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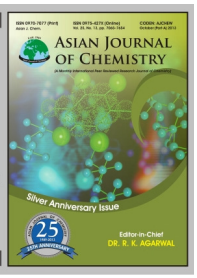

Quantitative Determination of Triterpenoidal Saponins in *Platycodi radix* and Its Variation in Different Regions of Korean Peninsula: A Herbal Plant Used as Traditional Medicine

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This survey was undertaken in different provinces in order to evaluate the contents of triterpenoidal saponins in *Platycodi radix* and its variation in different regions of Korea. HPLC and ESI-MS analysis were conducted for identification and quantification of ten know and four unknown compounds. Fourteen saponin compounds such as deapio-platycoside E, platycoside E, platycodin D3, platyconic acid A, platycodin D2, platycodin D, mixture of polygalacin D2 and 3'-O-acetyl-platycodin D, polygalacin D, 3'-O-acetyl-polygalacin D, 2'-O-acetyl-platycodin D and four unknown compounds widely ranged from 2,643 to 23,599 mg/kg dry weight (DW) in the 3-year-old roots and from 5,693 to 34,205 mg/kg DW in the 2-year-old roots, respectively. Since KFDA has not assigned any individual compounds for the quality control of *Platycodi radix*, we would suggest using *Platycodi radix* from the region of Jinan because the samples contain moderate total saponins contents.

Key Words: *Platycodi radix*, Platycodin D, Triterpenoidal saponins, HPLC-ELSD, LC/ESI-MS.

INTRODUCTION

Platycodi radix, the root of *Platycodon grandiflorum* A. DC (Companulaceae), is a perennial herb commonly known as balloon flower and widely spread in Northeast Asia including China, Japan and Korea. It has been used as a traditional oriental medicine for centuries due to several potent effects on the treatment of cough, cold, sore throats, tonsillitis, bronchitis and chest congestion[1]. The primary active constituents of *Platycodi radix* are well known as platycosides and triterpenoidal saponins. Approximately 20 types of platycosides have been reported from *Platycodi radix*[2]. The platycoside molecules are classified as bidesmosidic saponins with two sugar moieties; a glucose unit attached through an ether linkage at C-3 of a triterpene and others containing arabinose, rhamnose and xylose in sequence attached through an ester linkage between C-28 and arabinose. These bidesmosidic saponins were easily transformed into mono-desmosidic saponins, for example, glucosyl platycodigenin, by the alkaline hydrolysis of the esterified sugar at C-28[3]. Platycodin D and platycoside E were represented as the major platycosides of *Platycodi radix*, are potentially active against brain diseases and are cytotoxic to human tumour cells[4]. Platycodin A and C are used as a neuroprotective agent[5] whereas, platycodin D has antiobesity effects[6] and is a potent adjuvant of hepatitis B antigen[7]. Therefore, quality control of the active medicinal platycosides in *Platycodi radix* is necessary to achieve consistent medicinal activity. In South Korea, two kinds of commercial *Platycodi radix* are available from native, among that common *Platycodi radix* cultivated for 2 or 3 years; the other Jangseng (*P. grandiflorum*) is cultivated over 20 years by changing cultivation places in every 3 years. Up to now approximately 20 saponins from *Platycodi radix* have been separated by high performance liquid chromatography (HPLC) or HPLC-evaporative light scattering detector (ELSD) and identified by LC-electrospray ionization-mass spectrometry (ESI-MS) since 1972[8,9]. The chemical constituents of *Platycodi radix* are different based on the grown places and the length of cultivation

periods. However, there is still limited scientific information with individual saponins of *Platycodi radix* grown in different places and the length of cultivation periods in South Korea. In the present study, the saponin compositions of 30 *Platycodi radix* samples were collected and measured from 19 places of nationwide in Republic of Korea.

