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Application of accelerated solvent extraction coupled with high-performance counter-current chromatography to extraction and online isolation of chemical constituents from *Hypericum perforatum* L.

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ABSTRACT

Accelerated solvent extraction (ASE) coupled with high-performance counter-current chromatography (HPCCC) was successfully used for the extraction and online isolation of five chemical constituents from the plant *Hypericum perforatum* L. The upper phase of the solvent system of ethyl acetate–methanol–water (5:2:5, v:v:v) was used as both the ASE solvent and the HPCCC stationary phase. Two hydrophobic compounds including 28.4 mg of hyperforin with a HPLC purity of 97.28% and 32.7 mg of adhyperforin with a HPLC purity of 97.81% were isolated. The lower phase of ethyl acetate–methanol–*n*-butanol–water (5:2:2.5:12, v:v:v:v) was used as both the ASE solvent and CCC stationary phase. Three hydrophilic compounds of 12.7 mg of 3,4,5-*O*-tricaffeoylquinic acid with a HPLC purity of 98.82%, 15.2 mg of 1,3,5-*O*-tricaffeoylquinic acid with a HPLC purity of 99.46% and 42.5 mg of 3-*O*-caffeoylquinic acid with a HPLC purity of 96.90%, were obtained in a one-step extraction–separation process with less than 3 h from 10.02 g of raw material of *H. perforatum*. The targeted compounds isolated, collected and purified by HPCCC were analyzed by high performance liquid chromatography (HPLC), the chemical structures of all five compounds above mentioned were identified by UV, MS and NMR.

1. Introduction

Hypericum perforatum L., a typical traditional Chinese medicine, is used all over the world for the treatment of depression [1,2]. Additionally, it has been reported that the extracts of *H. perforatum* may have an inhibitory effect [3,4], on human bladder cancer cells [5], and act as an antioxidant [6] and anticonvulsant [7]. The extracts of *H. perforatum* contain many constituents with documented biological activity such as quinic acids [8], phloroglucinols [9] and a broad range of flavonoids [8–10]. Exposure of the extracts of *H. perforatum* to light may lead to the degradation of phloroglucinols, which are extremely sensitive to oxidation and unstable in solution on exposure to air [11,12], therefore phloroglucinols are difficult to separate and isolate by conventional method. In this case, a method combining an extraction system with an isolation system online to avoid the exposure of the extracts to air and light is urgently needed.

Quinic acids have previously been shown to possess a multitude of pharmacological activities [13–15]. Tricaffeoylquinic acids have strong antihyperglycemic [16] and antimutagenic effects. However, the supplies of tricaffeoylquinic acids have been limited due to their very low content in natural plants and difficulties in isolating their pure compounds from natural sources. More efficient extraction and separation methods to provide bioactive components with high sample recovery are also needed.

Accelerated solvent extraction (ASE) has several advantages over traditional solvent extraction methods, including shorter extraction time, lower solvent consumption, higher extraction yields, high reproducibility [17] and less extraction discrimination [18]. Many applications of ASE have been reported in food and pharmaceutical field [19–22]. Compared to conventional liquid–solid separation methods, counter-current chromatography (CCC) has the advantage of sample recovery as no solid phase is employed, thus preventing the irreversible adsorption of analytes and allowing for a theoretical recovery rate of 100% [23–27]. Counter current chromatography has been extensively used for the separation and purification of natural products and other researches [26–30].

This paper is the first to report the combination of ASE and HPCCC (ASE–HPCCC) online, and the two instrumental setups of ASE–CCC successfully applied in this experiment. As a consequence of using the two instrumental setups of online extraction–isolation

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Table 1
Description of ASE extractions.

No.	Extraction solvent	For hydrophobic compounds[a] (upper phase as extraction solvent)			For hydrophilic compounds[b] (lower phase as extraction solvent)		
		Temperature (°C)	Pressure (psi)	Content dry weight[c] (mg/g)	Temperature (°C)	Pressure (psi)	Content dry weight[c] (mg/g)
1	HEX–EtOAc–MeOH–water (1:5:1:5, v:v:v:v)	60	600	0.26 ± 30.01	100	900	–[d]
2	HEX–EtOAc–MeOH–water (1:4:1:5, v:v:v:v)	90	800	0.25 ± 0.01	120	1200	–
3	HEX–EtOAc–MeOH–water (0.5:3:1:5, v:v:v:v)	130	1000	0.62 ± 0.02	140	1500	–
4	HEX–EtOAc–MeOH–water (0.5:2.5:1:5.5, v:v:v:v)	150	1200	0.53 ± 0.02	160	1800	–
5	EtOAc–MeOH–water (4:1:7, v:v:v)	80	600	7.33 ± 0.12	100	900	8.31 ± 0.16
6	EtOAc–MeOH–water (4:2:5, v:v:v)	100	800	7.44 ± 0.14	120	1200	8.42 ± 0.16
7	EtOAc–MeOH–water (5:2:5, v:v:v)	130	1000	7.65 ± 0.14	140	1500	8.45 ± 0.15
8	EtOAc–MeOH–water (6:2:4, v:v:v)	150	1200	7.45 ± 0.16	160	1800	8.58 ± 0.14
9	EtOAc–MeOH–BuOH–water (5:2:5:15, v:v:v:v)	80	600	7.10 ± 0.17	100	900	8.31 ± 0.15
10	EtOAc–MeOH–BuOH–water (5:2:2.5:12, v:v:v:v)	100	800	7.20 ± 0.16	140	1200	8.57 ± 0.18
11	EtOAc–MeOH–BuOH–water (6:2:1:10, v:v:v:v)	130	1000	7.30 ± 0.16	160	1500	8.53 ± 0.17
12	EtOAc–MeOH–BuOH–water (4:3:2:8, v:v:v:v)	150	1200	7.21 ± 0.17	180	1800	8.60 ± 20.13
13	EtOAc–BuOH–water (5:1:8, v:v:v)	60	600	7.79 ± 0.21	120	900	4.35 ± 0.10
14	EtOAc–BuOH–water (5:2:5, v:v:v)	90	800	7.91 ± 0.21	140	1200	5.06 ± 0.09
15	EtOAc–BuOH–water (3:1:2, v:v:v)	130	1000	7.89 ± 0.21	160	1500	5.09 ± 0.09

