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Nontargeted Metabolomic Analysis of Four Different Parts of *Platycodon grandiflorum* Grown in Northeast China

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Abstract: Platycodonis radix is extensively used for treating cough, excessive phlegm, sore throat, bronchitis and asthma in the clinic. Meanwhile, the stems, leaves and seeds of *Platycodon grandiflorum* (PG) have some pharmaceutical activities such as anti-inflammation and anti-oxidation effects, etc. These effects must be caused by the different metabolites in various parts of herb. In order to profile the different parts of PG, the ultra-high performance liquid chromatography combined with quadrupole time-of- flight mass spectrometry (UPLC-QTOF-MS^E) coupled with UNIFI platform and multivariate statistical analyses was used in this study. Consequently, for the constituent screening, 73, 42, 35, 44 compounds were characterized from the root, stem, leaf and seed, respectively. The stem, leaf and seed contain more flavonoids but few saponins that can be easily discriminated in the root. For the metabolomic analysis, 15, 5, 7, 11 robust biomarkers enabling the differentiation among root, stem, leaf and seed, were discovered. These biomarkers can be used for rapid identification of four different parts of PG grown in northeast China.

Keywords: *Platycodon grandiflorum*; nontargeted metabolomic analysis; different part; UPLC-QTOF-MS^E

1. Introduction

It is well-known that there are both chemical and pharmacological differences in different parts of herbs. Taking *Aristolochia mollissima* Hance as an example, the fruits are used to treat cough and asthma, the roots have obvious antihypertensive effects, while the stems and leaves are rheumatoid medicines. This phenomenon also exists in other herbs, such as *Lycium barbarum, Polygonum Multiflorum* Thunb., *Trichosanthes kirilowii* Maxim, *Ephedra sinice* Stapf, etc. [1].

As both food and medicine, *Platycodon grandiflorum* (Jacq.) A. DC. (PG) is known as "Jiegeng" in China, "Huridunzhaga" in Mongolia, "Kikyo" in Japan and "Doraji" in North Korea [2]. In clinical, the root of PG which has various biological activities, such as apophlegmatic and antitussive [3], anti-inflammation [4], immunoregulation [5], anti-oxidant [6], etc., has been widely used for the treatment of cough, excessive phlegm, and sore throat. In addition, the stem and leaf of PG also have anti-inflammatory [7] and anti-oxidant [8,9] activities, while research on the pharmacological effects of PG seed is currently non-existent.

PG is a rich source of different natural products with various structural patterns. Around 100 compounds have been isolated from the roots of PG, including steroidal saponins, flavonoids, phenolic acids, polyacetylenes, sterols, etc. [2]. Triterpenoid saponins, mainly of the oleanane family

pentacyclic type, are the active components of the root of PG [10]. Several flavonoids and phenolic acids were isolated from the aerial parts of PG [11]. Two glycosides and four flavonoids were isolated from the seeds of PG [12]. Recently, instead of traditional separation and identification method, a combination of ultra-high performance liquid chromatography (UHPLC) separation, quadrupole time-of-flight tandem mass spectrometry (QTOF-MS/MS) detection and automated data processing software UNIFI with scientific library was innovatively used for screening and identifying chemical components in herbal medicines [13,14] and traditional Chinese medicine formulas [15]. In 2015, Lee et al. reported the global profiling of various metabolites in PG by UPLC-QTOF/MS [16]. In that paper, a total of 20 metabolites were characterized from the roots, and 56 compounds from stems and leaves of PG grown in Korea. Herbs collected from different regions will show certain differences both in chemical constituents and in pharmacological activities [17]. For example, saponins in the root of PG from different sites in Gyeongnam Province, Korea showed different contents [18]. The ^{1}H-NMR-based metabolomics with OPLS-DA statistical models was used to cluster the ginseng samples from Korea and China, and the result suggested that the chemical profiles from two countries are quite different due to their different geographical origins [19]. Hence, in order to illustrate different chemical constituents from the different regions and from the different parts of the plants, and to better clarify the pharmacological fundamental substances of PG, the root, stem, leaf and seed of PG produced in Jilin Province, China were taken as samples in this paper.

