Biotransformation of Glycosylated Saponins in Balloon Flower Root Extract into 3-O-β-D-Glucopyranosyl Platycosides by Deglycosylation of Pectinase from Aspergillus aculeatus

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Introduction

Platycodon grandiflorum A.DC. (Campanulaceae), commonly known as “balloon flower” or “bell flower,” is a perennial flowering plant widespread in Northeast Asia. Platycodon grandiflorum root (Platycodi radix) is used in the preparation of side dishes, desserts, teas, and flavored liquors. Platycodi radix extract is also widely used as a dietary supplement for the treatment of pulmonary diseases and respiratory disorders. The saponins (platycosides) in Platycodi radix extract exhibit diverse pharmacological activities, including anti-obesity [1], anti-inflammatory [2, 3], anti-allergy [4], anti-oxidant [5], neuroprotective [6, 7], and antitumor effects [8]. Platycosides in Platycodi radix extract are composed of pentacyclic triterpenes with two side chains. One of these side chains comprises the β-glucose residue, which is linked by a glycosidic bond at C-3 in the aglycon, whereas the other side chain includes an oligosaccharide moiety (apiofuranosyl-xylopyranosyl-rhamnopyranosyl-arabinofuranosyl residue) attached to the ester linkage at C-28 (Fig. 1).

Deglycosylated saponins, which are obtained from the biotransformation of glycosylated saponins, have been used as health supplements and food items for the treatment of respiratory disorders and pulmonary diseases. Deglycosylated saponins have been known to exert stronger biological effects than their glycosylated forms. In the present study, glycosylated platycosides in Platycodi radix extract were biotransformed into deglycosylated 3-O-β-D-glucopyranosyl platycosides, including 3-O-β-D-glucopyranosyl platycodigenin, 3-O-β-D-glucopyranosyl polygalacic acid, and 3-O-β-D-glucopyranosyl platyconic acid, by pectinase from Aspergillus aculeatus. This is the first report on the quantitative enzymatic production of 3-O-β-D-glucopyranosyl platycosides. The chemical structures of 3-O-β-D-glucopyranosyl platycosides were identified with LC/MS. Moreover, the biotransformation pathways of the three types of platycosides in Platycodi radix into 3-O-β-D-glucopyranosyl platycosides were established.

Keywords: Balloon flower root extract, platycosides, pectinase, 3-O-β-D-glucopyranosyl platycosides, biotransformation
In addition, the biotransformation pathways of glycosylated platycosides in Platycodi radix into 3-O-β-D-glucopyranosyl platycosides were investigated.

**Materials and Methods**

**Materials**

*P. grandiflorum* root was purchased from a local market (Republic of Korea). Platycoside standards, including deapi-PE, PE, deapi-PD, PD3, polygalacin D3, PD, and platyconic acid A, were purchased from Ambo Institute (Republic of Korea), while pectinase from *Aspergillus aculeatus* as the commercial enzyme Pectinex Ultra SP-L was obtained from Novozymes (Denmark).

**Preparation of Platycoside Standards**

Platycodin A (PA), 3″-O-acetyl polygalacin D3, and deapi-PD standards were purified from glycosylated platycosides from Platycodi radix extract. To prepare 3-O-β-D-glucopyranosyl platycodigenin, 3-O-β-D-glucopyranosyl polygalacic acid, and 3-O-β-D-glucopyranosyl platyconic acid standards, the 3-O-β-D-glucopyranosyl platycoside product solutions were obtained from the reactions at 50°C with pectinase from *A. aculeatus* in 50 mM citrate-phosphate buffer (pH 5.0) containing 10 mg/ml enzyme and 1 mg/ml of reagent-grade PE, polygalacin D3, and platyconic acid A as substrates, respectively, after 24 h. Platycodin A (PA), 3″-O-acetyl polygalacin D3, deapi-PD, and deapi-PD, obtained from the purification of glycosylated platycosides from Platycodi radix extract to total area in HPLC chromatograms were approximately 90%. The 3-O-β-D-glucopyranosyl platycosides showed 98% purity, as calculated from the ratio of the molar amount obtained after the purification of the products to the molar amount of the substrates.

