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# Effects of the root of *Platycodon grandiflorum* on airway mucin hypersecretion in vivo and platycodin $D_3$ and deapi-platycodin on production and secretion of airway mucin *in vitro*

Jiho Ryu[a,1], Hyun Jae Lee[a,1], Su Hyun Park[a], Jinwoong Kim[b], Dongho Lee[c], Sang Kook Lee[b], Yeong Shik Kim[b], Jang-Hee Hong[a], Jeong Ho Seok[a], Choong Jae Lee[a,*]

[a] *Department of Pharmacology, School of Medicine, Chungnam National University, Daejeon 303-131, Republic of Korea*
[b] *Department of Pharmacy, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151-742, Republic of Korea*
[c] *Division of Biotechnology, College of Life Sciences & Biotechnology, Korea University, Seoul 136-713, Republic of Korea*

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ABSTRACT

We investigated whether aqueous extract of the root of *Platycodon grandiflorum* A. de Candolle (APG), platycodin$D_3$ and deapi-platycodin significantly affect the production and secretion of airway mucin using *in vivo* and *in vitro* experimental models. Effect of APG was checked on hypersecretion of pulmonary mucin in sulfur dioxide-induced bronchitis in rats. Confluent NCI-H292 cells were pretreated with platycodin$D_3$ or deapi-platycodin for 30 min and then stimulated with PMA (phorbol 12-myristate 13-acetate) for 24 h. The MUC5AC mucin production and secretion were measured by ELISA. The results were as follows: (1) APG stimulated the secretion of airway mucin in sulfur dioxide-induced bronchitis rat model; (2) platycodin$D_3$ and deapi-platycodin inhibited the production of MUC5AC mucin induced by PMA from NCI-H292 cells, respectively; (3) however, platycodin$D_3$ and deapi-platycodin did not inhibit but stimulated the secretion of MUC5AC mucin induced by PMA from NCI-H292 cells, respectively. This result suggests that aqueous extract of *P. grandiflorum* A. de Candolle and the two natural products derived from it, platycodin$D_3$ and deapi-platycodin, can regulate the production and secretion of airway mucin and, at least in part, explains the traditional use of aqueous extract of *P. grandiflorum* A. de Candolle as expectorants in diverse inflammatory pulmonary diseases.



## Introduction

Mucus in the pulmonary system is very important in defensive action against various particles, noxious chemicals and invading pathogenic microbes. This defensive action of pulmonary mucus is attributed to the physicochemical property of mucins, i.e. viscoelasticity. Mucins are high molecular weight glycoproteins present in the airway mucus and produced by goblet cells in the surface epithelium as well as mucous cells in the submucosal gland. However, hypersecretion of airway mucus is one of the major symptoms associated with severe pulmonary diseases including chronic bronchitis, cystic fibrosis, bronchiectasis and asthma (Lee et al., 2002; Heo et al., 2007, 2009; Voynow and Rubin, 2009; Kim et al., 2012). According to traditional oriental medicine, the root of *Platycodon grandiflorum* A. de Candolle has been used for controlling pulmonary inflammatory diseases (Jang, 2003). Platycodin D, a compound derived from the root of *P. grandiflorum* A. de Candolle, was reported to have diverse biological effects including anticancer effect (Kim et al., 2001, 2008; Shin et al., 2002; Ahn et al., 2006; Chung et al., 2008; Yu and Kim, 2010). Platycodin$D_3$ was reported to modulate nitric oxide production and TNF-α secretion from RAW 264.7 cells (Wang et al., 2004). Deapi-platycodin showed antiproliferative effect on cancer cells (Choi et al., 2010). However, to the best of our knowledge, there is no report about the potential effect of aqueous extract of *P. grandiflorum* A. de Candolle and, platycodin$D_3$ and deapi-platycodin, the two natural products derived from *P. grandiflorum* A. de Candolle, on production and secretion of airway mucin. Therefore, in this study, we checked whether aqueous extract of *P. grandiflorum* A. de Candolle, platycodin$D_3$ and deapi-platycodin significantly affect the production and secretion of airway mucin using in vivo and *in vitro* experimental models reflecting the hypersecretion and/or hyperproduction of mucus observed in inflammatory

*Abbreviations:* EGF, epidermal growth factor; PBS, phosphate-buffered saline; RT-PCR, reverse transcription-polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; PMA, phorbol 12-myristate 13-acetate.

* Corresponding author at: Department of Pharmacology, School of Medicine, Chungnam National University, 6 Munhwa-Dong, Joong-Gu, Daejeon, Republic of Korea. Tel.: +82 42 580 8255; fax: +82 42 585 6627.
*E-mail address:* LCJ123@cnu.ac.kr (C.J. Lee).

[1] These authors contributed equally to this work.

