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An efficient new method for extraction, separation and purification of psoralen and isopsoralen from Fructus Psoraleae by supercritical fluid extraction and high-speed counter-current chromatography

Xiao Wang[a,b], Yuqiang Wang[c], Jinpeng Yuan[b], Qinglei Sun[b], Jianhua Liu[b], Chengchao Zheng[a,*]

[a] *College of Life Sciences, Shandong Agricultural University, 18 Daizong Street, Taian, Shandong 271018, China*
[b] *Test Center, Shandong Academy of Sciences, 19 Keyuan Street, Jinan, Shandong 250014, China*
[c] *Panorama Research Inc., 2462 Wyandotte Street, Mountain View, CA 94043, USA*

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Abstract

Psoralen and isopsoralen were extracted from Fructus Psoraleae (*Psoralea corylitolia* L.) by supercritical CO_2. The effect of various parameters, i.e., pressure, temperature and sample particle size on yield was investigated with an analytical-scale supercritical fluid extraction (SFE) system to find the optimal conditions. The process was then scaled up by 50 times with a preparative SFE system under the optimized conditions of pressure (26 MPa), temperature (60 °C) and a sample particle size of 40–60 mesh. The yield of the preparative SFE was 9.1% and the combined yield of psoralen and isopsoralen was 2.5 mg/g of dry seeds. Psoralen and isopsoralen in the extract were separated and purified by high-speed counter-current chromatography with a two-phase solvent system composed of *n*-hexane–ethyl acetate–methanol–water (1:0.7:1:0.8, v/v), and the fractions were analyzed by HPLC, MS, ^{1}H NMR and ^{13}C NMR. The structures of the products were further confirmed by comparison with authentic samples (National Institute of the Control of Pharmaceutical and Biological Products, Beijing, China).

Keywords: Supercritical fluid extraction (SFE); Counter-current chromatography; *Psoralea corylitolia*; Psoralen; Isopsoralen; Preparative chromatography

1. Introduction

Fructus Psoraleae (*Psoralea corylitolia* L., Buguzhi in Chinese) is one of the most popular traditional Chinese medicines. This herb is used to treat a wide variety of human diseases including impotence, seminal emission, cold pain in the loins and knees, frequent urination and enuresis due to deficiency of the kidney [1]. The major bioactive components of Buguzhi are psoralen and isopsoralen (Fig. 1) [2]. Psoralens and isopsoralen can be activated by light; as a result, they are widely used as diagnostic agents in biological systems, and are also used as photo-therapeutic agents [3]. For example, psoralen is used as a treatment for topical skin diseases such as psoriasis and vitiligo [4–6] and as probes in nucleic acid research [7,8]. Recently, psoralen and isopsoralen have been shown to be effective against cutaneous T-cell lymphoma and other autoimmune diseases [6,9]. The biological role of psoralens is due to their activity towards DNA. Psoralens bind to DNA covalently after exposure to UV light and induce DNA interstrand cross-links [10,11].

The preparative separation and purification of psoralen and isopsoralen from plant materials by classical methods are tedious and usually require multiple chromatographic steps on silica gel [12,13]. Because of the important biological properties and broad applications, it is urgent to develop an efficient method to extract, separate and purify psoralens. Psoralens can be chemically synthesized; however, in view of the increasing environmental and health concerns about

* Corresponding author. Tel.: +86 538 8242894; fax: +86 538 8226399.
E-mail address: cczheng@sdau.edu.cn (C. Zheng).