**Extraction of Platycodi Radix**

The dried root of *P. grandiflorum* (100 g) was suspended in 1 l of 99.8% (v/v) methanol and incubated at 80°C for 24 h. After incubation, the precipitates were eliminated with vacuum filtration through a filter with a pore size of 0.2 μm.
0.45 μm. The methanol was removed by evaporation, and the dried residue was dissolved in 1 l of water. The methanol-free solution was applied to a column containing Diaion HP-20 resin (500 mm × 12 mm). Other hydrophilic compounds and free sugars were removed by washing the column with water, and the adsorbed platycosides in the resin were extracted by sequentially eluting with 2 l of methanol at a flow rate of 0.5 ml/min. The methanol in the extracted platycosides was removed by evaporation, and the dried residue was dissolved in 1 l of water. The dissolved solution was diluted to 7.4% (w/v) Platycodi radix extract by adjusting the concentration of PE to 1.0 mg/ml, which was used for the biotransformation of platycosides.

Biotransformation of Glycosylated Platycosides

The biotransformation into 3-O-β-D-glucopyranosyl platycodigenin, 3-O-β-D-glucopyranosyl polygalacic acid, and 3-O-β-D-glucopyranosyl platycconic acid in the presence of 10 mg/ml of pectinase from A. aculeatus were carried out at 50°C in 50 mM citrate-phosphate buffer (pH 5.0) with 1 mg/ml of reagent-grade PE, polygalacin D₃, and platyconic acid A for 24 h, respectively. The biotransformation into 3-O-β-D-glucopyranosyl platycosides was performed at 50°C in 50 mM citrate-phosphate buffer (pH 5.0) containing 10 mg/ml enzyme and 7.4% (w/v) Platycodi radix extract containing 1 mg/ml PE, 0.05 mg/ml polygalacin D₃, and 0.17 mg/ml platyconic acid A for 36 h.

Analytical Methods

The reaction was terminated and exacted with n-butanol at a ratio of 1:1, resulting in the separation of components into n-butanol and water fractions. The n-butanol fraction was evaporated until complete dryness, and the dried residue was treated with methanol. The concentrations of platycosides were determined using the HPLC system (Agilent 1100) equipped with a HydroSphere C18 column (4.6 × 150 mm, 5 μm particle size, YMC, Japan), which was eluted at 30°C with a gradient of acetonitrile from 10% to 40% (v/v) for 30 min, 40% to 90% for 30–45 min, 90% to 10% for 45–50 min, and 10% for 50–60 min at a flow rate of 1 ml/min. All platycosides were quantified from the calibration curves constructed using standard solutions of 0.2 to 0.8 mg/ml platycosides in triplicates.

LC/MS analysis of platycosides was performed to identify the chemical structures using a Thermo-Finnigan LCQ Deca XP Plus ion trap MS (Thermo Scientific, USA) at the NICEM (Seoul National University, Republic of Korea).

Fig. 2. HPLC chromatograms from the biotransformation of platycosides in 7.4% (w/v) Platycodi radix extract with pectinase from A. aculeatus. (A) Platycosides from Platycodi radix extract. Deapi-platycoside E (1), platycoside E (2), deapi-platycodin D₃ (3), platycodin D₄ (4), polygalacin D₃ (5), deapi-platycodin D₆ (6), platycodin D₇ (7), polygalacin D₈ (8), 3″-O-acetyl polygalacin D₉ (9), platycodin A (10), and platycanic acid A (11). (B) Platycosides at 12 h. Deapi-platycodin D₃ (1), deapi-platycodin D₆ (2), platycodin D₇ (3), intermediate platycoside 1 (4), polygalacin D₈ (5), intermediate platycoside 2 (6), intermediate platycoside 3 (7), intermediate platycoside 4 (8), intermediate platycoside 5 (9), intermediate platycoside 6 (10), intermediate platycoside 7 (11), unknown product 1 (12), unknown product 2 (13), and unknown product 3 (14). The biotransformation was performed at 50°C in 50 mM citrate-phosphate buffer (pH 5.0) containing 10 mg/ml enzyme and Platycodi radix extract containing 1 mg/ml PE, 0.05 mg/ml polygalacin D₃, and 0.17 mg/ml platycanic acid A for 36 h.
Korea). The samples were ionized using electrospray ionization under the conditions of 275°C capillary temperature, 30 psi nebulizer gas, 5 kV ion source voltage, 46 V capillary voltage in positive mode, 15 V fragmentor voltage in negative ionization mode, 0.01 min average scan time, 0.02 min average time to change polarity, and 35% abundant precursor ions at collision energy.