Metabolomics, including targeted and untargeted complementary approaches, is primarily concerned with identification and quantitation of small-molecule metabolites (<1500 Da) [20]. Recently, because of its ability to profile diverse classes of metabolites, untargeted metabolomics has been widely used to compare the overall metabolic composition of different samples [21]. An untargeted analysis approach is mainly applied in metabolite identification through mass-based search followed by manual verification [20] Being a sensitive, efficient, reliable, accurate and nondestructive method, UPLC-QTOF-MS has been widely used recently in this kind of analysis, such as exploring the early detection of mycotoxins in wheat [22], estimating compliance to a dietary pattern [23], exploring the bioavailability of the secoiridoids from a seed/fruit extract in human healthy volunteers [24], evaluating the enantioselective metabolic perturbations in MCF-7 cells after treatment with *R*-metalaxyl and *S*-metalaxyl [25].

In this study we focus on both the quickly chemical components' screening and the non-targeted metabolomic analysis of the root, stem, leaf and seed of PG. UPLC-QTOF-MS^{E}, UNIFI platform and multivariate statistical analyses, such as principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were used to profile the four different plant parts and to find the biomarkers among these four parts of PG grown in northeast China.

2. Results

2.1. Identification of Components from Different Parts of PG

As a result, a total of 159 compounds were identified or tentatively characterized in both positive and negative mode from the four parts of PG, the base peak intensity (BPI) chromatograms are shown in Figure 1, and their chemical structures are shown in Figure 2. More specifically, 73, 42, 35, 44 compounds were characterized from the root, stem, leaf and seed respectively (Table 1), including triterpenoid saponins, organic acids, steroids, phenols, flavonoids, alcohols, amino acids, coumarins, terpenoids, alkaloids and amides and so on.

For the compounds which have isomers, they may be distinguished by their characteristic MS fragmentation patterns reported in literature, or may be compared with the retention times of reference standards. Taking compounds 98 and 106 as example, both have the same protonated ion $[M + H]^{+}$ at m/z 1413.6530 and 1413.6530. In the results, they matched 3″-*O*-acetylpolygalacin D2 and 2″-*O*-acetylpolygalacin D2, respectively.

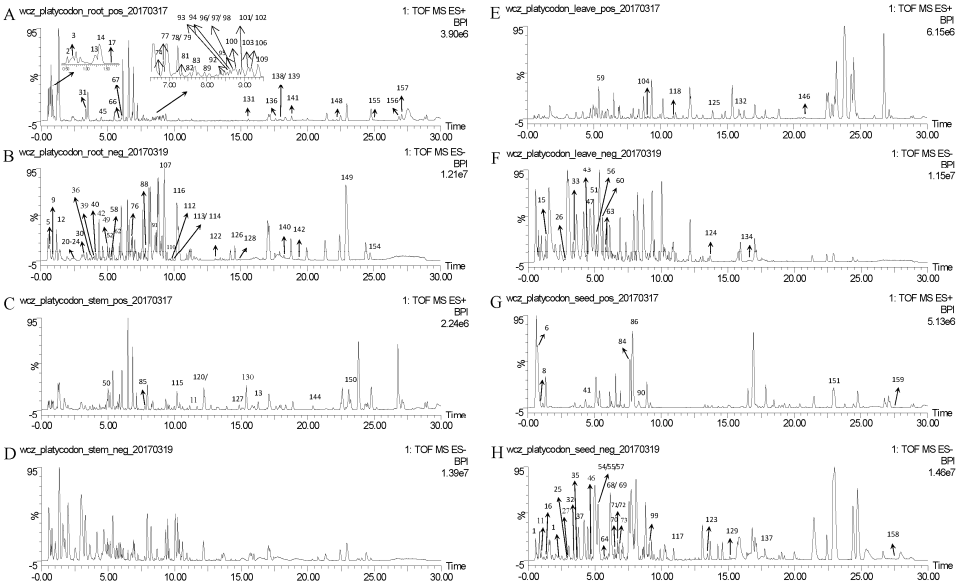

Figure 1. The representative base peak intensity (BPI) chromatograms of root in positive (**A**) and negative (**B**) modes; of stem in positive (**C**) and negative (**D**) modes; of leaf in positive (**E**) and negative (**F**) modes; of seed in positive (**G**) and negative (**H**) modes.