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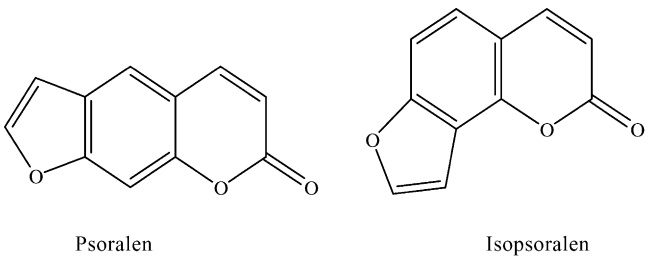

Fig. 1. Chemical structures of psoralen and isopsoralen.

the use of organic solvents and the production of toxic waste in organic synthesis, there has been growing interest in using supercritical fluids for extraction and isolation of products from natural sources since it requires less solvent, has a short extraction time and is capable of extracting thermally labile compounds under mild conditions. Although supercritical CO_2 has been used for extraction and isolation of valuable compounds from natural products over the past three decades [14–17], very little is known about the extraction and purification parameters of psoralens by SFE.

In the present study, the extraction condition was optimized first with an analytical-scale SFE system with an orthogonal test. Then, the extraction was scaled up by 50 times with a preparative-scale SFE system. The crude extract obtained was then purified by high-speed counter-current chromatography (HSCCC). HSCCC is a unique liquid–liquid partition chromatography technique that uses no solid support matrix. HSCCC eliminates the irreversible adsorptive loss of samples onto the solid support matrix as used in the conventional chromatographic column. This method has been successfully applied to the analysis and separation of various natural products [18–20]. However, no report has been published on the use of HSCCC for the separation and purification of psoralens from the Chinese herbs. We herein report an efficient new method for extraction, separation and purification of psoralen and isopsoralen from the Chinese medicinal plant *Psoralea corylitolia*.

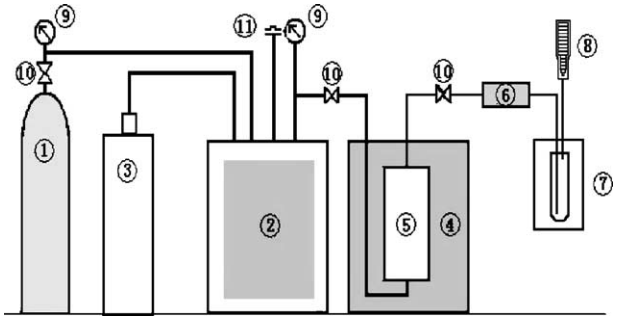

Fig. 2. SFE system: (1) carbon dioxide; (2) high-pressure cylinder and pressure control system; (3) compressed air; (4) thermostatic control system; (5) extraction vessel; (6) control and collection system; (7) collection vial; (8) flow-meter; (9) pressure gauge; (10) valve; (11) rupture disk.

2. Experimental

2.1. Reagents

Carbon dioxide (99.9%) was obtained from Tianhai Gas Company, Jinan, China. Organic solvents including ethanol, hexane, ethyl acetate and methanol were all of analytical grade and were purchased from Beijing Chemical Factory, Beijing, China. HPLC-grade methanol was from Siyou Tianjin Chemical Factory, Tianjin, China. Psoralen and isopsoralen were purchased from the National Institute of the Control of Pharmaceutical and Biological Products, Ministry of Health, Beijing, China. The dry seeds of *P. corylitolia* L. were obtained from Linyi, Shandong, China.

2.2. Optimization of SFE conditions

A *Spe-ed* SFE system (Applied Separations, Inc., USA**)** is illustrated in Fig. 2. A 50-ml extraction cell was used to optimize extraction conditions. In order to determine a suitable extraction condition in a wide range with a minimum number of trials, An orthogonal test design L_9 $(3)^3$ was employed where temperature, pressure and the sample's particle size were considered to be three major factors for effective extraction. Combinations of the three different levels of each factor were listed in Table 1. In each test, 10 g of the milled and sieved *P. corylitolia* L. seeds was placed into an extraction cell. Carbon dioxide with a purity of 99.9% was used as a solvent. After 1 h of static extraction (no liquid flow), the sample was then subjected to dynamic extraction by flowing gaseous carbon dioxide at a rate of 2 l/min for 1 h. The extract was trapped into a collection vessel containing about 100 ml of ethanol, and the sample was then analyzed by HPLC.