EXPERIMENTAL

HPLC-grade methanol used for extraction was purchased from Fisher Scientific (Pittsburg, PA). HPLC-grade mobile phase, acetonitrile (J.T. Baker, Phillipsburg, NJ) and water purified using a Milli-Q system (Millipore, Molsheim, France), was filtered through Millipore 0.45 µm membrane filters and degassed for 20 min by sonication before use. Ammonium acetate, formic acid, acetic acid, phosphoric acid, potassium phosphate dibasic and triethylamine were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ten pure saponins were isolated from the methanol extract of *Platycodi radix* following methods described in the literature[2]. The purity of the saponins was determined to be more than 95 % compared with the peak areas detected by HPLC-ELSD.

Two and three years old roots named as PR-1 and PR-2 (PR: *Platycodi radix*) were collected from 19 places of nationwide in Republic of Korea from middle to end of April, 2009. The roots were stored at less than 4 ºC in until the process of saponins extraction. They were washed with tap water, rinsed with distilled water and lyophilized.

Extraction of samples: Freeze-dried plant powder (1 g) was placed in a 50 mL centrifuge conical tube and extracted with 30 mL of HPLC-grade methanol. The mixture was vigorously vortexed for 3 min and allowed to stand for seven days at ambient temperature. The sample was monitored by vortexing for 1 min every day three times. The supernatant was separated by centrifugation at 1,000 g for 10 min and filtered through a No. 2 filter paper. The solvent is evaporated until dryness and re-dissolved in a minimum volume (2 mL) of H_2O. The solution was filtered through a 0.45 µm of syringe filter (PVDF, 25 mm, Millipore Co., Billerica, MA, USA) into a HPLC vial. All samples were analyzed with three replications.

HPLC-ELSD analysis: The filtrate was then analyzed by an HPLC NS-3000i system (Futecs Co., Ltd, Daejeon, Korea) equipped with a Model 100 ELSD (Softa Co., Westminster, CO, USA). *Platycodi radix* saponins were separated using a Chemcobond 5-ODS-H column (150 mm × 4.6 mm i.d., 3 µm particle size; Chemco Scientific, Osaka, Japan). The ELSD was set to a probe temperature of 100 ºC, a gain of seven and the nebulizer gas was nitrogen and adjusted to 36 psi (250 kPa). The column oven temperature of LC was set at 40 ºC. The injection sling was 20 µL. The solvent system was delivered at a rate of 1.0 mL/min and consisted of a mixture of (A) 30 mM acetic acid: 0.07 % formic acid:methanol (85:5:10, v/v/v) and (B) 25 mM acetic acid: 0.05 % formic acid:methanol (72:5:23, v/v/v). The initial mobile phase composition was 0 % solvent B, followed by a linear gradient from 0-15 % of solvent B in 5 min, to 38 % in 28 min, to 40 % in 33 min, to 43 % in 53 min, to 60 % in 63 min, to 100 % in 71 min and then holding at 100 % solvent B for an additional 24 min. The content of saponins was estimated by comparing the corresponding peak area of the chromatograms of the samples with that of the purified platycodin D in our laboratory.

HPLC/ESI-MS analysis for identification of saponins: The extracts were passed through a 0.45 µm filter and applied to an HPLC/ESI-MS system consisting of Agilent 1200 series HPLC (Agilent Technologies Inc., Palo Alto, CA, USA) and an ABI 3200 Q Trap (Applied Biosystems Inc, Foster City, CA, USA). The separation was carried out on an ODS-H column (100 mm × 2.1 mm i.d. 3.5 µm particle size; Shiseido, Tokyo, Japan) at a flow rate of 0.2 mL/min. The ion source was set to run in the negative ion mode (-3.5 kV) with N_2 sheath gas at a nebulizing pressure of 50 psi (344 kPa). The capillary temperature and voltage were set at 365 ºC and -15 V, respectively. The ion trap MS analysis was carried out with N_2 as the collision gas and the normalized collision energy was set to 30 %. The total ion chromatogram was obtained from 150-2,000 m/z. The LC solvent system was delivered at a rate of 1.0 mL/min and consisted of a mixture of (A) 30 mM acetic acid: 0.07 % formic acid: methanol (85:5:10, v/v/v) and (B) 25 mM acetic acid: 0.05 % formic acid: methanol (72:5:23, v/v/v).

