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Development of a simultaneous extraction and acid hydrolysis process for recovery of paclitaxel from plant cell cultures

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ABSTRACT

In this study, a microwave-assisted extraction (MAE)/acid hydrolysis simultaneous process was developed for recovering the anticancer agent paclitaxel from plant cell cultures of *Taxus chinensis*. The optimal pH of the extraction solution (90% aqueous methanol) for hydrolysis was 2.2 at fixed extraction time (6 min), ratio of extraction solution to biomass (1:1, v/w), and extraction temperature (40 °C). In the MAE/acid hydrolysis simultaneous process, the paclitaxel recovery was 2.2-fold higher than in the existing extraction methods. Regarding changes in the content of glycoside (7-xylosyl paclitaxel as sugar-binding paclitaxel) and paclitaxel depending on the inclusion of acid hydrolysis in the MAE process, the content of 7-xylosyl paclitaxel decreased after acid hydrolysis whereas the content of paclitaxel increased. Based on this result, it was confirmed that acid hydrolysis breaks down a glycosidic bond of glycoside (sugar-binding paclitaxel), and so the recovery of paclitaxel increases.

1. Introduction

Paclitaxel is an anticancer agent that is a diterpenoid found in the bark of the yew tree [1,2]. It is an FDA (Food and Drug Administration)-approved anticancer drug most widely used for treatment of ovarian cancer, breast cancer, Kaposi's sarcoma and non-small cell lung cancer [3]. Its application has also been expanded to the treatment of acute rheumatoid arthritis and Alzheimer's disease. Since clinical trials regarding its combined prescription with various other treatments are underway, the demand for paclitaxel is expected to increase [4,5]. The main paclitaxel production methods are direct extraction from the yew tree, semi-synthesis, and plant cell culture [6–8]. Among these methods, plant cell culture enables stable mass production of paclitaxel of consistent quality in a bioreactor without being affected by such external factors as climate and environment [7]. Most of the paclitaxel produced by plant cell culture is contained in plant cells and the debris [9], and it is important to effectively extract paclitaxel from cells in regard to increasing the recovery. The existing extraction methods used most frequently use an organic solvent to recover paclitaxel from the biomass, a plant cell [10–12]. However, the conventional solvent extraction (CSE) method requires a long extraction time and large amounts of organic solvents and has a low extraction efficiency. To overcome these disadvantages, microwave-assisted extraction (MAE) has been studied [13]. During MAE, microwaves heat the solvent or solvent mixture directly. In addition, the direct interaction of microwaves with free water molecules present in glands and vascular systems results in the subsequent rupture of plant tissue and the release of active compounds into the organic solvent, which increases recovery. Compared with CSE, MAE has many advantages, which include a shorter extraction time, a lower solvent requirement, a higher extraction rate, and production of a higher-quality product at lower cost [14]. Recently, research studies have been conducted on the development of MAE methods for the extraction of saponin from *Ganoderma atrum* [15], camptothecin from *Nothapodytes foetida* [16], essential oil from *Elettaria cardamomum* [17], ginsenoside from ginseng root [18], glycyrrhizic acid from licorice root [19] and polyphenol and caffeine from green tea leaves [20]. In our previous study [21], we confirmed the possibility that the extraction of paclitaxel from plant cell cultures using MAE could overcome the aforementioned problems with CSE.

In 2000, Kim et al. [22] reported that the recovery of paclitaxel from the supernatant of plant cell culture increased by hydrolysis. It was assumed that the increase in recovery of paclitaxel in the supernatant of plant cell culture by acid hydrolysis was caused by the cleavage of 7-xylosyl paclitaxel and 7-xylosyl-10-deacetylpaclitaxel, which are glycosides (sugar-binding paclitaxel) found in the cell culture supernatant (the cleavage of glycosidic bonds between sugar and paclitaxel) [23]. However, the

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cause of the increase in recovery of paclitaxel by acid hydrolysis has still not been clarified. Therefore, in this study, we developed the simultaneous MAE and acid hydrolysis process to increase the recovery of paclitaxel from plant cells of *Taxus chinensis*. In particular, the efficiency of the process was maximized by investigating and understanding the mechanism of acid hydrolysis of glycosides in the MAE/acid hydrolysis simultaneous process.

2. Materials and methods

2.1. Plant materials

A suspension of plant cells originating from *Taxus chinensis* was cultured in a bioreactor [24]. Following cultivation, biomass (plant cell and debris) was recovered using a decanter (CA150 Clarifying Decanter; Westfalia, Germany) and a high-speed centrifuge (BTPX 205GD – 35CDEFP; Alfa Laval, Sweden). The biomass was provided by Samyang Genex Company, South Korea.

2.2. Paclitaxel and glycoside (7-xylosyl paclitaxel, 7-xylosyl-10-deacetylpaclitaxel) analysis

An HPLC system (SCL-10AVP, Shimadzu, Japan) and a Capcell Pak C18 column (250 × 4.6 mm, Shiseido) were used to analyze the paclitaxel and glycoside contents. Acetonitrile/water (35:65–65:35, v/v gradient) for paclitaxel and acetonitrile/water (25:75–65:35, v/v gradient) for glycoside were used as the mobile phase. Using a UV detector, paclitaxel and glycoside were analyzed at 227 nm and 228 nm, respectively [25]. In addition, the flow rate and sample injection volume were 1.0 mL/min and 20 μL, respectively. Authentic paclitaxel, 7-xylosyl paclitaxel and the 7-xylosyl-10-deacetylpaclitaxel were purchased from Sigma–Aldrich (purity: 95%), Quality Phytochemicals (purity: 99%), and Santa Cruz Biotechnology (purity: 60%), respectively, and used as standards. Each sample was analyzed in triplicate.