2.3. SFE scaling up

After the extraction conditions were optimized, the extraction was then scaled up by approximate 50 times with a preparative-scale SFE system. The seeds (500 g, 40–60 mesh) were placed into a 1-l extraction vessel and were extracted statically for 1 h followed by another 5 h dynamically under the optimized conditions at 60 °C and 26 MPa. The flow-rate

Table 1
L_9 $(3)^3$ orthogonal test design

Test no.	A (pressure, MPa)	B (temperature, °C)	C (particle size, mesh)
1	A_1 26	B_1 40	C_1 10–20
2	A_1 26	B_2 50	C_2 20–40
3	A_1 26	B_3 60	C_3 40–60
4	A_2 30	B_1 40	C_2 20–40
5	A_2 30	B_2 50	C_3 40–60
6	A_2 30	B_3 60	C_1 10–20
7	A_3 34	B_1 40	C_3 40–60
8	A_3 34	B_2 50	C_1 10–20
9	A_3 34	B_3 60	C_2 20–40

of carbon dioxide supercritical fluid was set at 2 l/min, and the extract in the supercritical fluid was depressed directly into a separate vessel. The extract was washed with petroleum ether and was then subjected to purification by HSCCC.

2.4. Measurement of partition coefficient

Two millilitres of each phase of the equilibrated two-phase solvent system was added to approximately 1 mg of a test sample placed in a 10-ml test tube. The test tube was caped, and was shaken vigorously for 1 min to equilibrate the sample thoroughly. An equal volume of each phase was then analyzed by HPLC to obtain the partition coefficients (K). The K value was expressed as the peak area of the compound in the upper phase divided by the peak area of the compound in the lower phase.

2.5. HSCCC separation procedure

The preparative HSCCC instrument employed in the present study is a Model GS10A-2 multilayer coil planet centrifuge (Beijing Institute of New Technology Application, Beijing, China) equipped with a polytetrafluoroethylene multilayer coil of 110 m × 1.6 mm i.d. with a total capacity of 230 ml. The β value of the preparative column varied from 0.5 at internal to 0.7 at the external. The rotation speed is adjustable from 0 to 1000 rpm, and 800 rpm was used in the present study. The system was also equipped with a NS-1007 constant flow pump, a Model 8823A-UV monitor operating at 254 nm, a Yakogawa 3057 recorder and a manual injection valve with a 15-ml sample loop.

The two-phase solvent system utilized in the present study was composed of *n*-hexane–ethyl acetate–methanol–water (1:0.7:1:0.8, v/v). After the mixture in a separation funnel was thoroughly equilibrated at room temperature, the two phases were separated shortly before use, where the organic phase was used as the stationary phase, and the aqueous phase as the mobile phase.

In each separation, the multiplayer coiled column was first filled entirely with the upper organic phase as the stationary phase. Then, the lower aqueous phase was pumped into the head end of the column at a suitable flow-rate of 1.5 ml/min for Model GS10A-2, while the apparatus was rotated at an optimal speed of 800 rpm. After hydrodynamic equilibrium was reached, as indicated by a clear mobile phase eluting from the tail outlet, the sample solution was injected through the injection valve. The effluent from the tail end of the column was continuously monitored by UV detection at 254 nm, and the peak fractions were collected according to the chromatogram.

2.6. HPLC analyses and identification of HSCCC fractions

The HPLC system used throughout this study consisted of a Waters 660 pump, a Waters 660 controller (Waters, USA), a sample injector (Rheodyne, USA) with a 10-μl loop and a Waters 996 photodiode array detector. Evaluation and quantification were made on a Millenium 32 workstation (Waters).