RESULTS AND DISCUSSION

Identification of triterpenoidal saponins: A mixture of 14 platycosides was analyzed by gradient solution, containing different buffers with varying buffering condition, because pH of the mobile phase plays an important role in determining the chromatographic retention time of acidic or basic compounds[10]. The ESI-MS spectral data showed various triterpenoidal saponins quantified with ELSD; 14 saponins were separated (Fig. 1) and identified with 4 unknown compounds as deapio-platycoside E (peak No. 1 of HPLC-ELSD chromatogram), platycoside E (2), platycodin D3 (3), platyconic acid A (4), unknown-1 (5), platycodin D2 (6), platycodin D (7), unknown-2 (8), mixture of (polygalacin D2 and 3'-O-acetyl-platycodin D) (9), polygalacin D (10), 3'-O-acetyl-polygalacin D (11), unknown-3 (12), 2'-O-acetyl-platycodin D (13) and unknown-4 (14) (Table-1).

TABLE-1
SAPONINS SEPARATED FROM THE ROOT OF *Platycodi radix* COLLECTED IN SOUTH KOREA

No.	Compounds	m/z $[M+H]^+$
1	Deapio-platycoside E	1416
2	Platycoside E	1548
3	Platycodin D3	1386
4	Platyconic acid A	1238
5	Unknown-1	–
6	Platycodin D2	1386
7	Platycodin D	1224
8	Unknown-2	–
9	Mixture (polygalacin D 2+3'-O-acetyl-platycodin D)[g]	1370+1266
10	Polygalacin D	1208
11	3'-O-Acetyl-polygalacin D[g]	1266
12	Unknown-3	–
13	2'-O-Acetyl-platycodin D[g]	1266
14	Unknown-4	–

[g]Putative compound.

