Natural Product Sciences
10(4) : 141-151 (2004)

Review

Quality Control and Evaluation of *Platycodon grandiflorum*: Implications for Future Management

Bao-jun Xu[1], Yi-nan Zheng[2], and Chang-keun Sung*

Department of Food Science and Technology, College of Agriculture and Biotechnology, Chungnam National University, Taejon 305-764, Korea
[1]The Pharmaceutical Institute, Dalian University, Dalian 116622, China
[2]College of Chinese Material Medicine, Jilin Agricultural University, Changchun 130118, China

Abstract – Platycodi Radix, as one of the most important traditional oriental materia medica, is utilized frequently in clinical practice and dietary in China, Korea, and Japan. To evaluate definitely the quality of Platycodi Radix, and provide scientific data for commodity circulation, new product development and clinical application of Platycodi Radix, we summarized its medical history, application status, chemical compositions, bioactivities, cultivation, international circulation, quality evaluation, dosage and safety, and brought forward some constructive suggestions on further establishment of international criteria for quality control of *Platycodon grandiflorum*.
Keywords – *Platycodon grandiflorum*, Platycodi Radix, quality control, international criteria

Introduction

Platycodi Radix, the root of *Platycodon grandiflorum* A. DC (Campanulaceae), commonly known as Jiegeng in Chinese, Doraji in Korean, Kikyo in Japanese, has been used as a traditional oriental medicine. P. Radix has been used in China as an antiphlogistic, antitussivic and expectrantic agent since ancient times, and has also been widely used in Korea and Japan for centruries. Nowadays, P. Radix, as one of the most important traditional herbal medicines, is used to many TCM formulas, over 40 Chinese patent medicines, foods and cosmetics extensively in China. As a food and a folk remedy, P. Radix is used for hyperlipidemia, hypertension, and diabetes in Korea. Modern pharmacology and clinical medical research suggest that P. Radix possesses extensive pharmacological activity, such as anti-tussive, expectorant, anti-inflammatory, anti-ulcer, reducing blood pressure, expanding vascular, anti-febrile, analgesic, sedation, reducing blood sugar, anti-allergic, anti-tumor, immunostimulatory effect and so on.

As one of the most important traditional oriental materia medica, P. Radix is utilized frequently in clinical practice and dietary in China, Korea, and Japan. However, up to now, there are no universe novel standards of quality control that can be available in cultivation, international circulation, clinical application of *P. grandiflorum*. Although WHO monographs on selected medicinal plants (WHO, 1999) have provided some scientific information on the safety, efficacy, and quality control of P. Radix, the monographs are not pharmacopoeial monographs, rather they are comprehensive scientific references for drug regulatory authorities, physicians, traditional health practitioners, pharmacists, manufacturers, researchers; additionally, and present monographs were written on the basis of some relative earlier references. In order to promote application of some novel quality control methods and index, facilitate performance of good agricultural practice (GAP) in cultivation of *P. grandiflorum*, and provide abundant scientific data for commodity circulation, new product development, and clinical application, in present review, we summarized the medical history, application status, chemical compositions, bioactivities, cultivation, international circulation, quality evaluation, dosage and safety of *P. grandiflorum*.

*Author for correspondence
Fax: +82-42-822-2287; E-mail: kchsung@cnu.ac.kr

Medical history and application status of *P. grandiflorum*

P. Radix has been used frequently in the practice of Chinese medicine ever since some early descriptions were made. It was first referenced in the ancient Chinese medicine book *Shen Nongs Canon of Materia Medica*

(*Shennong Bencao Jing*), before Eastern Han Dynasty of China (about 100-180 A.D). On *Treatise on Exogenous Febrile Diseases* (*Shanghan Lun*) by Zhang Zhongjing (about 219A.D.) and *Jingui Yaolue* (about 220 A.D.), P. Radix was mainly used for treatment of abscesses. It is one of the ingredients in *Jiegeng Tang* (with licorice), *Painong Tang* (with ginger, licorice, and jujube), and in *Painong San* (with peony and chih-shih) for skin ailments marked by swelling and pus. The efficacy of "help five internal organs and intestines and stomach, benefit blood and vital energy, dispel chills and fever migratory arthralgia, warm the middle warmer, treat sore throat" was recorded on *A separate collection of famous Physicians* (*Mingyi Bielu*) by Tao Hongjing. The efficacy of "cure sore of mouth and tongue, red and swell of eyes" was described on *Compendium of Materia Medica* (*Bencao Gangmu*) by Li Shizhen at Ming Dynasty of China (1578 A.D).

P. Radix is still used in formulas to treat skin swellings, such as abscesses, and is also used for treatment of intestinal abscesses (used orally or by enema). In the treatment of intestinal disorders, it is commonly employed in the prescription called *Shenling Baizhu San* (Ginseng and Atractylodes Formula), developed during the Song Dynasty (about 1000 A.D.) to treat diarrhea; the formula has been adopted for relieving many inflammatory disorders of the intestines. Finally, P. Radix is said to soothe a sore throat or relieve hoarseness. These applications described its main medicinal uses in China (Bensky and Gamble, 1993; Dong and Yu, 1990; Huang and Wang, 1993).

Later in Chinese medical history, P. Radix became better known for its application to lung disorders and inflammatory conditions of the head and neck. In fact, it might now be described as the principal herb in Chinese medicine for diseases of the lungs and throat, and a herb commonly used for diseases of the eyes, ears, and sinuses. In the modern Chinese Materia Medica guides, P. Radix is always listed with the "phlegm-resolving" herbs. This herb can be used for both hot and cold phlegm. It assists the lung vital energy to expectorate phlegm and stop cough. Traditional prescriptions for respiratory tract disorders, especially those of the Ming Dynasty and Qing Dynasty periods and up to the present, usually include a combination of P. Radix with two or more other herbs from this phlegm-resolving section of the Materia Medica. It is also used for pulmonary abscesses, throat inflammations, and loss of voice.

In addition, there is a Chinese medicine theory that P. Radix, when used in a formula, helps guiding the action of the herbs to the chest area, where they can affect the entire upper body, including the head. Hence, it is used in a well-known formula *Xuefu Zhuyu Tang* (Persica and Achyranthes Combination), one of the most intensively studied and widely-used remedies of modern Chinese medicine, designed during the 19th century by Wang Qingren to treat injuries to the chest area. The inclusion of P. Radix in the formula is not because of its phlegm-resolving action, but because it focuses the herbs used for treating injuries to the chest area. This application of P. Radix is consistent with its indicated use for injuries to the chest, mentioned in the *Shen Nongs Canon of Material Medica.*

P. Radix is used in a very large number of Chinese prescriptions, illustrated by the fact that it was found in 6% of the formulas in the book *Thousand Formulas and Thousand Herbs of Traditional Chinese Medicine* (Huang and Wang, 1993). it has several indications that make it a favored ingredient, including treatment of abscesses, respiratory disorders, intestinal disorders, and injuries. In the practice of Chinese medicine, it is common to combine many herbs together. Many of the formulas that include P. Radix are quite large, possessing more than 15 ingredients, which makes it difficult to interpret the actual role of P. Radix or other individual ingredients in the formula. However, there are a number of commonly-used formulas, several of which have 15 or fewer ingredients, for which it is possible to assess the roles of P. Radix and other herbs. As an example, some formulas found in *Commonly Used Chinese Herb Formulas Companion Guide* were listed in Table 1, which shows how P. Radix is used and combined with other herbs.

