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Short communication

Extraction of berberine from rhizome of *Coptis chinensis* Franch using supercritical fluid extraction

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Abstract

Supercritical fluid was used to extract berberine from rhizome of *Coptis chinensis* Franch. The recovery of berberine was compared with various modifiers, i.e. methanol and 95% ethanol with and without surfactant Tween 80, and 1,2-propanediol. The results show that the yield obtained after 3 h extraction with 1,2-propanediol-modified supercritical carbon dioxide was the highest (from 6.91%, w/w at 200 bar to 7.53%, w/w at 500 bar), while that obtained with 95% ethanol modified-supercritical carbon dioxide was the lowest (from 0.15%, w/w at 300 bar to 0.19%, w/w at 600 bar). The recovery of berberine was not improved by adding 5% Tween 80 in supercritical fluid.

Keywords: Supercritical fluid extraction; Berberine; *Coptis chinensis* Franch; Modifiers; Sample preparation

1. Introduction

Rhizome of *Coptis chinensis* Franch has been used in traditional Chinese medicine for clearing away heat and depriving dampness for treatment of diarrhea, dysentery and jaundice, and purging the sthenic fire and clearing away toxic material for the cases of seasonal febrile diseases, carbuncle, sorethroat. The herbal medicine possesses broad-spectrum antibacterial and antiprotozoal effects [1]. The major active ingredient of the herb, berberine, is an isoquinoline derivative alkaloid (Fig. 1) and has many pharmacological effects including activation of the aryl hydrocarbon receptor [2], insulin sensitizing and insulinotropic action [3], inhibition of arylamine *N*-acetyltransferase activity [4], inhibition of HIF-1alpha expression as a potent anti-angiogenic agent [5], anxiolytic effect by acting at 5-HT receptors [6], anti-inflammatory and anti-cancer by inhibiting basal and 12-otetradecanoylphorbol-13-acetate mediated prostaglandin E2 level and cyclooxygenase-2 expression [7], and lowering blood cholesterol, and especially LDL-cholesterol [8].

For conventional extraction methods, such as solvent extraction, there are few adjustable parameters to control the selectivity of the extraction processes. Therefore, developing alternative extraction techniques with better selectivity and efficiency are highly desirable. Consequently, supercritical fluid extraction (SFE) as an environmentally responsible and efficient extraction technique for solid materials was introduced and extensively studied for separation of active compounds from herbs and other plants. Today, SFE has become an acceptable extraction technique used in many areas. SFE of active natural products from herbs or more generally, from plant materials has become one of the most important application areas [9]. Since clinical trails have indicated that berberine is a pharmacologically active ingredient, a great deal of research has been done to develop more effective and selective extraction methods for recovery of the compound from medicinal plants. At present, extraction of berberine in different raw materials using pressurized methanol extraction [10] and pressurized hot water from coptidis rhizoma [11], and ion-pair supercritical fluid extraction in phellodendri cortex with subsequent analysis by on-line ion-pair supercritical fluid chromatography has been reported [12]. However, no report has been done for extraction of berberine from *C. chinensis* Franch using SFE.

Various modifiers, such as methanol and surfactants have been used in attempts to compensate for the poor ability of

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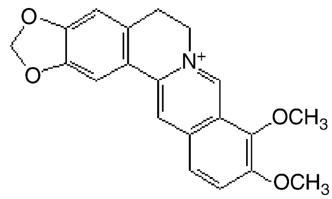

Fig. 1. Structure of berberine.

supercritical carbon dioxide to solvate polar organic compounds and to interrupt strong analyte–matrix interactions [13]. It was reported that non-ionic surfactant Tween 80 allow the efficient extraction of phenolic compounds from environmental solid matrices [14]. It is not clear whether the recovery of berberine from plant matrices can be improved by adding the surfactant in supercritical fluid. The purpose of this work was to study the use of SFE to obtain *C. chinensis* Franch extracts and to evaluate the effects of pressure and different modifiers, i.e. methanol and 95% ethanol with and without surfactant Tween 80, and 1,2-propanediol, on the yield of berberine of the extracts.