The crude extract and each purified fraction from the preparative HSCCC separation were analyzed by HPLC with a Shim-pack VP-ODS column (250 mm × 4.6 mm i.d.) at 247 nm and at a column temperature of 25 °C. The mobile phase, a solution of methanol and water (55:45, v/v), was set at a flow-rate of 1 ml/min. The effluent was monitored by a photodiode array detector [21]. All the calculations concerning the quantitative analysis were performed with external standardization by the measurement of peak areas.

The identification of HSCCC peak fractions was carried out, respectively, by MS on an Agilent 5973N mass spectrometer and by ^{1}H NMR and ^{13}C NMR spectra on a Varian 300 MHz NMR spectrometer.

3. Results and discussion

3.1. Optimization of temperature, pressure and sample particle size for maximum SFE efficiency

The products obtained from each L_9 $(3)^3$ test of the analytical SFE were quantitatively analyzed, and the results were shown in Table 2. The maximum extraction yield of psoralen and isopsoralen was 1.100 and 1.344 mg/g of dry seeds, respectively. In each test, the concentration of psoralen in the extract was lower than that of isopsoralen.

Extraction efficiencies at different sets of temperature, sample particle size and pressure were examined under L_9 $(3)^3$ test design. The results shown in Table 2 indicate that there are great yield differences among each set of SFE conditions. If the yield of psoralen and isopsoralen was expressed as a control index, the results in Table 2 are transformed to Table 3 after orthogonal analysis.

The sample particle size was found to be the most important determinant of the yield. The yield of psoralen and isopsoralen significantly increased as the particle size decreased

Table 2
L_9 $(3)^3$ test results

Test no.	A, 1	B, 2	C, 3	Yield (%)[a]	Yield (mg/g)[b]	
					Psoralen	Isopsoralen
1	A_1	B_1	C_1	5.0	0.380	0.463
2	A_1	B_2	C_2	6.7	0.580	0.756
3	A_1	B_3	C_3	9.8	1.100	1.344
4	A_2	B_1	C_2	7.3	0.553	0.678
5	A_2	B_2	C_3	9.3	0.956	1.159
6	A_2	B_3	C_1	5.1	0.361	0.456
7	A_3	B_1	C_3	9.9	1.035	1.244
8	A_3	B_2	C_1	5.8	0.403	0.498
9	A_3	B_3	C_2	8.9	0.607	0.763

[a] Extraction yield (%) = (the amount of extract/sample mass) × 100.
[b] Extraction yield (mg/g) = the amount of psoralen or isopsoralen in extract/sample mass.

Table 3
Analysis of L_9 $(3)^3$ test results

	Psoralen yield (mg/g)			Isopsoralen yield (mg/g)		
	A	B	C	A	B	C
K_1	2.060[a]	1.968	1.144	2.563	2.385	1.417
K_2	1.870	1.939	1.740	2.293	2.413	2.197
K_3	2.045	2.068	3.091	2.505	2.563	3.747
k_1	0.687[b]	0.656	0.381	0.854	0.795	0.472
k_2	0.623	0.646	0.580	0.764	0.804	0.732
k_3	0.682	0.689	1.030	0.835	0.854	1.249
R	0.064[c]	0.043	0.649	0.090	0.059	0.777
Optimal level	A_1	B_3	C_3	A_1	B_3	C_3

[a] K_i^A = Σextraction yield at A_i.
[b] $k_i^A = K_i^A/3$.
[c] $R_i^A = \max\{k_i^A\} - \min\{k_i^A\}$.

(Fig. 3). Pressure and temperature have no significant influence on the yield of psoralen and isopsoralen, the 26 MPa of pressure and 60 °C of temperature, however, seem favorable for the extraction of psoralens (Fig. 3 and Table 3). These results indicate that the optimal conditions for extraction of psoralen and isopsoralen by SFE were 26 MPa of pressure, 60 °C of temperature and 40–60 mesh of sample particle size.

