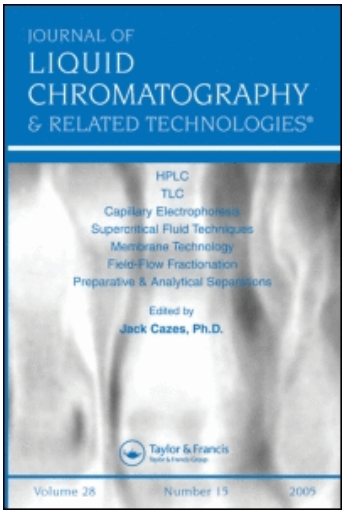

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Preparative Isolation of Mangiferin from *Anemarrhena asphodeloides* Rhizomes by Centrifugal Partition Chromatography

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Preparative Isolation of Mangiferin from *Anemarrhena asphodeloides* Rhizomes by Centrifugal Partition Chromatography

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Abstract: Mangiferin was separated from rhizomes of *Anemarrhena asphodeloides* extract using centrifugal partition chromatography (CPC) with a two-phase solvent system composed of ethyl acetate-isopropanol-water (3 : 2 : 5, v/v). Mangiferin (22.5 mg) was successfully isolated from methanolic extracts (957 mg) in only one step, and the purity of isolated compound was determined to be over 95% by HPLC analysis. The structure of mangiferin was identified by ^{1}H, ^{13}C NMR and ESI-MS spectral data analysis.

Keywords: Centrifugal partition chromatography, *Anemarrhena asphodeloides*, Preparative chromatography, Mangiferin, Xanthone glycoside

INTRODUCTION

Mangiferin, a glucosyl xanthone, was a major component of rhizomes of *Anemarrhena asphodeloides*.[1] Mangiferin (Figure 1) showed a wide range of pharmacological properties such as antioxidant, antidiabetic, anti-HIV, anticancer, and immunomodulatory activities.[2–7] It required a large amount of purified compounds to evaluate the biological activities and to control the quality of the herbal medicine. This necessitates the development of efficient, preparative separation methods for this compound.

Centrifugal partition chromatography (CPC) is a liquid-liquid partition chromatographic technique and its advantage is shorter separation time, no

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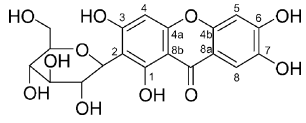

Figure 1. Structure of mangiferin.

sample adsorption, and various solvent systems to purify diverse compounds. These merits bring about the wide use of CPC for the preparative separation of natural products.[8–10]

In this paper, we describe the successful preparative separation and purification of mangiferin from methanolic extract of *A. asphodeloides* rhizomes using CPC with a two-phase solvent system composed of ethyl acetate-isopropanol-water (3 : 2 : 5, v/v).

EXPERIMENTAL

Apparatus

Preparative CPC was performed using a LLB-M high performance centrifugal partition chromatograph (Sanki Engineering Ltd., Kyoto, Japan). The total cell volume is 230 mL. A four-way switching valve incorporated in the CPC apparatus allows operating in either the descending or the ascending mode. This CPC system was equipped with a Hitachi 6000 pump (Hitachi, Japan), a Gilson UV/Vis-151 detector, a Gilson FC 203B fraction collector (Gilson, France), and a Rheodyne valve (Rheodyne, Cotati, CA, USA) with a 2 mL sample loop. The HPLC system used in this experiment consisted of a binary Gilson 321 pump, a Gilson UV/Vis 151 detector, a Gilson 234 auto-injector, and a 506C interface module (Gilson, France). The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker GPX 400 spectrometer (Germany) at 400 MHz and 100 MHz, respectively. ESI-MS was measured with a Finnigan LCQ ion trap mass spectrometer (San Jose, CA, USA).

Materials and Reagents

The rhizomes of *A. asphodeloides* Bunge (Liliaceae) were purchased from a Kyungdong oriental medicine market, Seoul, Korea, in July 2005. A voucher specimen was deposited in the Herbarium of College of Pharmacy, Seoul National University (SNURAA-0720). HPLC grade solvents were purchased from Burdick & Jackson (MI, USA). All other chemicals were analytical grade.

Preparation of the Methanolic Extract from the Rhizomes of *A. asphodeloides*

The rhizomes of *A. asphodeloides* (600 g) were extracted three times in an ultrasonic apparatus with methanol (1000 mL) for 1 h. The extract was concentrated by a rotary evaporator, and then the extract (108 g) was stored in a refrigerator for CPC separation.

CPC Separation Procedure

According to the partition coefficient of mangiferin in various solvent systems, the two-phase solvent system composed of ethyl acetate, isopropanol, and water in a 3 : 2 : 5 (v/v) ratio was chosen to separate mangiferin. The solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated before use. The column was first filled with the organic stationary phase and then rotated at 1000 rpm while mobile phase was pumped into the column in a descending mode at a flow rate of 2 mL/min. When the mobile phase emerged from the column and the hydrodynamic equilibrium was reached, the sample, dissolved in 4 mL mixture each of phase (1 : 1, v/v), was injected through the Rheodyne injection valve. The UV detection was performed at 254 nm from the outlet of the column, and each fraction was collected in an 8 mL test tube in a Gilson FC 203B fraction collector.