**Results and Discussion**

**HPLC Analysis for Biotransformation of Glycosylated Platycosides from Platycodi Radix Extract by Pectinase**

The glycosylated platycosides, namely, deapi-PE (1), PE (2), deapi-PD (3), PD (4), polygalacin D (5), deapi-PD (6), PD (7), polygalacin D (8), 3′′-O-acetyl polygalacin D (9), PA (10), and polytic acid A (11) from Platycodi radix extract were identified with HPLC at the same retention times of the standard platycosides (Fig. 2A). The biotransformation of the glycosylated platycosides from Platycodi radix extract into deglycosylated platycosides was performed with pectinase from *A. niger*. In the HPLC chromatogram at 12 h, deapi-PD (3), deapi-PD (6), PD (7), polygalacin D (8), polygalacin acid A (11), intermediate platycoside 1 (i1) intermediate platycoside 2 (i2), unknown product 1 (12), unknown product 2 (13), and unknown product 3 (14) were detected, whereas deapi-PE (1), PE (2), PD (4), polygalacin D (5), 3′′-O-acetyl polygalacin D (9), PA (10) were disappeared (Fig. 2B). In the HPLC chromatogram at 36 h, polygalacin acid A (11), intermediate platycoside 1 (i1), and intermediate platycoside 2 (i2) were disappeared, whereas deapi-PD (3), deapi-PD (6), PD (7), unknown product 1 (12), unknown product 2 (13) and unknown product 3 (14) were detected (Fig. 2C). The reagent-grade PD (4) and deapi-PD (6) were completely converted into unknown product 1 (12). However, PD (4) and deapi-PD (6) in Platycodi radix extract were not much decreased in Fig. 2. The results may be due to the inhibition of the enzyme activity by other platycosides in Platycodi radix extract [18].

The total concentration of platycosides in 7.4% (w/v) Platycodi radix extract was 2.76 mg/ml, while the concentrations of PE, polygalacin D, and PD as the main compounds were 1.00, 0.80, and 0.27 mg/ml, respectively, corresponding to the contents of 36.2, 29.0, and 9.8% (w/w) to total platycosides, respectively (Table 1). After 36 h, the concentrations of unknown product 1 (i12), unknown product 2 (i13), and unknown product 3 (i14) were 0.61, 0.21, 0.1 mg/ml, corresponding to the contents of 44.2, 15.2, and 7.2% (w/w), respectively.

**Identification of Unknown Products Obtained after Biotransformation of Glycosylated Platycosides from Platycodi Radix Extract by Pectinase**

For identification of unknown products 1, 2, and 3, LC/MS analyses of these compounds were performed. The total molecular masses of unknown products 1, 2, and 3 were indicated by distinct peaks at mass per charge (*m/z*) 683.7, 667.2, and 697.6 [M+H]+, respectively, in the LC/MS spectra (Fig. 2). Based on LC/MS data, the unknown products 1, 2, and 3 were identified as 3′′-O-β-D-glucopyranosyl platyconigenin, 3′′-O-β-D-glucopyranosyl polygalacic acid, and 3′-O-β-D-glucopyranosyl platycoside acid, respectively.

