Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Journal of Chromatography A, 988 (2003) 95–105
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Isolation and identification of four flavonoid constituents from the seeds of *Oroxylum indicum* by high-speed counter-current chromatography

Li-Juan Chen*, David E. Games, Jonathan Jones
Mass Spectrometry Research Unit, University of Wales Swansea, Singleton Park, Swansea SA2 8PP, UK

Received 10 October 2002; received in revised form 26 November 2002; accepted 26 November 2002

Abstract

Four flavonoids, chrysin, baicalein, baicalein-7-*O*-glucoside, baicalein-7-*O*-diglucoside (Oroxylin B) and one unknown flavonoid have been isolated and purified for the first time in the seeds of *Oroxylum indicum* by high-speed counter-current chromatography with a two-phase solvent system composed of chloroform–methanol–water (8:10:5, v/v). Then, 50 mg baicalein-7-*O*-glucoside, 10.5 mg baicalein-7-*O*-diglucoside, 4.5 mg chrysin-7-*O*-diglucoside, 25 mg baicalein and 45 mg chrysin could be obtained after injecting 20 mg/ml sample extract ten times and their purities were 96, 90, 85, 95 and 98%, respectively. All these constituents were identified by high-performance liquid chromatography–mass spectrometry and nuclear magnetic resonance.

Keywords: Plant materials; *Oroxylum indicum*; Counter-current chromatography; Flavonoids

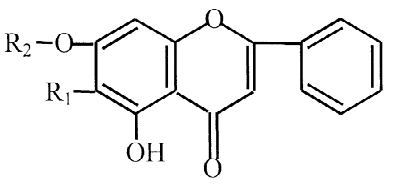
1. Introduction

Oroxylum indicum (*O. indicum*) is a traditional herbal medicine in China and Japan [1,2]. Its seeds have been used as an analgesic, antitussive and anti-inflammatory agent for the treatment of cough, bronchitis and other diseases. In recent years, more attention has been paid to the research of flavonoid chrysin, baicalein and baicalin due to their anti-inflammatory, anti-allergic [3], antioxidant and anti-cancer activities [4–7]. The major bioactive constituents in the seeds of *O. indicum* are: baicalein, baicalein-7-*O*-glucoside, chrysin and baicalin (structures shown in Fig. 1). However, there was not much reported on the separation and purification of these bioactive flavonoids of this plant. Separation and purification of these constituents are important in pharmaceutical research.

The preparative separation and purification of flavonoids from plant materials by classical methods were tedious and usually required multiple chromatographic steps on silica gel, polyamide column [8,9], etc. High-speed counter-current chromatography (HSCCC), being a support-free liquid chromatography method, eliminates complications such as irreversible adsorption onto the solid support, tailing of the solute peaks, etc. and, therefore, is considered a suitable alternative for the separation of flavonoids from natural products [10,11]. In this paper, HSCCC

*Corresponding author.
E-mail address: 183366@swansea.ac.uk (L.-J. Chen).

0021-9673/02/$ – see front matter
doi:10.1016/S0021-9673(02)01954-4



	R1	R2	M_r
Baicalin	OH	Glucuronide	446
Baicalein	OH	H	270
Baicalein-7-o-glucoside	OH	Glucoside	432
Chrysin	H	OH	254
Baicalein-7-o-diglucoside	OH	Diglucoside	594

Fig. 1. Structures of flavonoids in the seeds of *Oroxylum indicum*.

was applied to the isolation and purification of five flavonoids followed by the identification of these components using high-performance liquid chromatography (HPLC)–electrospray ionisation mass spectrometry (ESI-MS) and nuclear magnetic resonance (NMR).

2. Experimental

2.1. HSCCC separation

An experimental prototype J-type coil planet centrifuge high-speed preparative instrument was supplied by Brunel Institute for Bioengineering, Brunel University, Uxbridge, Middlesex, and UB8 3PH, UK. This machine was equipped with a HP1100 (Hewlett-Packard) HPLC system, a UV spectrophotometer detector and a sample collector. A manual sample injection valve was used to introduce the samples into the column. The HSCCC system has four coils that are wound tightly on two separate bobbins on one rotor; each bobbin containing two concentrically wound coils of PTFE tubing with a total volume of 495 ml, column I.D. 1.6 mm. In this study, a column of 49.9 ml volume, β range at 0.7 to 0.83 was used. β is the ratio of the holder radius to the revolution radius. The other three coils were filled with methanol–water (50:50) for balance. A mixture of chloroform–methanol–water (8:10:5) was prepared, and was thoroughly equilibrated in a separator funnel by repeated vigorous shaking and degassing at room temperature. The column was first filled with the lower phase of the biphasic mixture, then the coil was rotated in a reverse direction at a constant speed of 800 rev./min, and the system purged with lower (aqueous–methanol phase) mobile phase up to the head of the coil. The mobile phase was then pumped into the coil from tail to head at a flow-rate of 1.0 ml/min. The equilibration point of the system was determined when no more stationary phase was eluted (hydrodynamic equilibration). Then 20 mg of the sample dissolved in equal upper phase and lower phase was loaded. The retention volume of the system could then be calculated by subtracting the volume of stationary phase eluted at the end of the equilibration process from the total volume of the system and dead volume. The effluent from the head end of the column was continuously monitored with a UV detector at 275 nm and the retention of the stationary phase relative to the total column capacity was computed from the volume of the stationary phase collected from the column after the separation was completed. Peak fractions were collected into test tubes with a sampler controller (Gilson, France) for analysis by HPLC.