Aside from its medical uses, P. Radix is also a popular food in Korea, Japan and Northeast of China including Chinese-Korean inhabiting area. As an uncommon constituent in meals, P. Radix usually comes from young herb. Also, it is a source of dietary starch, serving the same role as potatoes and other foods obtained from storage roots. In addition, extracts of P. Radix have been used in the manufacture of bath soaps, cosmetics and remedies for hangovers in Korea.

Chemical compositions and their bioactivities of *P. grandiflorum*

Chemical compositions of *P. grandiflorum* – Chemical analysis of active constituents from *P. grandiflorum* was first carried out during the 1930s and in more detail during the 1970s in Japan, revealing that the root contains a large group of oleanane-type triterpenoid saponins, such as platycodin D, platycodin D_2 and D_3, Deapi-platycodin

Table 1. Commonly-used formulas combining with P. Radix

Disorder category	Commonly-used formulas
Cold/flu with cough	Morus and Chrysanthemum Combination (*Sangju Yin*) Lonicera and Forsythia Formula (*Yin Qiao San*) Bupleurum and Pueraria Combination (*Chai Ge Jieji Tang*) Ginseng and Perilla Formula (*Shen Su Yin*)
Bronchitis	Lily Combination (*Baihe Gujin Tang*) Aster Combination (*Ziwan Tang*) Platycodon and Schizonepeta Formula (*Zhi Sou San*) Platycodon and Fritillaria Combination (*Qingfei Tang*) Morus and Platycodon Formula (*Dun Sou San*)
Formulas for enteritis, diarrhea	Ginseng and Atractylodes Combination (*Shen Ling Baizhu San*) Agastache Formula (*Huoxiang Zhengqi San*)
Formula for injuries to the chest	Persica and Achyranthes Combination (*Xuefu Zhuyu Tang*)
Formulas for abscesses (skin diseases)	Astragalus and Platycodon Formula (*Qianjin Neituo San*) Gleditsia Combination (*Tuoli Xiaodu Yin*) Schizonepeta and Siler Formula (*Jingfang Baidu San*) Platycodon Combination (*Jiegeng Tang*) Platycodon and Jujube Combination (*Painong Tang*) Siler Combination (*Qingsheng Fangfeng Tang*)

D, D_3, polygalacin D and D_2, and their monoacetates, platycodin A, C, platycodin V (2"-*O*-acetyl-platycodin D_2), VI (3"-*O*-acetyl-platycodin D_2), polygalacin VIII (2"-*O*-acetylpolygalacin D), IX (3"-*O*-acetylpolygalacin D), XI (2"-*O*-acetylpolygalacin D_2), XII (3"-*O*-acetylpolygalacin D_2) were the main chemical components; platycodigenin and polygalacic acid were main sapogenin, and platycogenic acid A, B, and C were minor sapogenin components in the root (Kubota *et al.*, 1969; Elyakov *et al.*, 1972; Akiyama *et al.*, 1972; Tada *et al.*, 1975; Ishii *et al.*, 1978a; Ishii *et al.*, 1978b; Ishii *et al.*, 1978c; Konishi *et al.*, 1978; Ishii *et al.*, 1981a; Ishii *et al.*, 1981b; Ishii *et al.*, 1984). Moreover, five new triterpenoid saponins, platycoside A, B, C, D, and E were isolated from P. Radix

Table 2. Structures and chemical information of saponin compounds from P. Radix

Compounds	R_1	R_2	R_3	R_4	R_5	Molecular formula	M.W.	References
Platycoside A	OH	Lam	H	H	H	$C_{58}H_{94}O_{29}$	1254	Nikaido *et al.*, 1998
Platycoside B	OH	Glc	Ac	H	H	$C_{54}H_{86}O_{25}$	1134	Nikaido *et al.*, 1998
Platycoside C	OH	Glc	H	Ac	H	$C_{54}H_{86}O_{25}$	1134	Nikaido *et al.*, 1998
Platycoside D	H	Glc-Gen	H	H	Api	$C_{69}H_{112}O_{37}$	1532	Nikaido *et al.*, 1999
Platycoside E	OH	Glc-Gen	H	H	Api	$C_{69}H_{112}O_{38}$	1548	Nikaido *et al.*, 1999
Platycodin A	OH	Glc	Ac	H	Api	$C_{59}H_{94}O_{29}$	1266	Konishi *et al.*, 1978; Ishii *et al.*, 1981b
Platycodin C	OH	Glc	H	Ac	Api	$C_{59}H_{94}O_{29}$	1266	Elyakov *et al.*, 1972; Ishii *et al.*, 1981b
Platycodin D	OH	Glc	H	H	Api	$C_{57}H_{92}O_{28}$	1224	Tada *et al.*, 1975; Konishi *et al.*, 1978; Ishii *et al.*, 1978b
Platycodin D_2	OH	Lam	H	H	Api	$C_{63}H_{102}O_{33}$	1386	Ishii *et al.*, 1978c; Ishii *et al.*, 1984
Platycodin D_3	OH	Gen	H	H	Api	$C_{63}H_{102}O_{33}$	1386	Ishii *et al.*, 1978b
2"-*O*-acetyl-platycodin D_2 (Platyodin V)	OH	Lam	Ac	H	Api	$C_{65}H_{104}O_{34}$	1428	Ishii *et al.*, 1978b
3"-*O*-acetyl-platycodin D_2 (Platyodin VI)	OH	Lam	H	Ac	Api	$C_{65}H_{104}O_{34}$	1428	Ishii *et al.*, 1978b
Deapi-Platycodin D	OH	Glc	H	H	H	$C_{52}H_{84}O_{24}$	1092	Ishii *et al.*, 1978c
Deapi-Platycodin D_3	OH	Gen	H	H	H	$C_{58}H_{94}O_{29}$	1254	Ishii *et al.*, 1978c
Polygalacin D	H	Glc	H	H	Api	$C_{57}H_{92}O_{29}$	1208	Ishii *et al.*, 1978b
2"-*O*-acetylpolygalacin D (Polygalacin VIII)	H	Glc	Ac	H	Api	$C_{59}H_{94}O_{30}$	1250	Ishii *et al.*, 1978b
3"-*O*-acetylpolygalacin D (Polygalacin IX)	H	Glc	H	Ac	Api	$C_{59}H_{94}O_{30}$	1250	Ishii *et al.*, 1978b
Polygalacin D_2	H	Lam	H	H	Api	$C_{63}H_{102}O_{32}$	1370	Ishii *et al.*, 1978b
2"-*O*-acetylpolygalacin D_2 (Polygalacin XI)	H	Lam	Ac	H	Api	$C_{65}H_{104}O_{33}$	1412	Ishii *et al.*, 1978b
3"-*O*-acetylpolygalacin D_2 (Polygalacin XII)	H	Lam	H	Ac	Api	$C_{65}H_{104}O_{33}$	1412	Ishii *et al.*, 1978b

