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Process synthesis and optimization for the isolation and purification of paclitaxel from *Taxus chinensis*

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Abstract−Cavitation extraction, water/hexane washing, and cavitation precipitation-based process synthesis and optimization were performed for the isolation and purification of biomass-derived paclitaxel. The final purity, overall yield, and operating time were significantly improved when negative pressure cavitation extraction was followed by sequential washing with water and hexane, adsorbent treatment, and negative pressure cavitation fractional precipitation using acetone-pentane and methanol-water. This procedure showed a purity and yield increase of 11.0-45.6% and 2.2-29.6%, respectively, and an operating time reduction of 56.2-98.3% compared to the traditional methods.

Keywords: Paclitaxel, Purification, Negative Pressure Cavitation, Extraction, Fractional Precipitation, Process Synthesis

INTRODUCTION

Paclitaxel (Fig. 1) is a natural compound of the tetracyclic diterpenoid ($C_{47}H_{51}NO_{14}$, MW: 853.9) family isolated from the epidermis of *Taxus brevifolia*, the Pacific yew tree [1]. This molecule induces apoptosis of tumor cells by inhibiting depolymerization through improved stability of microtubules. With its unique pharmacological effect, paclitaxel is one of the most widely used chemotherapy drugs showing anticancer activity in ovarian, breast, cervical, non-small cell lung, and bladder cancer [2,3]. The demand for paclitaxel is expected to continue to increase due to the expansion of indications and the development of prescription methods [4-6]. As a method for producing paclitaxel, plant cell cultures that can stably produce a product of consistent quality in a bioreactor without the influence of external factors, such as climate and the environment, have recently been in the spotlight [7-9].

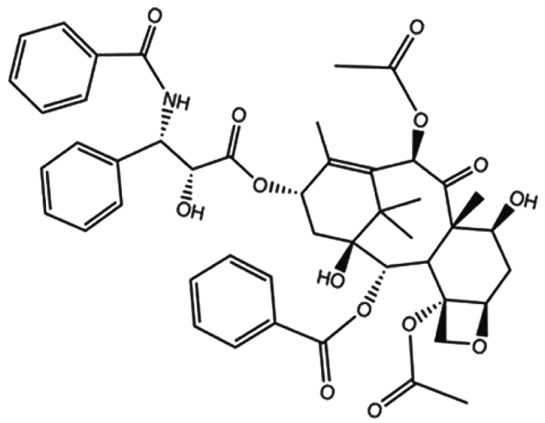


Fig. 1. The chemical structure of paclitaxel.

Paclitaxel, a secondary metabolite produced by plant cell cultures, is mostly accumulated in plant cells (biomass) [10]. In general, to produce high purity (>98%) paclitaxel from biomass, the biomass must first be extracted with an organic solvent, and then undergo a pre-purification process, such as liquid-liquid extraction, adsorbent treatment, hexane precipitation, or fractional precipitation, and a final purification process, such as Octadecylsilylated silica-HPLC or Silica-HPLC [11-14] (Fig. 2(a)). During biomass extraction using organic solvents, the sample purity is usually about 0.5-0.7%, and when it undergoes subsequent pre-purification steps, such as liquid-liquid extraction, adsorbent treatment, hexane precipitation, and fractional precipitation, the sample purity is improved to 6-9%, 9-10%, 21-27%, and 46-61%, respectively [14]. Through the pre-purification process, a sample suitable for final purification using HPLC is obtained. The processing time required for this isolation/purification is approximately 18.2-76.2 hr [14]. However, there is a limit to the efficient mass production of paclitaxel due to the multiple extractions and multiple steps in the pre-purification process [15]. Therefore, a more efficient and economical isolation and purification process is required to reduce the production cost of paclitaxel.

The first step for the isolation and purification of paclitaxel is solvent extraction, and methanol as a solvent is the most effective because it can obtain a high recovery rate using a small amount [14]. However, traditional solvent extraction is disadvantageous due to low extraction efficiency, a large amount of organic solvent and a long operating time. To overcome these problems, a cavitation-based extraction technique has been recently introduced [16-19]. Negative pressure cavitation extraction (−260 mmHg, room temperature, once) has been shown to recover most of the paclitaxel from *Taxus chinensis* in a short operating time (<20 min) [20]. If the negative pressure cavitation extraction method, which guarantees process efficiency, convenience, and feasibility, is applied to the existing paclitaxel production process, the isolation/purification effi-

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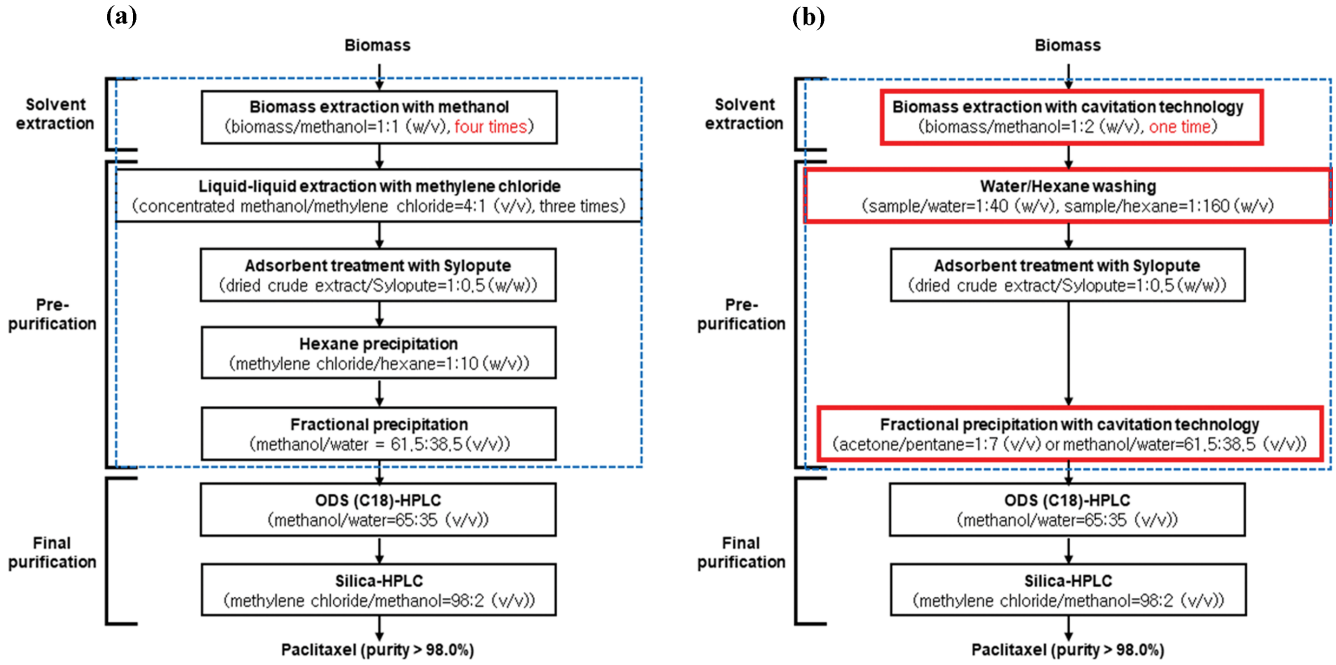