2.2. Extraction of plant sample

In this study, 250 g of seeds of *O. indicum* were extracted 3 days with 600 ml of 70% aqueous methanol, the extractions then filtered and evaporated.The residue was redissolved in 200 ml water and extracted three times with 250 ml 2-butanol. The 2-butanol layer was evaporated with evaporator at a temperature of 30 °C. A yellow powder of 25 g complex was obtained. This complexes was directly subjected to HSCCC

2.3. HPLC–ESI-MS–MS analysis and identification of unknown components

The peak fraction analyses and identification were carried out in a Hewlett-Packard 1100 HPLC apparatus, which was interfaced to a LCQ ion trap mass spectrometer (Finnigan MAT, San Jose, CA, USA). A Hypersil C_8 RP column (150×4.6 mm I.D.) was used (Phenomenex) at a temperature of 30 °C, a flow-rate of 1.0 ml/min and with a detection wavelength of 275.5 nm.

2.4. Nuclear magnetic resonance (NMR)

^{1}H NMR and ^{13}C NMR were performed using a Beckman 400 spectrometry with dimethyl sulfoxide (DMSO) as both solvent and internal standard.

3. Results and discussion

3.1. Measurement of partition coefficients

Successful separation necessitates the careful search for a suitable two-phase solvent system to provide an ideal range of partition coefficients for the applied sample. Generally speaking, the two phase solvent system should satisfy the following requirements: (1) for ensuring satisfactory retention of the stationary phase, the settling time of the solvent system should be considerably shorter than 30 s; (2) for efficient separation, the partition coefficient (k) value of target compounds should be close to 1, and the separation factor α, which is the ratio of k between two components, should be greater than 1.5. If the k value is much smaller than 1, the solutes are eluted close together near the solvent front, which will result in a loss of peak resolution; if k is larger than 1, the solutes are eluted in excessively broad peaks, requiring a long elution time. The k value for a pure compound can be determined simply by measuring the UV absorbance of each phase after partitioning between the two phases. When the compounds to be separated are not available in a pure form, as in the present case, their k values obviously cannot be determined by this method. In this case, HPLC could be used to measure the partition coefficients. Table 1 shows the partition coefficients of these compounds. Fig. 2 shows the separation of the 2-butanol extract from the seeds of *O. indicum* by HPLC.

Table 1 shows that the chloroform–methanol–water ratios (6:10:5), (7:10:5), (8:10:5) and (9:10:5) could be used to separate the sample. After trying all the above solvent systems, the ratio 8:10:5 (v/v) was the best to effect the separation of the extracts. Fig. 3 showed the separation of HSCCC using this solvent system.

Fig. 3 shows that compounds 4 and 5 did not resolve well in the normal-phase elution mode, but compounds 3 and 1 resolved very well. When the elution mode was changed into reversed-phase, a very good resolution could be obtained. Fig. 4 showed the separation result by reversed-phase chromatography.

Fig. 4 shows that five compounds were eluted in order of decreasing k value and good resolution could be obtained in the reversed-phase elution mode using this solvent system. In this solvent system, a minor compound 2, which was difficult to be purified by preparative HPLC, could also be enriched dramatically. No stationary phase wash off was

Table 1
The partition coefficients (K*) in different ratio of volume in chloroform–methanol–water system

Chloroform–methanol–water ratio of volume	Compound 1	Compound 2	Compound 3	Compound 4	Compound 5
5:3:5	58.4	3.58	35.0	0	0
5:5:5	43.1	4.45	12.6	0.09	0.02
5:6:5	16.28	5.43	2.09	0.09	0.03
5:7:5	12.21	7.41	1.71	0.13	0.05
6:10:5	2.60	8.79	1.06	0.31	0.14
7:10:5	3.28	12.64	1.10	0.25	0.18
8:10:5	3.26	16.85	0.98	0.22	0.13
9:10:5	3.68	19.25	1.10	0.27	0.11
10:10:5	4.0	22.35	1.12	0.19	0.09

Experimental procedure: approx, 1 mg of the test sample was weighted in a 10 ml test tube to which 2 ml each pre-equilibrated two-phase solvent system was added. The test tube was stopper and shaken vigorously for 1 min to thoroughly equilibrate the sample with two phase. Then, equal volumes (about 100ul) of the upper and lower phases were evaporated to dry separately. The residues were diluted with methanol to 1 ml and analyzed by HPLC to determine the partition coefficients (K) of each component. The K was defined as the peak area of compound in the upper phase divided by the peak area of compound in the lower phase.

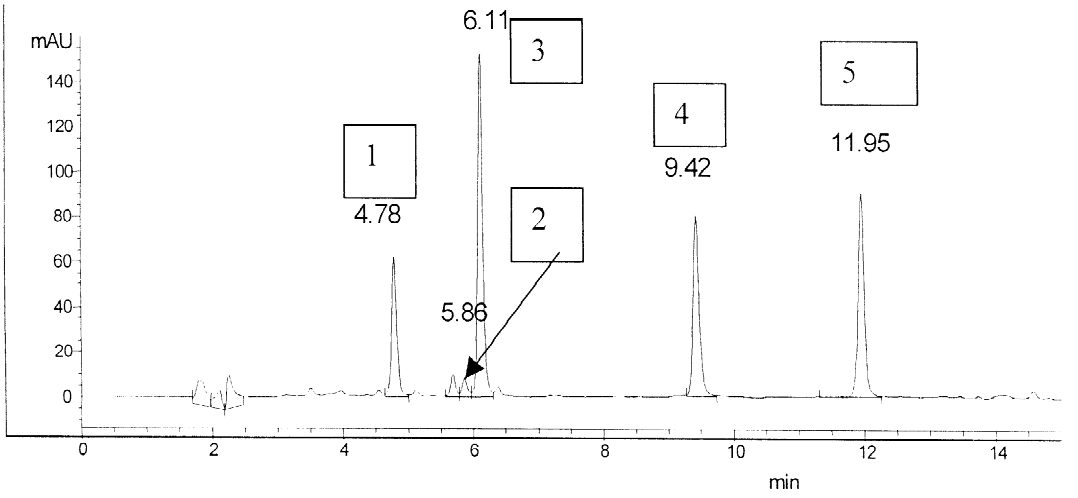


Fig. 2. Chromatograms of 2-butanol extract by HPLC analysis from the seeds of *O. indicum*. Chromatographic conditions: gradient elution: 0–15 min 0.2% formic acid from 80 to 35% and acetonitrile from 20 to 65%; 15–20 min 0.2% formic acid from 35% to 10% and acetonitrile from 65 to 90%; temperature 35 °C; Flow-rate 1.0 ml/min; $\lambda = 275.5$ nm.