2. Experimental

2.1. Materials

Rhizome of *C. chinensis* Franch was obtained from Wuhan Second Hospital (Wuhan, China). Berberine hydrochloride standard was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). CO_2 (food grade) was from Fangxin Gas Ltd. (Ningbo, China). Acetonitrile of HPLC grade was purchased from Tianjin Shield Company (Tianjin, China). Hydrochloric acid, methanol, 95% ethanol, and 1,2-propanediol were analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Celite (Chemical grade) was from Fengcheng Chemical Ltd. (Shanghai, China).

2.2. Supercritical fluid extraction system

A supercritical fluid extractor Spe-ed SFE-2 (Applied Separation, USA) was used, which operates with two pumps, a master pump fitted with a cooling jacket on the pump head and a second pump (Knauer pump, model K-501, Berlin, Germany) for the addition organic modifier. The Sped-ed SFE is a screening system extractor that allows a modularity of extraction but requires more handling of the controls. It is capable of pressure up to 680 bar and temperature up to 240 °C, static and dynamic extraction with flow from 0 to 10 L/min (gaseous carbon dioxide) and extraction vessels from 5 mL to 1 L. A metering valve is used to vary flow. Collection is at room temperature and atmospheric pressure. The extracted analytes are collected in glass vial (30 mL) with a rubber plug at the top. A metal extension to the metering valve is used to pierce the rubber plug and allow collection directly in the collection solvent (methanol). A hypodermic needle connected to a flow meter also pierces the plug for the mensuration of flow. Collection was at room temperature and atmospheric pressure.

2.3. HPLC conditions

A high-performance liquid chromatography system (Knauer, Berlin, Germany) equipped with a Knauer pump (model K-501) and an ultraviolet–visible detector (Knauer, model K2501) was used. The column used for separation was a Diamonsil C18 separation column (5 μm, 250 × 4.6 mm i.d., Dikma Technologies, Beijing, China). The mobile phase was 33 mM of potassium dihydrogen phosphate–acetonitrile (70: 30, v/v) at a flow-rate of 1 mL/min. Detection was at a wavelength of 345 nm. For all experiments, 20 μL of standards and sample extract were injected.

2.4. Preparation of reference standards

A series of standards of berberine hydrochloride in the range of 1.7–68 μg/mL were prepared in methanol. Quantification was done using external standard calibration. A linear response with a correlation coefficient of 0.999 ($n = 6$) was obtained for the standards. Following all content was calculated in berberine hydrochloride form.

2.5. Preparation of medicinal plant extracts

Rhizome of *C. chinensis* Franch was ground into powder using a herbal pulverizer (FW 100, Tianjin Taisite Instrument Co. Ltd., Tianjia, China) and sieved through a 250 μm filter. For extraction using SFE, a known quantity of grounded sample (1 g) was placed in a 10 mL of extraction vessel (60 × 15 mm, i.d.) and the void volume was filled with celite. Before the extraction was started, the extraction vessel was preheated in the oven for 10 min. The extraction conditions were as follows: extraction time, static extraction for 5 min and then dynamic extraction up to 3 h; temperature, 60 °C; pressure, from 200 to 500 bar; flow-rate of carbon dioxide (gaseous state), 1 L/min; flow-rate of modifier, 0.4 mL/min. The extract was collected in a glass vial containing 5 mL of methanol, and then quantitatively transferred to a 25 mL volumetric flask and made up to the mark with methanol. This solution was further diluted suitably prior to analysis.

For Soxhlet extraction, a known quantity of grounded sample (1.0 g) was accurately weighed into a thimble and was extracted in a 50 mL of extractor with 50 mL of hydrochloric acid:methanol (1:100) at a syphon rate of 1 cycle/5 min. Berberine and co-extract gave a yellow color with the extraction solvent. The yellowish color turned lighter and lighter through the course of the extraction. After extraction with the solvent for 8 h, the extraction solvent was essentially colorless and the extracts were transferred to a 50 mL volumetric flask and made up to the mark with methanol. This solution was further diluted suitably prior to analysis. The recovery of berberine in plant materials can be improved by addition of a little of hydrochloric acid in methanol and the method has been adopted by literature [15].

