Journal of Medicinal Plants Research Vol. 5(1), pp. 104-108, 4 January, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Preparative purification of coniferyl ferulate from *Angelica sinensis* oil by high performance centrifugal partition chromatography

Yue Yu, Qing-Wen Zhang* and Shao-Ping Li

Institute of Chinese Medical Sciences, University of Macau, Taipa, Macau SAR, China.

Accepted 18 November, 2010

A high performance centrifugal partition chromatography (HPCPC) method was successfully developed to purify coniferyl ferulate (CF) from the extracts of commercial *Angelica sinensis* oil by using *n*-hexane-ethyl acetate-ethanol-water (5:5:5:5, v/v) as the two-phase solvent system. The upper and lower phases of this system were used as stationary and mobile phases, respectively. The purity of coniferyl ferulate was over 98% as determined by high-performance liquid chromatography (HPLC). The structure of coniferyl ferulate was identified by MS, ^{1}H NMR and ^{13}C NMR.

Key words: *Angelica sinensis*, coniferyl ferulate, high performance centrifugal partition chromatography, solvent extraction.

INTRODUCTION

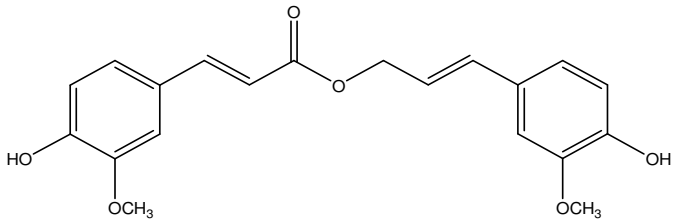
Angelica sinensis (Oliv.) Diels (Chinese name Danggui) is one of the most commonly used traditional Chinese medicines and used to enrich blood, activate blood circulation, regulate menstruation and amenorrhoea, relieve pain, relax bowels and so on (Pharmacopoeia Commission of PCR, 2005). Over 70 compounds such as phthalides, terpenes and aromatic compounds have been isolated and identified from Danggui (Lao et al., 2004). Its main components such as *Z*-ligustilide, ferulic acid and other phthalides are thought to be the biologically active components of *A. sinensis* (Lin et al., 1998). However, ferulic acid is rarely found in the free form in plants (Ou et al., 2004). Actually, its ester, coniferyl ferulate (CF, Figure 1) with activities of antioxidant (Li et al., 2007), anticancer (Zou et al., 2005), antibacterial (Chou et al., 2006), vasodilating (Naito et al., 1995) and inhibiting secretion of gastric acid (Chung et al., 2005), is rich in two well-known Chinese medicines of *A. sinensis* (*Danggui*) (Lu et al., 2005; Li et al., 2007) and *Ligusticum chuanxiong* (*Chuanxiong*) (Yan et al., 2005), and other plants (Kobayashi et al., 1984; Naito et al., 1995; Chou et al., 2006).

Centrifugal partition chromatography (CPC), which was first invented by K. Nunogaki (Foucault, 1994) is a kind of chromatography technique which uses a liquid-liquid biphasic system without solid support to maintain the stationary phase. Basically, a CPC instrument is a constant gravity field produced by a single axis rotation, together with rotatory seals for supply of solvent. Separation takes place in cartridge or disks (Berot et al., 2007).

Due to their liquid nature, the upper and lower phases of the biphasic system can be selected as mobile or stationary phases (ascending and descending modes) (Marston et al., 2006). Therefore, this type of chromatography does not suffer from irreversible sample adsorption or peak tailing and has the advantage of a great many choices of solvent systems, high recovery of the sample and relatively mild chromatographic conditions (Ingkaninan et al., 2000; Kim and Kim, 2007).

Furthermore, compared to conventional CCC (counter current chromatography), it offers shorter running time and higher loading capacity (Ingkaninan et al., 2000). In previous study, HPCPC and HSCCC have been successfully applied for separation and purification of

*Corresponding author. E-mail: qwzhang@umac.mo. Tel: +853-8397 4879. Fax: +853-2884 1358.



