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Short communication

High-speed counter-current chromatography separation and purification of resveratrol and piceid from *Polygonum cuspidatum*

Lei Chen[a,*], Yashan Han[a], Fuquan Yang[b], Tianyou Zhang[b]

[a]*College of Food Science, China Agricultural University, Beijing 100094, China*

[b]*Beijing Institute of New Technology Application, Beijing 100035, China*

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Abstract

High-speed counter-current chromatography (HSCCC) was applied to the separation and purification of resveratrol and piceid from the dried roots (20.0 g) of *Polygonum cuspidatium*. The EtOAc extracts were separated with chloroform–methanol–water (4:3:2, v/v). Resveratrol was identified in fraction 5. The water extracts were separated first with EtOAc–EtOH–water (10:1:10, v/v) and then with the same solvent system at the modified volume ratio of 70:1:70. Yields of resveratrol and piceid obtained were 2.18% and 1.07%. Chemical structures of the purified resveratrol and piceid were confirmed by electrospray ionization MS and ^{1}H nuclear magnetic resonance spectroscopy.

Keywords: Counter-current chromatography; *Polygonum cuspidatum*; Plant material; Resveratrol; Piceid; Polyphenols

1. Introduction

Resveratrol (3,4′,5-trihydrohydroxystilbene) and piceid (3,4′,5-trihydrohydroxystilbene-3-β-mono-D-glucoside) (Fig. 1) are phenolic compounds found in many families of plants. They can inhibit fungi pathogens of plants, and regulate plant–parasite interaction [1]. Resveratrol and piceid were first reported in the peel of grape berries for disease resistance and later in wines to benefit health. They were found to be the major polyphenols found in the root of *Polygonum cuspidatum*. Their contents were much higher in *P. cuspidatum* than in grape. The root of this plant was traditionally used in China and Japan as a folk medicine for the treatment of atherosclerosis and for other therapeutic purposes [2]. Resveratrol and piceid caused great interest after they were found to have effects in; inhibiting the copper-catalyzed oxidation of low-density lipoprotein (LDL) [3], inhibiting platelet clotting and arachidonate metabolism, reducing liver injury from peroxidized oil [4], and having cancer-chemopreventive activities, it had been suggested that resveratrol, a common constituent of the human diet, merits investigation as a potential cancer-chemopreventive agent in humans [5].

HPLC and column chromatography were used to separate and purify resveratrol and piceid from natural plant sources, but these two methods were time-consuming and used large amounts of reagent. Furthermore, these methods caused a great loss of the resveratrol and piceid [5]. In this paper, we described high-speed counter-current chromatography (HSCCC) to separate resveratrol and piceid

*Corresponding author.

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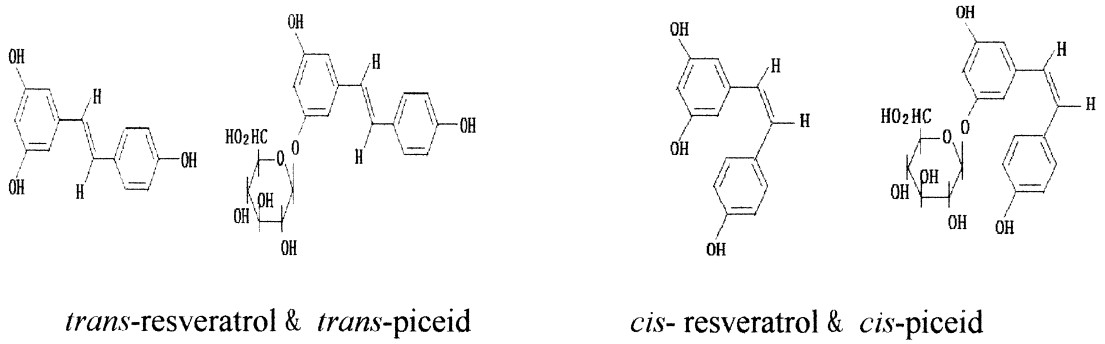

Fig. 1. Structures of resveratrol and piceid.

with a simple procedure and high purity. This special type of liquid–liquid fractionation chromatography used no solid support matrix and therefore eliminated irreversible adsorption of samples. HSCCC is increasingly used for component purification from herbs.