3.2. Preparative-scale SFE

Under the optimal conditions, 500 g of dry seeds was extracted by SFE, affording 45.3 g of extract. The extract contained 1.21% psoralen and 1.48% isopsoralen (Fig. 4A). The combined yield of psoralen and isopsoralen was approximately 2.5 mg/g of dry seeds. We obtained 2.0 g of crude psoralens after the extract was washed with petroleum ether, where psoralen and isopsoralen were present at 26.0% and 27.0%, respectively, according to the HPLC analysis (Fig. 4B).

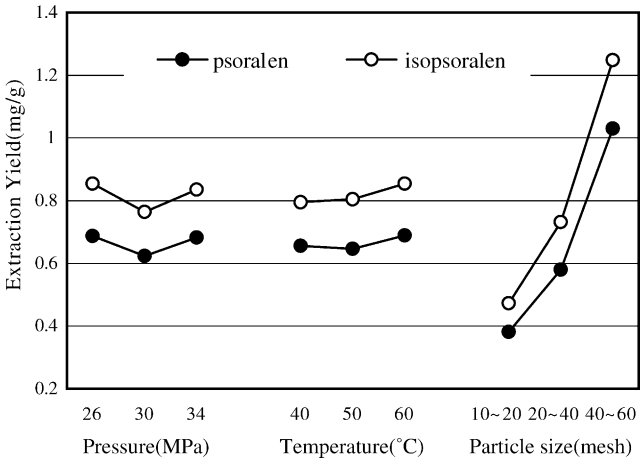

Fig. 3. Effects of pressure, temperature and sample particle size on yield of psoralen and isopsoralen.

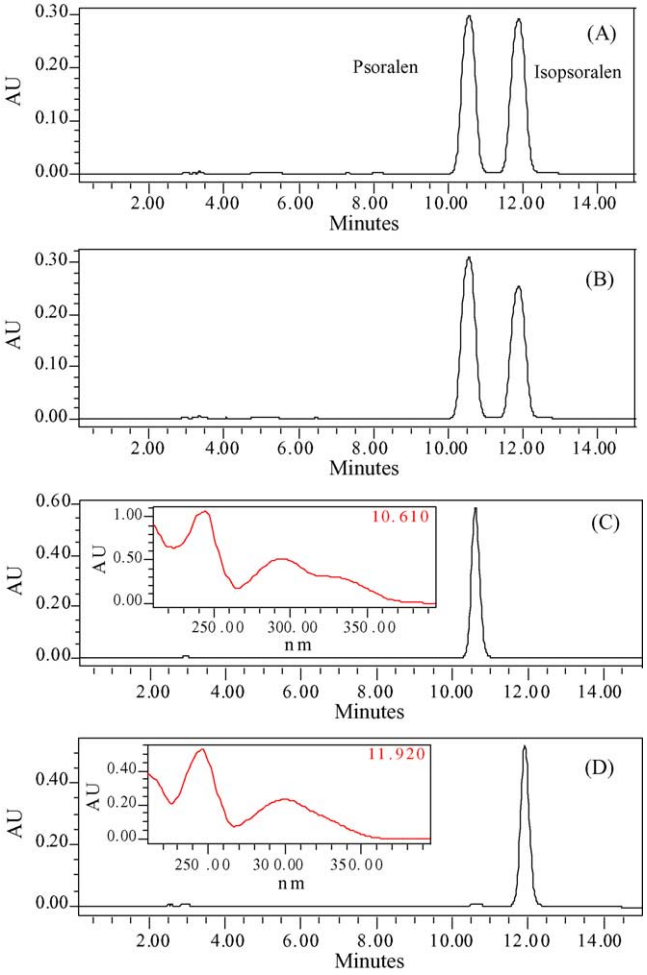

Fig. 4. (A) HPLC chromatogram of the extract from preparative SFE; (B) HPLC chromatogram of the extract from preparative SFE after cleaning up with petroleum ether; (C) HPLC analyses and UV spectrum of the psoralen purified with HSCCC; (D) HPLC analyses and UV spectrum of the isopsoralen purified with HSCCC. Conditions—a Shim-pack VP-ODS column (250 mm × 4.6 mm i.d.); column temperature: 25 °C; mobile phase: methanol–water (55:45, v/v); flow-rate: 1.0 ml/min; detection wavelength: 247 nm.