observed. This process could repeat injecting sample more than ten times and good resolution could also be obtained. After injecting ten times, 50 mg compound 3, 10.5 mg compound 1, 4.5 mg compound 2, 25 mg compound 4 and 45 mg compound 5 could be obtained. These entire peak fractions were subjected to HPLC analysis, the results and shown in Fig. 5. However, when increasing the flow-rate, the resolution will be poor. Fig. 6 showed the effect of varying the flow-rate on the elution time in this elution mode

After increasing the flow-rate, compounds 1 and 3 could not be resolved very well.

3.2. Identification of the compounds 1–5 by HPLC–ESI–MS and ESI–MS–MS

In crude plant extracts, flavonoids are often pres-

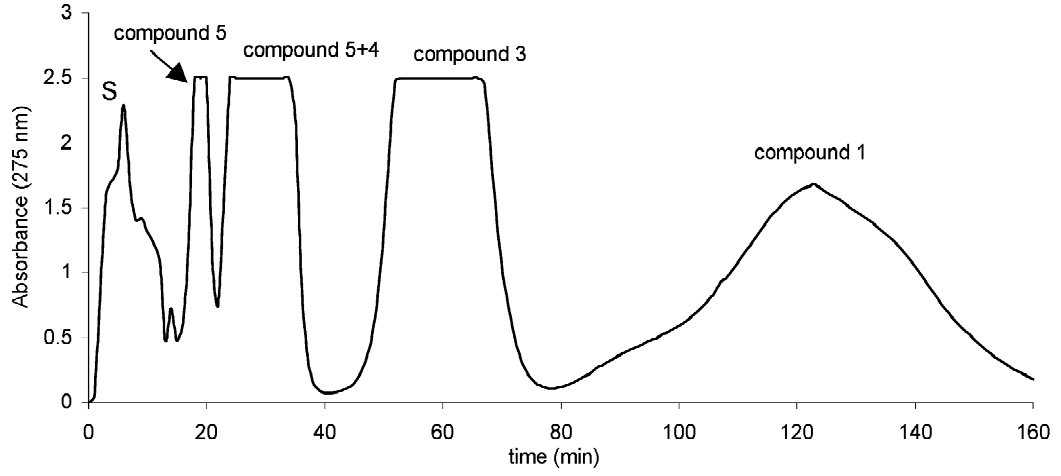


Fig. 3. Chromatograms of the 2-butanol extract by HSCCC separation in chloroform–methanol–water (8:10:5). Experimental conditions: isocratic elution; head to tail; coil volume: 49.9 ml; sample injection volume 1.0 ml; sample concentration 20 mg/ml; stationary phase: upper phase; stationary retention S_F (%) = 70; mobile phase, lower phase; stop rotation at 200 min; rotation speed: 800 rev./min.

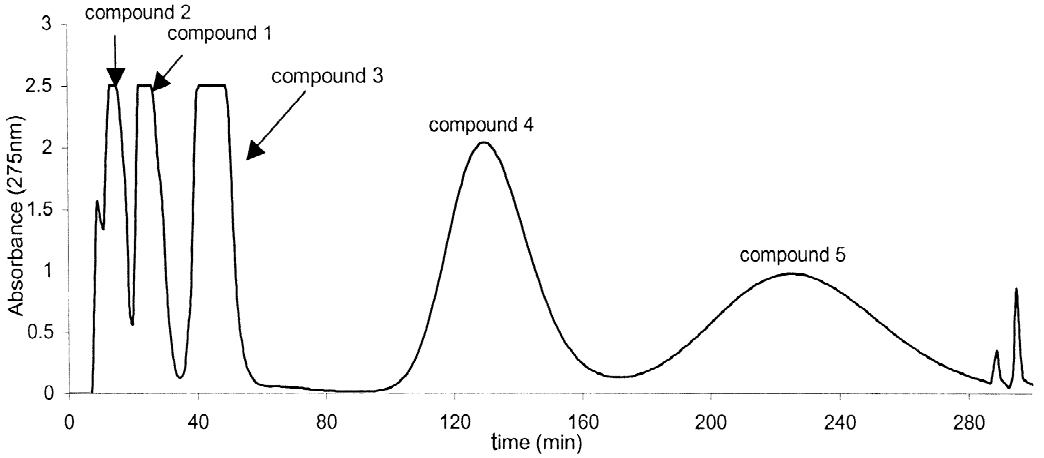


Fig. 4. Chromatograms of the 2-butanol extract by HSCCC separation in chloroform–methanol–water (8:10:5). Experimental conditions: isocratic elution; tail to head; coil volume: 49.9 ml; flow-rate: 1.0 ml/min; sample injection volume 1.0 ml (20 mg/ml sample); stationary phase: lower phase; stationary retention S_F (%) = 70, mobile phase, upper phase; stop rotation at 200 min; rotation speed: 800 rev./min.

ent as O- or C-glycosides [12,13]. The O-glycosides have sugar constituents bonded to a hydroxyl group of the aglycone, whereas the C-glycosides have sugar constituents bonded to a carbon of the flavonoid aglycone, generally at positions 6, 7 and 8. Contrary to flavonoid C-glycosides, the mass spectra of O-glycosides generate abundant aglycone ions by loss of neutral mass of sugar and C-glycosides do not generate abundant alycone ions but instead characteristic ions of the fragmentation of the C-glycoside unit itself [14]. So, it is easy to differ between them by MS–MS spectra. Hence, ESI-MS and ESI-MS–MS can provide important structure information for flavonoids and can be of particular value in the determination of the nature and site of attachment of the sugar in O-glycosides.