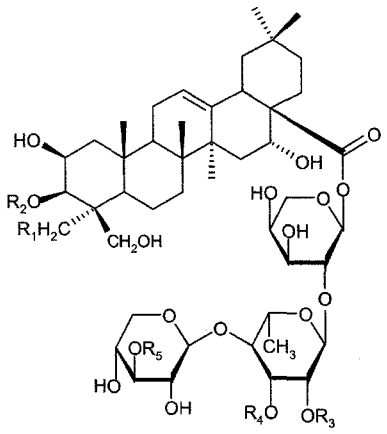

Fig. 1. Structures of compounds from P. Radix

(Nikaido *et al.*, 1998; Nikaido *et al.*, 1999). These compounds possess slight variations in chemical structures, and form many pairs of isomers, and their chemical information was illustrated in Table 2. and Fig. 1. In addition, two glycosides were isolated from the seed of *P. grandiflorum* (Inada *et al.*, 1992). A crude crystal blue pigment (platyconin) was obtained from the flower of *P. grandiflorum*. A number of other non-saponin chemical constituents have been isolated from *P. grandiflorum*, including α-spinasterol, α-spinasteryl-β-glucoside, stigmasterol, inulin (Chen, 1992; Oka *et al.*, 1992), pectin, alkaloids, etc.

Bioactivities of crude extract from *P. grandiflorum*

Platycodin yields pharmacological activities in laboratory experiments that parallel many of the clinical functions of the whole herb. While other non-saponin chemical constituents, such as plant sterols and inulin, are not responsible for the known activities of the herb, inulin may help to soothe sore throat due to their mucilaginous quality.

Traditionally, P. Radix has been used as an expectorant and to treat bronchitis, tonsillitis, sore throat and other respiratory conditions. In China, the medicinal properties of P. Radix have been recorded in many Chinese pharmacopoeias. P. Radix is generally used to treat coughs and upper respiratory tract infections, and its pharmacological effects include the ability of clearing the throat and lungs via the removal of phlegm and pus; heal sore throats and loss of voice; treat lung abscesses, and reduce the blood sugar level. It is also an anti-inflammatory and anti-allergenic agent. In Korea, P. Radix (4 years old) is used as a food and employed as a folk remedy for diseases of adulthood, such as bronchitis, asthma and pulmonary tuberculosis, hyperlipidemia, diabetes and inflammatory diseases, and as a sedative and analgesic (Takagi and Lee, 1972c; Lee, 1973; Lee, 1975; Kim *et al.*, 1995a).

Although the following components, triterpene saponins, inulin, sterols, etc, have been isolated from P. Radix by chemical analysis, crude platycodins were verified as main efficacy compositions (Takagi and Lee, 1972a; Takagi and Lee, 1972b; Takagi and Lee, 1972c; Lee, 1973; Lee, 1974; Lee, 1975). Several studies on immunopharmacological effects of P. Radix were reported (Takagi and Lee, 1972c; Lee, 1973; Lee, 1975; Kubo *et al.*, 1986; Nagao *et al.*, 1986). Presently, P. Radix is still a hot research topic in South Korea and Japan, because of its potential uses in health care. Many novel remarkable activities were discovered in succession. Extracts from P. Radix have been reported to have wide-ranging health benefits. It was observed that P. Radix (22 years old) helped preventing hypercholesterolemia and hyperlipidemia (Kim *et al.*, 1995a; Kim *et al.*, 1995b). It was reported that the aqueous extract from P. Radix cultivated for more than 20 years (Chang kil) (Lee, 1991), enhanced some of the functions of macrophages, including proliferation, spreading ability, phagocytosis, cytostatic activity and NO secretion, and induced the gene expression of TNFα, IL-1β and IL-6 (Choi *et al.*, 2001a; Choi *et al.*, 2001b). Moreover, it was reported that the P. Radix prevented obesity, hypertension, diabetes (Kim *et al.*, 2000; Arai *et al.*, 1997; Han *et al.*, 2000; Han *et al.*, 2002). In our previous studies, the aqueous extract from P. Radix showed to inhibit the intestinal absorption of dietary fat by inhibiting pancreatic lipase-induced fat hydrolysis; while inulin, a major component of P. Radix, had no effect on preventing obesity or the fat liver induced by the high fat diet (Han *et al.*, 2000). Study on immunostimulating effects of P. Radix showed polysaccharide isolated from *P. grandflorum* selectively activated B cells and macrophages (Han *et al.*, 2001). Further studies on the molecular mechanism responsible for the activation of macrophages by polysaccharide were performed, and nitric oxide production and iNOS gene expression as markers of macrophage activation was investigated, and the results suggested that the activation of macrophages by polysaccharide was mediated by activation of the membrane receptor, TLR4 (Yoon *et al.*, 2003). In addition, it was reported that the aqueous extract of Changkil (22 years old *P. grandiflorum*) showed hepatoprotective effects on chemicals-induced liver damage in mice (Lee *et al.*, 2001; Lee *et al.*, 2002). It was found that a methanol extract of P. Radix could protect

against mitomycin-induced mutagenesis (Shon *et al.*, 2001).

Bioactivities of single compounds from *P. grandiflorum*

Up to now, most of pharmacological experiments were focused on crude extract or crude saponins of P. Radix, there were a few pharmacological and pharmacodynamics experiments done on individual compound in the past three decades. Recently, pharmacological research at single compound level has been started, with some typical examples as shown below:

Arai *et al.* investigated the effective components of P. Radix and the mechanism of stimuli to pancreatic secretion of rats (Arai *et al.*, 1997). Animal experiments suggested that P. Radix served to stimulate pancreatic exocrine secretion mainly because platycodin D caused gastrointestinal hormones, particularly, CCK to be released from the duodenum. Crude saponins and platycodin D showed inhibitory effects on pancreatic lipase activity *in vitro* (Han *et al.*, 2000; Han *et al.*, 2002). Further research showed that the anti-obesity effect of P. Radix on mice fed a high fat diet might be due to the inhibition of intestinal absorption of dietary fat by crude saponins or platycodin D (Han *et al.*, 2000; Han *et al.*, 2002). To analyze the anti-inflammatory mechanism of P. Radix, the effects of platycodin D, platycodin D_3, and oleanolic acid on prostaglandin E_2 (PGE_2) production in rat peritoneal macrophages stimulated by kinase C activator were examined (Kim *et al.*, 2001). The results showed that platycodin D suppressed PGE_2 production at 10 and 30 μM in rat, while platycodin D_3 and oleanolic acid showed no effect at these concentrations. Further research was carried out to investigate the effects of platycodin D and D_3 on airway inflammation (Shin *et al.*, 2002), and the results showed that platycodin D and D_3 increased airway mucin release *in vivo* and *in vitro* in rats and hamsters, and the effect of platycodin D_3 was stronger than that of ATP, a mucolytic drug. These results suggested that platycodin D and D_3 were useful as expectorant agents in the treatment of various airway diseases. Choi *et al.* performed a preliminary study and found that platycodin D administered supraspinally had strong antinociceptive effect, and they suggested that platycodin D may act on the central nervous system; Further research (Choi *et al.*, 2002) was carried out to find antinociceptive mechanisms of platycodin D, and the results suggested that platycodin D had an antinociceptive effect when it was administered supraspinally, and supraspinal $GABA_A$, $GABA_B$, NMDA, and non-NMDA revceptors were involved in platycodin D-induced antinociception. Furthermore, platycodin D administered supraspinally produces antinociception by stimulating descending noradrenergic and serotonergic, but not opioidergic, pathways.