All extracts were filtered through a 0.45 mm membrane filter before injecting into the HPLC system.

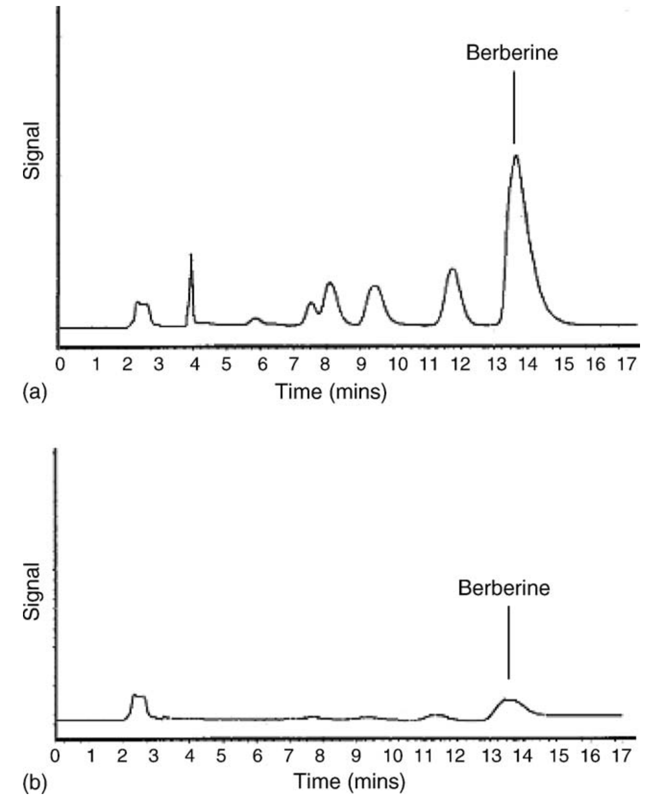

Fig. 2. High-performance liquid chromatograms of berberine using (a) 1,2-propanediol-modified supercritical carbon dioxide, and (b) ethanol-modified supercritical carbon dioxide.

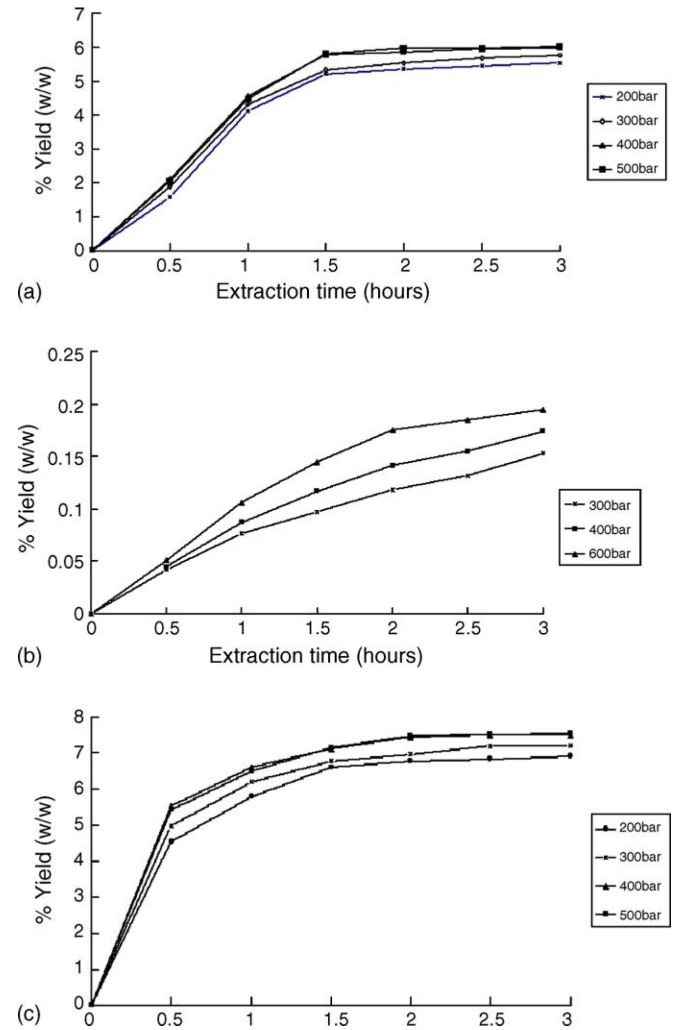

Fig. 3. Influence of pressure on the cumulative percentage yield of berberine extracted by several kind of modifiers at 60 °C. (a) methanol; (b) 95% ethanol; and (c) 1,2-propanediol.