For compound 1, the $[M–H]^-$ precursor ion at the m/z 593 molecule ion could be clearly observed, and the molecular mass of compound 1 was 594. The ESI-MS spectrum of compound 1 showed an intense aglycone ion $[A–H]^-$ at m/z 269 by loss of two neutral fragments of mass 324. The loss of 324 between these peaks confirmed the presence of two hexose residues. The daughter collision induced decomposition (CID) MS–MS spectra of the ion at m/z 269 exhibited five main diagnostic fragmentations, m/z 251.2, 223.3, 197.2, 169.2 and 142.9. The fragment at m/z 169 showed substitution of the A ring by three OH groups. These fragment ions were very important to identify the structure of compound 1. In order to identify the conclusion, compound 1 was refluxed for 2 h at 90 °C in 1.2 mol/ml hydrochloric acid (methanolic solution) and hydrolyzed in order to give the corresponding aglycones. After evaporatuion to dryness using a rotary evaporator at a 35 °C water bath, the residue was dissolved in methanol and then subjected to LC–MS–MS analysis. The hydrolyzed compound 1 gave the same retention time and fragmentations as that of standard baicalein at MS–MS m/z 269. In view of the common occurrence of particular sugar moieties in flavonoids and on the basis of Ref. [2], compound 1 was baicalein-7-*O*-diglucoside. The UV spectrum of compound 1 in methanolic solution resembled that of standard baicalin and the use of diagnostic shifts suggested the absence of a free hydroxyl at the 7-position. In the ^{13}C-NMR spectrum of hydrolyzed compound 1, the signals appeared at almost the same position as those of baicalein, indicating that compound 1 was baicalein-7-*O*-diglucoside (oroxylin B). This constituent has been isolated from the seeds of *Oroxylum indicum* [2]. Fig. 7 shows the negative ESI-MS–MS spectra of compound 1.

For compound 2, the $[M–H]^-$ precursor ion at m/z 577 molecule ion was very intensive, and the molecular mass of compound 2 was 578. The ESI-MS spectra of compound 2 in Fig. 8 showed an intense aglycone ion $[A–H]^-$ at m/z 253 by loss of

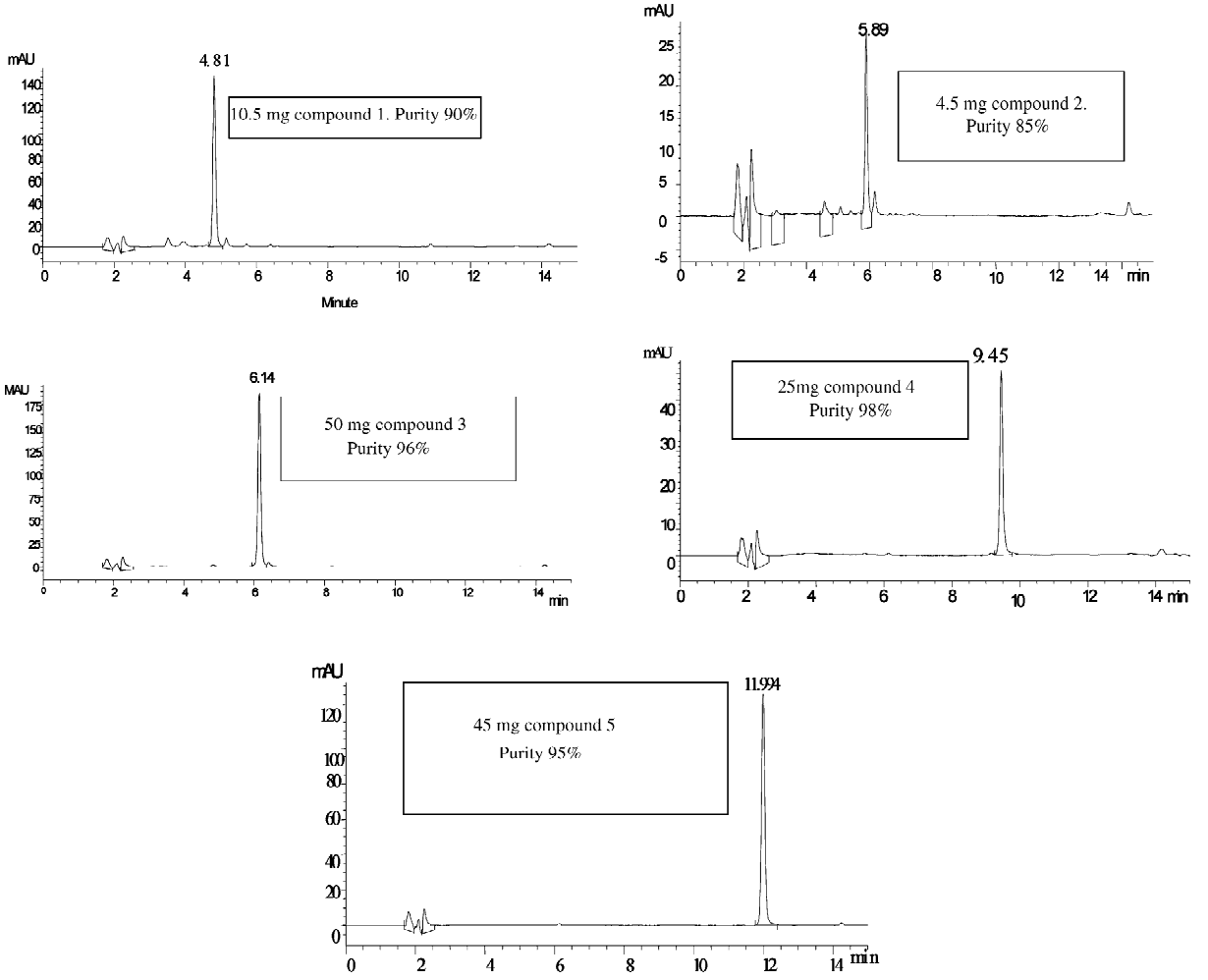


Fig. 5. HPLC analysis of all the fractions after HSCCC separation.