In the previous studies, the glycosylated platycoside PE was converted into PD by β-glucosidase of *A. niger* [14] and into deglu-PD by β-glucosidase of *D. turgidum* [16]. PD was converted into deapi-dexyl PD by crude enzyme of *A. niger* [15]. In this study, the biotransformation of glycosylated platycosides in Platycodi radix extract into 3′-O-β-D-glucopyranosyl platycosides by hydrolyzing oligosaccharide moiety (apiofuransyl-xylopyranosyl-rhamnopyranosyl-arabinofuranosyl) at C-28 was first reported.

| No. | Platycoside                  | Before reaction | After reaction |
|-----|------------------------------|----------------|---------------|
|     | Content (%) | Concentration (mg/ml) | Content (%) | Concentration (mg/ml) |
| 1   | Deapi-platycoside E         | 2.53           | 0.07          | 0              |
| 2   | Platycoside E               | 36.23          | 1.00          | 0              |
| 3   | Deapi-platycodin D3         | 0.36           | 0.01          | 0              |
| 4   | Platycodin D3               | 1.45           | 0.04          | 7.24           |
| 5   | Polygalacin D3              | 1.81           | 0.05          | 0              |
| 6   | Deapi-platycodon D          | 0.72           | 0.02          | 7.97           |
| 7   | Platycodon D                | 9.78           | 0.27          | 18.1           |
| 8   | Polygalacin D               | 28.98          | 0.80          | 0              |
| 9   | 3′′-O-Acetyl polygalacin D3 | 5.80           | 0.16          | 0              |
| 10  | Platycodon A                | 6.15           | 0.17          | 0              |
| 11  | Platycodon A                | 6.15           | 0.17          | 0              |
| 12  | Intermediate 1              | ND             | ND            | ND             |
| 13  | Intermediate 2              | ND             | ND            | ND             |
| 14  | Unknown product 1           | ND             | ND            | 44.20          |
| 15  | Unknown product 2           | ND             | ND            | 15.22          |
| 16  | Unknown product 3           | ND             | ND            | 7.24           |

Total: 100 2.76 100 1.38

ND: not detected.
To determine the pathways involved in the biotransformation of glycosylated platycosides in Platycodi radix extract by pectinase from Aspergillus aculeatus, we carried out the biotransformation of the reagent-grade glycosylated platycosides PE, polygalacin D3, and platyconic acid A. In the biotransformation, PE was completely converted into 3-O-β-D-glucopyranosyl platycodigenin via PD and deapi-PD (Figs. S1A-S1C), while polygalacin D3 was completely transformed into 3-O-β-D-glucopyranosyl polygalacic acid via polygalacin D and intermediate 1 (Figs. S2A-S2C). Platyconic acid A was completely converted into 3-O-β-D-glucopyranosyl platyconic acid via intermediate 2 (Figs. S3A-S3C).

Deapi-PD was confirmed as an intermediate formed during the biotransformation of PE into 3-O-β-D-glucopyranosyl platycodigenin with LC/MS. The total molecular mass of deapi-PD was indicated by the main peak at m/z 1093.3 [M+H]⁺ in the LC/MS spectrum. The fragment peaks resulted from the cleavage of xylose, rhamnose, and arabinose at C-28 and glucose at C-3, indicating that the intermediate was deapi-PD (Fig. S1D). The intermediates 1 and 2 were also identified by LC/MS. The total molecular mass of intermediates 1 and 2 were represented by peaks at m/z 1077.6 and 1107.3 [M+H]⁺, respectively. These results indicate that the intermediates 1 and 2 are deapi-polygalacin D and deapi-platyconic acid A (Figs. S2D and S3D). The fragment peaks of these compounds resulted from the cleavage of xylose, rhamnose, and arabinose at C-28 and glucose at C-3.

The biotransformation pathways of PE, polygalacin D3, and platyconic acid A in Platycodi radix extract were determined by HPLC analysis (Figs. S3-S5). The pathways involved in the biotransformation of other glycosylated platycosides in Platycodi radix extract, including deapi-PE, deapi-PD3, PD3, PA, and 3″-O-acetyl polygalacin D3, were investigated using reagent-grade platycosides in the HPLC chromatograms (Fig. S4). Deapi-PE, deapi-PD3,
and PD₃ as well as PA were completely converted into 3-O-β-D-glucopyranosyl platycodigenin via deapi-PD and deapi-PA, respectively. The compound 3″-O-acetyl polygalacin D₃ was completely converted into 3-O-β-D-glucopyranosyl polygalacic acid via 3″-O-acetyl polygalacin D and deapi-3″-O-acetyl polygalacin D.

