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Rapid and efficient purification of chrysophanol in *Rheum Palmatum LINN* by supercritical fluid extraction coupled with preparative liquid chromatography in tandem

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ABSTRACT

Chrysophanol has high pharmaceutical values. However, it was difficult to use the traditional extraction method to extract high-concentration chrysophanol. Therefore, the purpose of this study is to purify and separate chrysophanol in traditional herb, *Rheum Palmatum LINN*, by using supercritical fluid extraction (SFE) and preparative high-performance liquid chromatography (P-HPLC) for rapid and large-scale isolation. The method is efficient for selective extraction of chrysophanol from the herbs, which have complex compositions. The extraction efficiency of chrysophanol with SFE is 25× higher than that of boiled water extraction under the same extraction time. The optimal conditions for SFE were 210 atm and 85 °C for 30 min; for P-HPLC, a C18 column was used with a gradient elution of methanol and 1% acetic acid at a flow rate of 10 mL/min. According to ^{1}H NMR and LC–MS analyses, the purity of the isolated chrysophanol was as high as 99%. The recovery for chrysophanol in *Rheum* after SPE/PHPLC processing was in the range of 88–91.5%. Compared with other extraction and purification methods, the sequential system (SFE/P-HPLC) achieved the highest amount of extracted chrysophanol from *Rheum Palmatum LINN* (0.38 mg/g) and the shortest run time (3 h). Hence, this rapid and environmentally friendly method can separate compounds based on polarity with high efficiencies and, coupled with P-HPLC, it may be applicable in the large-scale production of foods and medicines in the future.

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1. Introduction

Rheum Palmatum LINN (*Rheum*), a well-known Chinese herbal medicine, has been used for thousands of years in China. It is a traditional purgative containing considerable amounts of hydrophilic glycosides, such as sennoside A, sennoside B, and emodin-6-monoglucoside [1,2]. Anthraquinones of *Rheum* have been used extensively since ancient times because of their therapeutic and biological properties, which include antioxidant [3–5], antifungal [6], antimicrobial [7,8], larvicidal [9], and anticancer activities [10–12]. Chrysophanol (1,8-dihydroxy-3-methylanthracenedione, Fig. 1) is an anthraquinone and has been identified as a metabolite responsible for antimicrobial activity, shortening of blood coagulation time, and anticancer action [13–16]. It is usually detected in the root of the plant *Rheum*. Anthraquinones are often used as a quality criterion of drug efficacy for medicinal herbs; therefore, numerous extraction and purification methods have been established for the isolation of anthraquinone derivatives, including aqueous methanol or acetonitrile extraction, high-speed countercurrent chromatography (HSCCC), and electrophoresis chromatography [17,18]. MS and UV have been used for the detection and quantification of anthraquinone derivatives [19,20]. Most studies on *Rheum* have focused on its hydrophilic components [21], whereas few studies have focused on its hydrophobic constituents. Hydrophilic glycosides are traditionally extracted with hot water or alcohol [22]. However, traditional extraction methods, such as stewing in hot water or alcohol immersion, cannot be used for efficient extraction of hydrophobic compounds (i.e., chrysophanol).

Supercritical carbon dioxide extraction is a promising technology that is a potentially more efficient alternative to conventional solvent extraction for bioactive components, such as for food and pharmaceutical applications, because it provides higher selectivity, shorter extraction times, and does not use toxic organic solvents. In the SFE process, by varying either pressure or temperature,

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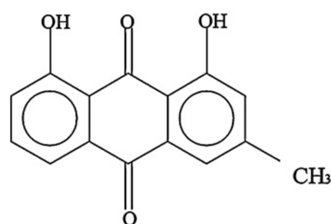

Fig. 1. Chrysophanol (1,8-dihydroxy-3-methylanthracenedione) structure, ($C_{15}H_{10}O_4$), molecular weight: 254.23.

supercritical fluid densities can be altered. In addition, extraction time and CO_2 flow rate can affect the efficiency of Sc-CO_2 extraction. Thus, a substance can be extracted selectively at a specific range of densities. SFE can also proceed at low temperatures to prevent the decomposition of thermally sensitive compounds [23–25]. In addition, Sc-CO_2 exhibits strong solvating power of a liquid and the fast exchange kinetics of a gas. When extraction is complete, liquid carbon dioxide can be removed by reducing the pressure and allowing the gas to evaporate. Carbon dioxide is chemically inert and is the solvent of choice for SFE in the manufacture of natural products, foods, flavoring, and medicine [26–30]. Therefore, in this study, we developed a system that combines supercritical CO_2 extraction (SFE) and preparative high-performance liquid chromatography (P-HPLC) in tandem to purify chrysophanol in large quantities and in a short period.

2. Experimental

2.1. Materials

The plant material of *Rheum Palmatum LINN* was collected from Sichuan, located in southwest China. Chrysophanol and the internal standard 1,8-dihydroxyanthraquinone were purchased from Sigma–Aldrich, USA. A known mass of solute was dissolved in ethanol to prepare different concentrations in 2, 5, 10, 15, 20, and 25 mg/L for the calibration curve. All chemicals and solvents were of analytical and HPLC grades (Sigma–Aldrich, USA).