neutral fragments of mass 324. The loss of 324 between these peaks confirmed the presence of two hexose residues. The daughter CID–MS–MS spectra of the ion at m/z 253 gave the same pattern as those of standard chrysin. According to the report of Tomimori et al. [2], compound 2 was possibly chrysin-7-*O*-diglucoside. However, due to the limited amount and impurity of compound 2, it's identity has not been completely confirmed. Further work on compound 2 would be required to fully characterise and this will be done in due course.

For compound 3, the ESI-MS mass spectrum gave flavonoid glycoside and its aglycone fragments with ions at m/z 431 $[M–H]^-$ and m/z 269 $[M–H\text{-glucoside}]^-$, the latter ion again corresponded to the $[M–H]^-$ ion of the standard baicalein. When the pseudomolecular ion m/z 431 was selected as parent ion and the daughter ions were recorded at a different CID energy, the negative ESI-MS–MS spectra showed the characteristic m/z 341 $[M–H\text{-}90]^-$ and m/z 311 $[M–H\text{-}120]^-$ ions due to the cleavage of the O-glycoside moieties [14]. Fig. 9 shows the negative ESI-MS–MS spectra at m/z 431 and the proposed fragments of spectra of compound 3. The existence of $[M–H\text{-}90]^-$ and $[M–H\text{-}120]^-$ ions indicated that a hexose glucoside was linked to the flavonoid. The hydrolyzed compound 3 also gave the same retention time and fragmentations as that of

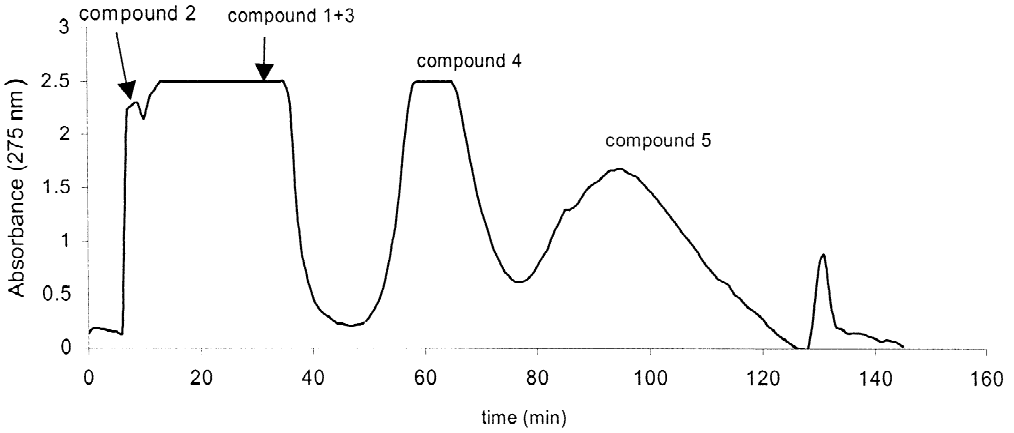


Fig. 6. The effect of varying the flow-rate on the separation of the 2-butanol extract by HSCCC. Experimental conditions: isocratic elution; tail to head; coil volume: 49.9 ml; flow-rate: 1.5 ml/min; sample injection volume 1.0 ml (20 mg/ml sample); stationary phase: lower phase; stationary retention S_F (%)=70.0, mobile phase, upper phase; stop rotation at 200 min; rotation speed: 800 rev./min.

standard baicalein at MS–MS m/z 269. Its UV spectrum and diagnostic shifts suggested the presence of a hydroxyl at the 5-position. ^{1}H NMR and ^{13}C NMR confirmed compound 3 as baicalein-7-*O*-glucoside. ^{1}H NMR ([$^{2}H_6$]DMSO): 4.59 (1 H, tri, anomeric H of terminal glucose), 8.49 (1 H, s, H-6), 7.9–8.0 (2 H, d, J=8.0 Hz, H-2′, 6′), 7.4–7.5 (3 H, m, H-3′, 4′, 5′), 6.95 (1 H, s, H-8), 6.91 (1 H, s, H-3), 12.46 (1 H, s, H-5). ^{13} C NMR ([$^{2}H_6$]DMSO): 182.9 (C-4), 163.8 (C-2), 151.9 (C-9), 149.6 (C-7), 146.8 (C-5), 132.4 (C-4′), 131.2 (C-6), 130.9 (C-1′), 129.5 (C-3′, 5′), 126.7 (C-2′, 6′), 106.4 (C-10), 105.1 (C-3), 101.2 (C-1″), 94.6 (C-8), 77.7 (C-3″), 76.2 (C-5″), 73.5 (C-2″), 70.0 (C-4″), 60.98 (C-6″).