Cultivation and international circulation of *P. grandiflorum*

P. grandiflorum is an extensively distributed species, and is distributed in the most of the provinces of China, far-east area of Russia, Korea hemi-island and Japan series islands. It has only one species and one variety in the world (Zhou, 1994). *P. grandiflorum* A.DC was enlisted in *Pharmacopoeia of the Peoples Republic of China*. *P. grandiflorum* A.DC. var *album* Hort (white flower) is one variety distributed in the eastern mountain area of Jilin Province of China.

In China, as one of common use staple Chinese materia medica with both medicinal and dietary value, P. Radix is demanded in large scale, there is an increasing demand for P. Radix in the drug market and food market. Nowadays, P. Radix, as one of tonic foods in South Korea and Japan, is imported in large scale from China. Attributing to domestic and international demand for medicine and food, commodity supply from wild *P. grandiflorum* is far from market requirements, additionally, wild resources reduced gradually in China, therefore the cultivation were increased year after year. Presently, P. Radix is cultivated in a large scale in most of provinces in China, especially in eastern, northern and northeastern provinces in China. In general, commercial P. Radix has been categorized into various grades in China, 70% of grade 1 and grade 2 from Zhengjiang Province have been exported to South Korea, which in turn, exports it to Japan and the US.

In South Korea, two kinds of commercial P. Radix are available from native, one is common P. Radix cultivated for two or three years; another is Jang-kil. *P. glandiflorum* cultivated over 20 years old is called as Jang-kil in Korean. Korea researchers thought that common P. Radix does not have valuable effects. Therefore, they have developed a special commercial *P. glandiflorum* that lives for several decades. This kind of *P. glandiflorum* (Jang-kil) became a useful and marvelous medical plant to the human body as above-mentioned. In Japan, currently about 150 tons of P. Radix is used for medicinal use and other commercial purpose, every year in the Japanese market.

Quality evaluation of *P. grandiflorum*

Due to medicinal and commercial importance of *P.*

grandiflorum, it is imperative to set up a rapid and accurate analysis method to evaluate the quality of the commercial P. Radix. Taditionally, the quality of herbal medicine can be judged by carefully inspecting the morphological and histological characteristics and surface features. However, it is found that there are no recognized differences between the commercial P. Radix collected from different sources. In the past two decades, the absorbency methods (Pharmacopoeia of China, 1988, 1995, 2000), the TLC-Densitometry method (Long, 1989; Hosoda *et al.*, 1992) were reported in the quantity and quality study of P. Radix.

In recent three versions of *Pharmacopoeia of China* (1988, 1995, 2000), it is stated that the official material should contain not less than 6% saponins. An evaluation of platycodon saponin content from several samples reported (Long, 1989) showed that the wild roots of *P. grandiflorum* contained 7.3 – 11.8% saponins; cultivated roots had 1.7 – 14% saponins (the very low figure of 1.7% was from an immature plant, which is the food-grade material). Evidently, obtaining a minimum yield of 6% in the Chinese plants is not difficult, and is usually accomplished by using the wild stock, and can be easily accomplished with cultivated material of appropriate age. In one Korean report (Kim *et al.*, 1995a), the level of crude saponins in *P. grandiflorum* was said to be 2% in a medicinal grade material (as opposed to the food grade material which is younger and has less); it is possible that the growing conditions in Korea differ from those in China, yielding the lower figure. Similarly, in a report from Japan on the effect of different cultivation techniques for *P. grandiflorum*, the saponin content of the collected root was only 1.3 – 1.7%; the roots were collected after less than one year of growth (Katsuko *et al.*, 1992).

In Korea, the root extract of *P. grandiflorum* is used in traditional herbal medicine as expectorant, antitussive and anti-inflammatory agent. Currently, the *Korean Pharmacopoeia* (KP) includes only a simple test for identification. Korean Pharmacopoeia does not give sufficient results in quality control. In Japan, commercial P. Radix is usually imported from China or Korea. The commercial products on the market are quite complicated with respect to their origin from different sources. In the *Japanese Pharmacopoeia XIV* (2001), P. Radix (Kikyou) is listed as the root of *P. grandiflorum* A. De Candolle, no description on quality control in detail, either.

For better quality control, in recent decades, various HPLC methods based on platycodin D (or A, C) as a marker substance were developed (Kim *et al.*, 1990; Higashi *et al.*, 1997; Chung *et al.*, 1997; Piao *et al.*, 1998;

Table 3. HPLC conditions of quality evaluation for *P. grandiflorum*

Authentic compounds	Sample sources	Stationary phase	Mobile phase	Detector	References
Platycodin D	Cultivated samples from Jaewon, Bonghwa, Euseung Province of Korea	Nucleosil-ODS (250×4 mm, 5 μm)	H_2O/CH_3CN (70:30 V/V) 1.0 ml/min	UV-detecter at 214 nm	Kim *et al.*, 1990
	Cultivated samples from Korea	Uchrosorb NH_2 (250×4 mm, 10 μm)	H_2O/CH_3CN/*n*-butanol (20:80:10)	Differential refractometer	Chung *et al.*, 1997
Platycodin D	Cultivated, Peeled and intact root	Develosil ODS-P-5 250×4.6 mm, 40°C	20 mM KH_2PO_4/CH_3CN (75:25 V/V) 1.0 ml/min	UV detector at 206 nm	Higashi *et al.*, 1997
Platycodin D	Market samples from Seoul, Korea	μBondpark-ODS 300×3.9 mm	H_2O/CH_3CN Gradient solvent 2.0 ml/min	UV-photodiode array (PDA) detecter at 210 nm	Piao *et al.*, 1998
Platycodin A, C, D	Market, cultivated, wild samples from Japan, China, Korea	PEGASIL-ODS 250×4.6 mm, 5 μm, 40°C	20 mM KH_2PO_4/CH_3CN (75:25 V/V) 0.8 ml/min	UV-PDA detecter at 210 nm	Saeki *et al.*, 1999
Platycodin D	Cultivated samples from Northeast, North, Central China	Microsorb-ODS 250×4.6 mm, 5 μm, 35°C	50 mM KH_2PO_4/CH_3CN, pH=3.0 regulated by H_3PO_4 (70:30 V/V) 0.7 ml/min	UV detector at 210 nm	Xu *et al.*, 1999
Platycodin D	Cultivated samples from Jilin Province of China	Hypersil-NH_2 200×4.4 mm room temp.	MeOH/2-propanol (38/62) 0.8 ml/min	UV detector at 210 nm	Xu *et al.*, 2001
Platycodin D	Cultivated samples from Korea	YMC-Pack ODS-AM 250×4.6 mm, 5 μm 25°C	H_2O/CH_3CN (70:30 V/V)	ELSD detector Drift tube: 105°C N_2 gas:2.85SLPM	Kim *et al.*, 2002

Saeki *et al.*, 1999; Xu *et al.*, 1999; Xu *et al.*, 2001; Kim *et al.*, 2002; Saeki *et al.*, 2003) (see to Table 3) for quality evaluation from different sources. Among various HPLC methods, general HPLC methods are based on reversed phase system with ODS column and acetonitrile-water (with or without phosphate) system as a mobile phase, monitored by UV detector (with or without PDA) (Kim *et al.*, 1990; Higashi *et al.*, 1997; Piao *et al.*, 1998; Saeki *et al.*, 1999; Xu *et al.*, 1999). Other HPLC methods based on reversed phase system with NH_2 column (Chung *et al.*, 1997; Xu *et al.*, 2001), monitored by special detector, such as differential refractometer (Chung *et al.*, 1997), evaporative light scattering detector (ELSD) (Kim *et al.*, 2002), were used for determination of platycodin D successfully.