Based on the HPLC data, the hydrolytic pathways of the three typical platycosides, including the platycodigenin-type platycosides deapi-PE (1), PE (2), deapi-PD₃ (3), PD₃ (4), deapi-PD (6), PD (7), and PA (10), polygalacic acid-type platycosides polygalacin D₃ (5), polygalacin D (8), and 3″-O-acetyl polygalacin D₃ (9); and the platyconic acid-type platycoside platyconic acid A (11) of major glycosylated platycosides in Platycodi radix extract into 3-O-β-D-glucopyranosyl platycodigenin (12), 3-O-β-D-glucopyranosyl polygalacic acid (13), and 3-O-β-D-glucopyranosyl platyconic acid (14), respectively, by pectinase from A. aculeatus were newly established as shown in Fig. 4. In the hydrolytic pathways, the enzyme hydrolyzed the glucose molecules, leaving one glucose

![Image](image_url)

**Fig. 4.** Pathways involved in the biotransformation of glycosylated platycosides from Platycodi radix extract into 3-O-β-D-glucopyranosyl platycosides with pectinase from *A. aculeatus*. (A) Pathways involved in the biotransformation of platycodigenin-type platycosides, including deapi-platycoside E, platycoside E, platycodin D₃, and platycodin A, into 3-O-β-D-glucopyranosyl platycodigenin. (B) Pathways involved in the biotransformation of polygalacic acid-type platycosides, including polygalacin D₃ and 3″-O-acetyl polygalacin D, into 3-O-β-D-glucopyranosyl polygalacic acid. (C) Pathways involved in the biotransformation of platyconic acid-type platycoside, platyconic acid A, into 3-O-β-D-glucopyranosyl platyconic acid. Unnumbered platycosides were not found in the HPLC chromatograms of Fig. 2. However, they were confirmed with reagent-grade platycosides (Fig. S4).
residue at C-3 and the oligosaccharide moiety (apiofuranosyl-xylopyranosyl-rhamnopranosyl-arabinofuranosyl residue) at C-28.

The biotransformation pathway of PE into PD by β-glucosidase of *A. niger* [14] and that of PE into deglu-PD by β-glucosidase of *Caldicellulosiruptor bescii* [19] were previously reported. In addition, the biotransformation pathways of glycosylated platycosides by human intestinal bacteria were suggested [20]. However, the biotransformation pathways of glycosylated platycosides in Platycodi radix extract into 3-O-β-D-glucopyranosyl platycosides by pectinase was first identified in this study.

**Biotransformation of Platycoside E, Polygalacin D₃, Platyconic Acid A, and Glycosylated Platycosides in Platycodi Radix Extract into 3-O-β-D-Glucopyranosyl Platycosides**

The time-course reactions for the biotransformation of reagent-grade PE as a platycodigenin-type platycoside were performed by pectinase from *A. aculeatus*. After 24 h, the enzyme completely converted 1 mg/ml PE into

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**Fig. 5. Biotransformation of reagent-grade platycosides and glycosylated platycosides from Platycodi radix extract into 3-O-β-D-glucopyranosyl platycosides by pectinase from *A. aculeatus*.**

