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Isolation of Acanthoside-D from *Acanthopanax senticosus* Using Supercritical Fluid Extraction

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Supercritical fluid extraction with co-solvent scheme for the extraction of acanthoside-D from *Acanthopanax senticosus* was developed in this work. Supercritical carbon dioxide was used as the extraction solvent at the flow rate of 20 L/min and water was chosen as the co-solvent in a range from 0.5 to 3.0 mL/mg. Different pressure from 20 to 30 MPa under the extraction temperature 333.15 K were investigated to see their effects on extraction results. The results showed that the yield of acanthoside-D was a little larger under higher pressure. Water additives could improve the yield of acanthoside-D and the best yield was obtained when the water content was 1.0 mL/mg. Moreover, with the pressure of supercritical carbon dioxide increasing, the purity of concentrated components increased.

Key Words: Supercritical fluid extraction, Acanthoside-D, *Acanthopanax senticosus*.

INTRODUCTION

The pharmaceutical studies of natural products are one of the most interesting and active research areas. Usually the pharmacologically active compounds in medical plants are in low concentrations, therefore developing new extraction technique with better selectivity and efficiency is highly desirable. Supercritical fluid extraction is a new technique and has been proven to be alternative method of the traditional liquid-liquid extraction. Supercritical fluid extraction offers several advantages over conventional extraction methods, such as, increased selectivity, expeditiousness, automaticity, environmental safety, dramatically decreased use of organic solvents, higher speed and better reproducibility[1].

The significant advantages of supercritical fluid are (a) good solvating power (which is related to density), (b) high diffusivity, (c) low viscosity, (d) minimal surface tension. Carbon dioxide as the frequently used supercritical fluid for most analytical applications because of its moderate critical parameters (critical pressure Pc = 7.68 MPa, critical temperature Tc = 304.23 K) and other highly desired properties, such as non-toxicity,

non-flammability, non-explosiveness, low reactivity, low cost, high purity and environmentally compatibility[2]. These advantages have attracted increasing interest from researchers, especially from food, pharmacy and environmental engineering industries fields[3].

Acanthopanax senticosus belongs to *Acanthopanacis cortex*, which not only shows medical benefit for treating tonic, lumbago, neuralgia and palsy, but also shows acceleration activities of metabolism[4]. It is a typical plant medicine in Korea and widely used in many fields, such as food, medicine, tea, drink and cosmetics[5,6]. Acanthoside-D is regarded as the index component of *Acanthopanax senticosus* and has been reported to show activities of increasing T-cell level, reducing cholesterol, increasing liver function, inhibiting stomach ulcer, improving the immunity of the organism and inhibiting leukemia[7-12].

Usually the isolation of acanthoside-D from *Acanthopanax senticosus* was performed by solvent extraction, in which an aqueous solution of 80 and 100 % ethanol was often used. The goal of this study is to investigate the feasibility of supercritical fluid extraction of Acanthoside-D from *Acanthopanax senticosus* using carbon dioxide at supercritical state and the additive of water and analyzed by reversed-phase high performance liquid chromatography. The yield of Acanthoside-D in supercritical carbon dioxide with different pressures and amounts of additive was investigated.

EXPERIMENTAL

The *Acanthopanax senticosus* was purchased from Korea Siberian Ginseng Association. Acnathoside-D was prepared in the laboratory by coupling of solvent extraction and preparative HPLC[8]. Fig. 1 shows the molecular structure of acanthoside-D. Methanol, ethanol and acetonitrile were purchased from Duksan Co. (HPLC grade, Kyungki-Do, Korea). Twice distilled water was filtered by decompressing pump (Division of Millipore, Waters, Milford, MA, USA) and filter (FH-0.5 μm, Waters, Milford, MA, USA).

M930 solvent delivery pump (Young Lin Co., Korea), UV detector (M720 Absorbance Detector, Young-In Scientific Co., Korea), colume oven (CTS30 HPLC Column Oven, Young Lin Co., Korea), a Reodyne injection valve with a 5.0 mL sample loop and integrated data system (Autochrowin. Ver. 1.42, Young Lin Co., Korea). The equipment for the extraction of acanthoside-D from *Acanthopanax senticosus* is shown in Fig. 2 (From Korean Institute of Science Technology, Seoul, Korea). Rotary-evaporator was LABO-THERM SW-200 (Buchi, New Castle, USA). The Waters HPLC column (3.9 × 300 mm, packed with Lichrospher 100RP-C18 particle, 15 μm).