Fig. 2. Traditional separation/purification process (a) [14] and the proposed separation/purification process (b) of this study for paclitaxel from biomass.

ciency can be remarkably improved [20].

The pre-purification process after extraction affects the final purification cost considerably [21,22]. After solvent extraction to recover the biomass-derived paclitaxel, liquid-liquid extraction and hexane precipitation are performed to remove polar and non-polar impurities contained in large amounts in the extract. This process requires many liquid-liquid extractions and has a complicated process (concentration and drying), so the isolation/purification has a long operating time [14]. Another pre-purification process, fractional precipitation, is a simple purification method using the solubility difference of paclitaxel [23]. High-purity paclitaxel can be obtained in high yield by optimizing operating conditions for fractional precipitation [24,25]. However, since fractional precipitation has a long operating time (~3 days) at a low temperature (0-4 °C), it has limited economic feasibility for the mass production of paclitaxel. Studies have attempted to resolve this problem by improving the precipitation efficiency by increasing the surface area per working volume of the precipitator using glass beads or ion exchange resin [26-28]. Although the long precipitation time has been shortened to some extent through these studies, it is still insufficient for the mass production of paclitaxel. Additionally, the precipitation time (~30 min) was shortened by controlling the polarity of the precipitation solution using a methanol-distilled water mixture, but the purity of paclitaxel decreased due to the excessive addition of distilled water [29]. Recently, the long precipitation time was remarkably shortened by fractional precipitation using ultrasonic waves [9,30]. By introducing ultrasonic waves to the precipitation process, the nucleation rate increased, shortening the precipitation time. This result arises from the ultrasonic cavitation, collapsing of the cavitation bubbles during the compression/expansion process to generate microjets of the precipitation solution, intense local heating, and high-pressure shock waves [31]. However, even though ultrasonic cavitation fractional precipitation can reduce the operating time, it requires additional equipment and energy costs, and the process can be complicated. However, the inefficiency of this process may be remarkably improved by introducing negative pressure instead of ultrasound during fractional precipitation [32].

Therefore, the present study addresses the problems of low efficiency, high organic solvent requirements, and the long operating time in the existing extraction process by using negative pressure cavitation technology. In addition, liquid-liquid extraction and hexane precipitation to remove polar and non-polar impurities derived from biomass were replaced with simple water and hexane washing methods. The operating time was shortened remarkably by introducing negative pressure cavitation technology to the currently time consuming fractional precipitation process. Ultimately, as shown in Fig. 2(b), the synthesis and optimization of cavitation extraction, water/hexane washing, adsorbent treatment, and cavitation fractional precipitation processes not only improved the paclitaxel isolation/purification efficiency (purity and yield), but also remarkably shortened the operating time. The results of this study can be utilized for the environmentally friendly commercial mass production of the anticancer substance paclitaxel extracted from a plant cell culture.

MATERIALS AND METHODS

1. Plant Materials

Suspension cells originating from *Taxus chinensis* were cultured at 24 °C in dark conditions and stirred at 150 rpm in modified Gamborg's B5 medium. The cell culture medium was replaced with a fresh one every two weeks, and in order to extend the culture time,

1-2% (w/v) maltose was added on the 7th and 21st days and 4 mM $AgNO_3$ was added at the beginning of the culture as an elicitor [14]. After the plant cell culture, the plant cells were recovered from the culture medium using a decanter (CA150 Clarifying Decanter, GEA Westfalia Separator, Oelde, Germany) and a high-speed centrifuge (BTPX 205GD-35CDEFP, Alfa Laval, Lund, Sweden).

2. Negative Pressure Cavitation Extraction

As extraction conditions, the biomass/methanol ratio was 1 : 2 (w/v), the extraction temperature was 25 °C, the number of extractions was one, and the stirring speed was 500 rpm [33]. The extraction pressure was controlled using a vacuum controller unit (NVC-3000, EYELA, Tokyo, Japan) and a diaphragm vacuum pump (NVP-1000, EYELA, Tokyo, Japan). Considering excessive energy consumption at a high extraction temperature, the extraction was performed for 10 min while maintaining a negative pressure of −260 mmHg at room temperature (25 °C) [20]. After extraction, the extraction filtrate was recovered by vacuum filtration (filter paper: 185 mm, ADVANTEC, Tokyo, Japan), was concentrated using a concentrator (CCA-1100, EYELA, Japan), and was vacuum-dried at 40 °C overnight at −760 mmHg. The content of paclitaxel was measured through high performance liquid chromatography (HPLC). The traditional extraction method and the negative pressure cavitation extraction method are shown in Fig. 3. In addition, the yield of paclitaxel was calculated using Eq. (1).

$$\text{Yield (\%)} = \frac{\text{Quantity of paclitaxel in extract}}{\text{Quantity of paclitaxel in biomass}} \times 100 \quad (1)$$

3. Water/Hexane Washing

Washing with water and hexane was performed to remove a large amount of polar and non-polar impurities derived from biomass. The effect of washing order and drying between the two washing steps on process efficiency was investigated. Water washing was performed for 10 min by adding distilled water to the crude extract (crude extract/water ratio=1 : 40 (w/v)) and stirring at room temperature (~300 rpm) [34]. After washing with water, it was filtered (filter paper: Whatman Grade 5, 2.5 mm, particle retention, 150 mm diameter) to obtain a filter cake. Hexane washing was performed for 20 min by adding hexane to the crude extract (crude extract/hexane ratio=1 : 160 (w/v)) and stirring at room temperature (~300 rpm) [34]. After washing with hexane, a filter cake was obtained by filtration (filter paper: Whatman Grade 5, 2.5 mm, particle retention, 150 mm diameter). After washing, the cake obtained through filtration was vacuum-dried (−760 mmHg) at 40 °C for 12 hr.