(A) Biotransformation of reagent-grade PE. (B) Biotransformation of platycodigenin-type platycosides from Platycodi radix extract. (C) Biotransformation of reagent-grade polygalacin D₃. (D) Biotransformation of polygalactic acid-type platycosides from Platycodi radix extract. (E) Biotransformation of reagent-grade platyconic acid A. (F) Biotransformation of platyconic acid-type platycosides from Platycodi radix extract.
0.42 mg/ml 3-O-β-D-glucopyranosyl platycodigenin via PD and deapi-PD (Fig. 5A). The quantitative biotransformation of platycodigenin-type platycosides in Platycodi radix extract into 3-O-β-D-glucopyranosyl platycodigenin was also performed. The enzyme converted platycodigenin-type platycosides, including 0.07 mg/ml deapi-P, 1.0 mg/ml PD, 0.01 mg/ml deapi-PD, 0.04 mg/ml PD, 0.02 mg/ml deapi-PD, 0.27 mg/ml PD, and 0.17 mg/ml PA, in Platycodi radix extract into 0.61 mg/ml 3-O-β-D-glucopyranosyl platycodigenin as the main product and 0.1 mg/ml PD, 0.11 mg/ml deapi-PD, and 0.25 mg/ml PD as intermediates after 36 h (Fig. 5B).

The quantitative production of 3-O-β-D-glucopyranosyl polygalacys was attempted using the reagent-grade polygalacys acid-type polygalacin D. After 24 h, the enzyme completely converted 1 mg/ml polygalacin D into 0.43 mg/ml 3-O-β-D-glucopyranosyl polygalacys acid via polygalacin D and deapi-polygalacin D (Fig. 5C). The enzyme was also used for the quantitative biotransformation of polygalacys acid-type platycosides in Platycodi radix extract, including 0.05 mg/ml polygalacin D, 0.8 mg/ml polygalacin D, and 0.16 mg/ml 3-O-acetyl polygalacin D, which were converted into 0.21 mg/ml 3-O-β-D-glucopyranosyl polygalacys acid as a single product via 3-O-acetyl polygalacin D and deapi-polygalacin D after 36 h (Fig. 5D).

The time-course reactions for the biotransformation of reagent-grade platycosic acid A as a platycosic acid-type platycoside were performed. The enzyme completely converted 1 mg/ml platycosic acid A into 0.55 mg/ml 3-O-β-D-glucopyranosyl platycosic acid after 24 h via deapi-platycosic acid A (Fig. 5E). The time-course reactions for the biotransformation of platycosic acid A in Platycodi radix extract into 3-O-β-D-glucopyranosyl platycosic acid were also performed. The enzyme completely converted 0.17 mg/ml platycosic acid A as a platycosic acid-type platycoside in the Platycodi radix extract into 0.1 mg/ml 3-O-β-D-glucopyranosyl platycosic acid as a single product via deapi-platycosic acid A after 36 h (Fig. 5F).

The quantitative enzymatic production of 3-O-β-D-glucopyranosyl platycosides was carried out for the first time in the present study. Although the biotransformation of reagent-grade platycosides resulted in the complete conversion of the substrate into 3-O-β-D-glucopyranosyl platycosides after 24 h, we failed to observe the complete conversion of the platycosides in Platycodi radix extract after 24 h. The retardation effect may be attributed to the inhibition of the enzyme activity by other platycosides in the extract [18].

Saponins have been reported to improve in functionality as they are deglycosylated. For example, the anti-inflammatory activities of the platycodigenin-type platycosides followed the order deglu PD (three glycosides) > PD (four glycosides) > PD (six glycosides) > PE (seven glycosides) [16] and as an antioxidant activity, the peroxynitrite-scavenging capacities followed the order platycodigenin (no glycosides) > deapi-PE > PE > PD [21]. Thus, 3-O-β-D-glucopyranosyl platycosides (one glycoside) are expected to have high functionality and further study is needed in the functional properties of the deglycosylated platycosides.

In summary, the pectinase from A. auleatus converted glycosylated platycosides into the deglycosylated 3-O-β-D-glucopyranosyl platycosides by the hydrolysis of the glucose molecules at C-3, leaving one glucose residue, and the hydrolysis of the oligosaccharide moiety (apiofuranosyl-xylopyranosyl-rhamnopyranosyl-arabinofuranosyl residue) at C-28. This is the first report on the quantitative enzymatic production of 3-O-β-D-glucopyranosyl platycosides and the establishment of the biotransformation pathways.

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**Conflict of Interest**

The authors have no financial interests to declare.

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