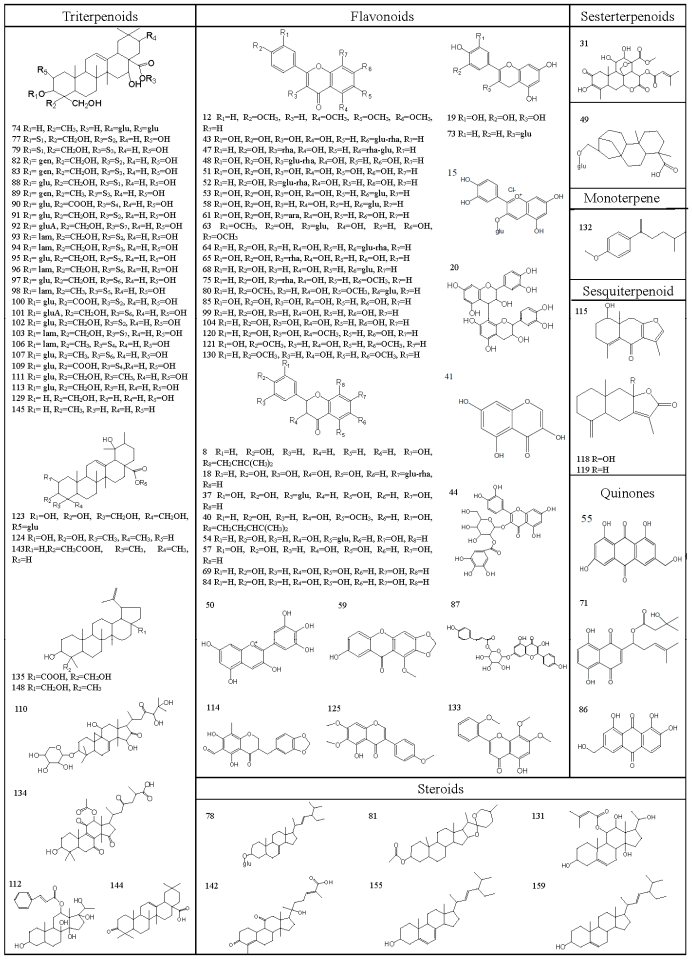

Figure 2. *Cont.*

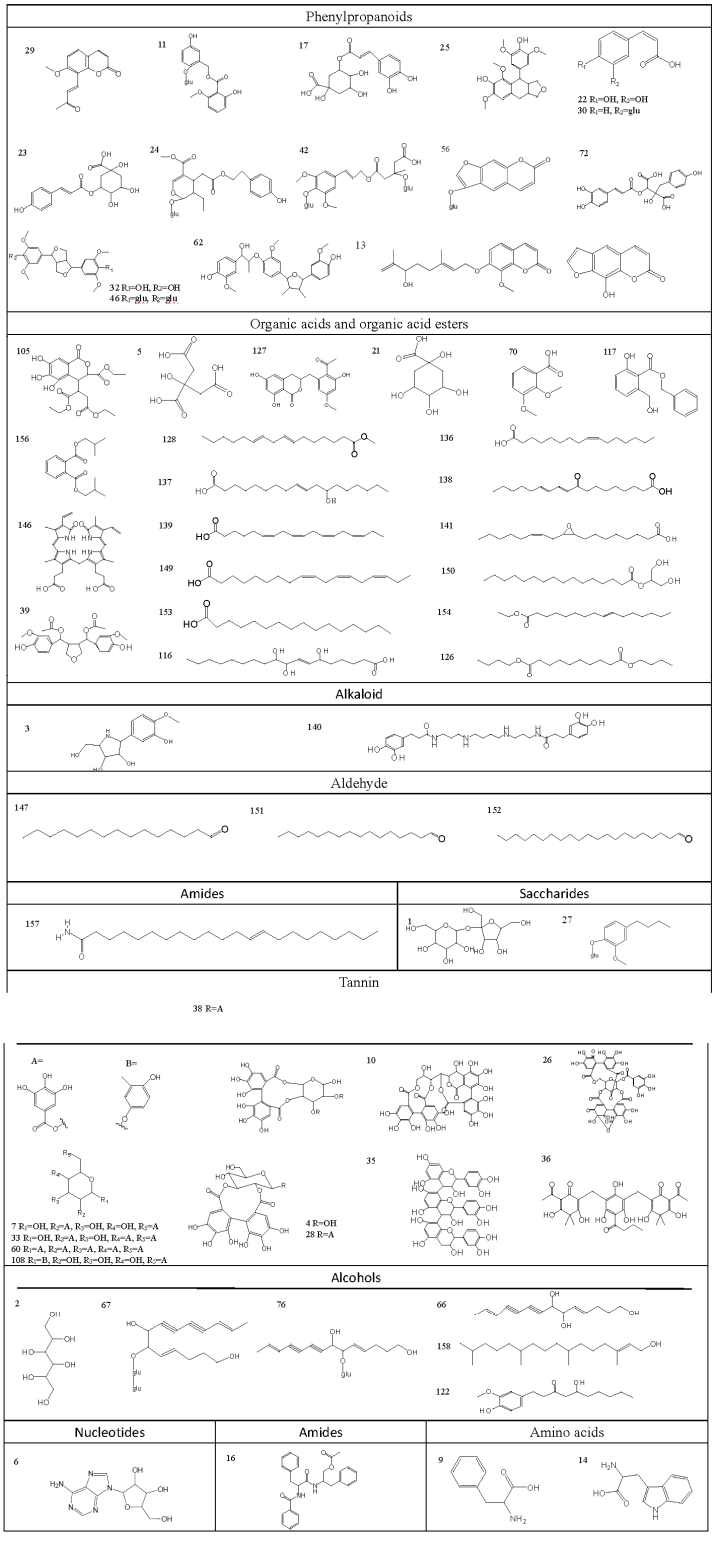

Figure 2. Chemical structures of compounds identified in PG.

Table 1. Compounds identified from different parts of PG by UPLC-QTOF-MS^E.

