Effect of Plant Matrix and Fluid Ethanol Concentration on Supercritical Fluid Extraction Efficiency of Schisandrin Derivatives

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Abstract

An investigation of the effect of plant matrix on the supercritical fluid extraction efficiency of five schisandrin derivatives is reported, exhibiting a great difference with respect to extraction efficiency depending on the matrix. Pure supercritical CO_2 at 60°C and 34.0 MPa cannot fully recover schisandrin derivatives from the leaves as much as from the other matrices. Only 36.9% of these compounds are extracted from leaves of *Schisandra chinensis* by supercritical CO_2 in comparison with organic solvent extraction. However, more than 80% of schisandrin derivatives are obtained from both stem and fruit parts. Ethanol addition also shows a different effect depending on plant matrix; that is, CO_2 modified with 10% ethanol could enhance the yield of schisandrin derivatives from leaves by four times when compared with that of pure CO_2, but it has little effect on both stems and fruits.

Introduction

Schisandra chinensis Baillon (Schisandraceae) is used as an antitussive, tonic, and sedative agent and has been found in traditional medicine to improve the liver function of patients with viral hepatitis (1,2). The main components of *S. chinensis* are schisandrin derivatives, the lignans having a dibenzocyclooctadiene skeleton, that have also been known as the active principles of *S. chinensis*. Among these lignans, the important ones are schisandrol A (**1**) and B (**2**) and schisandrin A (**3**), B (**4**), and C (**5**) (Figure 1). These compounds were found to prevent liver injuries, stimulate liver regeneration, and inhibit hepatocarcinogenesis (3–5).

Schisandrin derivatives of *S. chinensis* have been extracted from plants by several organic solvents such as methanol, chloroform, *n*-hexane, and petroleum ether (6–8). However, these organic solvents are potentially hazardous to one's health and should be eliminated from the final extract. Therefore, an alternative method should be evaluated for the extraction of these compounds.

As noted in the literature (9–11), supercritical fluids are being increasingly used as an alternative to conventional organic solvents for the extraction of natural products, because they possess some advantages such as shorter extraction times, enhanced selectivity, and lack of residual solvent in the final extracts in comparison with a conventional organic solvent. Among the candidate supercritical solvents, CO_2 has been widely used because it is nontoxic, nonflammable, environmentally acceptable, and shows low critical properties.

Recently, the effect of temperature, pressure, and density of supercritical CO_2 on the supercritical fluid extraction (SFE) efficiency of five shisandrin derivatives from *S. chinensis* fruits (one of the plant parts containing bioactive schisandrin derivatives) was investigated. As a result, the optimum SFE conditions for these compounds was determined as 60°C and 34.0 MPa. In this condition, the yields could reach approximately 80% of MeOH extraction. Moreover, the SFE method could greatly enhance the selectivity of schisandrin derivatives. Thus, SFE

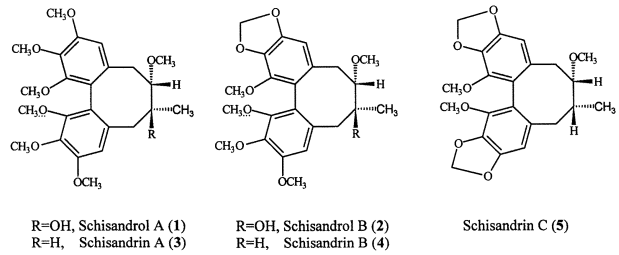

Figure 1. Chemical structures of the schisandrin derivatives employed in this study.

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could probably provide an alternative to the conventional organic solvent extraction for schisandrin derivatives from fruits of *S. chinensis* (12).

Other than the fruit, it was found that the leaves and stems of *S. chinensis* contain significant amounts of schisandrin derivatives (13). In this paper, the SFE method was applied to the extraction of these compounds from leaves and stems. Among numerous phenomena affecting SFE, the matrix is known to potentially influence the SFE efficiency (14). Some workers have reported that solubility does not completely explain recoveries of some solutes from a complex matrix (15,16). However, there have been some reports of the effect of plant matrix on SFE efficiency. In addition, the effect of ethanol concentration as a modifier was evaluated in order to improve the yield of schisandrin derivatives from each matrix.

Experimental

Plant material

Leaves, stems, and fruits of *S. chinensis* were collected from Medicinal Herb Garden (College of Pharmacy, Seoul National University) in October of 1995. The authenticity was confirmed by one of the authors (J. Kim). The plant materials were dried at 40°C for 24 h and then pulverized.