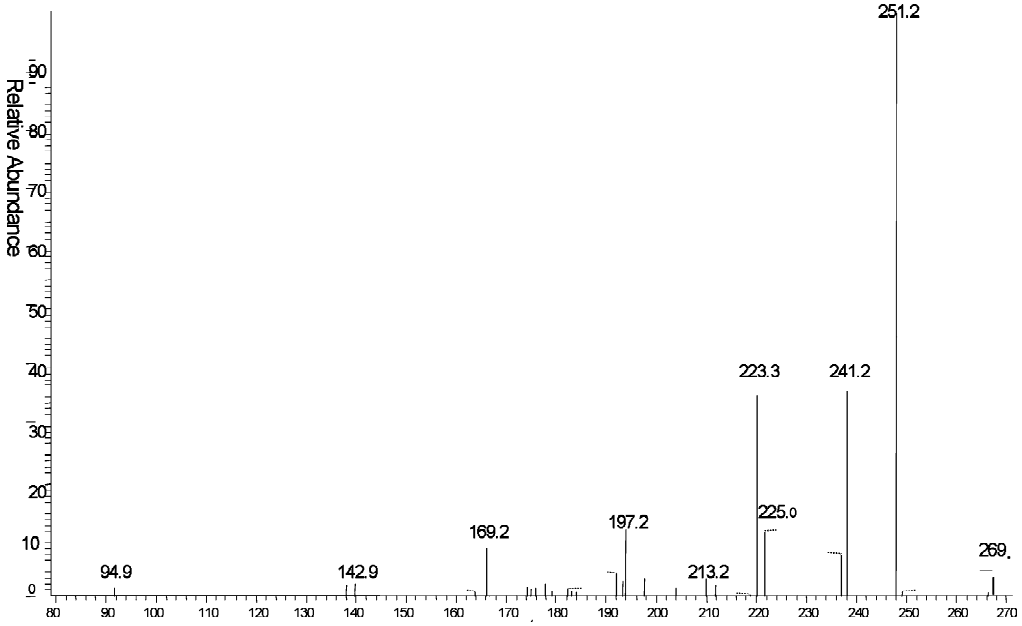


Fig. 7. The negative ESI-MS–MS spectrum of compound 1.

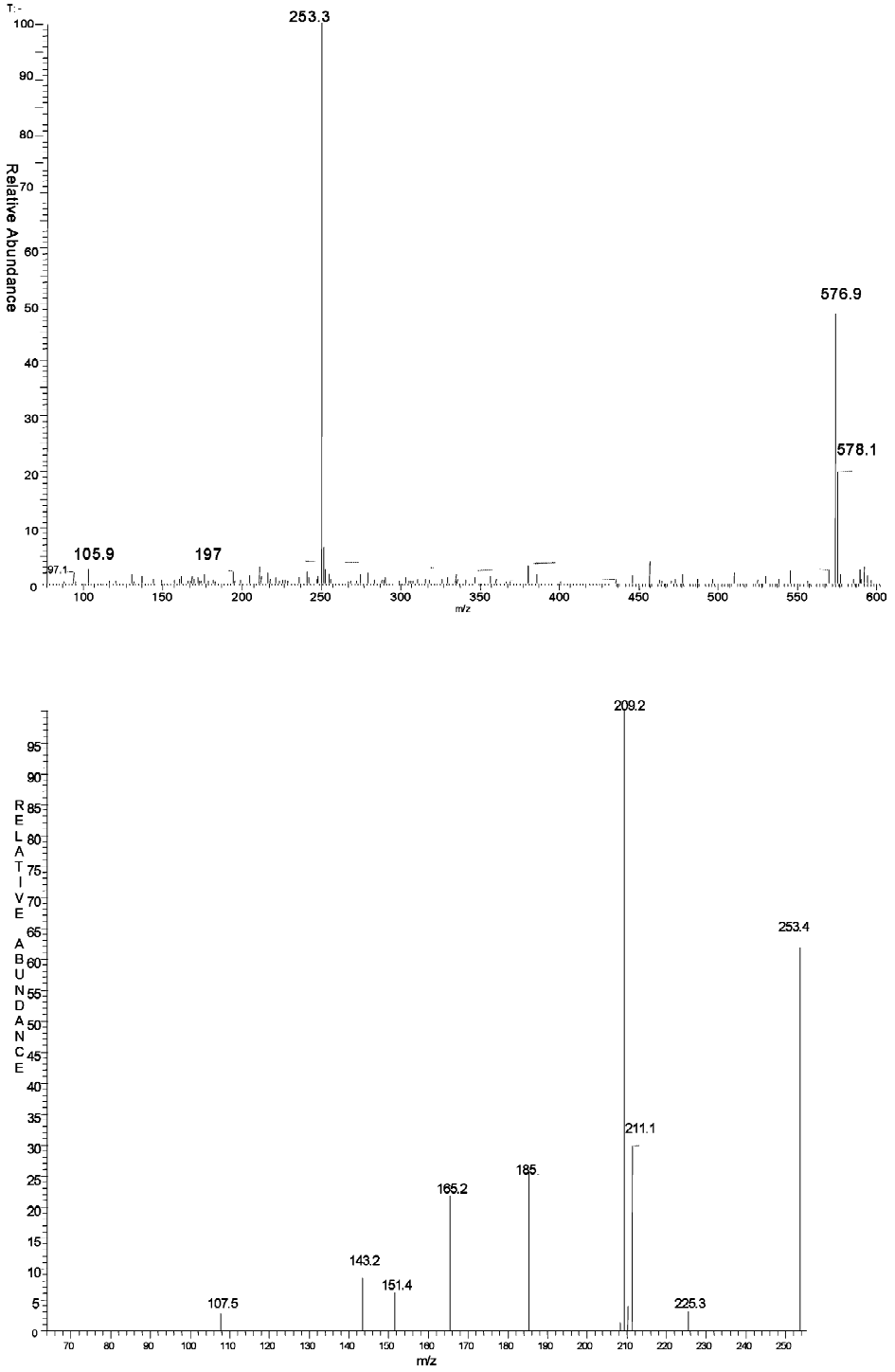


Fig. 8. The negative ESI-MS and ESI-MS–MS spectra of compound 2.