No.	t_R (min)	Formula	Experimental (Da)	Theoretical (Da)	Mass Error (ppm)	Adducts	MS^E Fragmentation	Component Name	Source
1 *	0.59	$C_{12}H_{22}O_{11}$	342.1169	342.1162	2.04	−H	323.0984, 195.0510, **161.0465**	Sucrose	D
2 *	0.60	$C_6H_{14}O_6$	182.0797	182.0790	3.04	+Na	205.0689, **152.0713**	Mannitol	R
3	0.67	$C_{12}H_{17}NO_5$	255.1114	255.1107	2.91	+H	256.1114, **226.1074**, 122.0375	Radicamine A	R
4	0.68	$C_{20}H_{18}O_{14}$	482.0682	482.0697	−2.95	−H	**343.0676**, 301.0007, 274.0119, 191.0554, 152.0124	2,3-(*S*)-Hexahydroxydiphenoyl-D-glucose [a]	S, L
5 *	0.71	$C_6H_8O_7$	192.0278	192.0270	3.93	−H	191.0205, **173.0077**, 111.0089	Citric acid	R
6 *	0.75	$C_{10}H_{13}N_5O_4$	267.0974	267.0968	2.23	+H	**218.1020**, 136.0634	Adenosine	D
7	0.82	$C_{20}H_{20}O_{14}$	484.0857	484.0853	0.78	−H	313.0568, 183.0308, **169.0156**, 152.0123	2,6-Di-*O*-Galloyl-β-D-glucose [a]	S, L
8	0.85	$C_{20}H_{20}O_4$	324.1347	324.1362	−4.38	+H	**203.0708**, 175.0758, 164.0463, 149.0602, 103.0556	Isobavachin [a]	D
9 *	0.86	$C_9H_{11}NO_2$	165.0796	165.0790	3.98	−H	164.0724, 147.0456, 103.0549	Phenylalanine	R
10	0.95	$C_{34}H_{24}O_{22}$	784.0751	784.0759	−1.05	−H	421.0417, **337.0214**, 249.0416, 182.0223, 168.0074, 149.9967	Casuariin [a]	S
11	0.97	$C_{21}H_{24}O_{11}$	452.1341	452.1319	4.86	−H	**299.0771**, 289.0737, 271.0611, 165.0206, 137.0257	Curculigoside B [a]	D
12	1.02	$C_{19}H_{18}O_6$	342.1089	342.1103	−4.14	−H	211.0628, 181.0506, **179.0349**, 161.0240, 151.0404	5,6,7,4′-Tetramethoxyflavone [a]	R
13	1.24	$C_{20}H_{24}O_5$	344.1609	344.1624	−3.98	+Na	222.0916, **194.0973**, 182.0611, 127.0394	Schininallylol [a]	R
14 *	1.35	$C_{11}H_{12}N_2O_2$	204.0903	204.0899	2.29	+H	**188.0706**, 144.0808, 132.0813, 118.0661	Tryptophan	R
15	1.36	$C_{21}H_{21}ClO_{11}$	484.0775	484.0772	0.45	−H	309.0630, 287.0594, 124.0163, **109.0291**	Cyanidin 3-glucoside [a]	L
16	1.37	$C_{27}H_{28}N_2O_4$	444.2034	444.2049	−3.41	−H	235.1215, **175.0626**, 173.0464, 131.0364, 105.0356	Aurantiamide acetate [a]	D
17 *	1.73	$C_{16}H_{18}O_9$	354.0950	354.0951	−0.22	+H	192.0663, **163.0396**, 145.0294, 135.0452	Chlorogenic acid	R
18	2.15	$C_{27}H_{32}O_{16}$	612.1712	612.1690	3.51	−H	593.1511, 461.1313, **303.0532**, 285.0428, 177.0209, 151.0052	(2*R*,3*R*)-Taxifolin7-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside	D
19	2.30	$C_{30}H_{26}O_{12}$	578.1430	578.1424	0.98	−H	449.0876, 425.0875, 407.0777, **289.0718**, 125.0257	Procyanidin B_1 [a]	D
20 *	2.31	$C_{15}H_{14}O_7$	306.0738	306.0740	−0.53	+HCOO	**179.0349**, 167.0343, 163.0406, 161.0241, 109.0315	Gallocatechin	R
21 *	2.34	$C_7H_{12}O_6$	192.0637	192.0634	1.53	−H	173.0480, **127.0406**, 116.0514, 111.0456	Quinine acid	R
22 *	2.35	$C_9H_8O_4$	180.0425	180.0423	1.60	−H	161.0241, 135.0451, **133.0297**, 109.0315, 108.0224	Caffeic acid	R
