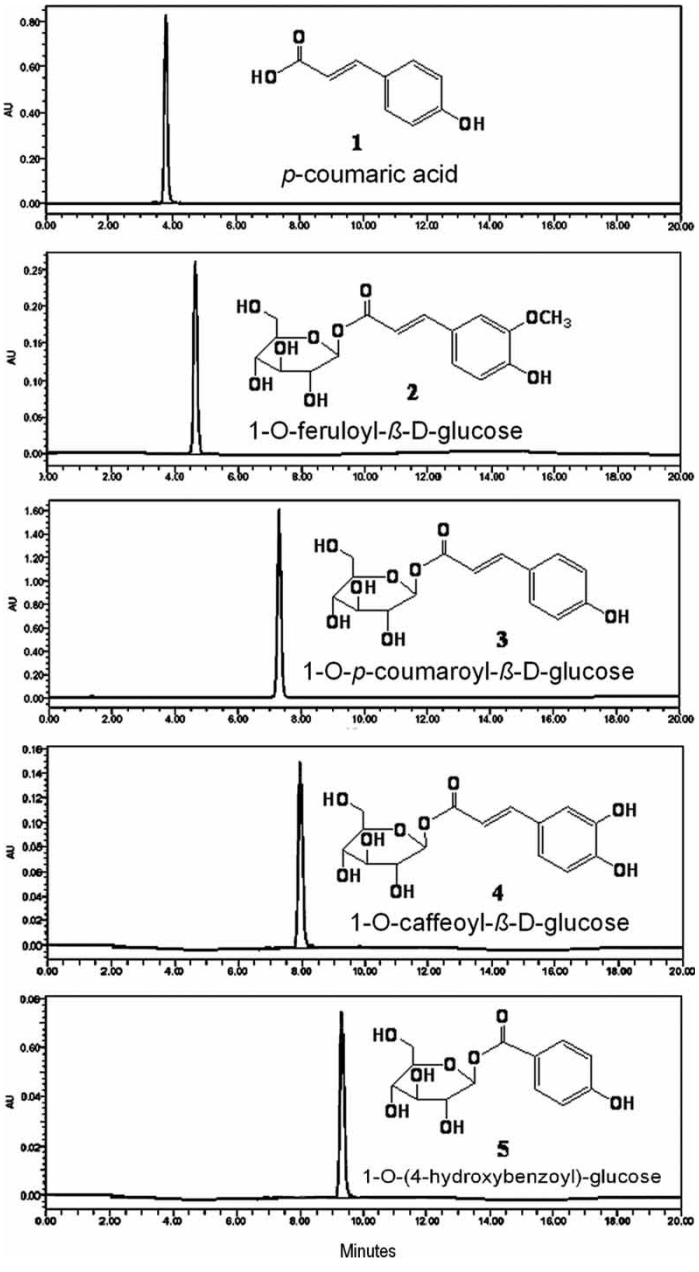

Figure 2. HPLC analysis of the compounds from SRCCC separation of Fr30. Column: symmetry C-18 column (5 μm, 150 × 3.9 mm); detection wavelength: 254 nm, gradient (A: 0.05% formic acid in water, B: 0.05% formic acid in methanol): 95% A to 85% A from 0 to 10 min, 85% A to 40% A from 10 to 20 min, B; flow rate: 1.0 mL/min.

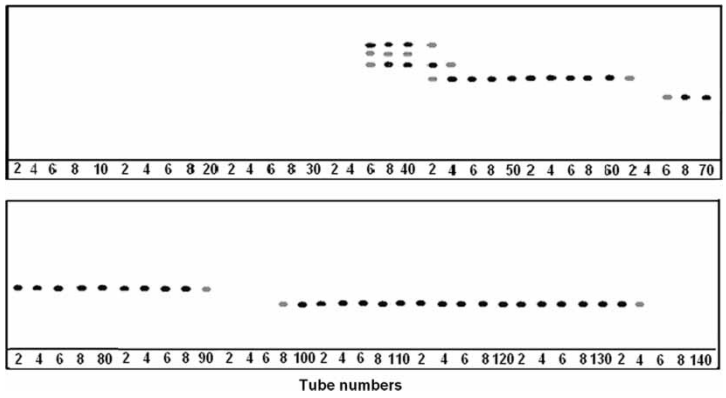

Figure 3. TLC analysis of fractions from SRCCC separation of Fr50. SRCCC conditions: solvent system: chloroform-methanol-water (13:7:8, v/v); stationary phase: upper phase; flow rate of mobile phase: 5 mL/min; rotation speed of column: 70 rpm; fraction volume: 50 mL; tubes 46–52: **6**; tubes 56–90: **7**; tubes 98–134: **8**.