HPLC Analysis

HPLC analysis was achieved on a reversed phase column (Capcell pak UG 120, 5 μm particle size, 250 × 4.6 mm I.D, Shiseido, Japan). The mobile phase was acetonitrile-0.1% acetic acid in gradient mode as follows: acetonitrile-0.1% acetic acid (10 : 90 v/v at 0 min to 50 : 50 v/v at 30 min). The flow rate was 1 mL/min with UV absorbance detection at 254 nm.

RESULTS AND DISCUSSION

Selection of a Suitable Two-Phase Solvent System

Separation of natural products using CPC is based on the partition behaviors of target compounds between immiscible solvents that are used as a mobile phase and stationary phase, respectively. In order to choose a suitable two-phase solvent system, several two-phase solvent systems were tested and their partition coefficients (K values) were measured. The two-phase solvent

Table 1. The *K* (partition coefficient) values of mangiferin in different solvent systems

Solvent system	*K* value
n-hexane-ethyl acetate-acetonitrile-methanol-water (10 : 10 : 10 : 4 : 8)	0.06
Ethyl acetate-acetonitrile-water (4 : 1 : 5)	0.06
Ethyl acetate-acetonitrile-water (3 : 2 : 5)	0.09
Ethyl acetate-methanol-water (5 : 1 : 4)	0.07
Ethyl acetate-isopropanol-water (4 : 1 : 5)	0.27
Ethyl acetate-isopropanol-water (3 : 2 : 5)	0.74

system composed of ethyl acetate-isopropanol-water (3 : 2 : 5, v/v) showed appropriate partition behavior between upper and lower phase (Table 1).

CPC Separation

A. asphodeloides extract (957 mg) was separated and purified by CPC with ethyl acetate-isopropanol-water (3 : 2 : 5, v/v) as a solvent system. Separation of the methanolic extract by CPC is shown in Figure 2. The purification of

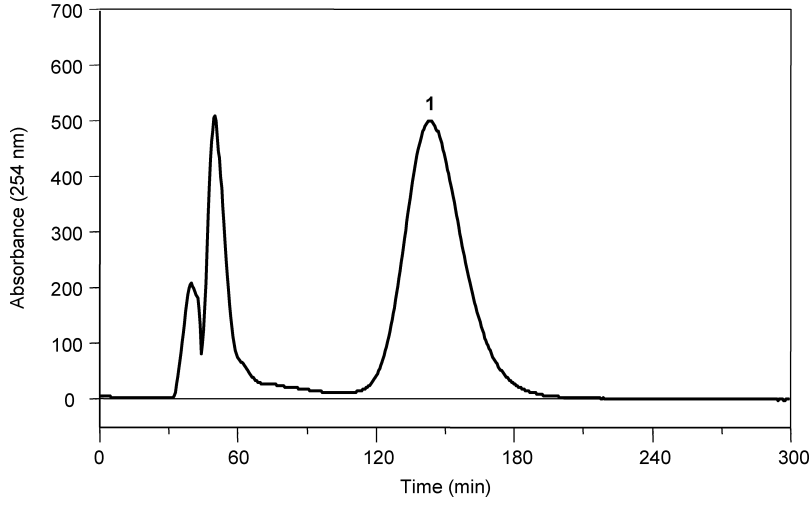

Figure 2. Preparative CPC separation of methanolic extract from *A. asphodeloides*. CPC solvent system: ethyl acetate-isopropanol-water (3 : 2 : 5); stationary phase: upper organic phase; mobile phase: lower aqueous phase; flow rate: 2 mL/min; rotation speed: 1000 rpm; sample: 957 mg dissolved in 4 mL mixture of lower phase and upper phase (1 : 1, v/v) of the solvent system.

mangiferin was achieved in only 200 min with this solvent system, and the retention of the stationary phase was favorable. The methanolic extract of *A. asphodeloides* rhizomes was analyzed by HPLC, and the results indicated that it contained several compounds including mangiferin (**1**) and some unknown compounds (Figure 3A). However, a one step operation of CPC was able to isolate mangiferin, and the HPLC chromatogram in Figure 3B illustrated that the purity of the collected fraction peak 1 was 96%.

