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Short communication

Increasing the efficiency of the separation and purification process for paclitaxel by pre-treatment with water

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ABSTRACT

In this study, a water pre-treatment method for the separation and purification of an anticancer agent paclitaxel from plant cell cultures was developed. When the methanol extract obtained by biomass extraction was pre-treated with water, a high yield (>99%) of high-purity (~12.1%) paclitaxel was observed within a short period of time (~10 min). In addition, in the follow-up pre-purification process, using a crude extract obtained by water pre-treatment, high-yield (~87.9%), high-purity (50% or higher) paclitaxel was obtained in a short operating time (~2.9 h). Thus, the efficiency of the separation and purification process for paclitaxel was improved dramatically by water pre-treatment, particularly in terms of the reduction of operating times and the simplification of processes.

1. Introduction

Paclitaxel is a diterpenoid anticancer agent found in the bark of the yew tree. The chemical structure and the anticancer mechanism were investigated by Wani et al. [1] in 1971 and Schiff et al. [2] in 1979. Since its discovery, paclitaxel has been used as an important anticancer drug, with the approval of the U.S. Food and Drug Administration, to treat ovarian cancer, breast cancer, Kaposi's sarcoma and non-small cell lung cancer. The main production methods for paclitaxel are; 1) paclitaxel is directly extracted from yew trees [3]; 2) precursors (baccatin III, 10-deacetylbaccatin III, 10-deacetylpaclitaxel, etc.) are obtained from the leaves of yew trees to combine side chains chemically for semi-synthesis [4]; and 3) callus is induced into yew trees and then the plants' cells are cultured in a bioreactor after seed culture [5]. In the latter case, the plant cell culture is not affected by external factors, such as climate or the environment, and can be stably produced in a bioreactor. This makes the mass production of paclitaxel of a consistent quality possible, allowing production to meet with increasing demand [6].

Many separation and purification steps are required to obtain high-purity paclitaxel from plant cell cultures. In general, paclitaxel is extracted from the biomass by using organic solvents and then high-purity paclitaxel is obtained through pre-purification and final purification steps. However, in previous studies, expensive chromatography has been applied to the pre-purification process for the final purification stage, or a crude extract has been directly used in final purification by HPLC. In particular, if a low-purity sample is used for final purification by HPLC without pre-purification, a large amount of organic solvent is consumed and the lifetime of column packing materials (resin) and throughput are reduced. This is very uneconomical, rendering this process unsuitable for mass production [7–10]. Therefore, efficient and economical pre-purification is definitely required to obtain a high-yield of high-purity paclitaxel. According to previous studies, the pre-purification process for the mass production of paclitaxel consists of liquid-liquid extraction, adsorbent treatment, hexane precipitation and fractional precipitation (Fig. 1) [11–14]. The purity of paclitaxel extracted from biomass using an organic solvent (methanol) generally ranges from 0.5 to 0.7%. If liquid-liquid extraction, adsorbent treatment, hexane precipitation and fractional precipitation, which are included in the pre-purification process, are performed, the purity is improved to 6–9%, 9–10%, 21–27% and 46–61%, respectively [11,12,15–21]. Where a lot of impurities are removed by the pre-purification process, the purity of crude paclitaxel increases greatly. Thus, the sample becomes suitable for final purification using HPLC. However, many steps in the pre-purification process require a long operating time and a large amount of organic solvent, so the efficient mass production of paclitaxel is not easy and possible reductions in production costs are limited [11,12,16,22–24]. In this study, we adopted the water pre-treatment in an attempt to dramatically improve the efficiency