3.3. Measurement of partition coefficients

With HSCCC, a successful separation necessitates a careful search for a suitable two-phase solvent system, which provides an ideal range of the partition coefficient (K) for the applied sample. Generally, the two-phase solvent system needs to satisfy the following requirements [22,23]: (1) for ensuring a satisfactory retention of the stationary phase, the settling time of the solvent system should be considerably shorter than 30 s; (2) for an efficient separation, the partition coefficient (K) value of the target compounds should be close to 1, and the separation factor between the two components ($\alpha = k_2/k_1$, $k_2 > k_1$) should be greater than 1.5. A smaller K value may result in a loss of peak resolution, while a larger one produces excessive sample band broadening. In this case, the K value was determined after partitioning the

Table 4
Partition coefficients (K) and separation factors (a) of these compounds

Solvent systems	Psoralen K_1	Separation factors (a)	Isopsoralen K_2
Chloroform–methanol–water			
4:4:2	0.10	1.2	0.12
n-Hexane–ethyl acetate–methanol–water			
1:0.4:1:0.4	0.16	1.50	0.24
1:0.5:1:0.5	0.65	0.98	0.64
1:0.6:1:0.6	0.58	1.12	0.65
1:0.7:1:0.8	0.98	1.51	1.48

sample between the two solvent phases, and aliquots of the upper and lower layers were analyzed by HPLC. From these two chromatograms, the K value of each component was determined by computing the ratio of the peak heights (or areas) between the corresponding peaks.

Firstly, we used chloroform–methanol–water (4:4:2, v/v), but the resulted K value was too small. Then we used a two-phase solvent system composed of *n*-hexane–ethyl acetate–methanol–water because the latter solvents provided a broad range of hydrophobicity by modifying the volume ratio of the four solvents [22]. The solvents with *n*-hexane–ethyl acetate–methanol–water ratios ranging from 1:0.4:1:0.4, 1:0.5:1:0.51:0.6:1:0.6 and 1:0.7:1:0.8 can be used to separate the samples (Table 4). The optimal solvent system for separation contained *n*-hexane–ethyl acetate–methanol–water in a ratio of 1:0.7:1:0.8 (v/v) (Fig. 5).

3.4. Purification of psoralen and isopsoralen by HSCCC

The crude extract (160 mg) was separated and purified by HSCCC. The retention of the stationary phase was 70.0%, and the separation time was about 180 min in each separation run. Separation of the crude extract by the preparative HSCCC with *n*-hexane–ethyl acetate–methanol–water (1:0.7:1:0.8, v/v) as a solvent system was shown in Fig. 5. The HSCCC fractions were analyzed by HPLC, and the absorbance was measured at 254 nm to draw an elution curve (Fig. 5). As expected, the components were eluted in the order of peak a (psoralen) followed by peak b (isopsoralen). The fractions were pooled (Fig. 4C and D). The separation produced 39 and 40 mg of psoralen and isopsoralen with recovery of 94% and 93%, respectively. In the crude psoralens, the content of psoralen and isopsoralen was 26.0% and 27.0%, respectively (Fig. 4B). After only one-step of operation by HSCCC, the purity of the components was increased to 99.2% (Fig. 4C) and 99.0% (Fig. 4D), respectively. These results demonstrate the high resolving power of HSCCC.

