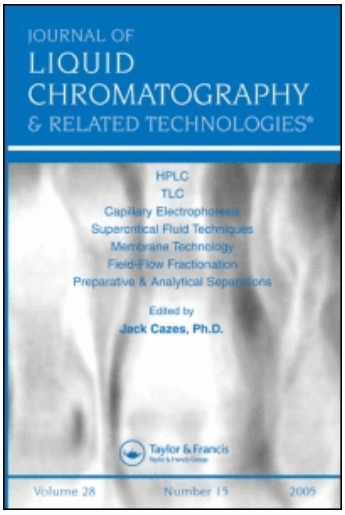


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# Isolation and Purification of Psoralen and Bergapten from *Ficus carica* L. Leaves by High-Speed Countercurrent Chromatography

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**Abstract:** High-speed countercurrent chromatography (HSCCC) was successfully applied for the first time for the isolation and purification of psoralen and bergapten from the plant *Ficus carica* L leaves. The crude extract was obtained by extraction with light petroleum (b.p.: 60°C–90°C) from the dried leaves of *Ficus carica* L. *n*-Hexane-ethyl acetate-methanol-water (1:1:1:1, v/v) was used as the two-phase solvent system. Each peak fraction was analyzed by high performance liquid chromatography (HPLC). The method yielded 4.4 mg of psoralen at 99.1% purity and 2.1 mg of bergapten at 98.2% purity from 400 mg of the crude extract in a single run. The structures of the two compounds were identified by $^{1}$H-NMR, $^{13}$C-NMR, and MS.

**Keywords:** Bergapten, *Ficus carica* L leaves, High-speed countercurrent chromatography, Psoralen

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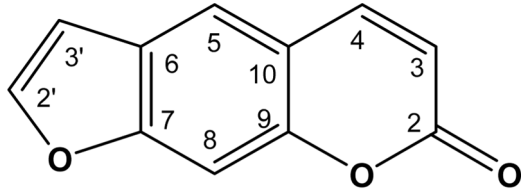
## INTRODUCTION

*Ficus carica* L., one of the oldest plant species in China, is abundant in Shandong, Xinjiang, Jiangsu Province and elsewhere. Its fruits are delicious and can be eaten by humans. Its leaves are commonly used to cure hemorrhoids and eliminate heart pain.[1] Furthermore, it has also been shown to possess antihypertension and anticancer effects.[2,3] Psoralen and bergapten are two of the major active components of *Ficus carica* L. leaves. The chemical structures of these two compounds are shown in Figure 1. Psoralen has anti-tumor, antibacterial, and antiviral activities. Bergapten is used to cure vitiligo, psoriasis, and alopecia areata.[1]

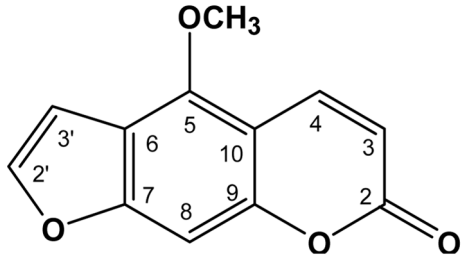
The preparative separation of psoralen and bergapten from *Ficus carica* L. leaves by classical methods is tedious and time consuming, requiring multiple chromatographic steps on silica gel column chromatography.[4] Therefore, an efficient method for the preparative isolation and purification of psoralen and bergapten from *Ficus carica* L leaves is needed.

High-speed countercurrent chromatography (HSCCC), a support free liquid-liquid partition chromatographic technique, eliminates irreversible adsorption of the sample onto the solid support and has an excellent sample recovery.[5] It has been successfully applied to isolate and purify a number of natural products.






( I ) Psoralen




( II ) Bergapten

***Figure 1.*** Structures of psoralen and bergapten.

The present paper describes a HSCCC method for separation of psoralen and bergapten from a light petroleum crude extract of *Ficus carica* L. leaves. The optimum conditions were obtained, which led to the successful separation of psoralen and bergapten with the purity of each at over 98%. The identifications of psoralen and bergapten were performed with $^{1}$H-NMR, $^{13}$C-NMR and MS.

## EXPERIMENTAL

### Materials

The dried *Ficus carica* L. leaves were provided by Henan Hengxiang Inc., Xiangcheng City, Henan province.