4. Adsorbent Treatment

To remove the biomass-derived tar/wax components, the dried crude extract obtained from washing with water and hexane was dissolved in methylene chloride at a ratio of 20% (w/v) and treated with an adsorbent. The treatment temperature was 40 °C, the time was 30 min, and the crude extract/Sylopute ratio was 1 : 1 (w/w). The main component of Sylopute (Fuji Silysia Chemical Ltd., Japan) was SiO_2 (>99.8%), and the surface area, pore volume, and pore diameter were 331 m^2/g, 1.81 cm^3/g, and 40-60 nm, respectively. After treatment with the adsorbent, the extract was filtered (filter paper: Whatman Grade 4, 20-25 μm, particle retention, 150 mm diameter), and the filtrate was dried at 40 °C and reduced pressure [29].

5. Negative Pressure Cavitation Fractional Precipitation

Schematic diagrams for traditional acetone-pentane fractional precipitation (Fig. 4(a)), acetone-pentane negative pressure cavitation fractional precipitation (Fig. 4(b)), traditional methanol-water fractional precipitation (Fig. 4(c)) and the methanol-water negative pressure cavitation fractional precipitation (Fig. 4(d)) are shown in Fig. 4. For acetone-pentane fractional precipitation, crude paclitaxel (purity: 20.47%) was dissolved in acetone (16.7 g crude paclitaxel/mL acetone) and pentane was added dropwise while stirring (335 rpm) until the acetone/pentane ratio (v/v) became 1 : 7 at room temperature [28,35]. Fractional precipitation was performed for 15 min while maintaining a negative pressure of −200 mmHg at a precipitation temperature of 5 °C [32]. For methanol-water fractional precipitation, crude paclitaxel (purity: 20.47%) was dissolved in methanol (pure paclitaxel content in methanol: 0.5%, w/v) and distilled water was added dropwise while stirring (335 rpm) until the methanol/water ratio (v/v) was 61.5 : 38.5 at room temperature [8,9]. Fractional precipitation was performed for 15 min while maintaining a negative pressure of −200 mmHg at a precipitation temperature of 5 °C [32]. The precipitation pressure was controlled using a vacuum controller unit (NVC-3000, EYELA, Tokyo, Japan) and a diaphragm vacuum pump (NVP-1000, EYELA, Tokyo, Japan). After precipitation, the precipitate obtained through vacuum filtration (185 mm, ADVANTEC, Tokyo, Japan) was dried at 40 °C for 24 h in a vacuum oven (UP-2000, EYELA, Tokyo, Japan). The pacli-

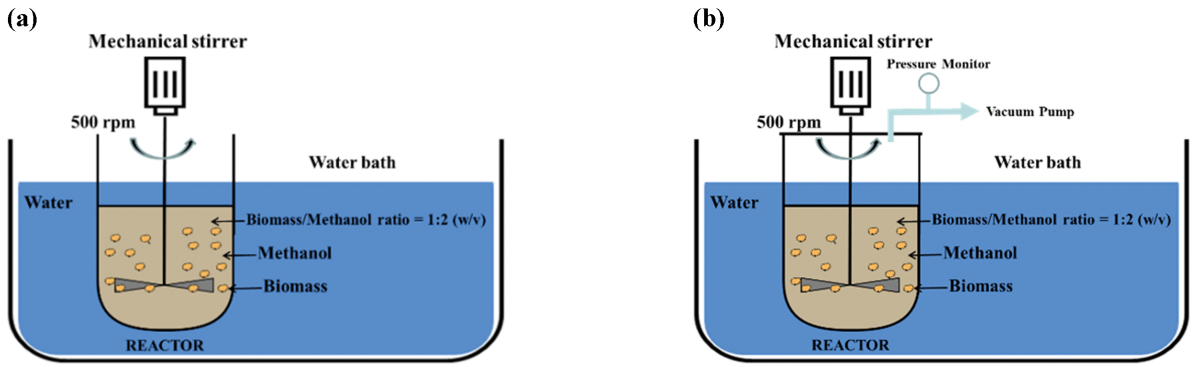


Fig. 3. Schematic diagrams of different extraction methods for paclitaxel recovery from biomass. (a) Conventional extraction; (b) Negative pressure cavitation extraction.