Due to no commercially available authentic compounds, Piao *et al.* introduced a purification method of platycodin D and a standardization method for evaluation of *P. grandiflorum* (Piao *et al.*, 1998). Platycodin D was isolated from *P. grandiflorum* using HPLC with a gradient (water and acetonitrile) program. The purified platycodin D was characterized with spectroscopic analysis. The purified compound was used as a standard in evaluating the quality of *P. grandiflorum* from various sources. The result indicated that the wild *P. grandiflorum* contained 1.8 times more platycodin D than the cultured one.

Saeki *et al.* performed systematic comparative study (Saeki *et al.*, 1999; Saeki *et al.*, 2003) of commercial samples from China, Korea and Japan. A qualitative and quantitative method using HPLC with photo-diode array detector was set up. Totally twelve saponin peaks were positively identified by referring to the authentic compounds isolated earlier. The identification was made on the basis of their ultraviolet absorption spectra and retention time. The peak of identified platycodins D, A, and C, were relatively rich in P. Radix and well separated from other peaks. Platycodin A, C, D were chosen as the reference standards to evaluate the commercial P. Radix. By carefully examining the peak/area ratio of platycodin A, C, and D, it was found that all the samples from China gave a ratio of about 1:2:3. The samples from Korea gave a ratio of about 2:4:1. Platycodin D was the main saponin in the sample from China, while platycodin C was relatively abundant in the sample from Korea. On the other hand, platycodins A, C, and D were all rich in the samples cultivated in Japan and the ratio of platycodins A, C, D was about 1:2:1. Thus, it seemed appropriate to use platycodin A, C, and D as the index of the quality and quantity of the commercial P. Radix. After careful comparison of a total of twenty-four samples collected from China, Korea and Japan, it was found that samples from China and Korea had different patterns, and the samples purchased from the Japanese market resembled those either from China or from Korea, which indicated that commercial P. Radix sold in Japan was imported from China or Korea based upon their HPLC patterns. The samples from various botanical gardens or the wild within Japan gave a different pattern with much higher total saponin contents compared to those from China or Korea. The data showed significant differences in the total saponin contents among the marked samples, ranging from the lowest at 20.04 mg/g to the highest at 28.65 mg/g. But no big variation was found in the total saponin content among the commercial P. Radix collected from different sources.

More recently, Kim *et al.* established a new HPLC method (Kim *et al.*, 2002) for determination of platycodin D based on using a reversed phase system with YMC-Pack ODS-AM column and 30% acetonitrile as a mobile phase. ELSD was used as detector. The contents of platycodin D was highest as 0.083% when platycodon roots were dried at 60°C in oven.

During our research on bioactivity and chemical composition of *P. grandiflorum*, electrospray ion trap multiple-stage tandem mass spectrometry ($ESI\text{-}MS^n$) technique was successfully applied for qualitative determination of platycodin D and other platycodins (Xu *et al.*, 1999; Xu *et al.*, 2000). The multiple-stage tandem approach enabled structural determination of platycodins to a detailed level. The structures derived from multiple-stage tandem mass spectrometry were deduced from typically 200 ng of samples and might significantly reduce the time needed for complete structure elucidation by NMR and chemical methods. In addition, electrospray ionization mass spectrometry technique also can be applied to characterize total saponins from *P. grandiflorum* (Xu *et al.*, 1999). The mass spectrum profile of total saponins was shown in Fig. 2. One grade excitation figure of electrospray-MS of total saponin suggested that total saponin contained at least 17 kinds of individual saponins (there are 17 molecular ion peaks which conform to the law of $[M+23]^+[504+23]^+$ or $[M+23]^+[519+23]^+$ on one grade excitation figure, 504 and 519 are molecular weight of polygalacic acid and platycodigenin, respectively). There are some characteric figures, such as $[1232]^+=[1208+23]^+$, $[1247]^+=[1224+23]^+$, $[1261]^+=[1238+23]^+$, $[1273]^+=[1250+23]^+$, $[1289]^+=[1266+23]^+$, $[1304]^+=[1281+23]^+$, $[1321]^+=[1298+23]^+$, $[1335]^+=1312+23]^+$, $[1347]^+=[1324+23]^+$, $[1361]^+=[1338+23]^+$, $[1377]^+=[1354+23]^+$, $[1393]^+=[1370+23]^+$, $[1409]^+=[1386+23]^+$,