### Preparation of Crude Extract Sample

The dried *Ficus carica* L. leaves were ground to powder (about 30 mesh). The powder (400 g) was marinated with 2000 mL light petroleum (b.p.: 60°C–90°C) for 48 h at room temperature. After filtration, the extract was evaporated to paste by rotary vaporization at 45°C under reduced pressure. About 6 g of the crude extract was obtained and stored in a refrigerator at 4°C for the subsequent HSCCC separation.

### Selection of the Two-Phase Solvent System

The solvent system was selected according to the partition coefficients (K) of the target compounds. The K values were determined by HPLC as follows: add a small amount of the sample (typically a few milligrams or less depending on its extinction coefficient or absorptivity) to the two mutually equilibrated solvent phases (2 mL each) in a test tube. Thoroughly mix with a vortex to equilibrate the contents. After settling, pipette and deliver an equal volume of each phase into a separate test tube, and dilute with an equal volume of methanol. Analyze each phase by HPLC to determine the peak area of the target compound. The partition coefficient (K) is expressed as the solute absorbance in the upper phase divided by that of the lower phase, or $K = A_{upper}/A_{lower}$.

### Isolation and Purification of Psoralen and Bergapten with HSCCC

A preparative HSCCC instrument (Model CCC-1000, Pharma-Tech Research Corp., Baltimore, Maryland, USA) was used for the

separation of *Ficus carica* L. leaves extract. It holds three multiplayer coil separation columns connected in series (diameter of tube: 2.6 mm and total volume: 325 mL) and a 10 mL sample loop. The revolution radius or the distance between the holder axis and the central axis of the centrifuge (R) was 7.5 cm, and the β-value from 0.5 at the internal terminal to 0.8 at the external terminal ($\beta = r/R$, where r is the distance from the coil to the holder shaft). The revolution speed of the apparatus can be regulated with a speed controller in the range between 0 and 1600 rpm. The system was also equipped with a constant flow pump (Pharma-Tech Research Corp.) and a model SPD-10A UV monitor (SHIMADZU, Kyoto, Japan). The data were collected with a model EC-2000 chromatography workstation (Dalian Yilite Apparatus CO. Ltd., Dalian, China).

A two-phase solvent system composed of *n*-hexane-ethyl acetate-methanol-water (1:1:1:1, v/v) was applied for the HSCCC separation with the upper phase as the stationary phase and the lower phase as the mobile phase. In each separation, the separation coil tube was first filled with the stationary phase at the rate of 6 mL/min, then the mobile phase was pumped into the head end of the column at the rate of 2 mL/min, while the HSCCC apparatus was run at a revolution speed of 800 rpm. After hydrodynamic equilibrium was established, as indicated by a clear mobile phase eluting at the tail outlet. The *Ficus carica* L. leaves crude extract of 400 mg in 5 mL of upper phase and 5 mL of lower phase was injected into the separation column through the sample loop. Afterward, the mobile phase was delivered into the column to elute the component. The effluent from the tail end of the column was continuously monitored with a UV detector at 254 nm, and collected by an ADVANTEC SF-3120 model fraction collector, collecting 4 mL of each fraction. Each peak fraction was collected according to the chromatogram and evaporated with $N_2$ gas. The residue was sealed and stored in a refrigerator at 4°C for the subsequent HPLC analysis and identification by MS and NMR.



## HPLC Analysis

The HPLC equipment used in the study was Waters Alliance series HPLC system including a Waters 2695 Separations Module with an automated injector, a Waters 2996 photodiode array detector, a Waters HPLC workstation, and an Agilent reverse $C_{18}$ column (150 mm × 4.6 mm id,10 μm). The crude extract of *Ficus carica* L. leaves and each HSCCC peak fraction were performed at 30°C, eluting with acetonitrile-water (30:70 v/v) and the flow rate was 1.0 mL/min. The effluent was monitored at 254 nm.

## EIMS and NMR

EIMS experiments were performed on a ZAB-HS mass spectrometer (Micromass Inc., UK) analyzing ions up to $m/z$ 220. $^{1}$H- and $^{13}$C-NMR spectra were recorded in $CDCL_3$ on a Bruker ARX-400 (Bruker Inc., Switzerland) with 400 MHz for $^{1}$H- and 100.5 MHz for $^{13}$C-measurements, respectively.