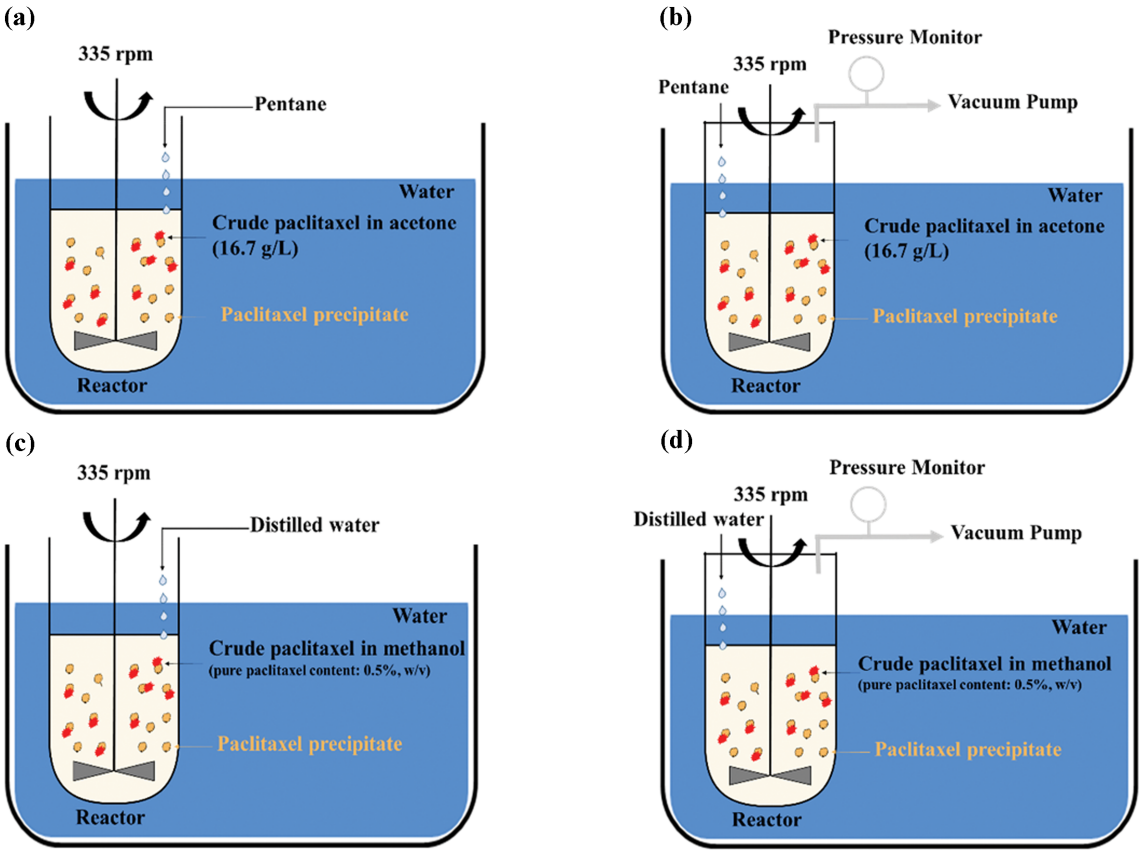


Fig. 4. Schematic diagrams of different fractional precipitation methods for the purification of paclitaxel. (a) Conventional acetone-pentane fractional precipitation; (b) Negative pressure cavitation acetone-pentane fractional precipitation; (c) Conventional methanol-water fractional precipitation; (d) Negative pressure cavitation methanol-water fractional precipitation.

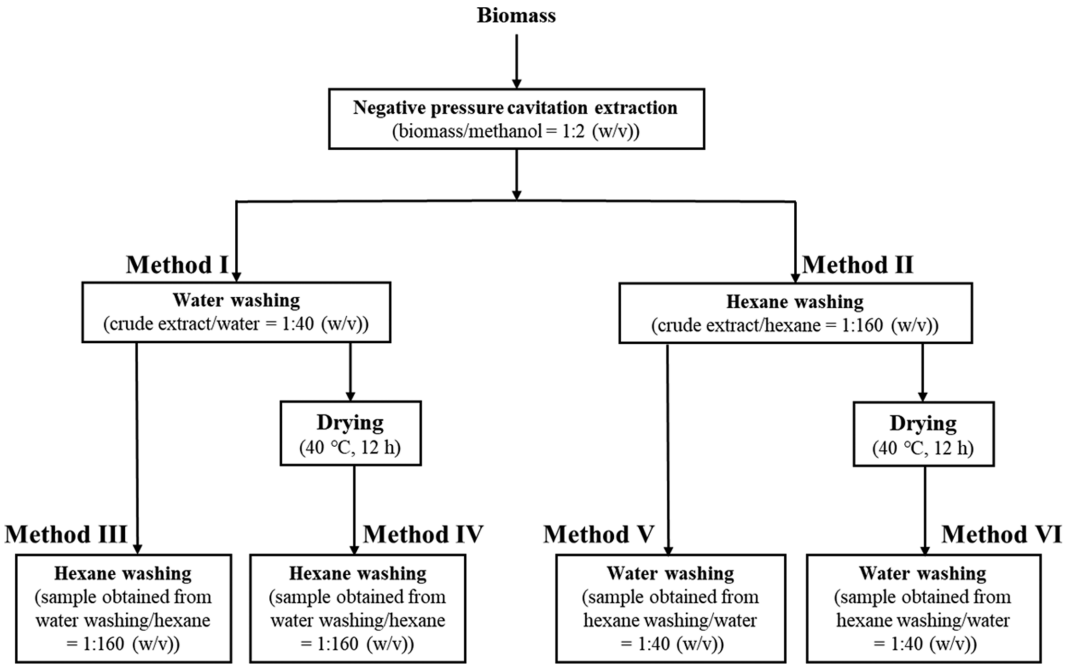


Fig. 5. Water and hexane washing methods. Method I: Water washing, Method II: Hexane washing, Method III: Water and hexane washing without drying, Method IV: Water and hexane washing with drying, Method V: Hexane and water washing without drying, Method VI: Hexane and water washing with drying.

taxel content of the dried precipitate was analyzed by HPLC.

6. Paclitaxel Analysis

For the paclitaxel content analysis, an HPLC system (SCL-10AVP, Shimadzu, Japan) and a Capell Pak C_{18} (250×4.6 mm, Shiseido, Japan) column were used. The mobile phase was a mixed solution of distilled water and acetonitrile (65/35-35/65, v/v, gradient mode), and the flow rate was 1.0 mL/min. The sample injection volume was 20 μL and was detected by ultraviolet (UV) at 227 nm [32]. Authentic paclitaxel (purity: 97%) was purchased from Sigma-Aldrich to use as a standard.

RESULTS AND DISCUSSION

1. Development of a Pre-purification Process for Fractional Precipitation: Washing with Water and Hexane

A previous study showed that most paclitaxel could be recovered from biomass in a single extraction using methanol at room temperature (25 °C) and a short operating time (<20 min) through negative pressure cavitation extraction (−260 mmHg) [20]. Washing was performed using the sample (purity: 4.4%) obtained through the extraction process. Washing with either water or hexane alone as well as two-step washing with water and hexane were performed to analyze the effect of each washing strategy. Furthermore, the effect of drying or not drying between the two washes was investigated during the two-step washings. As shown in Fig. 5, six washing strategies were performed for the experiment. Methods I and II represent washing with water or hexane alone, respectively. Methods III and IV represent sequential washes of water and hexane, but Method IV involves drying between the two washes. Methods V and VI represent sequential washes of hexane and water, but Method VI includes drying between the two washes.

Fig. 6(a) and (b) shows the HPLC chromatograms of the extract before and after the washing process (Method I). The purity of paclitaxel before and after washing with water was 4.4% and 10.2%, respectively. The HPLC chromatogram of the sample after washing using Method II is shown in Fig. 6(c). The purity of paclitaxel was 4.7%; the change in purity before and after washing was insignificant.