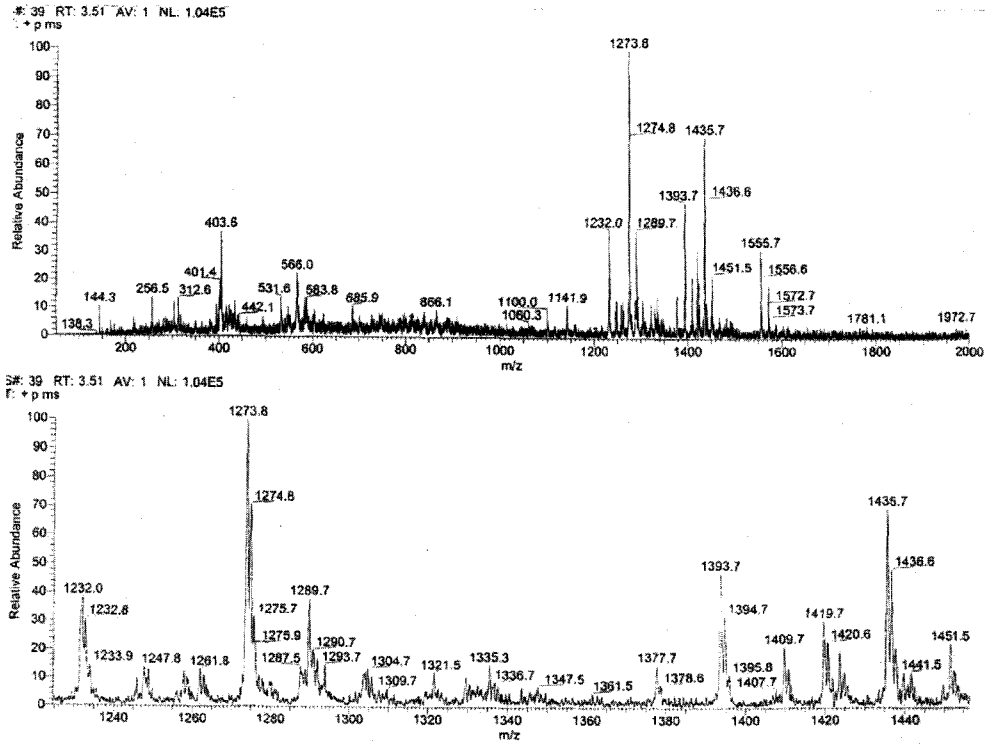

Fig. 2. Mass spectrum of total saponin from *P. grandiflorum.*

$[1419]^+$=$[1396+23]^+$, $[1435]^+$=$[1412+23]^+$, $[1451]^+$=$[1428+23]^+$ etc. and among these molecular ion peaks, $[1232]^+$ is attributed to polygalacin-D, $[1247]^+$ is platycodin-D, $[1273]^+$ is polygalacin-VIII or polygalacin-IX, $[1289]^+$ is platycodin-A or platycodin-C, $[1393]^+$ is polygalacin-D_2, $[1409]^+$ is platycodin-D_2 or platycodin-D_3, $[1435]^+$ is polygalacin-XI or polygalacin-XII, $[1451]^+$ is platycodin-V or platycodin-VI. Above molecular ion peaks are known structure components, and other unknown peaks remain to be further studied. These data suggested that electrospray ionization mass spectrometry was a powerful tool to provide abundant information for qualitative analysis of individual saponins among total platycodins, it also suggested that quantitative analysis might be carried out to compare the fingerprint information provided by ESI-MS from various sources.

Dosage and safety of *P. grandiflorum*

In traditional Chinese practice, the root slices are boiled along with other herbs to make a decoction. In some cases, the herbal combination is powdered and boiled to make a tea. The usual dosage of P. Radix is about 3 – 9 grams for one day dose, which may be taken in divided doses. This amount of herb could theoretically yield about 180 – 540 mg of saponins (assuming 100% extraction and 6% content; the actual extraction would likely be slightly less, but the saponin content would likely be somewhat higher than this minimum figure). Dosages of isolated crude platycodin given orally in laboratory animal experiments to show medicinal effects ranged from 25 mg/kg to 100 mg/kg (rough correspondence in humans by body weight: about 1750 mg to 7000 mg per day). Crude extracts of P. Radix, using water or an ethanol extract, were administered orally in doses equivalent to 100 mg/kg to 1,000 mg/kg of the crude herb material (rough correspondence to humans: about 7 – 70 g/day). Thus, the laboratory studies generally involved higher doses of the herb and its saponins than one would encounter using P. Radix as the sole saponin source in clinical practice.

There are two well-known adverse effects of saponins when the dosage is high enough: hemolytic action (causes red blood cells to break) and nauseant effect (causes loss of appetite and vomiting). Both of these adverse effects of saponins were observed in laboratory experiments with platycodin (Chang and But, 1986; Perry, 1980), but have not been reported from human use of P. Radix. The hemolytic action of the saponins from P. Radix has prevented their use by injection, which is one of the routes of administration used for herbs in China. The hemolytic effect is reduced by degradation in the alimentary tract, so that platycodon is safe at normal doses. The nauseant effect can be observed at very high doses, but these are

doses beyond those normally prescribed in clinical practice.

Numerous animal studies were conducted with P. Radix. Toxicity studies such as LD_{50} evaluations showed that the toxic dose in animal models is very high, consistent with a non-toxic status for normal human dosage. For example, the LD_{50} in mice of P. Radix (as decoction) was reported to be 24 g/kg (Chang and But, 1986). The decoction was used in a trial of expectorant activity in mice at a dosage of 20 g/kg (Zhang *et al.*, 1987). In rabbits given 20 g/kg, all survived (at 40 g/kg, all died). Therefore, the LD_{50} is rated in excess of 20 g/kg. Although a direct comparison is not possible, a 50 kg human being would need to consume 1 kg of platycodon as decoction in order to get this dosage per unit body weight. Human dosing of platycodon (in decoction) is never recommended at greater than about 12 – 15 g/day for short term use by practitioners of traditional Chinese Medicine. This corresponds to a platycodin level of about 750 – 1000 mg/day. The LD_{50} of purified platycodin was greater than 400 mg/kg: in one report, the LD_{50} for mice was listed as 420 mg/kg and in rats was listed as being greater than 800 mg/kg (Lee *et al.*, 1973). It was reported that at large oral doses (amount not stated), platycodin stimulated the vomiting center in animals (Chang and But, 1986). Crude platycodin was shown to have an inhibitory action on the central nervous system in laboratory animals (Takagi and Lee, 1972a) in the oral dosage range of 50 – 200 mg/kg, which is similar to the range used in the study of other pharmacological actions (25 – 100 mg/kg). P. Radix does not have the reputation of being a sedative, but this action could clearly be expected when the dosage is high, based on the laboratory animal experiments.

In the practice of Chinese medicine, P. Radix is almost always prescribed in complex formulas. P. Radix by itself, therefore, has not been the subject of clinical trials. Thus clinical studies of P. Radix were limited.

Perspectives

Up to now, studies on *P. grandiflorum* have been extended deeply in many aspects. However, more extensive and in-depth study still should be done at single compound level to discover molecular mechanisms of known activity. Clinical trials of known pharmacological activity need to be furtherly studied to verify efficacy and safety. As major pharmaceutically active components, commercially available single saponins, such as platycodin D, should be provided as authentic compounds for research and quality evaluation. A universal international criteria for quality control and evaluation of *P. grandiflorum* should be established as early as possible in order to effectively promote production, circulation and application of *P. grandiflorum.*

References

Akiyama, T., Tanaka, O., and Shibata, S., Chemical studies on the oriental plant drugs. XXX. Sapogenins of the roots of *Platycodon grandiflorum* A. De Candolle (1) Isolation of the sapogenins and the stereochemistry of polygalacic acid. *Chem. Pharm. Bull.* **20**(9), 1945-1951 (1972).

Arai, I., Komatsu, Y., Hirai, Y., Shingu, K., Ida, Y., Yamaura, H., Yamamoto, T., Kuroiwa, Y., Sasaki, K., and Taguchi, S., Stimulative effects of saponin from kikyo-to, a Japanese herbal medicine, on pancreatic exocrine secretion of conscious rats. *Planta Med.* **63**(5), 419-424 (1997).

Bensky, D. and Gamble, A., *Chinese Herbal Medicine: Materia Medica*, rev. ed., Eastland Press, Seattle, WA (1993)

Chang, H.M. and But, P.P.H., *Pharmacology and Applications of Chinese Materia Medica.* World Scientific Publishing, Singapore (1986)

Chen, Y.H., The extract of inulin from *Platycodon grandiflorum. Journal of LanZhou Medical College* **18**(4), 253 (1992).

Choi, C.Y., Kim, J.Y., Kim, Y.S., Chung, Y.C., Seo, J.K., and Jeong, H.G., Aqueous extract isolated from *Platycodon grandiflorum* elicits the release of nitric oxide and tumor necrosis factor-α from murine macrophages. *Int. Immunopharmacol.* **1**(6), 1141-1151 (2001a).