[a] Amounts of hyperforin plus adhyperforin.
[b] Amounts of 3,4,5-*O*-tricaffeoylquinic acid plus 1,3,5-*O*-tricaffeoylquinic acid and 3-*O*-caffeoylquinic acid.
[c] Data are expressed as mean ± SD. For each sample $n = 3$.
[d] Not determined.

process, five compounds, including hyperforin, adhyperforin, 3,4,5-*O*-tricaffeoylquinic acid, 1,3,5-*O*-tricaffeoylquinic acid and 3-*O*-caffeoylquinic acid were separated and purified from *H. perforatum*.

2. Experimental

2.1. Reagents and materials

Ethyl acetate, *n*-hexane, *n*-butanol, methanol and ethanol used were of analytical grade (Beijing Chemicals, Beijing, China). Water was purified on a Milli-Q water purification system (Millipore, Boston, USA). Acetonitrile and acetic acid were of HPLC grade (Fisher Scientific, Pittsburg, PA, USA). *H. perforatum* L. was harvested from Qiannan autonomous region of Guizhou province (Qiannan, China) and identified by Yuchi Zhang (Changchun Normal University, Changchun, China).

2.2. Apparatus

Accelerated Solvent Extraction 150 System (Dionex, Sunnyvale, CA, USA) with 100 ml stainless steel ASE vessels was used for the pressurized liquid extraction. High-performance countercurrent chromatography was performed on a DE Spectrum HPCCC (Dynamic Extractions, Slough, UK). The multilayer coil separation column was prepared by winding a 28 m × 2.6 mm I.D. PTFE tube directly onto one of the holders forming multiple coiled layers to give a total capacity of 125 ml. The β-value varied from 0.33 at the internal terminal to 0.58 at the external terminal ($R = 8$ cm, $\beta = r/R$, where r is the distance from the coil to the holder shaft and R is the revolution radius or the distance between the holder axis and the central axis of the centrifuge). The rotation speed was adjusted in a range of from 0 to 1600 rpm and 1400 rpm was used in the present study. The HPCCC system was equipped with a solvent delivery module of BT 8100 (Biotronic, Maintal, Germany), an integrator of D-2500 (Merck Hitachi, Darmstadt, Germany), and an injection valve with a sample loop of 10 ml. Electrospray ionization mass spectrometry (ESI-MS) was performed on a Finnigan LCQ ion-trap mass spectrometer (Thermo Finnigan, San Jose, USA). High-performance liquid chromatography (HPLC) was carried out on a Waters 2695 coupled with a Waters 2998 Diode array detector (DAD) (Milford, USA). Nuclear magnetic resonance spectra were recorded on a Bruker AV 500 spectrometer (Bruker BioSpin, Rheinstetten, Germany).

2.3. Accelerated solvent extraction

An ASE 150 System with 100 ml stainless steel ASE vessels was used for the pressurized liquid extraction. About 10.02 g of *H. perforatum* powder was mixed homogeneously with the same weight of diatomaceous earth and placed into the extraction cell. The extraction cells were placed into the ASE system and the extraction conditions and process were as follows: firstly, static time of 5 min, followed by a flush elution with 60% volume, and followed by the nitrogen purge of 60 s, and extract one time [31]. The extraction pressure and the extraction temperature were optimized in the subsequent experiments (provided in Table 1).

2.4. Selection of the two-phase solvent systems of HPCCC and ASE extraction solvent

In view of the upper phase and lower phase of the two-phase solvent system of HPCCC used as ASE extraction solvent, we investigated the extraction solvent and isolation solvent system simultaneously. A series of solvent systems was selected as the ASE solvent and HPCCC separation solvent systems with the extraction conditions summarized in Table 1. First, the upper phase of the solvent system was used as the ASE solvent for extracting the hydrophobic compounds, and after extraction, 3 ml of ASE solution was added into a test tube, and then the same volume of the