For two-step washing, the effect of washing sequence was first investigated. After washing with water, the sample obtained through filtration was sequentially washed with hexane (Method III) without drying. The cake was dried after filtration and the purity of paclitaxel was measured through HPLC analysis. The HPLC chromatogram of the sample is shown in Fig. 6(d), and the purity of paclitaxel was 26.6%. For Method V, the sample obtained through filtration after washing with hexane was sequentially washed with water without drying. The cake was dried after filtration and the purity of paclitaxel was measured through HPLC analysis. The HPLC chromatogram of the sample is shown in Fig. 6(e), and the purity of paclitaxel was 13.8%. Depending on the washing sequence, the purity of paclitaxel was approximately doubled, and the optimal two-step washing sequence was water washing followed by hexane washing.

The effect of drying or not drying between the two washing steps was also investigated. After washing with water, the sample obtained through filtration was dried, washed with hexane (Method IV), and filtered. The cake was dried and the purity of paclitaxel was measured through HPLC analysis. The HPLC chromatogram of the sample is shown in Fig. 6(f). The purity of paclitaxel was 25.1%, similar to the purity of paclitaxel in the process without drying (26.6%). This shows that the drying step between the two washing steps had a negligible effect on the process efficiency and could increase the process cost rather than reduce it. Similarly, after wash-

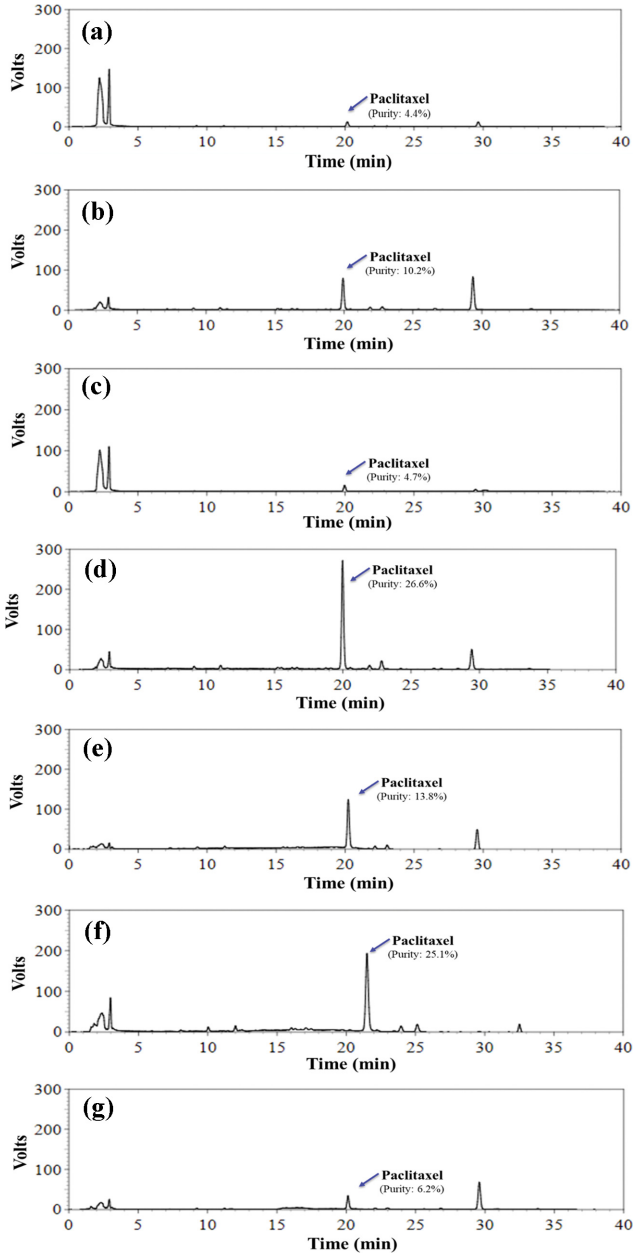


Fig. 6. Chromatograms of water and hexane washing steps. (a) Crude extract obtained from negative pressure cavitation extraction; (b) Sample (cake) obtained from water washing; (c) Sample (cake) obtained from hexane washing; (d) Sample (cake) obtained from water and hexane washing without drying; (e) Sample (cake) obtained from hexane and water washing without drying; (f) Sample (cake) obtained from water and hexane washing with drying; (g) Sample (cake) obtained from hexane and water washing with drying.

Table 1. Comparison of paclitaxel purity according to water and hexane washing methods

Method	Washing	Drying*	Purity (%)**
I	Water	-	10.2
II	Hexane	-	4.7
III	Water→Hexane	Without	26.6
IV	Water→Hexane	With	25.1
V	Hexane→Water	Without	13.8
VI	Hexane→Water	With	6.2

*Drying between two types of washing

**Purity of starting sample (sample obtained from negative pressure cavitation extraction): 4.4%

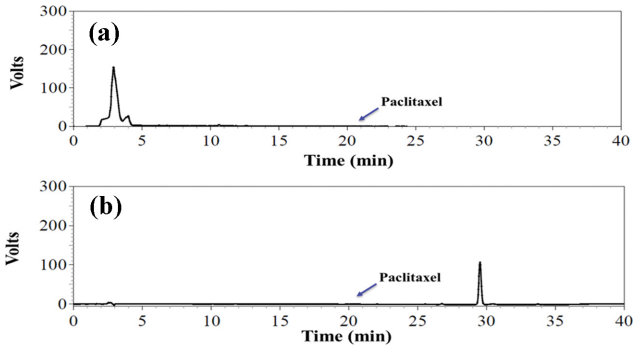


Fig. 7. Chromatograms of filtrate from water and hexane washings. (a) Filtrate obtained from water washing; (b) Filtrate obtained from hexane washing.

ing with hexane, the sample obtained through filtration was dried and sequentially washed with water (Method VI). After washing, the cake obtained through filtration was dried and the chromatogram was analyzed by HPLC as shown in Fig. 6(g). The purity of paclitaxel was 6.2%, which was approximately two times lower than the purity of paclitaxel in the process without drying (Method V) (13.8%). Thus, drying between the two washing steps lowered the washing efficiency. Comparisons of the paclitaxel purity according to the washing method are arranged in Table 1. The highest paclitaxel purity (26.6%) was shown after sequential washing with water and hexane without a drying step between the two washing steps. As a result, it was possible to shorten the process time and reduce the cost of the process by sequentially performing a second step hexane washing without drying the cake obtained after filtering the first step water-washed sample.