Choi, C.Y., Kim, J.Y., Kim, Y.S., Chung, Y.C., Hahm, K.S., and Jeong, H.G., Augmentation of macrophage functions by an aqueous extract isolated from *Platycodon grandiflorum. Cancer Lett.* **166**(1), 17-25 (2001b).

Choi, S.S., Han, E.J., Lee, T.H., Lee, J.K., Han, K.J., Lee, H.K., and Suh, H.W., Antinociceptive mechanisms of platycodin D administered intracerebroventricularly in the mouse. *Planta Med.* **68**, 794-798 (2002).

Chung, J.H., Shin, P.G., Ryu, J.C., Jang, D.S., and Cho, S.H., Pharmaceutical substances of *Platycodon grandiflorum* (jacquin) A.De Candolle. *Agric. Chem. Biotechnol.* **40**(2), 152-156 (1997).

Dong, Z.L. and Yu, S.F., *Modern Study and Application of Materia Medica.* China Ocean Press, Beijing (1990)

Elyakov, G.B., Chemical studies on platycodoside C, a new glycoside from *Platycodon grandiflorum* (Jacg.) A.De. *Tetrahedron Lett.* **35**, 3651-3652 (1972).

Han, L.K., Xu, B.J., Kimura, Y., Zheng, Y.N., and Okuda, H., Platycodi Radix affects lipid metabolism in mice with high fat dietinduced obesity. *J. Nutr.* **130**, 2760-2764 (2000).

Han, L.K., Zheng, Y.N., Xu, B.J., Okuda, H., and Kimura, Y., Saponins from Platycodi Radix ameliorate high fat diet-induced obesity in mice. *J. Nutr.* **132**(8), 2241-2245 (2002).

Han, S.B., Park, S. H., Lee, K. H., Lee, C.W., Lee, S.H., Kim,

H.C., Kim, Y.S., Lee, H.S., and Kim, H.M., Polysaccharide isolated from the radix of *Platycodon grandiflorum* selectively activates B cells and macrophages but not T cells. *Int. Immunopharmacol.* **1**(11), 1969-1978 (2001).

Higashi, A., Arimoto, K., Iwata, Y., Ujita, K., Kawai, T., Kobayashi, Y., Sakurai, K., Shimada, Y., Takagi, A., Taniyama, T., Nakajima, K., Hisata, Y., Hosoda, K., Yamamoto, K., Yamamoto, Y., and Noguchi, M., Studies on the effect of preparation method of platycodon root on its quality. *Natural Medicines* **51**(1), 56-62 (1997).

Hosoda, K., Noguchi, M., Tomonari, K., and Nishijima, A., Studies on the cultivation and preparation of Platycodon root III. Effect of picking flower and fruit on the quality of skin peeled root. *Chem. Pharm. Bull.* **40**(7), 1946-1947 (1992).

Huang, B.S. and Wang, Y.X., *Thousand Formulas and Thousand Herbs of Traditional Chinese Medicine*, Heilongjiang Education Press, Harbin (1993)

Inada, A., Murata, H., Somekawa, M., and Nakanishi, T., Phytochemical Studies of Seed of Medicinal Plants. II. A new dihydroflavonol glycoside and a new 3-methyl-1-butanol glycoside from seeds *Platycodon grandiflorum* ADe Candolle. *Chem. Pharm. Bull.* **40**(11), 3081-3083 (1992).

Ishii, H., Tori, K., Tozyo, T., and Yoshimura, Y., Structures of prosapogenins from roots of *Platycodon grandiflorum*, determined by carbon-13 and hydrogen-1 nuclear magnetic resonance spectroscopy. *Chem. Pharm. Bull.* **26**(2), 671-673 (1978a).

Ishii, H., Tori, K., Tozyo, T., and Yoshimura, Y., Structures of polygalacin-D and -D_2 platycodin-D and -D_2, and their monoacetaes, saponins isolated from *Platycodon grandiflorum*, determined by ^{13}C-NMR spectroscopy. *Chem. Pharm. Bull.* **26**(2), 674-677 (1978b).

Ishii, H., Tori, K., Tozyo, T., and Yoshimura, Y., Structures of platycodin-D_3, platycodinic-A, and their derivatives, saponins isolated from root of *Platycodon grandiflorum* A. De determined by ^{13}C-NMR spectroscopy. *Chem. Lett.* 719-722 (1978c).

Ishii, H., Kitagawa, I., Matsushita, K., Shirakawa, K., Tori, K., Tozyo, T., and Yoshimura, Y. *Tetrahedron Lett.* **22**, 1529-1532 (1981a).

Ishii, H., Matsushita, K., Shirakawa, K., Tozyo, T., and Yoshimura, Y., Saponins from roots of *Platycodon grandiflorum.* Part 1. Structure of prosapogenins. *J. Chem. Soc. Perkin Trans. I.* 1928-1933 (1981b).

Ishii, H., Tori, K., Tozyo, T., and Yoshimura, Y., Saponins from roots of *Platycodon grandiflorum.* Part 2. Isolation and structure of new triterpene glycosides. *J. Chem. Soc. Perkin Trans. I.* 661-668 (1984).

Kim, G.S., Kim, H.T., Seong, J.D., Park, H.S., and Kim, S.D, Quantitative analysis of platycodin D from *Platycodon grandiflorum by* HPLC-ELSD. *Korean J. Medicinal Crop Sci.* **10**(3), 200-205 (2002).

Kim, K.S., Ezaki, O., Ikemoto, S., and Itakura, H., Effects of *Platycodon grandiflorum* feeding on serum and liver lipid concentrations in rats with diet-induced hyperlipidemia. *J. Nutr. Sci. Vitaminol.* **41**(4), 485-491 (1995a).

Kim, K.S., Ezaki, O., and Ikemoto, S., Effects of *platycodon grandiflorum* feeding on serum and liver lipid concentrations in rats with diet-induced hyperlipidemia. *Yakugaku Zasshi* **41**, 485-491 (1995b).

Kim, K.S., Seo, E.K., Lee, Y.C., Lee, T.K., Cho, Y.W., Ezaki, O., and Kim, C.H., Effect of dietary *Platycodon grandiflorum* on the improvement of insulin resistance in obese Zucker rats. *J. Nutr. Biochem.* **11**(9), 420-424 (2000).

Kim, T.J., Lee, S.I., Lee, T.H., and Ko, J.S., Isolation and determination of platycodin D from Platycodi radix. *Analytical Sci. & Technol.* **3**(3), 399-404 (1990).

Kim, Y.P., Lee, E.B., Kim, S.Y., Li, D.W., Ban, H.S., Lim, S.S., Shin, K.Y., and Ohuchi, K., Inhibition of prostaglandin E_2 production by platycodin D isolated from the root of *Platycodon grandiforum. Planta Med.* **67**, 362-364 (2001).

Konishi, T., Tada, A., Shoji, J., Kasai, R., and Tanaka, O., The structures of platycodin A and C, monoacetycated saponins of the roots of *Platycodin grandiflorum. Chem. Pharm. Bull.* **26**(2), 668-670 (1978).