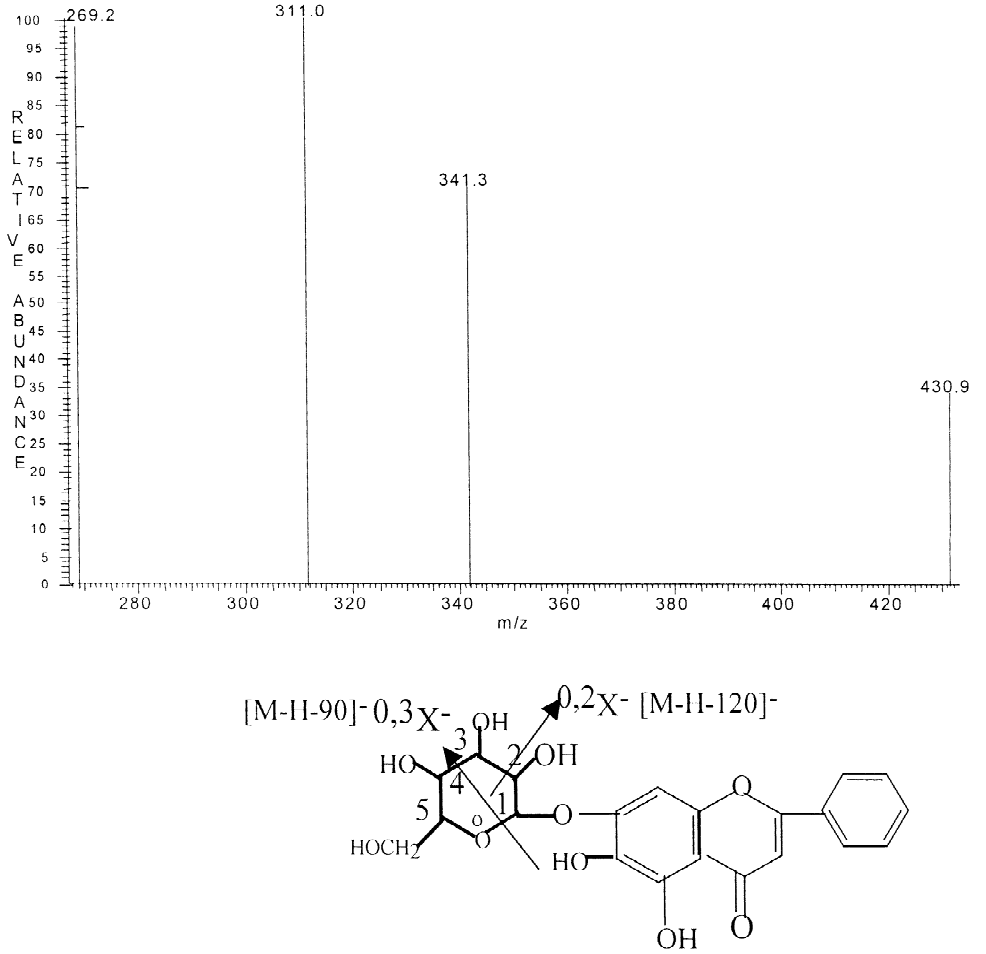


Fig. 9. The ESI-MS–MS spectra at *m*/*z* 431 at 30% CID energy and 7-*O*-glucoside unit fragmentation for compound 3.

For compound 4, the daughter MS–MS spectra at *m*/*z* 269 displayed exactly the same fragmentation patterns as standard baicalein sample. The LC retention time of compound 4 also confirmed its identity as baicalein. Its ^{1}H NMR ($C^{2}H_{3}O^{2}H$, tetramethylsilane) δ ppm: 7.8 (2 H, H-2′, H-6′); 7.3 (3 H, H-3′, H-4′, H-5′); 6.5 (1 H, H-3); 6.4 (1 H, H-8). ^{13}C NMR ([$^{2}H_{6}$]DMSO) δ ppm: 95.45 (C-8), 105.75 (C-10), 106.24 (C-3), 127.77 (C-2′, C-6′), 130.61 (C-3′, C-5′), 131.15 (C-6′), 133.32 (C-4′), 133.13 (C-1′), 148.30 (C-9), 152.56 (C-5), 155.55 (C-2), 165.98 (C-7), 184.64 (C-4).

For compound 5, from the MS–MS data and retention time analysis, compound 5 was identified as chrysin. Its ^{1}H NMR ($C^{2}H_{3}C^{2}H$, tetramethylsilane) δ ppm: 7.9 (2 H, H-2′, H-6′); 7.5 (3 H, H-3′, H-4′, H-5′); 6.4 (1 H, H-3); 6.5 (1 H, H-8); 6.1 (1 H, H-6) ^{13}C NMR ($C^{2}H_{3}O^{2}H$) δ ppm: 95.45 (C-8), 99.27 (C-6), 104.31 (C-10), 104.90 (C-3), 126.30 (C-2′, C-6′), 129.10 (C-3′, C-5′), 131.43 (C-1′), 131.93 (C-4′), 158.44 (C-9), 162.16 (C-5), 164.50 (C-2), 165.57 (C-7), 182.74 (C-4). Its ^{13}C NMR and ^{1}H NMR signals were completely the same as those of standard chrysin. So, compound 5 was identified as chrysin. Table 2 gave all the spectra of compounds 1–5 in negative ESI-MS and ESI-MS–MS. Table 3 gave all the signals of ^{13}C NMR spectra of compounds 1–5 and standard flavonoids. Table 3 gave all the signals of ^{13} C NMR for compounds 1–5.