Coniferyl ferulate

Figure 1. Chemical structure of coniferyl ferulate.

Table 1. K values of coniferyl ferulate in *n*-hexane-ethyl acetate-ethanol-water.

Solvent system(V/V)	K (CF)
5:5:5.5:4.5	0.43
5:5:5:5	0.67
5:5:4.5:5.5	1.37

active natural products (Foucault et al., 1998). To date, HSCCC has been successfully applied for separation and purification of ferulic acid from *A. sinensis* (Liu et al., 2006). However, no report has been published on the use of HPCPC and/or HSCCC for isolation and purification of coniferyl ferulate. This paper describes an HPCPC method for separation and purification of coniferyl ferulate from commercial essential oil of *A. sinensis*.

EXPERIMENTAL

Chemicals and materials

Acetic acid, ethyl acetate and acetonitrile for liquid chromatography were purchased from Merk (Damstadt, Germany). *n*-hexane (Beichen, China) and *n*-heptane (Xilong, China) were analytical grade. Water was prepared using a Millipore Milli-Q Plus system (Millipore, Bedford, MA, USA).

Essential oil of Radix *A. sinensis* (AS oil) extracted using supercritical fluid extraction (SFE), was purchased from Guangzhou Honsen Sunshine BioScience and Technology Co. Ltd.

Apparatus

High performance liquid chromatography instrument employed in the present study is CPC240 (Everseiko Corporation, Tokyo, Japan). The solvent was pumped into the rotor with S1021 solvent delivery system (Syknm German) with a 5 ml sample loop; the flow rate is up to 30 ml/min. The continuous monitoring of the effluent was achieved with a WAD monitor (Agilent Technologies, USA), a multi-wavelength UV-Vis monitor for simultaneous monitoring of up to five wavelengths in the range of 190 to 800 nm and a CF-1 fraction collector (Spectrum, USA) was also employed.

HPLC-DAD-MS analysis was performed on an Agilent series 1100 (Agilent Technologies, USA) liquid chromatograph, equipped with a vacuum degasser, a quaternary pump, an autosampler and a diode array detection (DAD) system and an ion-trap mass spectrometer with electrospray ionization interface, controlled by Agilent LC/MSD Trap software.

Enrichment of CF by solvent extraction

AS oil (5.0 g) was extracted five times in an ultrasonic apparatus (1300 W, 45 Hz) with *n*-heptane (50 ml) for 10 min. The *n*-heptane extract was removed and the rest of oil which named coniferyl ferulate rich fraction was stored in a refrigerator (-20℃) for HPCPC separation.

Preparation of two-phase solvent system

N-hexane-ethyl acetate-ethanol-water solvent system with the volume ratios showed in Table1 was prepared by adding the solvents to a separation funnel according to the volume ratios and thoroughly equilibrated by shaking repeatedly. Then upper and lower phases were separated and degassed by sonication for 30 min before use.

Separation and purification of CF by HPCPC

100 mg coniferyl ferulate rich fraction was dissolved in 5 ml mixture of upper and lower phases (1:1, v/v). Switched the separation mode as descending mode, adjusted the rotation speed at 300 rpm, and pumped the upper organic phase (stationary phase) of solvent system at a flow rate of 5 ml/min. After totally filling the rotor with the stationary phase, the lower phase was pumped at a flow rate of2 ml/min, and at the same time, the HPCPC rotor was run at a revolution speed of 1500 rpm. After hydrodynamic equilibrium was reached (about 1 h), the sample solution was injected through the injection valve. The effluent was on-line monitored with UV detector at 318 nm from the outlet of the rotor, and each fraction was collected in a 5 ml test tube. The fractions were subsequently analyzed by HPLC.

HPLC analysis and MS, ^{1}H NMR, ^{13}C NMR identification.