2. Experimental

2.1. Apparatus

Preparative HSCCC was performed with a Model GS10A2 multilayer coil planet centrifuge (Beijing Institute of New Technology Application, Beijing, China) equipped with a PTFE 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 0.8 at the external terminal ($\beta=r/R$ 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 central axis of the centrifuge). The system was also equipped with one NS-1007 constant flow pump, a Model 8823A-UV monitor operating at 254 nm, a Yokogawa 3057 recorder and a manual injection valve with a 10-ml loop. The HPLC system was from LKB, equipped with a HPLC 2150 pump, a 2151 variable-wavelength monitor, a 2152 LC controller, a 2155 column oven and a 2156 solvent conditioner.

2.2. Reagents

All solvents used for HSCCC were of analytical grade or above. Methanol used for HPLC was purchased from Fisher (USA), the standard of *trans*-resveratrol was from Sigma (USA).

2.3. Sample preparation

A 20.0-g dry mass of *Polygonum cuspidatum* was extracted by 200 ml methanol, the mixture was centrifuged and the supernatant was washed with light petroleum (b.p. 60–90°C). The remaining methanol phase was evaporated to form a syrup. The syrup was then dissolved and fractionated in 20 ml water and 20 ml EtOAc. Resveratrol existed in the EtOAc phase and piceid in the water phase. Both EtOAc and water solution were vacuum evaporated at 40°C, and 1.5122 g residue of EtOAc and 1.8801 g residue of water were obtained.

2.4. HSCCC separation

In each separation process, the coiled column was first entirely filled with the upper organic stationary phase, then the apparatus was rotated at 80 rpm while the lower aqueous mobile phase was pumped into the column at flow-rate of 2.0 ml/min. After the mobile phase front emerged and the system established a steady state hydrodynamic equilibrium, the sample solution was injected through the injection valve. The effluent from the outlet of the column was continuously monitored with a UV detector at 254 nm. Peak fractions were separately collected for analysis.

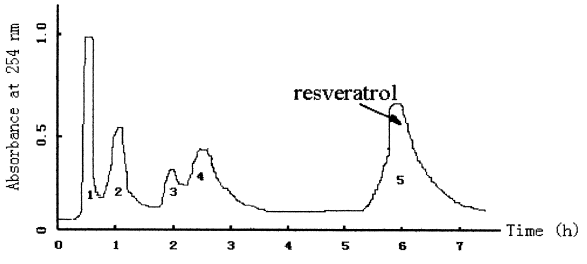

Fig. 2. HSCCC separation of resveratrol in *Polygonum cuspidatum*. Solvent system: chloroform–methanol–water (4:3:2).

2.4.1. Separation of trans-resveratrol by HSCCC

A solvent system consisting of chloroform–methanol–water (4:3:2) was used for resveratrol HSCCC separation, and five fractions were obtained. Each fraction was analyzed by thin-layer chromatography (TLC) with precoated silica G-25 UY_{254} plates. TLC plates were developed with hexane–EtOAc–formic acid (30:10:0.5), and detected at 254 nm. Fraction 5, a completely separated peak, was supposed to be resveratrol (Fig. 2), 72.5 mg dried resveratrol was obtained from the peak.

2.4.2. Separation of trans-piceid by HSCCC

Two steps of separation with EtOAc–EtOH–water (10:1:10 and 70:1:70, v/v) were used. Among the five fractions of step one (EtOAc–EtOH–water, 10:1:10, v/v), fraction 2 was supposed to be an enriched fraction of piceid with 58.5 mg yield (Fig. 3). The fractionally purified fraction was separated by HSCCC using the same solvent at a volume ratio of 70:1:10. Fraction 2 was collected and gave a yield

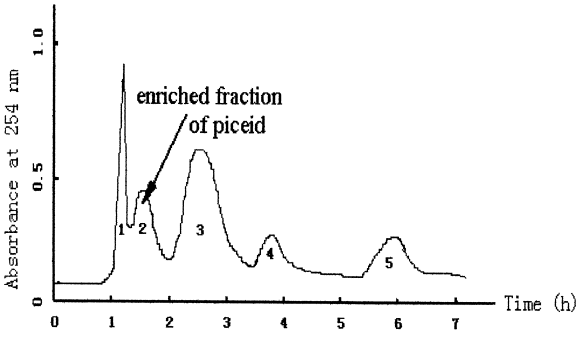

Fig. 3. HSCCC separation of piceid in *Polygonum cuspidatum* – step one. Solvent system: ethyl acetate–ethanol–water (10:1:10).