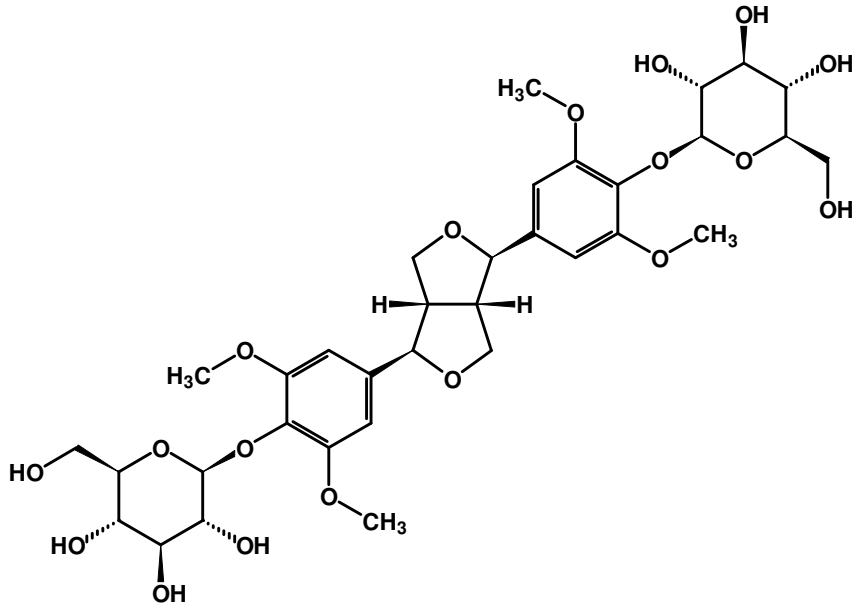

Fig. 1. Molecular structure of acanthoside-D

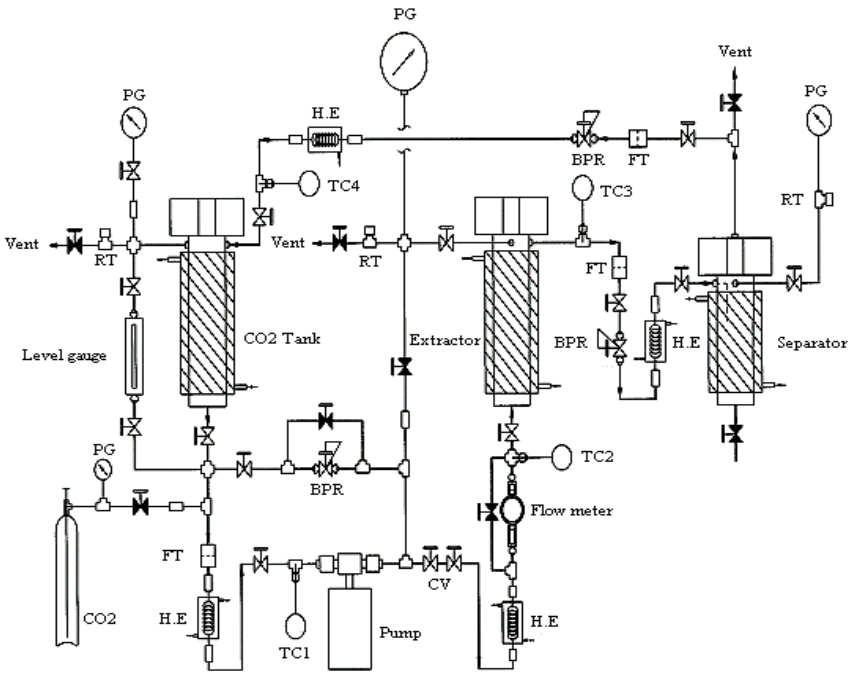

Fig. 2. Schematic diagram of supercritical fluid extraction system used in this study (BPR: back pressure regulator, CV: check valve, FT: filter, HE: heat exchanger; PG: pressure gauge, RT: rupture, TC: thermal controller)

Isolation and analysis of acanthoside-D: 5 g of trunk powder of *Acanthopanax senticosus* was extracted with 100 mL ethanol for 3 h. The ethanol was concentrated using the rotary-evaporator and purified by preparative HPLC system. Acanthoside-D standard was obtained by freeze-dryer after collection from the preparative HPLC and dissolved into water to make a concentration of 1 mg/mL for further use.

The mobile phase was water-acetonitrile-methanol (80:14:6, v/v) and flow rate was 1 mL/min. The UV wavelength was set at 210 nm and column temperature was ambient temperature.

Supercritical fluid extraction: The supercritical fluid extraction procedure was shown in Fig. 3. The extractor was loaded with 120 g Acanthopanax senticosus trunk powder. Supercritical fluid extraction time was 6 h and the flow rate of carbon dioxide was 20 L/min. Temperature of extractor was kept at 333.15 K. HPLC analyses was performed to quantitatively determine the content of acanthoside-D in the supercritical fluid extraction.

