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SUPERCRITICAL FLUID EXTRACTION OF TAXOL AND BACCATIN III FROM NEEDLES OF *TAXUS CUSPIDATA*

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SUMMARY

Taxol and baccatin III were extracted from the ground needles of *Taxus cuspidata* using supercritical carbon dioxide mixed with 3 wt % ethanol as a cosolvent. The pressure and temperature ranges used to attain supercritical fluid condition are 100~300 bar and 40~70 °C, respectively. However, the amount of taxol and baccatin III in the extract obtained at 100 bar was not noticeable, while the major portion of extract was found to be the waxy compounds. The highest selectivity of taxol and baccatin III were about 0.094 and 0.158 wt %, respectively, at 40 °C and 300 bar. At the same pressure and temperature condition, taxol and baccatin III selectivities in the extract obtained from the ground seeds of *Taxus cuspidata* was about 0.198 and 0.157 wt %, respectively.

INTRODUCTION

Taxol isolated from Pacific yew, *Taxus brevifolia*, is the first compound with a taxane ring that has been demonstrated to possess antineoplastic activity, and it has become one of the most important anticancer agents. While the demand for taxol is steadily increasing, availability of the drug is limited due to the restricted nature of the source. Taxol is currently supplied from the bark of the slow-growing yews, which is provided by cutting large numbers of trees. Limitation of bark as a main source of taxol prompted the search for this drug in all parts of the tree as well as in other *Taxus* spp.. The needles of *Taxus* spp. have been very strongly recommended as a possible alternative source since the needles are renewable without damaging forest. Recently, it is indicated that *Taxus* spp. needles also contain amounts of taxol comparable to the bark of *Taxus brevifolia* (Witherup *et al.*, 1990). Moreover, it should be noted that the needles possess relatively high amounts of baccatin III and 10-deacetylbaccatin III used as precursors of efficient semi-synthetic method of taxol. However, as the taxane content in the intact plant is quite small, their extraction using organic solvents and purification by the column chromatography on a large scale is tedious and time-consuming. Recently, It was reported that supercritical fluids can be used to extract taxol from the bark of *Taxus brevifolia* (Jennings *et al.*, 1992). Castor reported the extraction of

taxol from the needles of the ornamental yew using supercritical carbon dioxide (Castor, 1992). The objective of this study was to test the applicability of supercritical fluid extraction (SFE) process for selectively extracting taxol and baccatin III from the needles of *Taxus cuspidata*. In addition, the ground seeds was also tested in order to compare with the extraction results of needles and barks.

MATERIALS AND METHODS

The needles and seeds of *Taxus cuspidata* cultivated in Korea were dried for 24 hrs in the oven maintained at 45 ℃ and ground to 300 mesh size in order to increase the mass transfer efficiency between supercritical fluids and the sample particles. Carbon dioxide used in this study was 99.99 % purity and 99.5 % HPLC grade ethanol was supplied from Aldrich.

A continuous flow-through SFE system was used for this study. Carbon dioxide was supplied from a gas cylinder and was directed to an electrically driven diaphragm-type compressor (Model 554-2121, Nova Werke Ag., Effretikon, Switzerland). A μLC-500 micro flow syringe pump (Model 1240-018, ISCO Inc., Lincoln NE) was used to inject the cosolvent, ethanol, into the solvent gas, carbon dioxide, at a constant volumetric flow rate. The flow rates of ethanol and carbon dioxide used in this experiment were 46 std μL/min and 0.5 std L/min, respectively. In order to maintain a constant pressure within a system, a back pressure regulator (Model 26-1700, Tescom Co., Elk River, MN) with a stated accuracy of ± 1 % of the relief pressure range was employed. The equilibrium cell is a 200 cm^3 high-pressure stainless steel vessel packed with 3 mm glass beads. Approximately 5 g of ground needles and seeds were charged in this cell. To increase the extraction efficiency between carbon dioxide and the sample powder, a metal filter was installed at the end of the inlet tube. The temperature inside the air bath was controlled to within ± 0.1 ℃ by using a proportional temperature controller (Model 4202PC2, Omega Engineering Inc., Stanford, CT). The carbon dioxide-solute mixture leaving the top of the extractor was expanded to atmospheric pressure through a micrometering valve into cold traps where the solute was condensed. The flow rate and volume of carbon dioxide were measured by a flow meter and a dry test meter (Model 63115, Precision Scientific Inc., Chicago, IL). The extraction time of each sample was 4 hrs. A more detailed descriptions for the experimental apparatus and procedure are given elsewhere (Lee *et al.*, 1992; Song *et al.*, 1992).

Solvent extraction using a liquid mixture CH_3OH/CH_2Cl_2 (1:1) was performed in order to approximately estimate the total amounts of taxol and baccatin III originally contained in the sample powders of ground needles. The ground needles were extracted in a glass percolator with CH_3OH/CH_2Cl_2 (1:1) for 3 hrs at 40 ℃ at three times. A crude residue was subsequently partitioned between CH_2Cl_2– and H_2O–rich phases to collect taxane compounds in the CH_2Cl_2–rich phase.