## RESULTS AND DISCUSSION

### Optimization of HSCCC Conditions

As shown in Figure 2, HPLC analysis of the crude extract from *Ficus carica* L. leaves contained two major compounds. In order to achieve excellent separation of these two compounds by HSCCC, a two-phase solvent system composed of *n*-hexane-ethyl acetate-methanol-water was tested at various volume ratios to optimize K values. With a volume ratio at 1:1:1.2:0.8, the $K_{psoralen}$ was 0.56 and the $K_{bergapten}$ was 0.67, which gave too short a separation time to produce good resolution between the two peaks. The stationary phase retention at flow rate of 2 mL/min was 74% (Figure 3a). When the volume ratio of 1:1:1:1 was used, the $K_{psoralen}$ was 1.33 and the $K_{bergapten}$ was 1.67, which gave a longer separation time, but yielded much better peak resolution. The stationary phase retention at flow rate of 2 mL/min was 70% (Figure 3b). The crude extracts from *Ficus carica* L. leaves were separated and purified under the



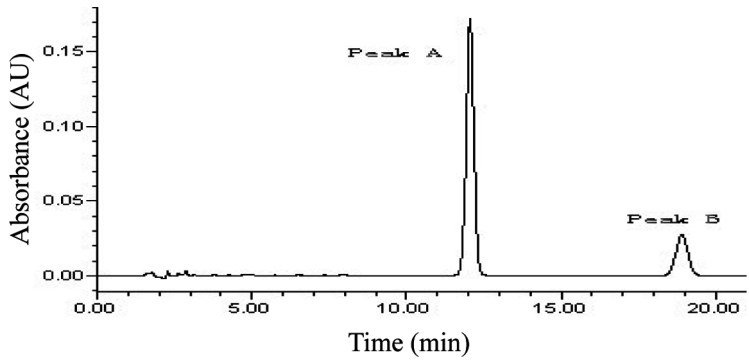



***Figure 2.*** HPLC chromatogram of crude extract with light petroleum from *Ficus Carica* L. leaves. Conditions: column: Agilenti reverse $C_{18}$ column(150 × 4.6 mm id,10 μm); column temperature: 30°C; mobile phase: acetonitrile-water(30:70 v/v); flow rate: 1.0 mL/min; detection wavelength: 254 nm.

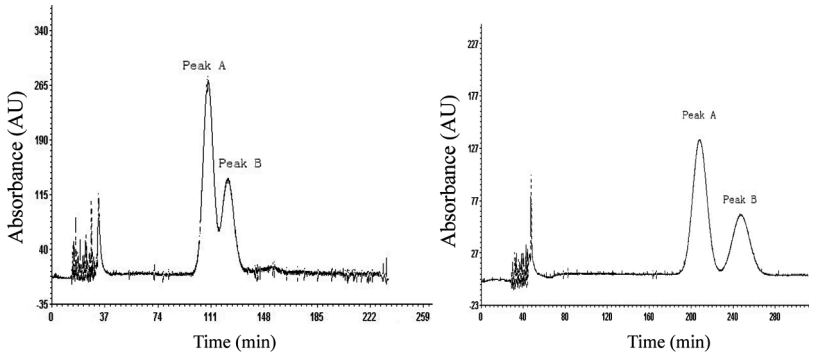



***Figure 3.*** HSCCC chromatograms of crude extract from *Ficus Carica* L leaves. Two-phase solvent system: *n*-hexane-ethyl acetate-methanol-water at 1:1:1.2:0.8 (Figure 3a) and 1:1:1:1 (Figure 3b); stationary phase: upper phase; mobile phase: lower phase; the flow rate of mobile phase: was 2 mL/min; revolution speed was 800 rpm; retention of the stationary phase was about 74% (*n*-hexane-ethyl acetate-methanol-water at 1:1:1.2:0.8) and 70% (*n*-hexane-ethyl acetate-methanol-water at 1:1:1:1); detection wavelength: 254 nm; sample size: 400 mg crude extract sample dissolved in 5 mL up phase and 5 mL low phase; separation temperature: room temperature.



above optimum HSCCC conditions. Psoralen was obtained at 180 min to 230 min, while bergapten was obtained at 240 min to 270 min (Figure 3b). The purity of psoralen and bergapten was determined by HPLC.