Furthermore, in the sequential washing of water and hexane, the filtrate of each step was analyzed by HPLC. The HPLC chromatograms of the filtrate after washing with water and hexane are shown in Fig. 7(a) and Fig. 7(b), respectively. As a large amount of polar impurities were detected in Fig. 7(a), it was found that the polar impurities (retention time: 1-5 min) were effectively removed by washing with water. In addition, there was almost no paclitaxel (retention time: 20 min) in the filtrate, indicating that most of the paclitaxel was recovered with the cake obtained through filtration after washing with water. Because the principle of water washing utilized the insolubility of paclitaxel in water and the solubility of polar impurities, a high paclitaxel yield could be obtained [34]. On the other hand, a large amount of non-polar impurities were detected in Fig. 7(b), meaning the non-polar impurities (retention time: 30-35 min) were effectively removed by washing with hexane. In addition, there was almost no paclitaxel (retention time: 20 min) in the filtrate, indicating that most of the paclitaxel was recovered with the cake obtained through filtration after washing with hexane. According to a previous study [14], liquid-liquid extraction was mainly used to remove polar impurities derived from biomass, and the purity and operating time of paclitaxel obtained through liquid-liquid extraction were 6.0-8.8% and 1.5 hr, respectively. In addition, hexane precipitation was used to remove non-polar impurities derived from biomass, and the purity and operating time of paclitaxel obtained through hexane precipitation were 21.0-27.1% and approximately 0.2 hr, respectively. Therefore, the water/hexane se-

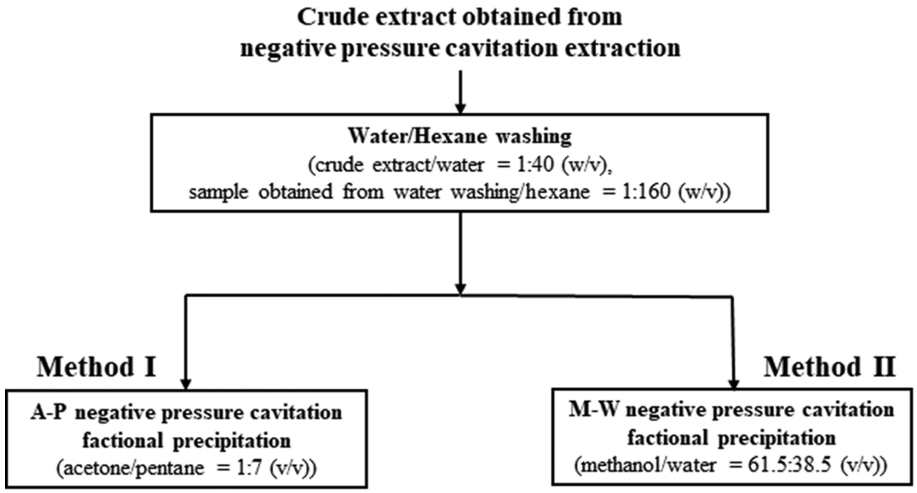


Fig. 8. Strategies for the separation and purification of paclitaxel with cavitation extraction, water/hexane washing, and negative pressure cavitation fractional precipitation. Method I: With acetone-pentane fractional precipitation, Method II: With methanol-water fractional precipitation.

quential washing method (paclitaxel purity: 26.6%, operating time: 0.5 hr) established through this study is not only much simpler and more convenient compared to the conventional method, but the process efficiency is also higher as it does not require a separate concentration and drying step.

2. Process Synthesis Optimization

Several stages of extraction, pre-purification and final purification are needed to obtain high-purity paclitaxel from the biomass recovered from plant cell culture. Developing an efficient isolation and purification process is vital for reducing the production cost. Traditional extraction requires a large amount of solvent for more than four extractions and has a long operating time [14]. In general, when a low-purity sample obtained from pre-purification undergoes the final purification via HPLC, the process is uneconomical and unsuitable for mass production due to the large amount of organic solvent, the shortened lifespan of the column packing material (resin), and the reduced throughput. Therefore, it is very important to reduce possible impurities by producing a sample with as high a purity as possible (50% or more) before final purification using HPLC [36]. In the present study, negative pressure-based green cavitation extraction and fractional precipitation techniques that guarantee process convenience and feasibility as well as process efficiency were used for process synthesis. Negative pressure cavitation extraction and fractional precipitation offer advantages including faster and more effective extraction and precipitation with less capital investment while utilizing energy efficiently.

As shown in Fig. 8, when the isolation and purification were performed using only three processes (negative pressure cavitation extraction, water/hexane washing, negative pressure cavitation fractional precipitation), the process efficiency was investigated through two methods. In Method I, negative pressure cavitation extraction, water/hexane washing, and negative pressure cavitation acetone-pentane fractional precipitation were performed. The final purity and overall yield were 36.0% and 61.0%, respectively (data not shown). In the present study, the purity and yield were very low compared to the negative pressure cavitation-based acetone-pentane fractional precipitation (purity and yield: 54.8%, 98.6%) performed using the adsorbent-treated sample. In Method II, negative pressure cavitation extraction, water/hexane washing, and negative pressure cavitation methanol-water fractional precipitation were performed. The final purity and overall yield were 31.1% and 74.2%, respectively (data not shown). In the present study, the purity and

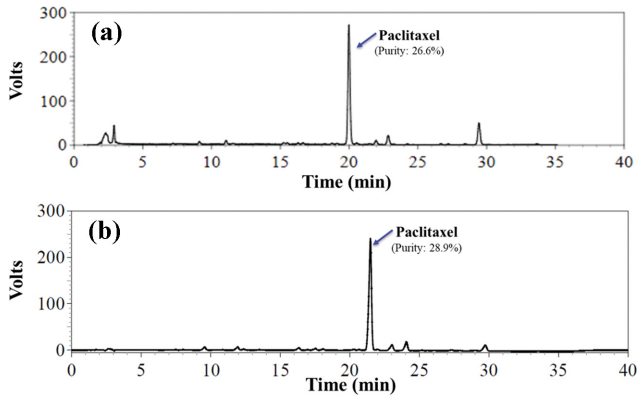


Fig. 9. Sample chromatogram (a) before and (b) after adsorbent treatment.