3. Result and discussion

Five organic solvent modifier systems, i.e. ethanol-modified supercritical carbon dioxide, methanol-modified supercritical carbon dioxide, 1,2-propanediol-modified supercritical carbon dioxide, 5% Tween 80 in methanol-modified supercritical carbon dioxide, 5% Tween in ethanol-modified supercritical carbon dioxide, were used to extract berberine from powdered Rhizome of *C. chinensis* Franch in order to evaluate the feasibility of SFE, and the effect of methanol and ethanol with and without surfactant Tween 80, and 1,2-propanediol used as modified on the yield of berberine. The HPLC chromatograms of the extract using 1,2-propanediol-modified supercritical carbon dioxide and ethanol-modified supercritical carbon dioxide are showed in Fig. 2a and b, respectively. It is possible to identify the berberine peak, which appears at a retention time of approximately 13.7 min. (*Note*: all other extracts show chromatograms similar with Fig. 2a). It can been seen in Fig. 2a and b that fewer peaks appear for the extract obtained with ethanol-modified supercritical carbon dioxide than that obtained with 1,2-propanediol-modified supercritical carbon dioxide.

Berberine is a polar compound and it is ineffective to extract the ingredient using supercritical CO_2 only. As a result, polar modifier should be considered. Usually, addition of a small amount of a liquid modifier can enhance significantly the extraction efficiency and, consequently, reduce the extraction time [9] and the method can be used to improve yield of some alkanoids, such as indole alkanoids [16] and sinomenine [17] from plant materials. Fig. 3 shows the effect of extraction pressure on the yield of berberine at 60 °C using (a) methanol-modified supercritical carbon dioxide; (b) 95% ethanol-modified supercritical carbon dioxide; and (c) 1,2-propanediol-modified supercritical carbon dioxide. It can be seen that the yield, after 3 h, is increased slightly by change of pressure from 5.53% (w/w, 200 bar) to 6.02% (w/w, 500 bar) with methanol-modified supercritical carbon dioxide, 6.91% (w/w, 200 bar) to 7.53% (w/w, 500 bar) with 1,2-propanediol-modified supercritical carbon dioxide, and 0.15% (w/w, 300 bar) to 0.19% (w/w, 600 bar) with 95% ethanol-modified supercritical carbon dioxide. It means that the yield of berberine can be varied by change of pressure, but the influence of pressure range from 200 to 500 bar on yield is small when methanol, 95% ethanol or 1,2-propanedio is used as polar modifier of supercritical CO_2.

The extraction using surfactant is an attractive method, which has been utilized to extract organic compounds, such as pyridine derivatives [18] and quaternary ammonium compounds

Table 1
Extraction yield of berberine from *Coptis chinensis* Franch

Modifier	Extraction condition	% Yield (w/w) mean ± S.D. ($n = 5$)
95% Ethanol	Temperature, 60 °C; pressure, 300 bar, flow-rate, 1 L/rnin CO_2 and 0.4 rnL/rnin modifier, dynamic extraction time, 1.5 h	0.10 ± 0.02
5% Tween in 95% ethanol	Temperature, 60 °C; pressure, 300 bar, flow-rate, 1 L/min CO_2 and 0.4 mL/min modifier; dynamic extraction time, 1.5 h	1.29 ± 0.19
Methanol	Temperature, 60 °C; pressure, 300 bar, flow-rate, 1 L/rnin CO_2 and 0.4 rnL/min modifier; dynamic extraction time, 1.5 h	5.34 ± 0.24
5% Tween in methanol	Temperature, 60 °C; pressure, 300 bar, flow-rate, 1 L/min CO_2 and 0.4 mL/min modifier; dynamic extraction time, 1.5 h	3.43 ± 0.35
1,2-Propanediol	Temperature, 60 °C; pressure, 30 bar; flow-rate, 1 L/min CO_2 and 0.4 rnL/min modifier; dynamic extraction time, 1.5 h	6.95 ± 0.38
	Soxhlet extractor; extraction solvent, hydrochloric acid: methanol (1:100); extraction, 8 h	7.21 ± 0.34