Reagents and chemicals

The standards of schisandrol A (**1**) and B (**2**) and schisandrin A (**3**), B (**4**), and C (**5**) were obtained from the Korean Research Institute of Bioscience and Biotechnology (KIST, Taejon, Korea). The stock solutions of the standard compounds were prepared in the range of 8.0–800 µg/mL to generate a calibration curve. Ethanol (99.9% purity) was obtained from Merck (Darmstadt, Germany). The high-performance liquid chromatography (HPLC)-grade methanol, chloroform, water, and acetonitrile were purchased from J.T. Baker (Phillipsburg, NJ). Petroleum ether and *n*-hexane were obtained from Duksan Chemical Co. (Yongin, Kyungki Province, Korea).

Organic solvent extraction

The dried and pulverized plant materials (100 mg) were extracted with *n*-hexane, petroleum ether, chloroform–methanol (2:1), or methanol under reflux (1 h × 3). The solvent was evaporated under reduced pressure, and each extract was dissolved in 2 mL methanol for the HPLC analysis.

SFE

SFE was performed on an ISCO (Lincoln, NE, USA) model SFX 3560 supercritical fluid extractor equipped with two syringe pumps using pure CO_2 and CO_2 modified with 1, 5, and 10% ethanol (v/v) at 60°C and 34.0 MPa. In each experiment, 100 mg of plant material was loaded into an extraction cell (57 × 20-mm i.d., Isco), and the remaining volume was filled with glass wool. The restrictor was kept at 80°C, and the static extraction time was set at 5 min. The flow rate was 1.0 mL/min for each experimental condition, and the dynamic extraction was maintained for 30 min. In each extraction step, the extract was collected in methanol every 6 min. The preparation of the sample solution for the HPLC analysis was the same as that of the organic solvent extraction.

HPLC analysis

The HPLC instrument consisted of a Hitachi (Tokyo, Japan) L-6200 pump, an L-4000 ultraviolet detector fixed at 240 nm, and a D-2500 integrator. The sample solution was injected through a

Table I. Yields of Schisandrin Derivatives Obtained by Extraction with *n*-Hexane, Petroleum Ether, Chloroform–Methanol (2:1), Methanol, and Supercritical CO_2 at 60°C and 34.0 MPa from Leaves, Stems, and Fruits of *S. chinensis**

Matrix	Solvent	Yield (mg/g) ± standard deviation				
		Schisandrol A	Schisandrol B	Schisandrin A	Schisandrin B	Schisandrin C
Leaves	*n*-hexane	0.3 ± 0.02	0.1 ± 0.01	0.2 ± 0.04	0.9 ± 0.1	0.4 ± 0.04
	petroleum ether	0.2 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.7 ± 0.01	0.3 ± 0.01
	chloroform–methanol (2:1)	1.2 ± 0.1	0.4 ± 0.03	0.9 ± 0.04	2.3 ± 0.1	0.9 ± 0.1
	methanol	1.3 ± 0.2	0.5 ± 0.1	1.0 ± 0.04	2.5 ± 0.1	1.0 ± 0.1
	SFE†	0.4 ± 0.04	0.1 ± 0.01	0.4 ± 0.02	1.0 ± 0.1	0.04 ± 0.03
Stems	*n*-hexane	1.1 ± 0.1	1.1 ± 0.1	0.8 ± 0.1	2.3 ± 0.2	1.1 ± 0.1
	petroleum ether	1.0 ± 0.02	1.0 ± 0.02	0.8 ± 0.03	2.2 ± 0.1	1.0 ± 0.04
	chloroform–methanol (2:1)	1.7 ± 0.1	1.4 ± 0.02	0.9 ± 0.1	2.6 ± 0.1	1.2 ± 0.04
	methanol	1.7 ± 0.2	1.4 ± 0.1	0.9 ± 0.1	2.4 ± 0.2	1.1 ± 0.1
	SFE†	1.7 ± 0.1	1.0 ± 0.1	0.8 ± 0.04	2.1 ± 0.2	1.1 ± 0.1
Fruits‡	*n*-hexane	5.1 ± 0.5	1.5 ± 0.1	1.5 ± 0.2	5.9 ± 0.1	1.1 ± 0.1
	petroleum ether	5.0 ± 0.1	1.5 ± 0.2	1.5 ± 0.1	5.8 ± 0.1	1.1 ± 0.1
	chloroform–methanol (2:1)	5.8 ± 0.4	1.6 ± 0.1	1.4 ± 0.1	5.6 ± 0.3	1.1 ± 0.1
	methanol	7.4 ± 0.2	2.0 ± 0.2	1.8 ± 0.3	6.7 ± 0.3	1.3 ± 0.1
	SFE†	5.8 ± 0.4	1.6 ± 0.1	1.3 ± 0.2	6.0 ± 0.5	1.1 ± 0.1

* All experiments were performed in triplicate.
† SFE using pure CO_2.
‡ Taken from reference 12.