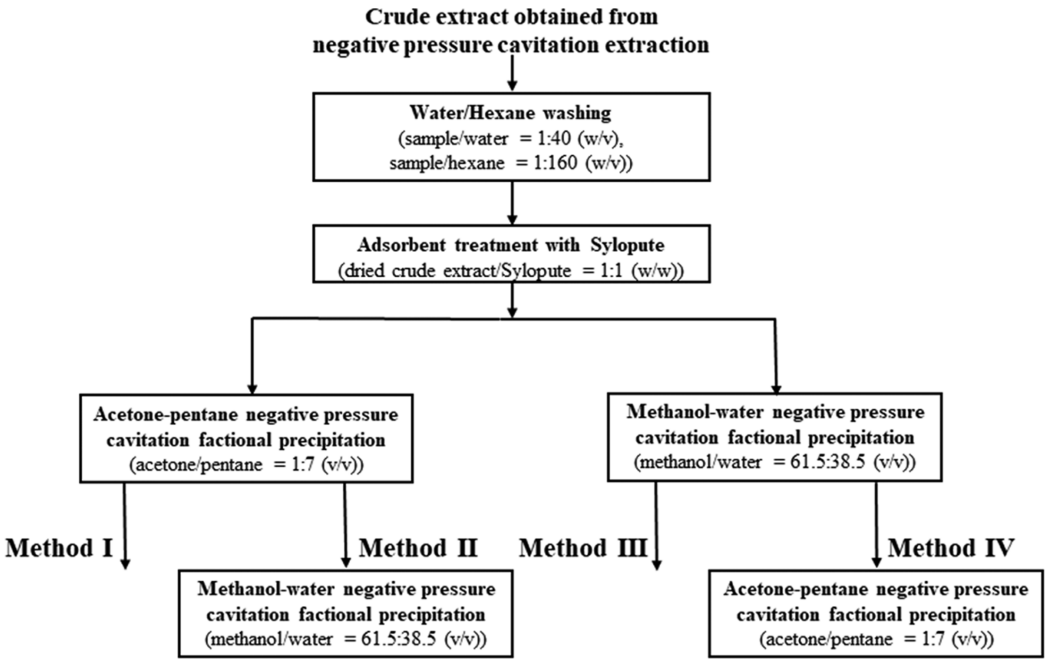


Fig. 10. Strategies for the separation and purification of paclitaxel with negative pressure cavitation extraction, water/hexane washing, adsorbent treatment, and negative pressure cavitation fractional precipitation. Method I: With one-step negative pressure cavitation fractional precipitation (acetone-pentane). Method II: With two-step negative pressure cavitation fractional precipitation (acetone-pentane and methanol-water). Method III: With one-step negative pressure cavitation fractional precipitation (methanol-water). Method IV: With two-step negative pressure cavitation fractional precipitation (methanol-water and acetone-pentane).

Table 2. Summary of pre-purification for paclitaxel from biomass

Process	Purity (%)	Step yield (%)	Overall yield (%)	Step operating time (hr)	Overall operating time (hr)	Ref.
Traditional method I						[14]
Biomass	-	100.0	100.0	-	-	
Biomass extraction	0.5-0.7	97-99.0	97.0-99.0	2.0	2.0	
Liquid-liquid extraction	6.0-8.8	96.5-99.0	93.6-98.0	1.5	3.5	
Adsorbent treatment	8.8-9.8	95.6	89.5-93.7	0.5	4.0	
Hexane precipitation	21.0-27.1	95.2-99.0	85.2-92.7	0.2	4.2	
Fractional precipitation	46.5-61.0	81.3	69.3-75.4	14.0-72.0	18.2-76.2	
Traditional method II						[12]
Biomass	-	100.0	100.0	-	-	
Biomass extraction	2.5±0.3	99.2±0.4	99.2	2.0	2.0	
Water pre-treatment	12.1±1.0	99.4±0.8	98.6	0.2	2.2	
Liquid-liquid extraction	28.6±2.1	98.2±1.7	96.8	1.5	3.7	
Adsorbent treatment	36.8±1.8	93.9±3.1	90.9	0.5	4.2	
Fractional precipitation	56.5±1.5	94.8±3.0	86.2	0.2	4.4	
Traditional method III						[12]
Biomass	-	100.0	100.0	-	-	
Biomass extraction	2.5±0.3	99.2±0.4	99.2	2.0	2.0	
Water pre-treatment	12.1±1.0	99.4±0.8	98.6	0.2	2.2	
Adsorbent treatment	30.0±1.2	92.7±2.7	91.4	0.5	2.7	
Fractional precipitation	50.0±2.3	96.2±2.0	87.9	0.2	2.9	
Method I in this study						
Biomass	-	100.0	100.0	-	-	
Biomass extraction	4.4	99.9	99.9	0.17	0.17	
Water-Hexane washing	26.6	99.9	99.8	0.50	0.67	
Adsorbent treatment	28.9	93.7	93.5	0.50	1.17	
A-P fractional precipitation	54.8	98.6	92.2	0.08	1.25	
Method II in this study						
Biomass	-	100.0	100.0	-	-	
Biomass extraction	4.4	99.9	99.9	0.17	0.17	
Water-Hexane washing	26.6	99.9	99.8	0.50	0.67	
Adsorbent treatment	28.9	93.7	93.5	0.50	1.17	
A-P fractional precipitation	54.8	98.6	92.2	0.08	1.25	
M-W fractional precipitation	67.7	97.4	89.8	0.02	1.27	
Method III in this study						
Biomass	-	100.0	100.0	-	-	
Biomass extraction	4.4	99.9	99.9	0.17	0.17	
Water-Hexane washing	26.6	99.9	99.8	0.50	0.67	
Adsorbent treatment	28.9	93.7	93.5	0.50	1.17	
M-W fractional precipitation	46.9	99.2	92.8	0.02	1.19	
Method IV in this study						
Biomass	-	100.0	100.0	-	-	
Biomass extraction	4.4	99.9	99.9	0.17	0.17	
Water-Hexane washing	26.6	99.9	99.8	0.50	0.67	
Adsorbent treatment	28.9	93.7	93.5	0.50	1.17	
M-W fractional precipitation	46.9	99.2	92.8	0.02	1.19	
A-P fractional precipitation	65.8	92.1	85.5	0.08	1.27	