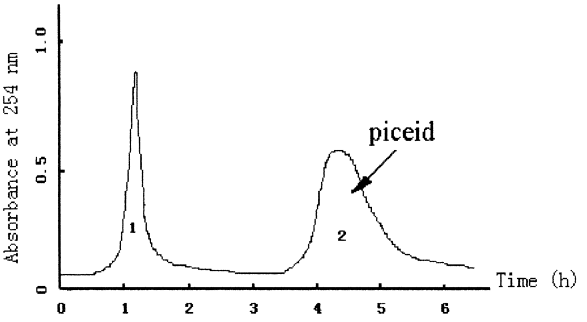

Fig. 4. HSCCC separation of piceid in *Polygonum cuspidatum* – step two. Solvent system: ethyl acetate–ethanol–water (70:1:70).

of 35.45 mg of piceid (Fig. 4). During the two steps, TLC analysis was carried out on precoated silica G-25 UV_{254} plates. Visualization of TLC plates was performed using 10% phosphomolybdic acid in absolute ethanol as a spray reagent. Spots were visualized by spraying the plates and then heating them at 110°C for 3 min in an oven. A solution of EtOAc–hexane–acetic acid (8:1:1, v/v) was used to develop the TLC plates.

2.5. Analysis of trans-resveratrol and trans-piceid by HPLC

Resveratrol and piceid obtained as described in Sections 2.4.1 and 2.4.2 were qualitatively analyzed by HPLC. Peaks in Fig. 5 indicated that the HSCCC separated fractions were *trans*-piceid and *trans*-resveratrol with purity higher than 99% comparing with *trans*-resveratrol standard.

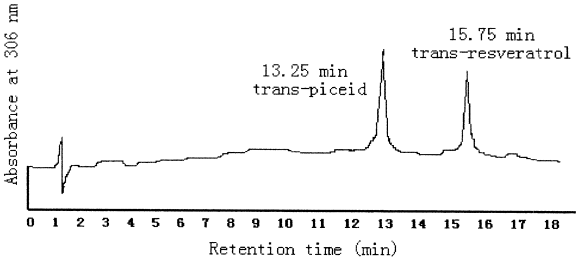

Fig. 5. HPLC separation of *trans*-piceid and *trans*-resveratrol. HPLC conditions: solvent A: 10% methanol in water, solvent B: 90% methanol in water. A Nova-Pak C_{18} (150×3.9 mm, 5 μm) column was used at a 0.60 ml/min flow-rate with a linear gradient from 0% B to 90% B in 25 min.

2.6. Analysis of trans-resveratrol and trans-piceid by ^{1}H nuclear magnetic resonance spectroscopy (NMR)

In dimethyl sulfoxide (DMSO), the ^{1}H-NMR data were as following. Resveratrol: 6.11 (d, 1H), 6.37 (d, 2H), 6.76 (d, 2H), 6.90 (d, 1H), 6.92 (d, 1H), 7.39 (d, 2H), 9.17 (s, 2H), 9.52 (s,1H). Piceid: 3.16~3.70 (H of glucoside), 4.62~5.27 (OH of glucoside), 6.33 (d, 1H), 6.56 (s, 1H), 6.72 (d, 2H), 6.86 (d, 1H), 7.03 (d, 1H), 7.40 (d, 2H), 9.46 (s, 2H). From the data, it was easy to confirm resveratrol, but for piceid, mass spectrometric analysis was necessary.

2.7. Analysis of trans-piceid by mass spectrometry (MS)

Electrospray ionization (ESI) MS was adopted to analyze *trans*-piceid, the data shown here. m/z 391.1 (M+1), 229.1 ($C_{14}H_{13}O_3^+$). The molecular mass of *trans*-piceid is 390.

3. Results and discussion

3.1. trans and cis isomers

Both resveratrol and piceid have *trans* and *cis* isomers; *trans* isomers are much more stable than *cis* ones, thus most of the resveratrol and piceid exist in *trans* form. However, *trans* isomers could transform into *cis* ones under UV light. The activity of the resveratrol glucoside was observed to be quite different from that of the aglycon, but the human digestive tract was known to have glucosidase activity [6], so it was possible that the glucoside of resveratrol could release the aglycon on ingestion.

3.2. Yields of trans-resveratrol and trans-piceid

After sample preparation, from 20.0 g *P. cuspidatum*, 1.5122 g coarse extract was gained, 252 mg of which was experimented by HSCCC to give 72.5 mg *trans*-resveratrol. On the other hand, 1.8801 g of aqueous extract was obtained, 310 mg of which was used for HSCCC, and after two analyses 35.45 mg of *trans*-piceid was left. Therefore, in this study, 2.18% and 1.07% of *trans*-resveratrol and *trans*-piceid, respectively, can be obtained from *P. cuspidatum*.

The results of our experiments indicate that HSCCC can be used as a suitable method for the preparative separation and purification of *trans*-resveratrol and *trans*-piceid.

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