[19]. It is not clear whether the recovery of berberine from *C. chinensis* Franch can be improved by addition of surfactant Tween 80 in methanol-modified supercritical carbon dioxide or ethanol-modified supercritical carbon dioxide. So the recovery of berberine was determined, using 5% Tween 80 in methanol-modified supercritical carbon dioxide and 5% Tween 80 in 95% ethanol-modified supercritical carbon dioxide over periods of extraction extending to 3 h and it was compared with that obtained only methanol, 95% ethanol or 1,2-propanediol used as modifier in Fig. 4. No experiment was done for extraction with Tween 80 in 1,2-propanediol modified-supercritical carbon dioxide due to too viscidity for the mixture of Tween 80 and 1,2-propanediol. It is obvious that improvement of recovery of berberine was marginal when addition of Tween 80 in 95% ethanol (0.15% for 95% ethanol and 1.63% for 5% Tween in 95% ethanol, while addition of the surfactant in methanol has an adverse effect on recoveries (5.76% for methanol and 3.81% for 5% Tween in methanol). The case is similar with the extraction of nitro-aromatic compounds with non-ionic surfactant as modifier, in which the recovery was not significantly improved by adding surfactant to methanol-modified fluid [13]. Small synergetic effect occurs for addition of 5% Tween in ethanol-modified or methanol-modified supercritical carbon dioxide. Fig. 4 shows also that the highest recovery was obtained using 1,2-propanediol as polar modifier, and methanol used as polar modifier was also effective, while 95% ethanol used as polar modifier was ineffective for the extraction of berberine from the powdered sample. As displayed in Fig. 4, it is possible to extract over 90% of berberine within 1.5 h except the extraction using ethanol or Tween plus ethanol as modifier (all yield being based on the recovery obtained after 3 h). The results for extraction of berberine from powdered rhizome of *C. chinensis* Franch with different modifiers, as well as using hydrochloric acid:methanol (1:100, v/v) in Soxhlet extractor are summarized in Table 1. It shows that extraction with 1,2-propanediol-modified supercritical CO_2 at 300 bar for extraction of 1.5 h gives lower yield than with Soxhlet extraction.

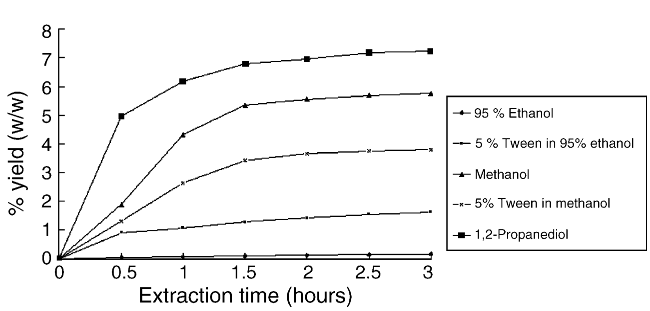

Fig. 4. Influence of different modifiers on the cumulative extraction yield of berberine at 62 °C and 300 bar.

4. Conclusion

The study shows that the recovery of berberine from the powdered rhizome of *C. chinensis* Franch was influenced by using various modifiers in supercritical CO_2, among which the highest recovery was obtained by using 1,2-propanediol as modifier, while the recovery obtained using 95% ethanol as modifier was the lowest. It is suitable for extraction of berberine from *C. chinensis* Franch to use 1,2-propanediol as a polar modifier in supercritical CO_2. The recovery of berberine obtained with ethanol-modified or methanol-modified supercritical carbon dioxide was not improved by adding Tween 80 in the supercritical fluid.

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