## HPLC Analysis and Identification of HSCCC Peak Fractions

The purity of each peak fraction of HSCCC separation was determined by HPLC. The HPLC chromatogram of each fraction was shown in Figure 4 (a and b). The purity of psoralen (peak A in Figure 4a) and bergapten (peak B in Figure 4b) was 99.1% and 98.2%, respectively.

The identification of each peak fraction in Figure 4 was performed with $^{1}$H-NMR, $^{13}$C-NMR, and MS. $^{1}$H-NMR, $^{13}$C-NMR and MS data of each fraction were given as follows: HSCCC peak A:$^{1}$H-NMR, (400 MHz, $CDCL_3$) δ(ppm):6.38(1H,d,J = 9.6Hz,H-3), 6.84(1H,dd,J = 0.6,1.3 Hz,H-3′), 7.47(1H,s,H-8), 7.67(1H,d,J = 3.0 Hz,H-5),7.70(1H,d,J = 2.2 Hz,H-2′), 7.81 (1H,d,J = 9.6 Hz,H-4); $^{13}$C-NMR, (100.5 MHz,$CDCL_3$) δ (ppm): 161.0 (s,C-2),114.6 (d,C-3),144.0 (d,C-4),119.8 (d,C-5),124.8 (s,C-6), 156.4 (s,C-7),99.8 (d,C-8),152.0 (s,C-9), 115.4 (s,C-10),146.9 (d,C-2′),106.3 (d,C-3′);EIMS $m/z$ 186$[M]^+$ (80),158 $[M\text{-}CO]^+$ (100),130 $[M\text{-}2CO]^+$ (18),102 $[M\text{-}3CO]^+$ (40),76 (16).

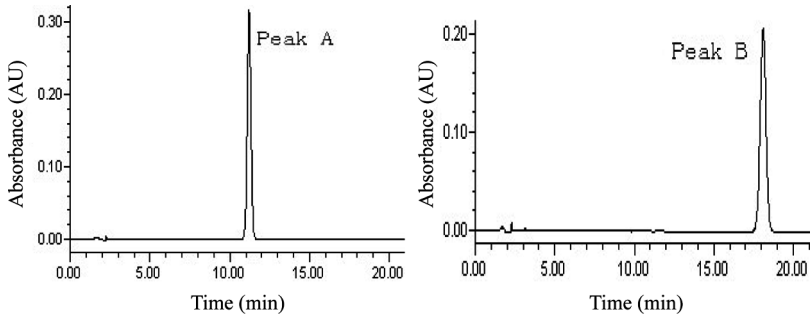



***Figure 4.*** HPLC chromatograms of HSCCC peak A and peak B fractions. Conditions: column: Aglient reverse $C_{18}$ column($150 \times 4.6$ mm id,10 μm); column temperature: 30°C; mobile phase: acetonitrile-water (30:70 v/v); flow rate: 1.0 mL/min; detection wavelength: 254 nm.



HSCCC peak B:$^{1}$H-NMR, (400 MHz,$CDCL_3$) δ (ppm):4.27 (3H,s,-$OCH_3$),6.27 (1H,d,J = 9.8 Hz,H-3),7.59 (1H,d,J = 2.1 Hz,H-2′),7.13 (1H, s,H-8),7.02 (1H,s,H-3′),8.15 (1H,d,J = 9.8 Hz,H-4); $^{13}$C-NMR, (100.5 MHz,$CDCL_3$) δ (ppm):161.2 (s,C-2),112.5 (d,C-3),139.2 (d,C-4),149.5 (d,C-5),112.6 (s,C-6),158.3 (s,C-7),93.8 (d,C-8), 152.7 (s, C-9),106.4 (s,C-10),144.7 (d,C-2′),105.0 (d,C-3′),60.0 (q,-$OCH_3$); EIMS *m/z* 216$[M]^+$ (100),201(36),188 (14),173 (65),145 (30).

According to the References,[6,7] peak A and peak B in Figure 3 were identified as psoralen and bergapten, respectively.

In conclusion, HSCCC was successfully used for the isolation and purification of psoralen and bergapten from *Ficus carica* L. leaves. Psoralen, 4.4 mg, at 99.1% purity and 2.1 mg of bergapten at 98.2% purity can be obtained from 400 mg of the crude extract in one step separation.

## ACKNOWLEDGMENT

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