The crude taxane extracts obtained from both solvent extraction and SFE were analyzed by a HPLC (Model 8800, Spectra-Physics, CA). The SFE extracts were dissolved in a small volume of methanol and filtered through a 0.2 μm FH-type Millipore filter. These samples were then loaded onto a reversed-phase LiChrosorb RP-18 column (250 × 4.6 mm, 5 μm). The run was performed using CH_3OH/H_2O (68:32) as a carrier solvent with a flow rate of 0.5 mL/min. The compounds were detected by absorbance at 230 nm with a UV detector. The quantitative analysis was determined by comparing the peak areas of the sample with those of authentic taxane compounds, taxol and baccatin III.

RESULTS AND DISCUSSION

The present studies examined whether the SFE can be highly recommended as a potential and efficient technique for the extraction of taxol and baccatin III from *Taxus cuspidata*. We used supercritical carbon dioxide with 3 wt % ethanol as a cosolvent for the extraction of two target compounds, taxol and baccatin III, from the needles. In Table 1, we demonstrate the SFE results of extracted taxol and baccatin III at several pressures and temperatures. In the supercritical extracts of ground needles, the taxol compositions ranged from 0.06 to 0.094 wt % and the baccatin III compositions

Table 1. Compositions and yields of taxol and baccatin III extracted by using supercritical carbon dioxide.

No.	Experimental condition	Source	Composition (wt %)		Yield (%)	
			Taxol	Baccatin III	Taxol	Baccatin III
# 1	200bar, 40°C	Needle	0.084	0.131	40.37	56.98
# 2	300bar, 40°C	Needle	0.094	0.158	46.84	70.64
# 3	300bar, 70°C	Needle	0.060	0.091	37.04	50.46
# 4	300bar, 40°C	Seed	0.198	0.157	31.21	27.14

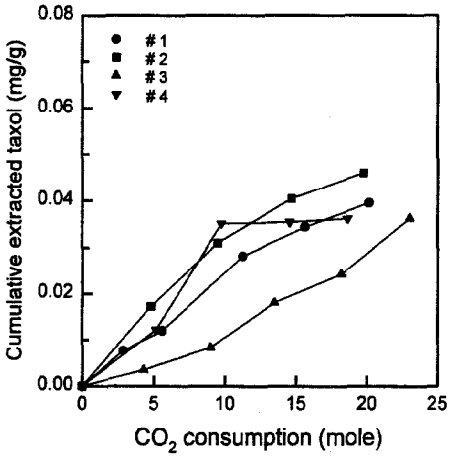

Figure 1. Amounts of cumulative extracted taxol as a function of CO_2 consumption for several pressure and temperature conditions.

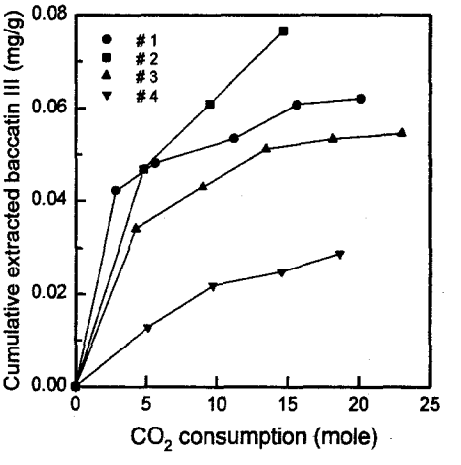

Figure 2. Amounts of cumulative extracted baccatin III as a function of CO_2 consumption for several pressure and temperature conditions.

from 0.091 to 0.158 wt %. The needles of intact *Taxus cuspidata* contain large amounts of waxy and nonpolar components that is more easily extracted than other components by supercritical carbon dioxide. As expected, the SFE extracts showed the higher selectivity than those of the organic solvent extraction. The yields resulted from SFE were calculated on the basis of the total amounts of taxol and baccatin III extracted by the conventional organic solvent extraction method. In the organic solvent extraction, the contents of taxol and baccatin III in the needles were 0.098 and 0.109 mg per gram of ground needles, respectively. The highest baccatin III yield was found to be about 70.64 % at 300 bar and 40 ℃. Figure 1 shows the cumulative extracted amount of taxol as a function of the amount of carbon dioxide passed through the cell at several pressure and temperature conditions. This figure also roughly shows that both taxol and baccatin III yields increase with pressure and decrease with temperature, which is the same behavior appearing in many other similar systems. The best extraction result in both yield and selectivity was obtained at 40 °C and 300 bar. The similar SFE results for baccatin III were also presented in Figure 2. The ground seeds of *Taxus cuspidata* were also tested at this temperature and pressure condition in order to compare with the extraction result of the ground needles. Taxol selectivity in the seed extract was found to be 0.198 wt %, which is almost two times higher than that in the needle extract. On the other hand, baccatin III selectivities were almost same in both cases. Detailed SFE experiments for various ground seed samples are in progress in our laboratory.

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