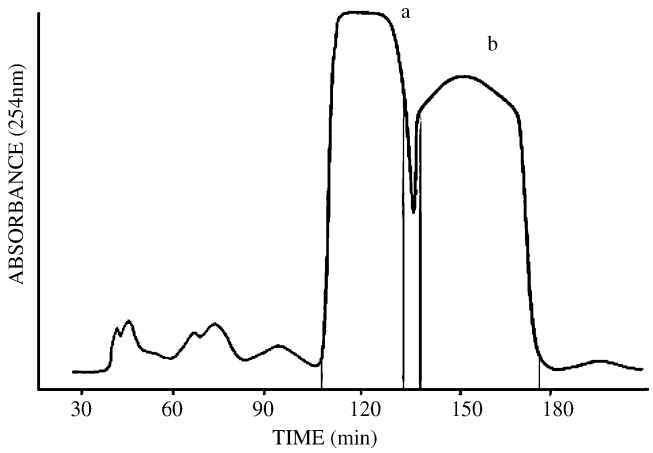

Fig. 5. Chromatogram of the crude extract by preparative HSCCC. Conditions—column: multilayer coil of 1.6 mm i.d., PTFE tube with a total capacity of 230 ml; rotation speed: 800 rpm; solvent system: *n*-hexane–ethyl acetate–methanol–water (1:0.7:1:0.8, v/v); stationary phase: upper organic phase; mobile phase: lower aqueous phase; flow-rate: 1.5 ml/min; detection wavelength: 254 nm; sample size: 160 mg; injection volume: 15 ml; retention of stationary phase: 70.0%; a: psoralen; b: isopsoralen.

3.5. Identification of psoralen and isopsoralen

The structural identification of the psoralen (peak a) was carried out by MS, ^{1}H NMR and ^{13}C NMR spectra. The MS data are as follows: *m*/*z*: 186 (M^+, 100), 158, 130, 102, 76, 51, 50. ^{1}H NMR (300 MHz, C^2HCl_3) δ ppm: 6.37 (1H, d, J = 9.3, H-3), 6.83 (1H, m, H-11), 7.48 (1H, s, H-8), 7.68 (1H, s, H-5), 7.69 (1H, d, J = 2.4, H-12), 7.87 (1H, d, J = 9.9, H-4). ^{13}C NMR (300 MHz, C^2HCl_3) δ ppm: 161.0 (C-2), 114.6 (C-3), 144.1 (C-4), 119.8 (C-5), 124.9 (C-6), 156.4 (C-7), 99.9 (C-8), 152.0 (C-9), 115.4 (C-10), 106.3 (C-11), 146.9 (C-12).

The structural identification of the isopsoralen (peak b) was similarly carried out as follows: *m*/*z*: 158 (M^+, 100), 186, 130, 102, 76, 75, 51, 50. ^{1}H NMR (300 MHz, C^2HCl_3) δ ppm: 6.38 (1H, d, J = 9.3, H-3), 7.13 (1H, m, H-3′), 7.37 (1H, d, J = 8.4, H-5), 7.43 (1H, d, J = 8.4, H-6), 7.70 (1H, m, H-2′), 7.80 (1H, d, J = 9.3, H-4). ^{13}C NMR (300 MHz, C^2HCl_3) δ ppm: 160.8 (C-2), 114.1(C-3), 144.5 (C-4), 123.8 (C-5), 108.7 (C-6), 157.3 (C-7), 116.9 (C-8), 148.5 (C-9), 113.5 (C-10), 145.9 (C-2′), 104.1 (C-3′).

The structures of the products were further confirmed by comparison with authentic samples.

4. Conclusion

Psoralen and isopsoralen from the traditional Chinese medicine *P. corylitolia* L. were extracted, separated and purified by the supercritical fluid extraction technique. Under optimal conditions i.e., a pressure of 26 MPa, a temperature of 60 °C and a sample particle size of 40–60 mesh, the combined yields of psoralen and isopsoralen were 2.5 mg/g of dry seeds. From a crude SFE extract, psoralen and isopsoralen were obtained with greater than 99% purity by HSCCC with a two-phase solvent system composed of *n*-hexane–ethyl acetate–methanol–water (1:0.7:1:0.8, v/v) in one step. The results of the present study demonstrate that SFE and HSCCC are very useful techniques for the extraction, separation and purification of psoralen and isopsoralen from *P. corylitolia* L.