The fraction of coniferyl ferulate from HPCPC separation was analyzed by HPLC-DAD-MS. HPLC analysis was performed on a Zorbax ODS C_{18} column (250×4.6 mm I.D., 5 μm) with a Zorbax ODS C_{18} guard column (12.5×4.6 mm I.D., 5 μm); mobile phase: 1% aqueous acetic acid (A) and acetonitrile (B) using a gradient program of 50% B in 0 to 15 min, 50 to 100% B in 15 to 18 min and100% B in 18 to 23 min. The flow-rate was 1 ml/min and the injection volume was 10 μl. The column was operated at 25℃. The analytes were monitored with DAD at 318 nm. ESI-MS conditions were as follows: drying gas N_2, 10 l/min, temperature, 350℃; pressure of nebulizer, 40 psi; source voltage, 4.0 kV; Scan range, 50 to 800 m/z, positive mode. The pure coniferyl ferulate was characterized by NMR (Bruker, Switzerland, referenced to TMS).

RESULTS AND DISCUSSION

Enrichment of CF from AS oil by solvent extraction

Solvent extraction is one of the oldest techniques for isolating metabolites from natural material. The technique can be used for the isolation and enrichment of analytes

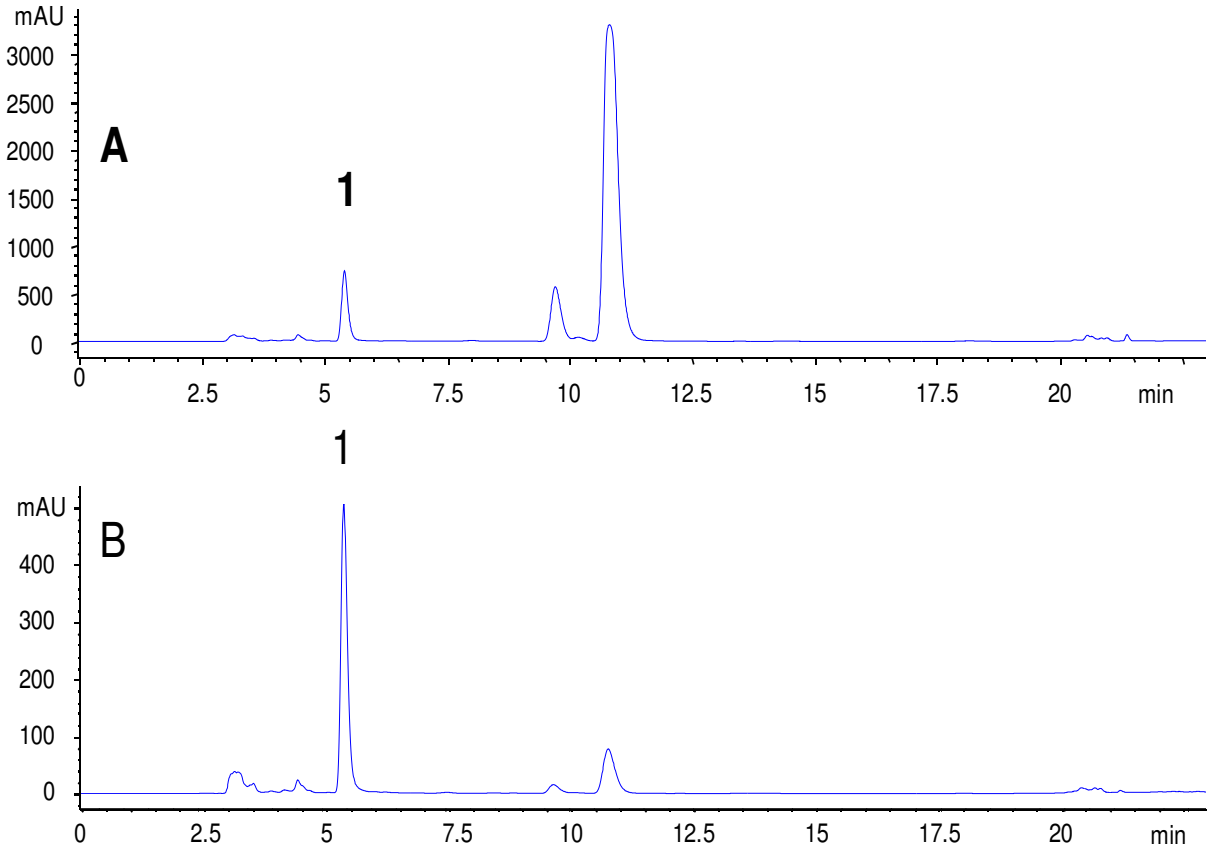


Figure 2. HPLC chromatography of commercial Danggui oil (A) and coniferyl ferulate rich fraction (B). 1 coniferyl ferulate. HPLC analysis was performed on a Zorbax ODS C_{18} column (250×4.6 mm I.D., 5 µm) with a Zorbax ODS C_{18} guard column (12.5×4.6 mm I.D., 5 µm); mobile phase: 1% aqueous acetic acid (A) and acetonitrile (B) using a gradient program of 50% in 0 to 15 min, 50 to 100% B in 15 to 18 min and 100% B in 18 to 23 min. The flow-rate was 1 ml/min and the injection volume was 10 µl. The column was operated at 25°C. The analytes were monitored with DAD at 318 nm.