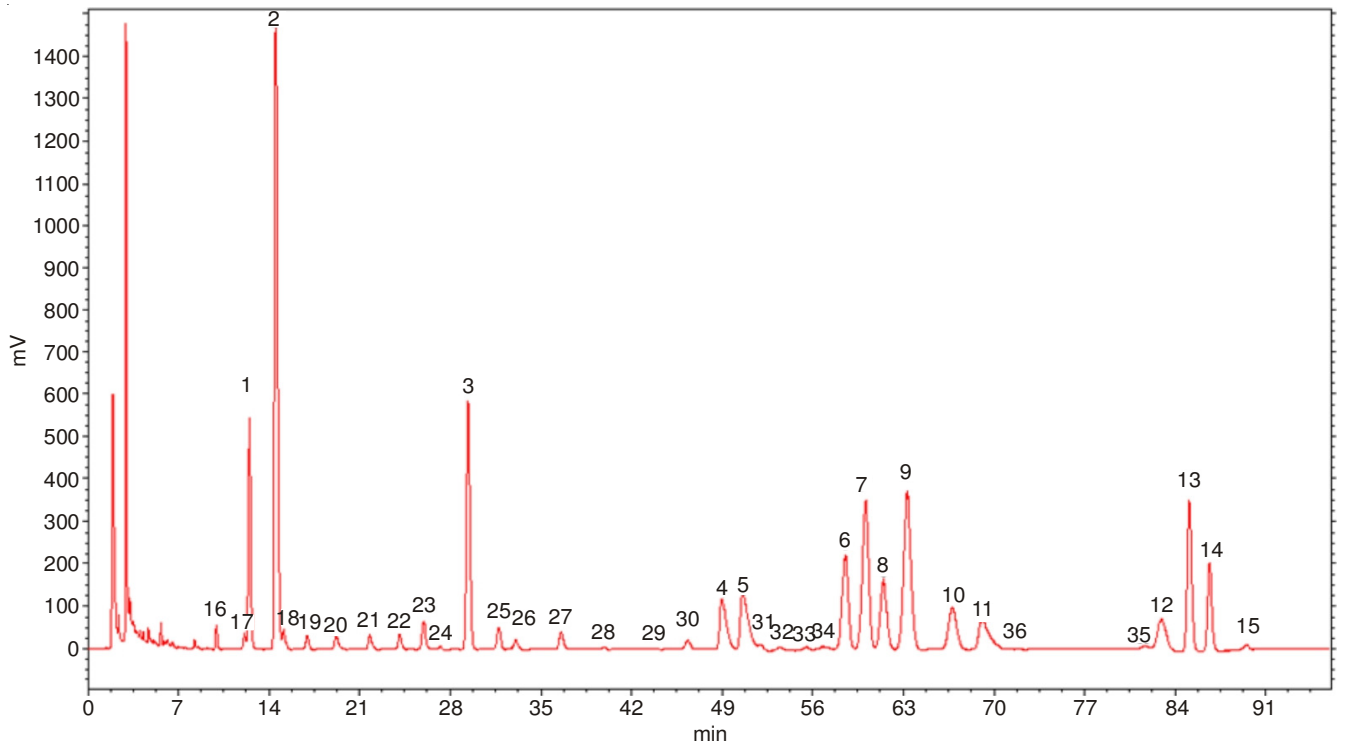

Fig. 1. HPLC profiles of saponins isolated from 2-year-old-root of *Platycodi radix*. 21. Peak numbers refer to the saponins listed in Table-1. Peak No. 1. Deapio-platycoside E; 2. Platycoside E; 3. Platycodin D3; 4. Platyconic acid A; 5. Unknown-1; 6. Platycodin D2; 7. Platycodin D; 8. Unknown-2; 9. Mixture (polygalacin D2+3'-O-acetyl-platycodin D); 10. Polygalacin D; 11. 3'-O-Acetyl-polygalacin D; 12. Unknown-3; 13. 2'-O-Acetyl-platycodin D; 14. Unknown-4

Variation of triterpenoidal saponin contents: *Platycodi radix* was collected from different regions of Korea, so it was expected that the platycoside contents in the samples would be varied with different cultivation areas. Samples collected from 19 different places were analyzed and the components were separated and identified. The triterpenoidal saponin contents ranged from 2,643 to 23,599 mg/kg dry weight (DW) in the 3-year-old roots and from 5,693 to 34,205 mg/kg DW in the 2-year-old roots, respectively (Tables 2 and 3). On the average, the content (16,613) in the 2 year-old roots was higher 52 % than that (10,914 mg/kg DW) of the 3-year-old roots.

platycodin D were notice in all the samples. Platycoside E and mixture of polygalacin D2 and 3'-O-acetyl-platycodin D found as the highest compounds in both 2- and 3-year-old roots. Platyconic acid A, platycodin D2 and platycodin D were only detected in 2-5 places and found less than 140 mg/kg DW. Platycodin D, a main compound of biological activity, was found very little amounts as 128 (1.17 % of the total) and 138 (0.83 %) mg/kg DW in 2- and 3-year-old roots, respectively and only detected limited collecting places. Moreover,

TABLE-2
SAPONIN CONTENTS IN THE 3-YEAR-OLD-ROOT OF *Platycodi radix* COLLECTED FROM THE 15 LOCAL CULTIVATION AREAS IN SOUTH KOREA (mg/kg DW)