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References

[1] Z.Q. Lei (Ed.), The Chinese Materia Medica, Shanghai Science and Technology Press, Shanghai, 1995, p. 289.
[2] L. Ji, Z.L. Xu, J. Chin. Materia Med. 20 (1995) 120.
[3] V. Boyer, E. Moustacchi, E. Sage, Biochemistry 27 (1988) 3011.
[4] J.A. Parrish, R.S. Stem, M. Pathak, T.B. Fitzpatrick, in: J.D. Regan, J.A. Parrish (Eds.), The Science of Photomadicine, Plenum Press, New York, 1982, p. 595.
[5] K. Danno, in: A. Shima (Ed.), Frontiers of Photobiology, Elsevier, New York, 1993, p. 97.
[6] M.A. Pathak, T.B. Fitzpatrick, J. Photochem. Photobiol. B 14 (1992) 3.
[7] G.D. Cimino, H.B. Gamper, S.T. Isaacs, J.E. Hearst, Annu. Rev. Biochem. 54 (1985) 1151.
[8] M.E. Zolan, C.A. Smith, P.C. Hanawalt, Biochemistry 23 (1984) 63.
[9] J.Z. Wu, Z.Q. Situ, J.Y. Chen, F. Li, Y. Li, B. Liu, J. Fourth Mil. Med. Univ. 21 (2000) 911.
[10] E.J. Gunther, T.M. Yeasky, F.P. Gasparro, P.M. Glazer, Cancer Res. 55 (1995) 1283.
[11] E. Ben-Hur, A.C. Moor, H. Margolis-Nunno, P. Gottlieb, M.M. Zuk, S. Lustigman, B. Horowitz, A. Brand, J. Van Steveninck, T.M. Dubbelman, Transfus. Med. Rev. 10 (1996) 15.
[12] F. Huang, X.F. Huang, W.P. Liang, F.H. Ge, H.Q. Wu, Zhong Yao Cai 23 (2000) 266.
[13] Y. Yang, J. Zhang, Y.T. Chen (Eds.), Handbook of Compositions from Crude Drug, Extraction, Separation and Purification, Chinese Traditional Medicine Press, Beijing, 2002, p. 410.
[14] N.H. Mermelstein, Food Technol. 53 (1999) 78.
[15] E. Martinelli, K. Schulz, G.A. Mansoori, in: T.J. Bruno, J.F. Ely (Eds.), Supercritical Fluid Technology, CRC, Boca Raton, FL, 1991, p. 451.
[16] R. Hartono, G.A. Mansoori, A. Suwono, Chem. Eng. Sci. 56 (2001) 6949.
[17] J.M. del Valle, J.M. Aguilera, Food Sci. Technol. Int. 5 (1999) 1.
[18] Y. Ito, CRC Crit. Rev. Anal. Chem. 17 (1986) 65.
[19] T.Y. Zhang, Y. Ito, W.D. Conway (Eds.), High-Speed Countercurrent Chromatography, Chemical Analysis Series, vol. 132, Wiley-Interscience, New York, 1996, p. 225.
[20] X. Wang, Y. Wang, Y. Geng, F. Li, C. Zheng, J. Chromatogr. A 1038 (2004) 171.
[21] K.Y. Deng, Chin. Trad. Patent Med. 23 (2001) 643.
[22] H. Oka, K.I. Harada, Y. Ito, J. Chromatogr. A 812 (1998) 32.
[23] Y. Ito, in: N.B. Mandava, Y. Ito (Eds.), Countercurrent Chromatography: Theory and Practice, Marcel Dekker, New York, 1988, p. 443.