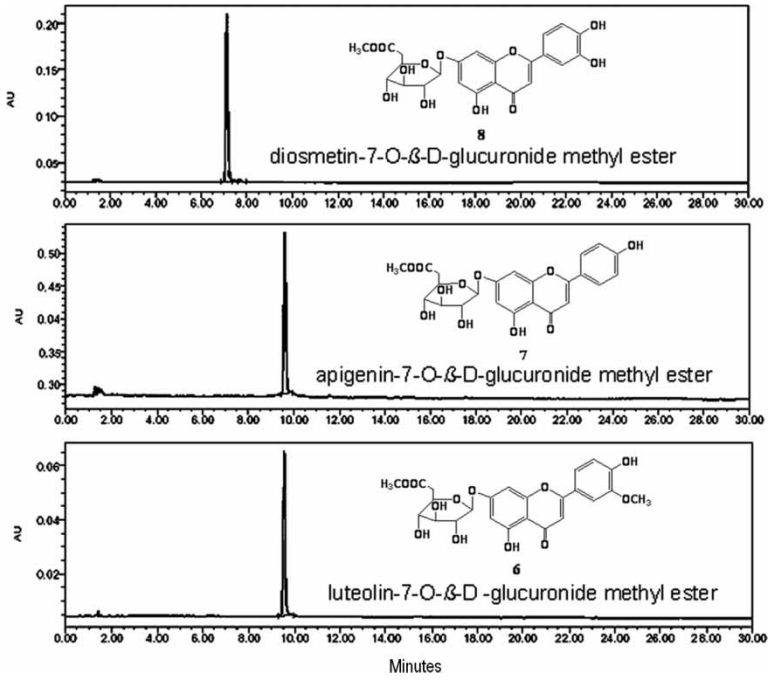

Figure 4. HPLC analysis of the compounds from SRCCC separation of Fr50. Column: Symmetry C-18 column (5 μm, 150 × 3.9 mm); detection wavelength: 254 nm; gradient (A: 0.05% formic acid in water, B: 0.05% formic acid in methanol): 95% A to 85% A from 0 to 10 min; 85% A to 40% A from 10 to 20 min; flow rate: 1.0 mL/min.

aigenin-7-O-β-D-glucuronide methyl ester, and 33 mg of luteolin-7-O-β-D-glucuronide methyl ester shown in ref. 2. The amount of each of the three compounds was approximately three times of those obtained from the HSCCC separation of a 1 g sample, which also implied that the SRCCC of 3 g of sample yielded a similar resolution of the HSCCC separation of 1 g sample.

The above results show that the sample loading amount in SRCCC can reach 3 times of that in HSCCC when the SRCCC column capacity is about three times of HSCCC column capacity. Our previous study showed that SRCCC can be scaled up to semi-industrial separation,[6] and its application in industrial separation is possible by adopting a larger CCC column made of larger i.d. convoluted tubing, which allows a larger sample load at a higher flow rate. Therefore, SRCCC is an effective way to scale up the separation of phenolic components in sponge gourds.

CONCLUSIONS

Slow rotary countercurrent chromatography (SRCCC) can be used for the preparative separations of a hundred milligrams of the phenolic compounds in sponge gourd extracts, which demonstrates that SRCCC is an efficient method to prepare larger amounts of natural products.

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REFERENCES

1. Kou, J.; Zhuang, S.; Tang, X.; Tong, C.; Yan, Y. Preliminary studies of Luffa-extract on allergy and acute inflammation. J. Chin. Pharm. Uni. **2001**, *23*, 293–296.
2. Du, Q.; Xu, Y.; Li, L.; Zhao, Y.; Jerz, G.; Winterhalter, P. Antioxidant constituents in the fruits of *Luffa cylindrica* (L.) Roem. J. Agri. Food Chem. **2006**, *54*, 4186–4190.
3. Du, Q.; Wu, P.; Ito, Y. Low-speed rotary countercurrent chromatography using a convoluted multilayer helical tube for industrial separation. Anal. Chem. **2000**, *72*, 3363–3365.
4. Du, Q.; Ito, Y. Low rotary countercurrent chromatography. J. Liq. Chromatogr. & Rel. Technol. **2003**, *26*, 1827–1838.
5. Du, Q.; Winterhalter, P.; Ito, Y. Large convoluted tubing for scale-up of slow rotary countercurrent chromatograph. J. Liq. Chromatogr. & Rel. Technol. **2003**, *26*, 1991–2002.

6. Du, Q.; Jerz, G.; He, Y.; Li, L.; Xu, Y.; Zhang, Q.; Zheng, Q.; Winterhalter, P.; Ito, Y. Semi-industrial isolation of salicin and amygdalin from plant extracts using slow rotary countercurrent chromatography. J. Chromatogr. A **2005**, *1074*, 43–46.

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