20-µL loop. A YMC-Pack (YMC Inc., Kyoto, Japan) ODS-A (250 × 4.6 mm, 5 µm) with an Econosphere C_{18} (7.5 × 4.6 mm, 5 µm) guard column (Alltech, Deerfield, IL) were eluted isocratically with acetonitrile–water (60:40). The flow rate was 1.0 mL/min.

Results and Discussion

HPLC analysis of schisandrin derivatives from leaves, stems, and fruits of *S. chinensis*

Prior to the SFE of five schisandrin derivatives from the *S. chinensis* leaves and stems, the preliminary quantitative analysis of these compounds was carried out on these matrices to confirm these plant parts as another resource of the bioactive lignans of *S. chinensis*. Table I shows the yields of these compounds from leaves, stems, and fruits using several solvents. Among the solvents, methanol could recover them more highly than any other solvent regardless of the selectivity (12). Generally, the yields of employed schisandrin derivatives were higher in fruits than leaves or stems. However, schisandrin C (**5**) was found to be as abundant in leaves and stems as in fruits. The yields of schisandrin C (**5**) were 1.0 and 1.1 mg/g in leaves and stems, respectively, with respect to 1.1 mg/g of the fruits. In addition, significant amounts of schisandrol B (**2**), which has been cited as having significant antitumor or hepatoprotective activity among the schisandrin derivatives (17,18), were obtained from stems at 70% those of fruits. These results suggest that leaves and stems of *S. chinensis* could be alternative sources for some schisandrin derivatives.

Comparison of the SFE efficiency of schisandrin derivatives from each plant matrix using pure CO_2

Recent experimental evidence demonstrated that the SF extractability of target compounds was highly dependent upon the characteristic of the matrix and the manner in which the analytes were incorporated into that matrix rather than the solubility of the target compound in extraction fluid (15,16). Therefore, the matrix effect on the extraction efficiency of schisandrin derivatives was investigated before reaching a general conclusion that SFE could be a reliable method for the extraction of these lignans from all parts of the plant matrix.

Figure 2 shows the percent recovery of the total schisandrin derivatives using supercritical CO_2 at 60°C and 34.0 MPa. The SFE recovery of schisandrin derivatives from leaves was clearly different from those of stems and fruits. Only 36.9% of these compounds were recovered from leaves using pure supercritical CO_2, whereas recovery from both stems and fruits were above 80% in comparison with methanol extraction. This result suggests that the SFE efficiency of lignans of *S. chinensisis* is highly dependent on the matrix containing them. The lower extraction efficiency from leaves by nonpolar supercritical CO_2 may be due to the constitutive structural difference of the composition of this matrix, such as in the epidermis, cuticle, or mesophyll cells.

In an extraction process, the selectivity of target compounds is an important factor of the yield. The composition of schisandrin derivatives in the CO_2 extract of stems was compared to that of

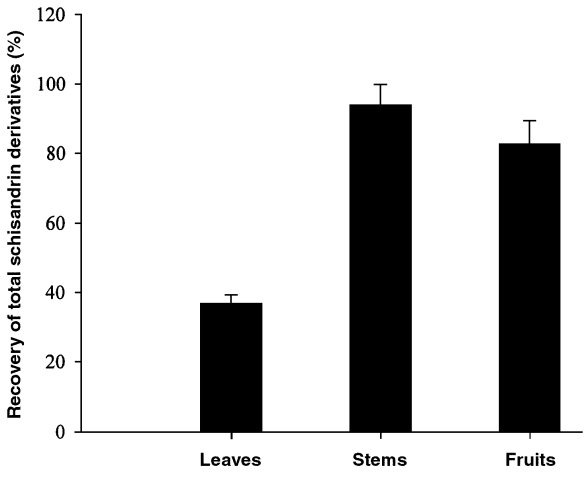

Figure 2. Recovery (%) of total schisandrin derivatives from leaves, stems, and fruits of *S. chinensis* using pure supercritical CO_2 at 60°C and 34.0 MPa in comparison with methanol extraction (mean ± standard deviation). All experiments were performed in triplicate.