Table 2
Negative ESI-multiple MS (MS^n) spectra (m/z value) of flavonoid compounds

	Compound	M_r	Retention time (min)	$[M-H]^-$	MS^n data of compounds
Sample	Compound 1	594	4.786	593	593, $\underline{MS^2}$ 269 $\underline{MS^3}$ 251, 241, 225, 197, 169, 141
	Compound 2	578	5.861	577	577, $\underline{MS}$ 253 $\underline{MS^2}$ 235, 151, 145, 127, 105
	Compound 3	432	6.114	431	431, $\underline{MS^2}$ 311 $\underline{MS^3}$ 269 $\underline{MS^4}$251, 241, 225, 197, 169, 141
	Compound 4	270	9.420	269	269, $\underline{MS^2}$ 251, 241, 225, 197, 169, 141
	Compound 5	254	11.950	253	253, $\underline{MS^2}$ 235, 151, 145, 127, 105
Standard	Chrysin	254	11.948	253	253, $\underline{MS^2}$ 235, 151, 145, 127, 105
	Baicalein	270	9.413	269	269, $\underline{MS^2}$ 251, 241, 225, 197, 169, 141
	Baicalin	446	6.231	445	445, $\underline{MS}$ 269 $\underline{MS^2}$ 269 $\underline{MS^3}$ 251, 241, 225, 197, 169, 141

Experimental conditions: spray voltage 3.5 kV; capillary temperature 200 °C; the isolation width for MS^n 1.0 u and the collision energy (%) was as follows: for MS^2 40%; MS^3 45% MS^4 45%.

4. Conclusions

Our study demonstrates that HSCCC is a valuable method in separating, purifying and identifying bioactive components from natural products. Using HSCCC, five flavonoids, one of which was not identified, baicalein-7-*O*-diglucoside, baicalein-7-*O*-glucoside, baicalein and chrysin are separated and purified from the *O. indicum* seeds with a two-phase solvent system comprising of chloroform–methanol–water (8:10:5). From 200 mg crude, 50 mg compound 3, 10.5 mg compound 1, 4.5 mg compound 2, 25 mg compound 4 and 45 mg compound 5 could be obtained in ten times run.

Table 3
The signals of ^{13}C NMR spectra of compounds 1–5 and standard flavonoids

^{13}C NMR signals	Compound 1	Compound 3	Compound 4	Compound 5	Chrysin	Baicalein
C-2	163.9	163.8	155.5	164.8	164.5	155.5
C-3	104.8	105.1	106.2	104.9	104.9	106.2
C-4	183	182.9	184.6	182.7	182.7	184.6
C-5	146.7	146.8	152.6	162.1	162.1	152.6
C-6	131.1	131.1	131.2	99.27	99.27	131.2
C-7	149.6	149.8	165.9	165.6	165.6	165.9
C-8	94.7	94.6	95.48	95.45	95.45	95.48
C-9	151.8	151.9	148.3	158.4	158.4	148.3
C-10	106.4	106.4	106	104.3	104.3	106
C-1′	130.8	130.8	133.1	131.4	131.4	133.1
C-2′, 6′	126.7	126.7	127.7	126.3	126.3	127.7
C-3′, 5′	129.5	129.5	130.6	129.1	129.1	130.6
C-4′	132.2	132.4	133.3	131.9	131.9	133.3
C-1″	162.7	101.2				
C-2″	73.4	73.5				
C-3″	77.1	77.7				
C-4″	70.5	70.6				
C-5″	75.9	76.2				
C-6″	61.4	60.98				
C-1‴	103.8					
C-2‴	73.7					
C-3‴	75.9					
C-4‴	69.8					
C-5‴	77.3					
C-6‴	61.4					

Acknowledgements

The author would like to thank Chinese government to give financial support and Brunel Institute for Bioengineering in Brunel University to give technical support in CCC.

References

[1] Chiang Su New Midical College in: Dictionary of Chinese Crude Drugs, Shanghai Scientific Technological, Shanghai, 1977, p. 368
[2] T. Tomimori, Y. Imoto, M. Ishida, Shoyakugaku Zasshi 42 (1988) 98.
[3] R. Kyo, N. Nakahata, I. Sakakibara, M. Kubo, Y. Ohizumi, J. Pharmacol. 50 (1998) 1179.
[4] S. Ikemoto, K. Sugimura, N. Yoshida, R. Yasumoto, S. Wada, K. Yamamoto, T. Kishimoto, Cancer Lett. 160 (2000) 219.
[5] H. Takizawa, A.M. DelliPizzi, A. Nasjletti, Hypertension 31 (1998) 866.
[6] Z.Y. Cehn, Y.L. Su, C.W. Lau, W.I. Law, Y. Huang, Eur. J. Pharmacol. 374 (1999) 41.
[7] J.H. Lin, Y.T. Lin, J. Food Drug Anal. 7 (1997) 185.
[8] L. Wei, K. Kazuo, A. Yoshihisa, Photochemistry 60 (2002) 351.
[9] Y. Ito, W.D. Conway, High Speed Countercurrent Chromatography, Wiley, New York, 1996.
[10] A. Berthod, K. Talabardon, F. De La Poype, J. Liq. Chromatogr. Rel. Technol. 21 (1998) 3003.
[11] T.Y. Zhang, X. Hua, R.X. Xiao, J. Liq. Chromatogr. 11 (1988) 233.
[12] Y.Y. Lin, K.J. Ng, S. Yang, J. Chromatogr. 629 (1993) 389.
[13] B. Ducrey, J.L. Wolfender, A. Marston, K. Hosttmann, Photochemistry 38 (1995) 129.
[14] J. Renee, N. Grayer, C. Veitch, Photochemistry 47 (1998) 779.