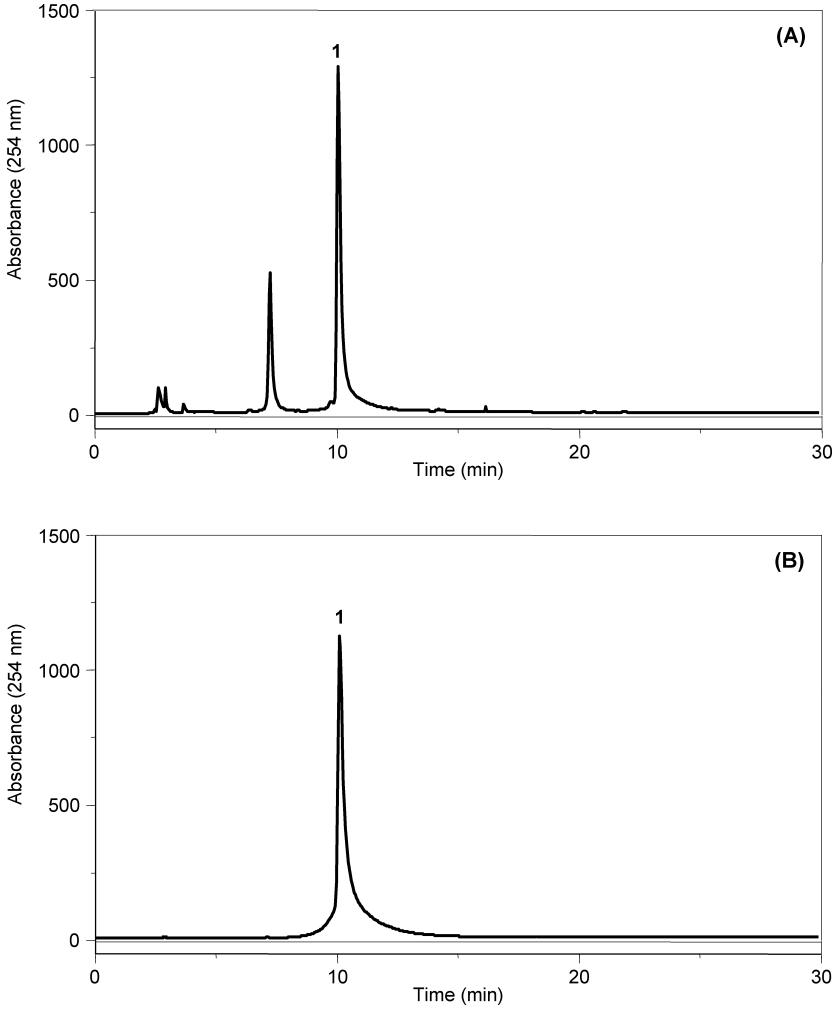

Figure 3. HPLC profiles of the methanolic extract of *A. asphodeloides* (A) and fraction peak 1 obtained from CPC operation (B). Column: Capcell pak UG120 column (250 mm × 4.6 mm i.d.); mobile phase: acetonitrile-0.01% acetic acid (10 : 90 v/v at 0 min to 50 : 50 v/v at 30 min); flow rate: 1 mL/min, monitored at 254 nm.

Mangiferin (22.5 mg) was successfully purified from methanolic extract of *A. asphodeloides* (957 mg) using CPC.

Structural Elucidation of Mangiferin

The structural identification of peak fraction 1 in Figure 2 was performed by ESI-MS, ^{1}H NMR, and ^{13}C NMR. ^{1}H NMR and ^{13}C NMR data were in good agreement with those reported in the literature.[11] Our data were listed as follows:

Mangiferin: ESI-MS m/z: 421 $(M\text{-}H)^{-}$;. negative ion mode. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 13.77 (1H, s, 1-OH), 7.37 (1H, s, H-8), 6.85 (1H, s, H-5), 6.37 (1H, s, H-4), 4.58 (1H, d, $J = 9.8$ Hz, H-1′), 4.04 (1H, t-like, $J = 9.0$ Hz, H-2′), 3.68 (1H, d, $J = 11.3$ Hz, H-6′a), 3.41 (1H, dd, $J = 11.3$, 5.7 Hz, H-6′b) 3.20 (1H, m, H-3′), 3.16 (1H, m, H-5′), 3.14 (1H, m, H-4′). ^{13}C NMR (DMSO-d_6): δ 179.1 (C═O), 163.9 (C-3), 161.8 (C-1), 156.2 (C-4a), 154.3 (C-6), 150.9 (C-4b), 143.3 (C-7), 111.6 (C-8a), 108.0 (C-8), 107.6 (C-2), 102.6 (C-5), 101.3 (C-8b), 93.3 (C-4), 81.6 (C-5′), 79.0 (C-3′), 73.1 (C-1′), 70.7 (C-2′), 70.2 (C-4′), 61.5 (C-6′).

CONCLUSION

Mangiferin (22.5 mg) was successfully separated from 957 mg of *A. asphodeloides* extract using CPC, with the two-phase solvent system composed of ethyl acetate-isopropanol-water (3 : 2 : 5, v/v), in only one step. The results of our studies demonstrated that CPC is a useful method for the preparative separation of mangiferin from *A. asphodeloides* extract.

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