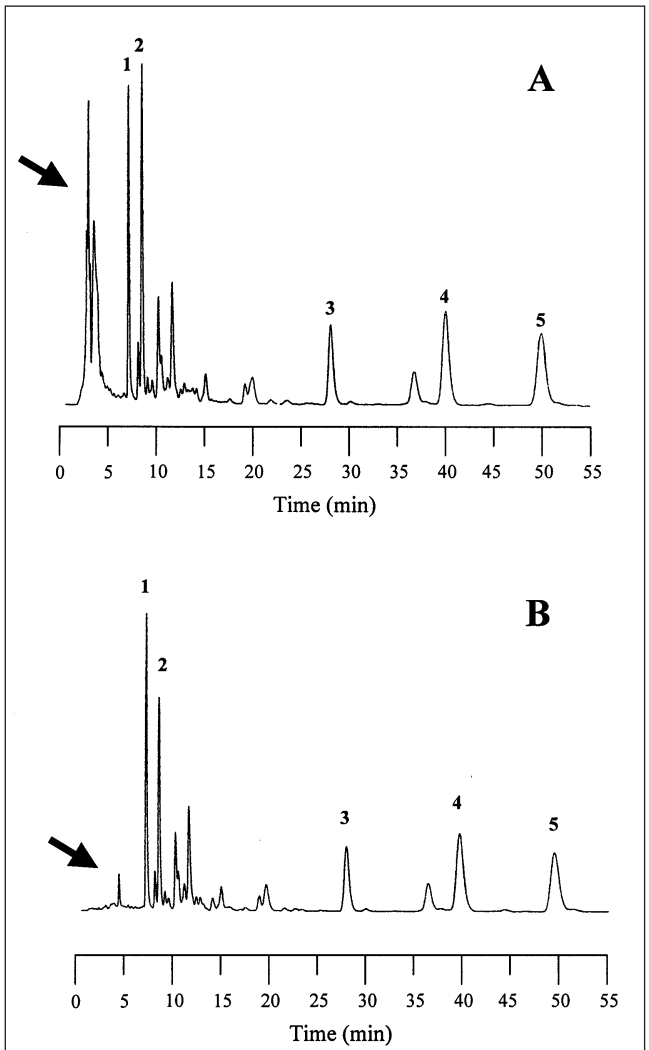

Figure 3. HPLC chromatograms of methanol (A) and supercritical CO_2 extracts (B) of *S. chinensis* stems. Peaks: 1, schisandrol A; 2, schisandrol B; 3, schisandrin A; 4, schisandrin B; 5, schisandrin C.

the methanol extract (Figure 3). In the HPLC chromatogram, supercritical CO_2 did not extract the polar components of *S. chinensis,* which were eluted within 5 min. This means that SFE could enhance the extraction selectivity of the lignans, as well as the extraction yield, when it is applied to the extraction of lignans in the stems of *S. chinensis*.

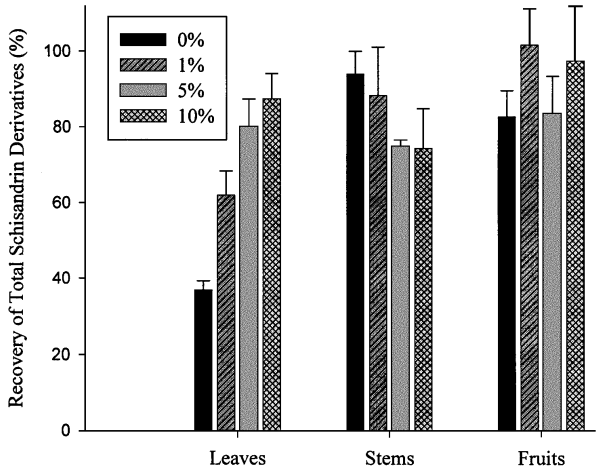

Figure 4. Effect of ethanol concentration on the SFE recovery (%) of schisandrin derivatives from leaves, stems, and fruits (mean ± standard deviation). All experiments were performed in triplicate.

Table II. Yields of Schisandrin Derivatives under Optimum SFE Conditions (60°C and 34.0 MPa) from Leaves, Stems, and Fruits of *S. chinensis**

Schisandrin derivatives	Yield (mg/g) ± standard deviation		
	Leaves (CO_2 + 10% ethanol)	Stems (CO_2)	Fruits (CO_2 + 1% ethanol)
Schisandrol A	1.8 ± 0.1	1.7 ± 0.1	8.1 ± 0.6
Schisandrol B	0.30 ± 0.04	0.98 ± 0.1	1.9 ± 0.2
Schisandrin A	0.70 ± 0.1	0.83 ± 0.04	1.8 ± 0.2
Schisandrin B	1.8 ± 0.2	2.3 ± 0.2	6.6 ± 0.8
Schisandrin C	0.8 ± 0.1	1.1 ± 0.1	1.3 ± 0.2

* All experiments were performed in triplicate.