2.2. Apparatus

The SPE/P-HPLC system was developed for the rapid and large-scale isolation of chrysophanol from the herbs. A schematic diagram of the tandem system is shown in Fig. 2. Supercritical fluid extraction was performed on an ISCO syringe pump (model 260D, Isco, Lincoln, NE, USA) equipped with a 50-mL stainless steel extraction vessel with two 0.45-μm filters, each positioned at the entrance and exit of the vessel. At the exit of the extraction vessel, 30 cm of stainless steel tubing (316 SS, 1/16-in. OD, 0.030-in. ID) was used as the pressure restrictor for the exit of CO_2. The entire extraction vessel and tubing were placed in a thermostatic oven. The extracts were extracted by supercritical CO_2 under proper conditions, and the released CO_2 was passed through a solvent to trap the target components in a 100-mL funnel-shaped collector with a stir bar at the bottom. The collector was connected with 2-mL sample loops of P-HPLC and was performed using a Prep-Star SD-1 pump linked to a Pro-Star 335 diode-array spectrophotometer (Hewlett-Packard, USA). Analytes were separated on a preparative C18 column (250 mm × 19 mm ID with a particle size of 5 μm, Waters, USA).

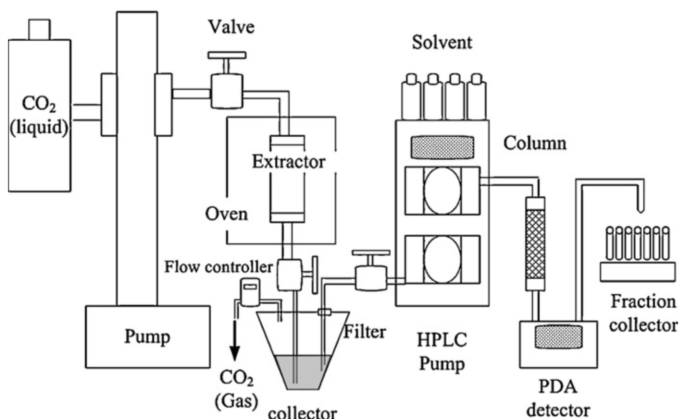

Fig. 2. Schematic diagram of the tandem system of supercritical fluid extraction and P-HPLC.

2.3. Procedure for the SPE/P-HPLC

All herbal samples were sun-dried, ground, and sieved, which resulted in a powder with particle sizes between 0.3 and 0.85 mm. A 5-g sample was placed in a stainless steel extraction vessel (50 mL) and was then extracted using the SFE procedure with Sc-CO_2. Optimal extraction conditions were achieved by varying the experimental parameters sequentially. The static extraction time, extraction pressure, and temperature were considered. The extraction conditions were altered as follows: temperatures of 40, 50, 60, 70, and 80 °C; pressures of 90, 120, 150, 180, 210, and 240 atm; and extraction times of 5, 10, 20, 30, 40, 50 and 60 min. After the appropriate extraction conditions were determined, the extracts were collected in 25 mL of ethanol at a CO_2 flow rate of 50 mL/L. Subsequently, 5 mL of the extractant in ethanol was injected into preparative P-HPLC for the separation and purification of chrysophanol from the hydrophobic fraction. A preparative C18 column was used under a gradient elution identical to the gradient used with the analytical column (Pursuit™ 250 mm × 4.6 mm ID with a particle size of 5 μm). The mobile phase was methanol and acetic acid (1%) in gradient mode as follows: 0–3 min: 50% methanol; 3–5 min: 50–10% methanol; 5–35 min: 10–85% methanol; and 35–40 min: 85% methanol. The flow rates through the analytical column and the preparative column were 1 mL/min and 10 mL/min, respectively.

2.4. Recovery

For percent-recovery experiments, 1 g samples of homogenized *Rheum* powder were spiked with 0.5, 1, and 2 mg of the chrysophanol standard reagent. These spiked samples were applied on the SFE/p-HPLC system, as described in Section 2.3. To calculate the recovery, the 1 g of *Rheum* was used as a blank sample. The recovery was calculated as follows:

$$\text{Recovery}\,(\%) = \frac{\text{chrysophanol in spiked sample} - \text{blank sample}}{\text{Amount of added chrysophanol}}$$

2.5. Characterization of chrysophanol

Chrysophanol identification was achieved by comparing the retention time and the wavelength of UV at 254 nm. Chrysophanol isolated from *Rheum* was identified by ^{1}H NMR and ESI-MS (Instrumentation Center, National Tsing Hua University).

3. Results and discussion

The experiments were performed to develop an SFE/P-HPLC tandem process for rapid obtainment of chrysophanol from *Rheum Palmatum LINN* and with a large yield. To determine the optimum

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