No.	Geumsan	Geumsan-	Gimhae	Non san	Muan	Muan-Wild[c]	Muan-White[d]	Muju	Boeun	Sachon (n = 2)	Yeosu (n = 2)	Icheon-Chinese[b]	Jeju- Blue[e]	Jeju-White[d]	Hoengsung	Average
1	1061	619	558	367	1138	744	581	480	181	823	1592	587	763	731	178	693
2	3270	4185	2446	1743	4969	3477	4174	1700	1222	6752	5456	3421	5194	4368	1319	3580
3	310	553	363	108	436	572	607	145	48	408	1093	221	718	530	176	419
4	N.D.[f]	19	3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	158	N.D.	N.D.	60
5	966	1646	847	883	1566	1830	1409	926	306	947	1913	998	2215	1692	755	1260
6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2	141	N.D.	N.D.	71
7	N.D.	30	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	15	24	525	45	N.D.	128
8	533	1287	488	413	964	1096	1165	477	134	958	1175	751	1509	684	122	784
9	769	2029	736	748	1610	1421	1358	840	293	1418	1980	2171	4927	1950	942	1546
10	298	549	437	333	620	525	737	171	56	579	780	415	1146	679	102	495
11	458	991	521	481	1073	1063	875	499	105	583	1070	639	1559	1113	420	763
12	40	428	68	N.D.	271	317	380	38	N.D.	294	384	168	500	99	N.D.	249
13	502	1821	537	559	1179	1022	924	635	255	1022	1278	1486	3348	1324	741	1109
14	155	416	320	286	451	421	468	187	44	479	611	276	895	595	200	387
Total	8361	14572	7323	5920	14278	12487	12678	6098	2643	14264	11565	11160	23599	13809	4954	10914

[a]Retention time, [b]Chinese seeds, [c]Wild type, [d]White flowers, [e]Blue flowers, [f]Not detected, [g]Putative compound.

TABLE-3
SAPONIN CONTENTS IN THE 2-YEAR-OLD-ROOT OF *Platycodi radix* COLLECTED FROM THE 15 LOCAL CULTIVATION AREAS IN SOUTH KOREA (mg/kg DW)

No.	Muan	Muan-White[b]	Sancheong	Andong	Yeosu	Yeoju-Chinese[c]	Yeongju Chinese[c]	Yeongju-Medicine[d]-Chinese[c]	Icheon-Chinese[c]	Icheon-Medicine[d]-Chinese[c]	Iksan	Jecheon	Jinan	Hongcheon	Hongcheon-Medicine[d]	Average
1	1418	939	1494	1337	1024	921	825	425	443	205	639	587	1377	693	192	834
2	5161	5648	5437	5018	8360	4492	6135	3167	3033	1821	4422	2770	6083	3093	1943	4439
3	646	610	1184	341	2654	1753	1170	310	329	79	914	206	708	242	242	759
4	N.D.[f]	N.D.	N.D.	N.D.	57	N.D.	105	N.D.	N.D.	N.D.	12	N.D.	N.D.	N.D.	N.D.	58
5	2130	2187	2937	1148	4034	3436	3119	687	1500	683	1557	1121	1808	647	1219	1881
6	N.D.	N.D.	N.D.	N.D.	113	N.D.	132	N.D.	17	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	87
7	N.D.	N.D.	N.D.	N.D.	126	142	335	14	128	N.D.	14	N.D.	205	N.D.	N.D.	138
8	1291	1769	1593	592	3715	2219	2854	747	1136	257	910	445	835	121	58	1236
9	1819	2174	3223	1273	4318	4761	5714	1317	2779	719	1855	1134	4379	976	1521	2531
10	992	1003	1441	364	1125	1165	1247	396	569	119	552	380	991	102	171	708
11	1443	1398	2094	634	2627	2529	2102	732	981	359	926	688	1385	341	747	1266
12	395	692	563	29	1776	897	1190	154	372	N.D.	257	N.D.	1637	N.D.	N.D.	724
13	1236	1618	2307	813	3311	3699	3981	883	2002	750	1268	769	1649	666	1159	1741
14	561	719	1062	289	965	968	877	403	521	701	491	401	498	232	235	595
Total	17091	18758	23336	11840	34205	26982	29786	9234	13809	5693	13816	8501	21553	7112	7486	16613

[a]Retention time, [b]White flowers, [c]Chinese seeds, [d]Medicine type, [e]Medicine type, [f]Not detected, [g]Putative compound.

it was not found in the 1- and 4-year-old roots (data not shown).