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| Peak | Rt (min.) | Compound |
|---|---|---|
| 1 | 6.1 | Platycoside E |
| 2 | 11.2 | Platycodin $D_3$ |
| 3 | 26.5 | Deapiplatycodin D |
| 4 | 30.5 | Platycodin D |
| 5 | 35.3 | Polygalacin D |
| 6 | 37.6 | Platycodin J |
| 7 | 40.1 | 2''-O-aectyl Platycodin D |
| 8 | 42.9 | Platycodin K |

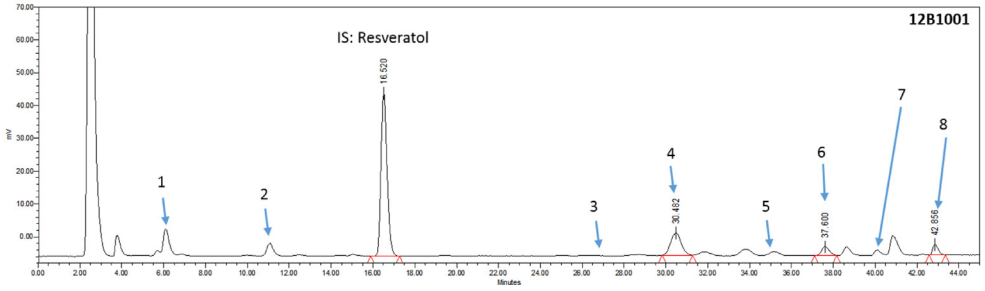


**Fig. 1.** HPLC-fingerprint analysis of the aqueous root extract of *Platycodon grandiflorum* A. de Candolle.

pulmonary diseases (Li et al., 1997; Takeyama et al., 1999; Shao et al., 2003).

## Materials and methods

### *Materials*

All the chemicals and reagents used in this experiment were purchased from Sigma (St. Louis, MO, U.S.A.) unless otherwise specified. Platycodin$D_3$ (purity: 98.0%), deapi-platycodin (purity: 98.0%) were isolated, purified and identified by analytical chemists in the Laboratory of Pharmacognosy, Department of Pharmacy, Seoul National University (Seoul, Korea). Briefly, small pieces of the roots of *P. grandiflorum* A. de Candolle 100 g were extracted twice with 1 L of distilled water for 3 h in water bath. Aqueous extract (41.0 g) were obtained after removing solvent by lyophilization. The HPLC-fingerprint analysis of the aqueous root extract of *P. grandiflorum* A. de Candolle was performed (Fig. 1). To isolate several compounds including platycodin$D_3$ and deapi-platycodin, the roots of *P. grandiflorum* A. de Candolle were extracted with 80% MeOH 3 l in ultrasonic bath and evaporated under reduced pressure. The extract was subjected to HP-20 column chromatography with water, 30%, 70% and 100% EtOH. 70% EtOH fraction was applied to RP-MPLC (20–40% MeOH) to give three fractions (Fr. 1–3). Fraction 3 was separated to 5 fractions (Fr. 3–1–3–5) by high speed counter current chromatography (HPCCC). A two-phase solvent system composed of BuOH–water–*n*-hexane [10:10:1 (v/v)] was used for the separation. A column was filled with upper stationary phase and the HSCCC instrument was revolved at 1.400 rpm while lower mobile phase was pumped in at a flow rate of 10.0 ml/min. Each fraction was purified by $C_{18}$ RP HPLC [$CH_3CN$–$H_2O$ (25:75–35:65)] to give platycodin $D_3$ and deapi-platycodin D (Fr. 3–2). Chemical structures of natural products derived from *P. grandiflorum* A. de Candolle including platycodin$D_3$ and deapi-platycodin can be seen in Fig. 2.

### *Animals*

Pathogen-free male Sprague-Dawley rats (Daehan Biolink, Seoul, Korea), 5 weeks of age weighing 200–220 g, were used. The animals were housed five per cage and were provided with the distilled water and food ad libitum. They were kept under a 12 h light/dark cycle (light on 08:00–20:00) at constant temperature (22.5 °C) and humidity (55%). Animals were cared through all of the experimental procedures in accordance with the Guide for the Care and Use of Laboratory Animals regulated by Chungnam National University, Daejeon, Korea.

### *Experimental design*

Twenty five rats were randomly divided into the following five groups: normal control; sulfur dioxide ($SO_2$)-only exposure; $SO_2$ exposure – aqueous extract of *P. grandiflorum* A. de Candolle (APG) 100 mg/kg; $SO_2$ exposure – APG 300 mg/kg; $SO_2$ exposure – dexamethasone 0.5 mg/kg. $SO_2$ was exposed to rats by inhalation and APG was administered per oral. A positive control, dexamethasone, was administered to rats via intraperitoneal injection. A 15% solution of sodium metabisulfite was aerosolized into a Plexiglas exposure chamber, using an ultrasonic humidifier (Samsung Electronics Inc., Seoul, Korea). The concentration of sulfur dioxide ($SO_2$) gas generated by this apparatus was measured to be 150 ppm. Rats were exposed to $SO_2$ for 3 h per day, 5 days per week, 3 weeks and APG was administered during the last 2 weeks out of 3 weeks in total. Normal control group were exposed to fresh air in a similar environment without $SO_2$ exposure.

### *Bronchoalveolar lavage fluid (BALF) collection and quantitation of in vivo mucins in BALF*

Rats were euthanized on the last day of experiment and the trachea was cannulated by using sterile polyethylene tube. BAL was performed four times with 5.0 ml of ice-cold PBS (pH 7.4) with 80% of recovery rate. Floating cells and cell debris were removed by centrifugation of BALF at 12,000 × *g* for 5 min. The BALF samples were stored at −70 °C until assayed for their mucin contents. The amount of mucins in each BALF sample was measured by using enzyme-linked immunosorbent assay (ELISA). The BALF samples were prepared with PBS at 1:10 dilution, and 100 μl of each sample was incubated at 42 °C in a 96-well plate, until dry. Plates were washed three times with PBS and blocked with 2% BSA (fraction V) for 1 h at room temperature. Plates were again washed three times with PBS and then incubated with 100 μl of 45M1 (NeoMarkers, CA, U.S.A.), a mouse monoclonal