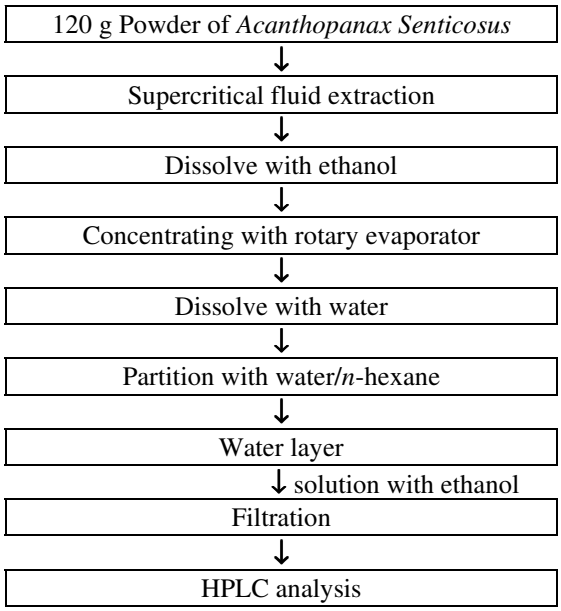

Fig. 3. Extraction and purification procedure of acanthoside-D from *Acanthopanax senticosus*

RESULTS AND DISCUSSION

Preparation of the standard of acanthoside-D: The standard of acanthoside-D was prepared by solvent extraction followed by preparative chromatographic separation. 5 g powder of *Acanthopanax senticosus* trunk

was extracted with 100 mL ethanol for 3 h at 333.15 K. The extraction was condensed using a reflux condenser and then dissolved in 100 mL water. By this way, a large amount of impurities were also co-extracted with acanthoside-D. In order to remove impurities, a partitioning step with water/hexane was undertaken for further refining. Non-polar components moved into hexane layer and polar components including acanthoside-D moved into water layer. The water layer was collected and added by ethanol to make the solution easily pass down through the filter. The effluents were separated by preparative HPLC and the peak of acanthoside-D was collected and dried by freeze-dryer. By this way, the standard of acanthoside-D was obtained (Fig. 4).

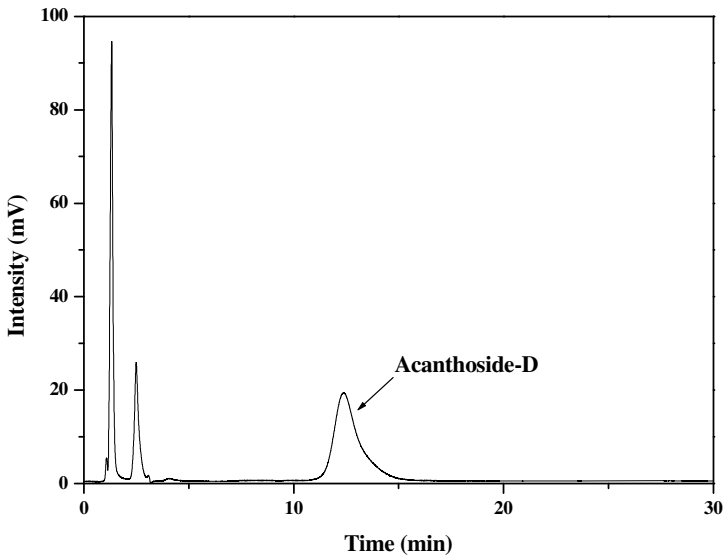

Fig. 4. Chromatogram of acanthoside-D standard (water-acetonitrile-methanol (80:14:6, %v/v), flow rate: 1.0 mL/min)

Quantitative analysis of acanthoside-D in the raw materials by HPLC: The standard solution of 1.0 mg/mL of acanthoside-D was prepared by dissolving the sample in ethanol. The injection volume ranged 2 through 20 μL. Calibration curve was obtained by plotting the peak area *vs.* different injection volumes can be seen in Fig. 5. The regression equation was:

$$Y = 0.0187\ X \quad (1)$$

where X and Y represent the peak area and different injection amount of the standard of acanthoside-D, respectively, the correlation coefficient was 0.9777. The content of acanthoside-D in raw materials of *Acanthopanax senticosus* was 2.98 mg/g.