23	2.36	$C_{16}H_{18}O_8$	338.0993	338.1002	−2.62	−H	**191.0567**, 177.0195, 161.0243, 119.0505, 105.0351	3-*O*-*trans*-Coumaroylquinic acid	R
24	2.70	$C_{25}H_{34}O_{12}$	526.2045	526.2050	−1.04	−H	**363.1452**, 315.1244, 179.0713, 167.0711, 149.0612	LucidumosideA [a]	R
25	2.78	$C_{22}H_{26}O_7$	402.1670	402.1679	−1.88	+HCOO	327.0884, **303.0885**, 297.0421, 209.0844, 137.0256	Neociwujiaphenol [a]	D
26	2.81	$C_{41}H_{28}O_{27}$	952.0809	952.0818	−0.97	−H	605.0777, 479.0469, **481.0642**, 453.0677, 246.0169	Geraniin [a]	L
27	2.98	$C_{17}H_{26}O_7$	342.1678	342.1679	−0.01	+HCOO	281.0651, **163.1130**, 121.0300	Citrusin C	D
28	2.99	$C_{27}H_{22}O_{18}$	634.0813	634.0806	1.16	−H	601.0460, **463.0518**, 419.0617, 301.0007, 291.0156, 275.0208	Sanguiin H-4 [a]	S
29	3.05	$C_{14}H_{12}O_4$	244.0745	244.0736	3.14	+HCOO	203.0721, **187.0402**, 161.0250, 123.0457, 109.0303	cis-Osthenone	D
30	3.24	$C_{15}H_{18}O_8$	326.1003	326.1002	0.33	+HCOO	**162.0552**, 129.0199, 121.0304	4-*O*-β-D-glucopyranosyl-*trans*-cinnamic acid [a]	R, D
31 *	3.28	$C_{26}H_{32}O_{11}$	520.1968	520.1945	4.46	+H	443.0984, 341.1392, **163.075**	Brusatol	R
32	3.42	$C_{22}H_{26}O_8$	418.1631	418.1628	0.72	−H	359.1465, 179.0726, 164.0477, 149.0251, **125.0254**	(+)-Syringaresinol	D
33	3.55	$C_{27}H_{24}O_{18}$	636.0969	636.0963	0.99	−H	483.0791, **465.0679**, 331.0667, 313.0578, 169.0163	2,4,6-Tri-*O*-galloyl-β-D-glucose [a]	S,L
34 *	3.65	$C_{11}H_6O_4$	202.0260	202.0266	−2.32	+HCOO	**163.0419**, 149.0244, 134.0373, 133.0304,	Xanthotoxol	S, L
35	3.67	$C_{45}H_{38}O_{18}$	866.2079	866.2058	2.37	−H	**575.1207**, 407.0781, 289.0730, 179.0356	Arecatannin A_1 [a]	D
36	3.76	$C_{32}H_{36}O_{12}$	612.2223	612.2207	2.59	−H	562.1866, 518.1583, **210.0880**, 135.0462	Filixic acid ABA [a]	R
37	3.78	$C_{21}H_{22}O_{12}$	466.1128	466.1111	3.59	−H	**285.0428**, 177.0208, 165.0568, 151.0053, 137.0257, 124.0178	Taxifolin-3-*O*-glucoside [a]	D
38	3.80	$C_{34}H_{26}O_{22}$	786.0915	786.0916	−0.08	−H	615.0646, 597.0511, 445.0416, **301.0021**, 125.0258	Collinin [a]	S
39	3.82	$C_{24}H_{28}O_9$	460.1739	460.1733	1.24	−H	414.1699, 389.1244, 193.0528, **137.0261**, 125.0258	Sanjidin A [a]	R
40	4.30	$C_{22}H_{24}O_6$	384.1560	384.1573	−2.96	+HCOO	**325.1065**, 313.1078, 310.0838, 150.0322	Sophoflavescenol [a]	R
41	4.33	$C_9H_6O_5$	194.0211	194.0215	−2.35	+H	177.0183, **153.0178**, 138.0309, 127.0398	3,5,7-Trihydroxychromone	D
42	4.38	$C_{29}H_{42}O_{18}$	678.2395	678.2371	3.54	−H	**497.1692**, 453.1789, 323.0997, 291.1258, 161.0471	TangshenosideI	R
43	4.48	$C_{27}H_{30}O_{16}$	610.1554	610.1534	3.24	−H	**463.0844**, 313.0580, 265.0370, 190.9983, 151.0043	Quercetin-7-*O*-rutinoside	L
44	4.50	$C_{28}H_{24}O_{16}$	616.1084	616.1064	3.27	−H	313.0580, 190.9983, 177.0206, **169.0158**, 151.0043	2′′-*O*-Galloylhyperoside [a]	S, L
45	4.53	$C_{11}H_{12}O_3$	192.0791	192.0786	2.21	+H	193.0863, 167.0703, **161.0603**	Myristicin	R