Kubo, M., Nagao, T., Matsuda, H., and Namba, K., Immune pharmacological studies on *Platycodi radix* (I): effect on the phagocytosis in mouse. *Shoyakugaku Zasshi* **40**, 367-374 (1986).

Kubota, T., Kitatani, H., and Hinoh, H., The structure of platycodigenic acid A, B, and C, Further triterpenoid constituents of *Platycodon grandiflorum* A. De Candolle. *Chem. Comm.* **22**, 1313-1314 (1969).

Lee, E.B., Pharmacological studies on *Platycodon grandiflorum* A. DC. IV. A comparison of experimental pharmacological effects of crude platycodin with clinical indications of *platycodi radix* (author's transl). *Yakugaku Zasshi* **93**(9), 1188-1194 (1973).

Lee, E.B., Pharmacological studies on *Platycodi radix. Kor. J. Pharmacog.* **5**(1), 49-60 (1974).

Lee, E.B., Pharmacological activities of crude platycodin. *J. Pharm. Soc. Korea* **19**, 164-176 (1975).

Lee, K.J., and Jeong, H.G., Protective effect of *Platycodi radix* on carbon tetrachloride-induced hepatotoxicity. *Food Chem. Toxicol.* **40**(4), 517-525 (2002).

Lee, K.J., You, H.J., Park, S.J., Kim, Y.S., Chung, Y.C., Jeong, T.C. and Jeong H.G., Hepatoprotective effects of *Platycodon grandiflorum* on acetaminophen-induced liver damage in mice. *Cancer Lett.* **174**(1), 73-81 (2001).

Lee, S.H., Patent on the method of cultivating the perennial balloonflower, Patent No. 045971, Korea (1991).

Long, Q.Q., Total saponin contents of the root of wild and cultivated *Platycodon grandiflorum. Zhong Yao Cai* **12**(3), 37-38 (1989).

Nagao, T., Matsuda, H., Namba, K., and Kubo, M., Immune pharmacological studies on *Platycodi radix* (II): anti-tumor activity of inulin from *Platycodi radix. Shoyakugaku Zasshi* **40**, 375-380 (1986).

Nikaido, T., Koike, K., Mitsunaga, K., and Saeki, T., Triterpenoid saponins from root of *Platycodon grandiflorum*. *Natural Medicines* **52**(1), 54-59 (1998).

Nikaido, T., Koike, K., Mitsunaga, K., and Saeki, T., Two new triterpenoid saponins from *Platycodon grandiflorum*. *Chem. Pharm. Bull.* **47**(6), 903-904 (1999).

Oka, M., Ota, N., Mino, Y., Iwashita, T., and Komura, H., Studies on the conformational aspects of inulin oligomers. *Chem. Pharm. Bull.* **40**(5), 1203-1207 (1992).

Perry, L., *Medicinal Plants of East and Southeast Asia.* MIT Press, Cambridge, MA, USA. (1980)

Pharmacopoeia Commission of PRC, *Pharmacopoeia of the People's Republic of China*, People's Medical Publishing House, Beijing (1988, 1995, 2000)

Piao, H.S., Kang, C.H., Kim, H.C., and Ahn, D.K., A study of standardization of *Platycodon grandiflorum* A.DC., Campanulaceae with platycodin D. *J. of Oriental Medicine* **3**(1), 125-130 (1998).

Saeki, T., Koike, K., and Nikaido, T., A comparative study on commercial, botanical gardens and wild samples of the roots of *Platycodon grandiflorum* by HPLC analysis. *Planta Med.* **65**(5), 428-431 (1999).

Saeki, T., and Nikaido, T., Evaluations of saponin properties of HPLC analysis of *Platycodon grandiflorum* A.DC. *Yakugaku Zasshi* **123**(6), 431-441 (2003).

Shin, C.Y., Lee, W.J., Lee, E.B., Choi, E.Y., and Ko, K.H., Platycodin D and D3 increase airway mucin release *in vivo* and *in vitro* in rats and hamsters. *Planta Med.* **68**, 221-225 (2002).

Shon, M.Y., Seo, J.K., Kim, H.J., and Sung, N.J., Antimutagenic effect of extract of *Platycodon grandiflorum*. *Korean J. Food Sci. Technol.* **33**(6), 651-655 (2001).

Tada, A., Kaneiwa, Y., Shoji, J., and Shibata, S., Studies on the saponins from roots of *Platycodon grandiflorum* A. De Candolle. I. Isolation and the structure of platycodin-D. *Chem. Pharm. Bull.* **23** (11), 2965-2972 (1975).

Takagi, K., and Lee, E.B., Pharmacological studies on *Platycodon grandiflorum* A. DC. I. Acute toxicity and central depressant activity of crude platycodin. *Yakugaku Zasshi* **92**(8), 951-960 (1972a).

Takagi, K., and Lee, E.B., Pharmacological studies on *Platycodon grandiflorum* A. DC. II. Anti-inflammatory activity of crude platycodin, its activities on isolated organs and other pharmacological activities. *Yakugaku Zasshi* **92**(8), 961-968 (1972b).

Takagi, K., and Lee, E.B., Pharmacological studies on *Platycodon grandiflorum* A. DC. III. Activities of crude platycodin on respiratory and circulatory systems and its other pharmacological activities. *Yakugaku Zasshi* **92**(8), 969-973 (1972c).

WHO, WHO monographs on selected medicinal plants: *Radix Platycodi*. Volume 1, WHO Geneva. p. 213-220 (1999).

Xu, B.J., Zheng, Y.N. Cui, M., and Song, F.R., Identification of platycodin-D by electrospray mass spectrum. *Chinese Journal of Medicinal Chemistry* **10**(3), 193-196 (2000).

Xu, B.J. Xu, C.L., Cui, M., Liu, M.X., and Zheng, Y.N., Identification on saponins composition of *Platycodon grandiflorum* applied mass spectral analyses method. *Journal of Jilin Agricultural University* **21**(1), 35-41 (1999).

Xu, C.L., Zheng, Y.N., Yang, L.H., Liu, M.X., and Xu, B.J., HPLC determination of platycodin D in different parts of *Platycodon grandiflorum* harvested at different time. *Journal of Jilin Agricultural University* **23**(1), 58-60, 64 (2001).

Xu, C.L., Yang, L.H., Zheng, Y.N., Liu, M.X., and Xu, B.J., determination of platycodin D of Radix Platycodi in different places by RP-HPLC. *Journal of Jilin Agricultural University* **21**(4), 35-38 (1999).

Yoon, Y. D., Han, S.B., Kang, J. S., Lee, C. W., Park, S.K., Lee, H.S., Kang, J.S., and Kim, H. M., Toll-like receptor 4-dependent activation of macrophages by polysaccharide isolated from the radix of *Platycodon grandiflorum*. *Int. Immunopharmacol.* **3**, 1873-1882 (2003).

Zhang, X.Y., A preliminary study on the expectorant action of Rheum plamatum and its mechanism of action. *Liangning Journal of Traditional Chinese Medicine* **11**(9), 44-46 (1987).

Zhou, R.H., Chinese medicine resource. Chinese medicine science and technology publishing House, Beijing, 486-489 (1994).

(Accepted June 28, 2004)