of medium (Romanik et al., 2007). Coniferyl ferulate could be enriched after *n*-heptane extraction. As shown in Figure 2, the content of CF was greatly enhanced after extraction (Figure 2B). After *n*-heptane extraction, coniferyl ferulate was enriched to 63.0% in extract from 9% in oil, which was evaluated with coniferyl ferulate peak percent in certain HPLC condition by normalization method; the recovery was about 82.0%. Therefore, this developed method has high recovery and selectivity of coniferyl ferulate from AS oil.

Selection of the two-phase solvent systems

N-hexane-ethyl acetate-ethanol-water two-phase system was used as solvent system. The composition of the two-phase system was selected according to the partition coefficient (*K*) of target compound of coniferyl ferulate rich fraction. The partition coefficients were determined by HPLC as follows: about 60 mg of coniferyl ferulate rich fraction was added to a separating funnel, to which 5 ml of each phase of the two–phase solvent system was added.

The funnel was shaken violently for several minutes. Then the upper and lower phases were analyzed by HPLC. The partition coefficient *K* was deter-mined form the HPLC peak area, as $K = A_{up}/A_{low}$ with A_{up} and A_{low} being the areas of the peaks corresponding to coniferyl ferulate in upper and lower phase, respectively. A solvent system with an appropriate partition coefficient (*K* value) ($0.5 \leq K \leq 2$) of target compound will produce a satisfactory running time and avoid band broadening in the counter-current chromatography (HSCCC or CPC).

Ideally, the partition coefficient has to be close to 1 (Cao, 2005; Foucault, 1994; Ito, 2005). According to *K* value shown in Table 1, two solvent systems (*n*-hexane-ethyl acetate-ethanol-water, 5:5:4.5:5.5 and 5:5:5:5, v/v) were selected for further study. The results indicated that the peak of coniferyl ferulate did not show up in 400 min, when *n*-hexane-ethyl acetate-ethanol-water (5:5:4.5:5.5 v/v) was used as the solvent system. Thus, *n*-hexane-ethyl acetate-ethanol-water (5:5:5:5, v/v) was used as