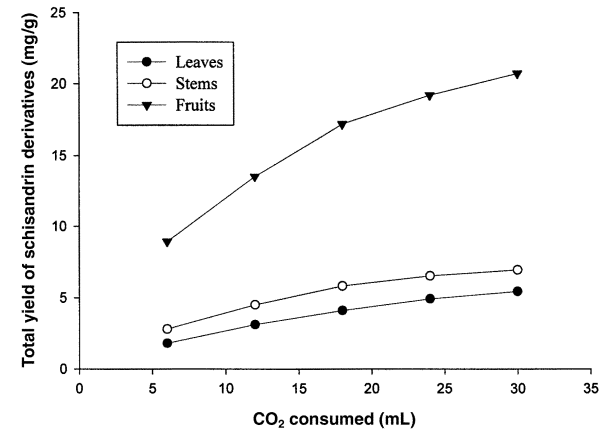

Figure 5. Yield (mg/g) of schisandrin derivatives from leaves, stems, and fruits versus CO_2 consumed (mL).

Table I shows each yield of the schisandrin derivatives employed in this study when using the several conventional organic solvents and supercritical CO_2 at 60°C and 34.0 MPa. All of the schisandrin derivatives in stems and fruits were easily recovered by any extraction solvents employed. In the case of leaves, however, the yields of these compounds from leaves showed great difference depending on the solvent employed. Supercritical CO_2, *n*-hexane, and petroleum ether could extract only 10–37% in comparison with methanol extraction. Therefore, polar modifier, which could increase the polarity of CO_2, should be utilized to enhance the yield of lignans in leaves of *S. chinensis*.

Effect of ethanol as a modifier on the SFE efficiency of schisandrin derivatives from leaves, stems, and fruits

Although CO_2 is a relatively good solvent for the extraction of lipophilic natural products, it has some limitations for the extraction of polar compounds from plant matrix due to its nonpolar character. Therefore, it appears that alternative fluids are required. Unfortunately, other supercritical fluids such as N_2O and CHF_3 have the severe disadvantage of possible ozone depletion or explosion hazard. Thus, modified fluids have been extensively used to enhance the extraction power of CO_2. Among the modifiers employed, water, methanol, and ethanol have been widely used for their extensive effects. Ethanol was employed as a modifier in this study, because water is not as miscible with CO_2 as ethanol (19), and methanol is environmentally hazardous and toxic to human health.

The effect of ethanol on the SFE efficiency of the lignans from leaves, stems, and fruits is shown in Figure 4. Ethanol, which was intended to enhance the polarity of supercritical CO_2, had different effects depending on the plant matrix employed. The extraction yield of the lignans in leaves was greatly increased with an increasing percentage of ethanol. The recovery of them reached 87.4% using 10% ethanol as a modifier in comparison with methanol extraction. However, in the case of stems and fruits, ethanol had little effect on the SFE extraction efficiency for these compounds as a whole. These results further indicate that the SFE efficiency of schisandrin derivatives largely depends on the type of plant matrix than on the solute solubility.

With respect to the concentration of modifier, the optimum condition of SFE for schisandrin derivatives from leaves, stems, and fruits were determined as 10% ethanol, neat supercritical CO_2, and 1% ethanol, respectively. Each yield of schisandrin derivatives under optimum SFE conditions is listed in Table II. Although all SFE yields of the compounds were slightly lower than methanol extraction, schisandrol A was more highly recovered by SFE. The cumulative extraction profile of schisandrin derivatives from each matrix under optimum conditions is shown in Figure 5, showing more than 60% of schisandrin derivatives obtained using 12 mL of supercritical solvent (1.0 mL/min flow rate).

Conclusion

This paper demonstrates that the SFE efficiency of schisandrin derivatives of *S. chinensis* was highly affected by the plant matrix

(leaves, stems, and fruits). Although the recovery of these compounds from both stems and fruits were more than 80% using neat supercritical CO_2 at 60°C and 34.0 MPa, only 36.9% was obtained from leaves. In addition, it was also found that the addition of ethanol as a modifier had different effects, depending on the plant matrix, on the SFE extraction efficiency of the compounds. These results reveal by example that not only the solubility of a target compound but also the desorption of the compound from the matrix are key factors in the SFE of natural products from plants.

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