Amongst the average platycoside values from the different cultivation areas platycoside E showed the highest content (3,580 mg/kg DW), followed by mixture of polygalacin D2 and 3'-O-acetyl-platycodin D (1,546 mg/kg DW) and three unknown-1 compound (1,260 mg/kg DW) 3-year-old roots. Two-year-old roots obtained from Yeosu region showed the highest total saponin contents (34,205 mg/kg DW). Deapio-platycoside E identified as the major saponin in both 2- and 3-years-old roots contributed 16-47 % of total saponins even though in some region (Yeoju-Chinese seeds) exhibited lower content (4,492 mg/kg DW). Han *et al.*[11] reported that the mixture of crude saponins obtained from *Platycodi radix* significantly decrease body weight and growth of adipose tissues by inhibiting the activity of pancreatic lipase, thereby fat absorption in the small intestine has been limited. Kwon *et al.*[12] claimed that the crude saponins decreased body weight and fat in mild type 2 diabetic rats. All the unknown compounds distributed comparatively similar in all region sample and shared average 24 % in total saponins, whereas sample from Iksan region showed significant amount of unknown compound and contributed 35 % of the total contents. It was suspected that this mixture of unknown compounds may have pharmacological effect since Han *et al.*[11] reported that the mixture of crude saponins significantly decrease body weight and growth of adipose tissues by inhibiting the activity of pancreatic lipase, thereby fat absorption in the small intestine has been limited. Kwon *et al.*[12] claimed that the crude saponins decreased body weight and fat in mild type 2 diabetic rats.

The relative content of the individual platycosides between the cultivated areas was widely varied, thereby indicating that the assessment of saponins content of *Platycodi radix* would be effective. KFDA has not assigned any individual compounds for the quality control of balloon flowers or *Platycodi radix*[4]. Considerably, we would like to suggest using *Platycodi radix* from the region of Jinan since the samples contains moderate total saponins contents. In our study the amount of deapio-platycoside E, platycosides E, mixture of polygalacin D2 and 3'-O-acetyl-platycodin D and polygalacin D2 were dominant in all the geographical places. The results showed that if one compound was high in content, the other compounds in same region exhibited very low in amounts, so that the sum of the four metabolites remained approximately constant. The average of deapio-platycoside E, platycosides E, mixture of polygalacin D2 and 3'-O-acetyl-platycodin D and polygalacin D2 for the fifteen cultivated regions were 693, 3,580, 1,546 and 763 mg/kg DW respectively in 3-year-old roots, whereas in two years root the average content were 883, 4,439, 2,531 and 1,266 mg/kg DW.

Conclusion

HPLC and ESI-MS analysis guided identification and quantification of 10 known and 4 unknown saponins from the roots of *Platycodi radix* collected from different cultivated in different the Korean peninsula. Known compounds such as deapio-platycoside E, platycoside E, platycodin D3, platyconic acid A, platycodin D2, platycodin D, mixture of polygalacin D2 and 3'-O-acetyl-platycodin D, polygalacin D, 3'-O-acetyl-polygalacin D and 2'-O-acetyl-platycodin D were observed in majority of the Platycodi radix roots. The triterpenoidal saponin contents ranged from 2,643 to 23,599 mg/kg DW in the 3-year-old roots and from 5,693 to 34,205 mg/kg DW in the 2-year-old roots, respectively. Platycoside E and the mixture of polygalacin D2 and 3'-O-acetyl-platycodin D were identified as the highest compounds in the 2 and 3-year-old roots. Individual platycosides between the cultivated areas were widely distinguished. However, Yeosu region exhibited high total saponins.

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