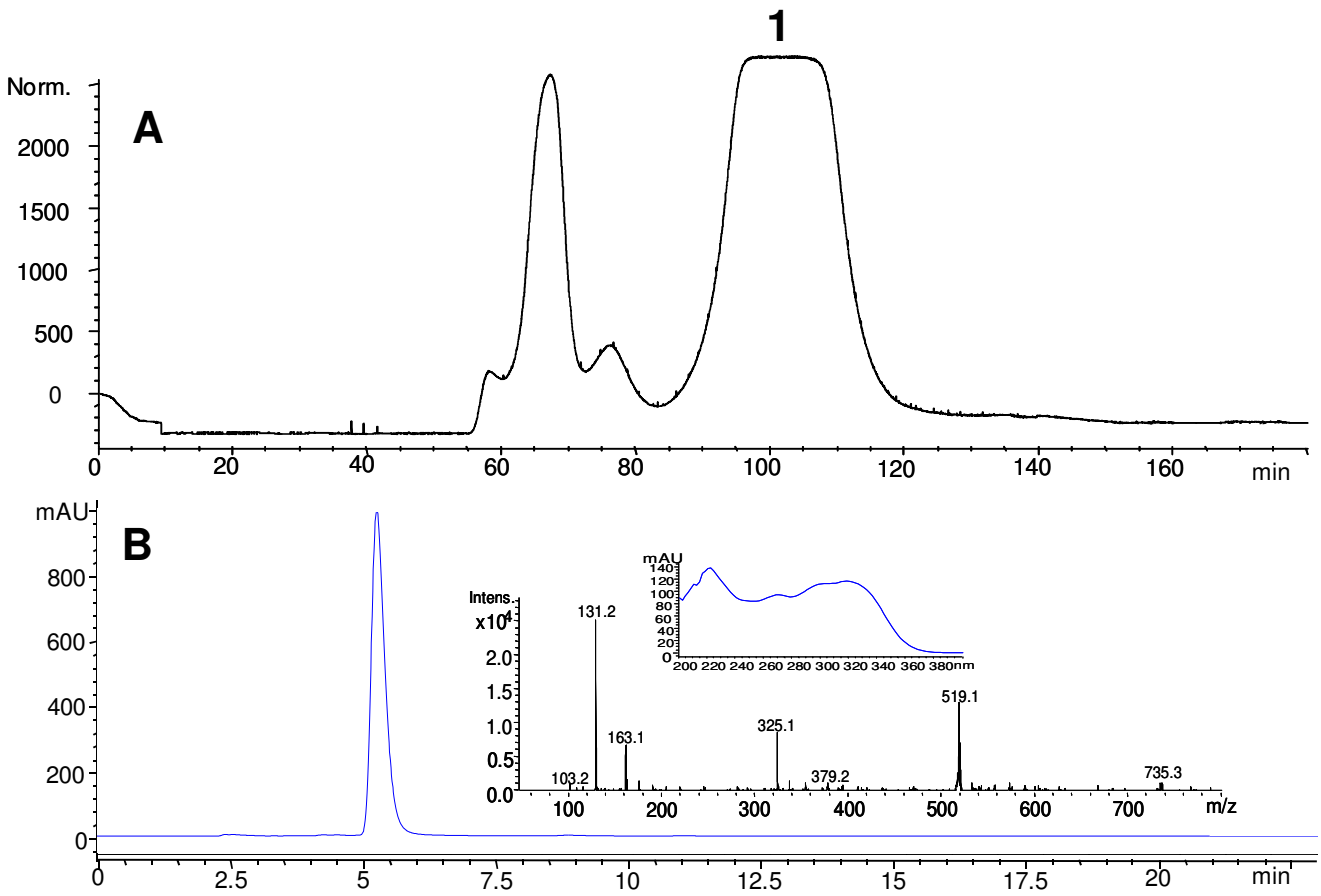


Figure 3. HPCPC chromatogram of coniferyl ferulate rich fraction (A), HPLC chromatogram of peak fraction 1(B) with UV spectra and MS data. HPCPC solvent system: *n*-hexane-ethyl acetate-ethanol-water (5:5:5:5, v/v) in elution; station phase: upper organic phase; mobile phase: lower aqueous phase; descending mode; flow rate: 2.0 ml/min; rotor speed 1500 rpm, detector wavelength: 318 nm. HPLC condition was as Figure 2 shown. ESI-MS conditions are as follows: drying gas N_2, 10 l/min; temperature, 350°C; pressure of nebulizer, 40 psi; source voltage, 4.0 kV; Scan range, 50 to 800 m/z, positive mode.