yield were very low compared to cavitation-based methanol-water fractional precipitation (purity and yield: 46.9%, 99.2%) performed using adsorbent-treated samples. This low process efficiency might be due to the lowering of the fractional precipitation efficiency by

biomass-derived tar and wax components not removed by only washing [37]. Tar and wax components have many adverse effects on the purification of paclitaxel and must be removed during the pre-treatment process, and these components can be easily removed by treatment with an adsorbent [14,37]. Therefore, an adsorbent treatment process was introduced between the water/hexane washing and the fractional precipitation. The HPLC chromatograms of samples before and after adsorbent treatment are shown in Fig. 9(a) and Fig. 9(b), respectively. The paclitaxel purity of the sample before adsorbent treatment (the sample after washing with water/hexane) was 26.6%, and the paclitaxel purity of the sample after adsorbent treatment was 28.9%. In addition, it was confirmed that a large amount of polar impurities (retention time: 1-5 min) and non-polar impurities (retention time: 30-35 min) were removed from the chromatogram after treatment with the adsorbent. Furthermore, the dark color, tar, and wax components derived from biomass were effectively removed through adsorbent treatment [14,37].

In the negative pressure cavitation extraction, water/hexane washing, adsorbent treatment, and negative pressure cavitation fractional precipitation process, the two-step fractional precipitation was performed with different solvent systems (acetone-pentane, methanol-water). As shown in Fig. 10, the experiment was performed with four strategies in order to investigate the process efficiency. A comparison of the pre-purification process of paclitaxel reported in previous studies [12,14] and the pre-purification process introducing negative pressure cavitation extraction, water/hexane washing, and negative pressure cavitation fractional precipitation established through this study is shown in Table 2. When liquid-liquid extraction, adsorbent treatment, hexane precipitation, and methanol-water fractional precipitation were sequentially performed after biomass extraction (Traditional method I), the final purity of the sample, overall yield, and operating time were 46.5-61.0%, 69.3-75.4%, and 18.2-76.2 hr, respectively. When water washing, liquid-liquid extraction, adsorbent treatment, and methanol-water fractional precipitation were sequentially performed after biomass extraction (Traditional method II), the final purity of the sample, overall yield, and operating time were 56.5%, 86.2%, and 4.4 hr, respectively. In addition, when Traditional method II was performed without liquid-liquid extraction (Traditional method III), the final purity of the sample, overall yield, and operating time were 50.0%, 87.9%, and 2.9 hr, respectively. On the other hand, when negative pressure extraction, water/hexane washing, adsorbent treatment, and negative pressure acetone-pentane fractional precipitation were sequentially performed (Fig. 10, Method I in this study), the final purity of the sample, overall yield, and operating time were 54.8%, 92.2%, and 1.25 hr, respectively. Compared to the three existing traditional methods, the final purity increased or decreased by −10.2-17.8%, the overall yield increased by 4.9-33.0%, and the operating time decreased by 56.9-98.4%. When negative pressure cavitation extraction, water/hexane washing, adsorbent treatment, and two steps of negative pressure fractional precipitation using acetone-pentane and methanol-water were sequentially performed (Fig. 10, Method II in this study), the final purity of the sample, overall yield, and operating time were 67.7%, 89.8%, and 1.27 hr, respectively. Compared to traditional methods, the final purity increased by 11.0-45.6%, the overall yield increased by 2.2-29.6%, and the operating time decreased by 56.2-98.3%. When negative pressure cavitation extraction, water/hexane washing, adsorbent treatment, and negative pressure methanol-water fractional precipitation were performed (Fig. 10, Method III in this study), the final purity of the sample, overall yield, and operating time were 46.9%, 92.8%, and 1.19 hr, respectively. Compared to the traditional methods, the final purity showed a −23.1-0.9% increase-decrease rate, an overall yield increase of 5.6-33.9%, and an operating time reduction of 59.0-98.4%. When negative pressure cavitation extraction, water/hexane washing, adsorbent treatment, and two steps of negative pressure fractional precipitation using methanol-water and acetone-pentane were sequentially performed (Fig. 10, Method IV in this study), the final purity of the sample, overall yield, and operating time were 65.8%, 85.5%, and 1.27 hr, respectively. Compared to the traditional methods, the final purity increased by 7.9-41.5%, the overall yield had an increase-decrease rate of −2.7-23.4%, and the operating time was reduced by 56.2-98.3%. As a result, high purity and high yield of paclitaxel could be obtained by effectively removing biomass-derived tar/wax components through the introduction of the adsorbent treatment process. In particular, the negative pressure cavitation extraction, sequential washing with water and hexane, adsorbent treatment, and two steps of negative pressure cavitation fractional precipitation using acetone-pentane and methanol-water (Method II in this study) developed through this study significantly improved the final paclitaxel purity, overall yield, and operating time compared to the existing processes [12,14].

CONCLUSIONS

The efficiency of the paclitaxel production process was remarkably improved by negative pressure cavitation extraction, water/hexane washing, and negative pressure cavitation fractional precipitation for the isolation and purification of biomass-derived paclitaxel. By effectively removing biomass-derived polar and non-polar impurities by sequential washing with water and hexane, paclitaxel with high purity (~26.6%) was obtained in a relatively short operating time (~30 min). Drying between washing with water and hexane did not improve the washing efficiency. In addition, it was possible to obtain high-purity paclitaxel in high yield by effectively removing biomass-derived tar and wax components using an adsorbent treatment before fractional precipitation. Furthermore, process synthesis and optimization for biomass-derived paclitaxel production were performed using negative pressure cavitation extraction, water/hexane washing, adsorbent treatment, and negative pressure cavitation fractional precipitation. The final purity, overall yield, and operating time were all dramatically improved by using negative pressure cavitation extraction, sequential washing with water and hexane, adsorbent treatment, and two steps of negative pressure cavitation fractional precipitation using acetone-pentane and methanol-water. These results show that the purity and yield increased by 11.0-45.6% and 2.2-29.6%, respectively, and the operating time was reduced by 56.2-98.3% compared to traditional methods.

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