2.3. Conventional solvent extraction (CSE)

The biomass from plant cell cultures was mixed with 90% aqueous methanol and stirred at a room temperature for 30 min. The mixture was filtered under vacuum in a Buchner funnel through filter paper (150 mm, Whatman), where the 90% aqueous methanol was preferably added to biomass at a ratio of 1:1 (mL/g, v/w). Extraction was performed four times with new 90% aqueous methanol [10]. Each methanol extract was collected, pooled, concentrated and dried at 40 °C under vacuum (635 mm Hg) for HPLC analysis.

2.4. Microwave-assisted extraction (MAE)

The microwave facility (2450 MHz Model 1501, Korea Microwave Instrument Co., Korea) used for MAE consisted of a microwave generator, cooling system, and extraction unit (Fig. 1) [14]. A thermocouple was installed to measure temperature changes continuously during extraction. The cooling system condensed vaporized solvent, thereby returning it to the reactor. The microwave power (150 W) supply was operated by a computer program to control the temperature. The extraction temperature, extraction time, and extraction solution (90% aqueous methanol)/biomass ratio were 40 °C, 6 min, and 1:1 (v/w), respectively [21]. After extraction, the mixture was filtered under vacuum in a Buchner funnel through filter paper (150 mm, Whatman). The methanol extract was collected, concentrated and dried at 40 °C under vacuum (635 mm Hg) for HPLC analysis.

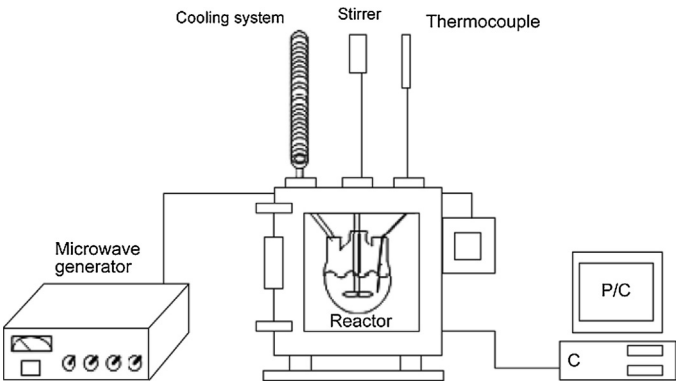

Fig. 1. Schematic diagram of the microwave-assisted extraction (MAE) process.

2.5. Simultaneous MAE and acid hydrolysis process

In our previous studies [21,26,27], the optimal extraction time, ratio of extraction solution to biomass, extraction temperature, and extraction solvent in MAE were found to be 6 min, 1:1 (v/w), 40 °C, and 90% aqueous methanol, respectively. The pH of extraction solution in the simultaneous process was optimized under these optimal conditions. The pH of the extraction solution (90% aqueous methanol) was adjusted to 1.8, 2.2, 2.6, and 3.0 for the experiment. Hydrochloric acid (HCl), sulfuric acid (H_2SO_4), or acetic acid (CH_3COOH) was used to adjust the pH of the extraction solution. In addition, the effects of the extraction time (3, 6, 9, 12 min), ratio of extraction solution to biomass (1:1, 2:1, 3:1, 4:1, v/w), extraction temperature (30, 35, 40, 45, 50 °C), and methanol concentration in the extraction solution (85, 90, 95, 100%, v/v) in the simultaneous process were investigated based on the optimal conditions in our previous studies [21,26,27].

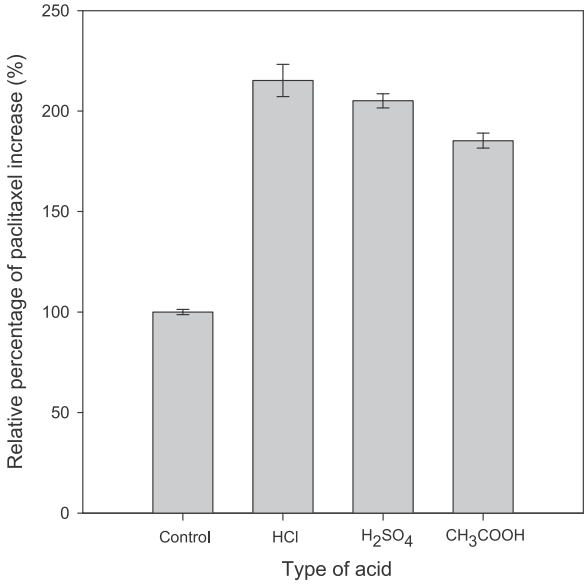

Fig. 2. Effect of type of acid on the simultaneous microwave-assisted extraction and acid hydrolysis process. The extraction solvent, temperature, time, and solvent/biomass ratio were 90% aqueous methanol, 40 °C, 6 min, and 1:1 (v/w), respectively. The control means the experimental result of CSE without acid hydrolysis.