HPCPC solvent system for separation coniferyl ferulate from AS oil and the target compound was separated in only 2 h.

HPCPC separation

100 mg sample of coniferyl ferulate rich fraction was separated by HPCPC in a 180 min run and the peaks were analyzed by HPLC. Figure 3 shows the chromatography of HPCPC separation of coniferyl ferulate rich fraction and HPLC chromatography with UV spectra and MS data of HPCPC peak fraction, and the purity of coniferyl ferulate was over 98% (HPLC).

The structural identification

The structural identification of peak 1 in (Figure 3) was carried out by MS, ^{1}H-NMR and ^{13}C NMR spectra as follows: ESI-MS: *m/z* 735 $[2M+Na]^+$; 379 $[M+Na]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz)δ: 3.90 (6H, s, - OCH_3×2), 4.84 (2H, d, *J*=6.5 Hz, 9'-H), 6.19 (1H, dt, *J*= 15.8, 6.5 Hz, 8'-H), 6.32 (1H, d, *J*=15.9 Hz, 8-H), 6.63 (1H, d, *J*=15.8 Hz, 7'-H), 6.85-7.10 (6H, Ar-H), 7.65 (1H, d, *J*=15.9 Hz, 7-H). ^{13}C NMR ($CDCl_3$, 100 MHz)δ: 134.4 (C-1), 109.5 (C-2), 148.1 (C-3), 146.9 (C-4), 121.1 (C-5), 128.9 (C-6), 145.1 (C-7), 114.5 (C-8), 167.1 (C-9), 123.1 (C-1'), 108.5 (C-2'), 146.0 (C-3'), 146.7 (C-4'), 120.7 (C-5'), 127.0 (C-6'), 115.4(C-7'), 114.8 (C-8'), 65.2 (C-9'), 55.4(OCH_3). All these data were in agreement with those of coniferyl ferulate reported (Yang et al., 2006; Lu et al., 2004).

Conclusion

The overall results indicate that HPCPC is successfully used for separation and purification of coniferyl ferulate form commercial AS oil and solvent extraction can selectively enrich the biological active substance.

ACKNOWLEDGEMENTS

We are grateful to Leon Lai and Fengqing Yang from our institute for their expert technical assistance. The research was supported by grants from the Science and Technology Development Fund of Macao (028/2007/A2) and the University of Macau (UL015/09-Y1) to S. P. Li.

REFERENCES

Berot S, Le Goff E, Foucault A, Quillien J (2007). Centrifugal partition chromatography as a tool for preparative purification of pea albumin with enhanced yields. J. Chromatogr., B 845: 205-209.

Cao XL (2005). The Method of High Speed Counter-current Chromatography. The Separation Technology and Application of High-speed Countercurrent Chromatography. Chemical Industry Press: Beijing.

Chou SC, Everngam MC, Sturtz G, Beck JJ (2006). Antibacterial Activity of components from *Lomatium californicum*. Phytother. Res., 20: 153-156.

Chung YJ, Kim YC, Lee KH, Namgung MA (2005). Peptic ulcer treating agent inhibiting activity of proton pump to inhibit secretion of gastric acid. *Korean patent* KR 2005023998.

Foucault AP (1994). Theory of Centrifugal Partition Chromatography. Centrifugal Partition Chromatography. Marcel Dekker: New York.

Foucault AP, Chevolot L (1998). Counter-current chromatography: instrumentation, solvent selection and some recent applications to natural product purification. J. Chromatogr. A, 808: 3-22.