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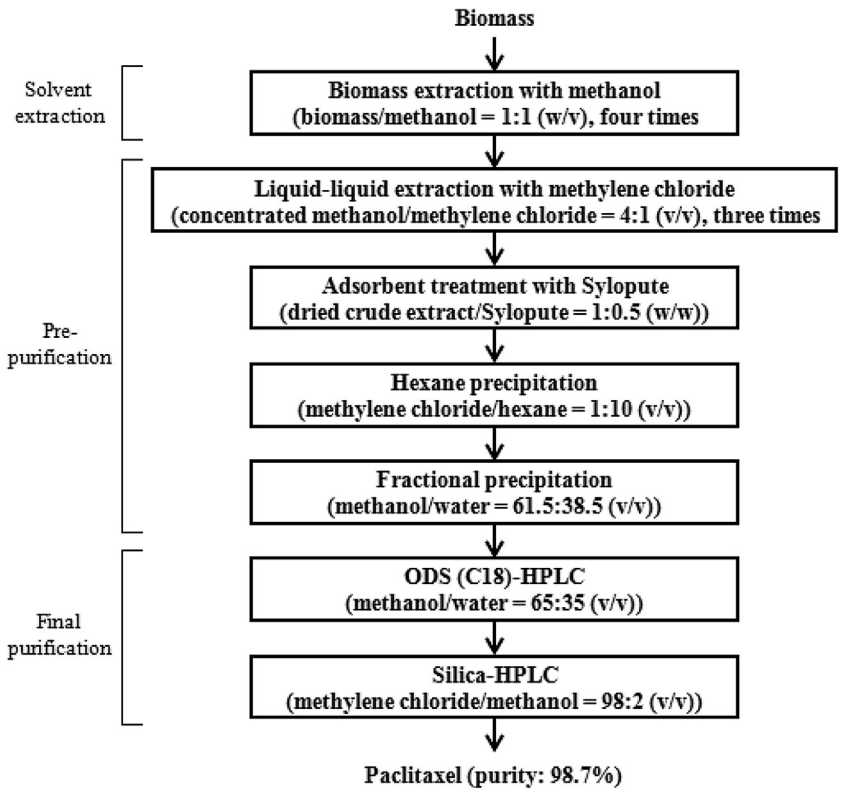

Fig. 1. Traditional purification process of paclitaxel from biomass [14].

of the pre-purification stage for paclitaxel. In other words, we optimized the conditions for water pre-treatment and then evaluated various follow-up pre-purification processes in order to improve efficiency, in terms of the enhancement of yield and purity, the reduction of operating time, and the simplification of processes for the separation and purification of paclitaxel.

2. Materials and methods

2.1. Plant materials and culture conditions

Suspension cells originating from *Taxus chinensis* were maintained under darkness at 24 °C with shaking at 150 rpm. The suspension cells were cultured in a modified Gamborg's B5 medium [25] supplemented with 30 g/l sucrose, 10 μM naphthalene acetic acid, 0.2 μM 6-benzylaminopurine, 1 g/l casein hydrolysate, and 1 g/l 2-(*N*-morpholino) ethanesulfonic acid. The cell cultures were transferred to a fresh medium every 2 weeks. In a prolonged culture for production, 1 and 2% (w/v) maltose were added to the culture medium on day 7 and day 21, respectively, and 4 μM of $AgNO_3$ was added on the initiation of a culture as an elicitor [26]. After plant cell culture, plant cells and cell debris (biomass) were recovered from the suspension using a decanter (Westfalia, CA150 Claritying Decanter) and high speed centrifuge (α-Laval, BTPX205GD-35CDEEP). The biomass was provided by the Samyang Genex Company, South Korea.

2.2. Sample preparation for water pre-treatment

The biomass was mixed with methanol at a ratio of 1:1 (w/v) and extracted at room temperature for 30 min. The mixture was filtered under vacuum in a Buchner funnel through filter paper. Extraction was repeated four times with new methanol. Following this, the extract was collected, pooled and concentrated in a rotary evaporator (CCA-1100, EYELA, Japan) under vacuum and dried in a vacuum oven (UP-2000; EYELA) at 35 °C for 24 h. The dried crude extract (purity: 2.5%) was crushed to powder for water-pre-treatment.

2.3. Water pre-treatment method

Distilled water was added to the powdered extract (purity: 2.5%) and the mixture was agitated (~300 rpm) for 10 min at room temperature. The crude extract/water ratio was changed to 1:5, 1:10, 1:20, 1:30, 1:40, 1:50 and 1:60 (w/v) in order to obtain the optimal water volume. After water pre-treatment, the precipitate was filtered with filter paper (Whatman Grade 5, 2.5 μm, particle retention, 150 mm diameter) and dried. Then, it was used in the follow-up pre-purification process.

2.4. Liquid-liquid extraction

The dried crude extract (purity: 12.1%) obtained by water pre-treatment was dissolved in methanol (crude extract/methanol ratio, 1:80, w/v) and methylene chloride (25% of the amount of methanol) and distilled water (70% of the amount of methanol) were added. The liquid–liquid extraction was performed three times for 30 min. After the upper methanol-water layer containing polar impurities was removed, the lower methylene chloride layer containing paclitaxel was collected. The methylene chloride layers were concentrated and dried under vacuum by a concentrator (CCA-1100, EYELA, Japan).

2.5. Adsorbent treatment

The dried crude extract (purity: 12.1, 28.6%) obtained by water pre-treatment and liquid-liquid extraction was dissolved in methylene chloride at the ratio of 20% (v/w) and synthetic adsorbent sylopute (Fuji Silysia Chemical Ltd., Japan) was added. The tem-