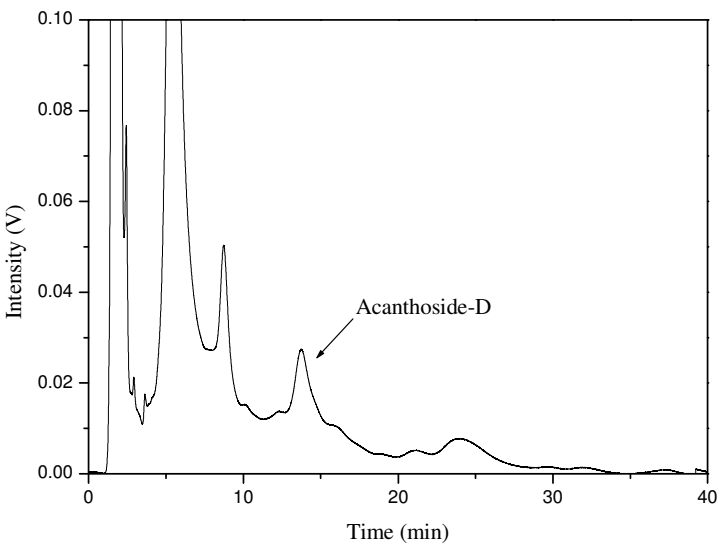

Fig. 5. Chromatogram of *Acanthopanax senticosus* after supercritical fluid extraction (water contents: 1.0 mL/min, mobile phase: water-acetonitrile-methanol (80:14:6, % v/v), flow rate: 1.0 mL/min)

Extraction of acanthoside-D by supercritical fluid extraction: First, different pressures range from 20 to 30 MPa were investigated when other supercritical fluid extraction conditions, *i.e.*, extraction time 6 h, flow rate of carbon dioxide 20 L/min, extractor temperature at 333.15 K were kept constant. The effect of pressure on the yield of acanthoside-D from the raw of *Acanthopanax senticosus* is listed in Table-1. The yield was calculated according to the following equation:

$$\text{Yield of acanthoside-D (\%)} = \frac{\text{Amount of extracted acanthoside} - \text{D}}{\text{Amount of raw material}} \times 100 \quad (2)$$

TABLE-1
YIELD OF ACANTHOSIDE-D FROM *Acanthopanax senticosus* BY SUPERCRITICAL FLUID EXTRACTION

No. of sample	Experimental conditions			Yield (%)
	Water content (mL/g)	Temperature (K)	Pressure (MPa)	
1	0.0	333.15	20	0. 01
2	0.0	333.15	25	0. 01
3	0.0	333.15	30	0. 02
4	0.5	333.15	30	0. 20
5	1.0	333.15	30	1.03
6	1.5	333.15	30	0. 93
7	3.0	333.15	30	0. 34

*Weight of *Acanthopanax senticosus* in the extractor: 120 g.

It can be seen from Table-1 that the yield of acanthoside-D increased with the increasing of supercritical fluid pressure. But the effect of pressure on the extraction yield is not so significant. It can be also seen under the current supercritical fluid extraction conditions, the yield of acanthoside-D is in a low degree when comparing with the content in the raw material. This is because carbon dioxide as the supercritical fluid extraction solvent mostly fit for non-polar materials because of good solubility and selectivity to these compounds. Therefore, polar material in natural materials was difficult to extract. In this case, as acanthoside-D is a moderately polar compound, the yield is low when only carbon dioxide was used as the extraction solvent. On the other hand, many neutral compounds could be extracted at the same time, which may interference the quantitative determination of acanthoside-D by HPLC. So after the collection from the supercritical fluid extractor, the extraction was partitioned with a water/*n*-hexane solvent system to remove the neutral compounds before HPLC analysis.

In order to improve the extracted yields of polar components in supercritical fluid extraction, small amount of liquid modifier can be added into the carbon dioxide fluids as co-solvent. The co-solvents usually show middle property of volatility between supercritical carbon dioxide and target material. The commonly used co-solvents are methanol, ethanol, water and acetone *etc.*[8,13]. In the case of extraction of acanthoside-D, because the compound show moderately polarity and solubility in water[14,15], water was chosen as the additives in the fluid as well as its safety and non-toxicity, which is especially important for extraction of natural plant.

Different water contents (0.5, 1.0, 1.5, 3.0 mL/g) were used as the additives when other supercritical fluid extraction conditions maintained constant. From Table-1 where relative standard deviation (RSD) values were less than 5 %, it can be seen that the yield of Acnathoside-D improved with the addition of water. When the water content was 1.0 mL/g, the yield of acanthoside-D is about 50 times higher than that without water additives. Further increase of water content above 1.0 mL/g cannot result in the increase of yield but decrease on the other hand. Fig. 5 show the HPLC chromatograms of supercritical fluid extraction with 1.0 mL/min water content.

Conclusion

In this work, supercritical fluid extraction of Acnathoside-D from the trunk of *Acathopanax senticosus* has been investigated and the yield of acanthoside-D increased slightly at higher pressure. Moreover, improved extraction yield can be obtained with the additives of water. The maximum yield was obtained when water additives was 1.0 mL/g in the fluid, which was about 50 times higher than that without water additives. Further, when

the polarity of the fluid increased, the yield of acanthoside-D did not inevitably increase, which indicates the solute-modifier interaction in the fluid is complex. The comparison of extraction efficiency by supercritical fluid extraction with that by solvent extraction will be made in a near future.

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