Ingkaninan K, Hazekamp A, Hoek AC, Balconi S, Verpoorte R (2000). Application of centrifugal partition chromatography in a general separation and dereplication procedure for plant extracts. J. Liq. Chrom. Rel. Technol., 23: 2195-2208.

Ito Y (2005). Golden rules and pitfalls in selecting optimum conditions for high-speed counter-current chromatography. J. Chromatogr. A, 1065: 145-168.

Kim CY, Kim J (2007). Preparative isolation and purification of geniposide from garadenia fruit by centrifugal partition chromatography. Phytochem. Anal., 18: 115-117.

Kobayashi M, Fujita M, Mitsuhashi H (1984). Components of *Cnidium officinale* makino: occurrence of pregnenolone, coniferyl ferulate, and hydroxyphthalides. Chem. Pharm. Bull., 32: 3770-3773.

Lao SC, Li SP, Kan KKW, Li P, Wan JB, Wang YT, Dong TTX, Tsim KWK (2004). Identification and quantification of 13 components in *Angelica sinensis* (Danggui) by gas chromatography-mass spectrometry coupled with pressurized liquid extraction. Anal. Chim. Acta, 526: 131-137.

Li SY, Yu Y, Li SP (2007). Identification of antioxidants in essential oil of Radix Angelicae sinensis using HPLC coupled with DAD-MS and ABTS-based assay. J. Agric. Food Chem., 55: 3358-3362.

Lin LZ, He XG, Lian LZ, King W, Elliott J (1998). Liquid chromatographic–electrospray mass spectrometric study of the phthalides of *Angelica sinensis* and chemical changes of Z-ligustilide. J. Chromatogr. A, 810: 71-79.

Liu ZL, Wang J, Shen PN, Wang CY, Shen YJ (2006). Microwave-assisted extraction and high-speed counter-current chromatography purification of ferulic acid from *Radix Angelicae sinensis*. Sep. Purif. Technol., 52: 18-21.

Lu GH, Chan K, Leung K, Chan CL, Zhao ZZ, Jiang ZH (2005). Assay of free ferulic acid and total ferulic acid for quality assessment of *Angelica sinensis*. J. Chromatogr. *A,* 1068: 209-219.

Lu XH, Zhang JJ, Liang H, Zhao YY (2004). Chemical constituents of *Angelica sinensis*. J. Chin. Pharm. Sci., 13: 1-3

Marston A, Hostettmann K (2006). Developments in the application of counter-current chromatography to plant analysis. J. Chromatogr. A, 1112: 181-194.

Naito T, Iketani Y, Kubota K. Shimoda Y. 1995. Peptic ulcer treating agent inhibiting activity of proton pump to inhibit secretion of gastric acid. Japan patent JP 07233060.

Ou SY, Kwok KC (2004). Review Ferulic aicd: pharmaceutical functions, preparation and applications in foods. J. Sci. Food Agric., 84: 1261-1269.

Pharmacopoeia Commission of PCR (2005). Radix Angelicae sinensis. Pharmacopeia of the People's Republic of China Vol. I. Chemical Industry Press: Beijing.

Romanik G, Gilgenast E, Przyjazny A, Kaminski M (2007). Techniques of preparing plant material for chromatographic separation and analysis. J. Biochem. Biophys. Methods, 70: 253-261.

Yan R, Li SL, Chung HS, Tam YK, Lin G (2005). Simultaneous quantification of 12 bioactive components of *Ligusticum chuanxiong* Hort. By high-performance liquid chromatography. J. Pharm. Biomed. Anal., 37: 87-95.

Yang F, Xiao YS, Zhang FF, Xue XY, Xu Q, Liang XM (2006). High performance liquid chromatography-mass spectrometry analysis of Radix Angelica sciensis. Acta Pharm. Sin., 41: 1078-1083.

Zou HF, Kong L, Sun NC, Wu LH, Chen XG (2005). Use of coniferyl ferulate in anticancer medicine. China patent CN 200310104929.0.