Table 1. *Cont.*

No.	t_R (min)	Formula	Experimental (Da)	Theoretical (Da)	Mass Error (ppm)	Adducts	MS^E Fragmentation	Component Name	Source
46	4.66	$C_{34}H_{46}O_{18}$	742.2707	742.2684	2.90	+HCOO	579.2040, **417.1564**, 181.0520, 149.0248	Syringaresinol-di-*O*-β-D-glucoside [a]	D
47	4.72	$C_{33}H_{40}O_{19}$	740.2178	740.2164	1.92	−H	593.1506, 575.1401, 429.0824, 335.0414, **284.0336**	Grosvenorine [a]	S, L
48 *	4.93	$C_{27}H_{30}O_{16}$	610.1550	610.1534	2.59	−H	401.0912, **301.0365**, 299.0205, 247.0609	Rutin	S, L, D
49	4.94	$C_{26}H_{42}O_8$	482.2874	482.2880	−1.13	+HCOO	**261.1352**,179.1074,149.0608, 125.0589	17-*O*-β-D-Glucopyra-nosyl-16β-H-ent-kauran-19-oicacid [a]	R
50 *	4.96	$C_{15}H_{10}O_7$	302.0427	302.0427	0.15	+H	161.0264, **123.0099**, 109.0306, 107.0153	Delphinidin	S, L
51	4.97	$C_{15}H_{10}O_8$	318.0368	318.0376	−2.25	+HCOO	**300.0266**, 264.0562, 176.0132, 148.0176	Quercetagetin	L
52	5.12	$C_{27}H_{30}O_{15}$	594.1609	594.1585	4.02	−H	**285.0403**, 161.0459, 151.0038, 135.0452	Kaempferol-3-*O*-neohesperidoside	R
53	5.14	$C_{21}H_{20}O_{12}$	464.0945	464.0955	−2.16	−H	313.0549, **300.0266**, 284.0330, 151.0041	Quercimeritrin	S,L,D
54	5.17	$C_{21}H_{22}O_{11}$	450.1178	450.1162	3.61	−H	193.0156, 179.0574, 175.0051, **151.0052**, 135.0468	Dihydrokaempferol-5-*O*-β-D-glucopyranoside	D
55	5.24	$C_{15}H_{10}O_6$	286.0463	286.0477	−4.95	−H	256.0372, 177.0180, 164.0487, **150.0300**, 123.0439, 107.0134	ω-Hydroxyemodin [a]	D
56	5.25	$C_{17}H_{16}O_9$	364.0780	364.0794	−3.53	+HCOO	337.0566, 278.0432, **202.0248**, 185.0254, 149.0251	Bergaptol-*O*-β-D-glucopyranoside	L
57	5.26	$C_{15}H_{12}O_7$	304.0568	304.0583	−4.92	−H	285.0366, 243.0329, 152.0099, **150.0300**, 125.0238	Dihydroquercetin	D
58	5.27	$C_{21}H_{20}O_{11}$	448.1005	448.1006	−0.19	−H	**285.0406**, 283.0256, 179.0569	Luteolin-7-*O*-glucopyranoside	R,D
59	5.40	$C_{15}H_{10}O_6$	286.0479	286.0477	0.70	+H	**149.0216**, 139.0371, 123.0433, 111.0439	7-Hydroxy-1-methoxy-2-methoxyxanthone [a]	S, L
60	5.57	$C_{41}H_{32}O_{26}$	940.1163	940.1182	−1.96	−H	**769.0887**, 617.0782, 313.0565, 291.0150, 169.0158	1,2,3,4,6-Penta-*O*-galloyl-β-D-glucopyranoside [a]	S, L
61	5.72	$C_{20}H_{18}O_{11}$	434.0853	434.0849	0.80	−H	**300.0301**, 195.0321, 151.0050, 109.0305	Quercetin-3-*O*-arabinoside	S
62	5.76	$C_{30}H_{36}O_8$	524.2409	524.2410	−0.19	+HCOO	453.1908, 339.1256, **195.0667**, 165.0570	Saucerneol C [a]	R
63	5.79	$C_{23}H_{24}O_{13}$	508.1224	508.1217	1.36	−H	315.0519, **207.0291**, 193.0506, 151.0044, 137.0246	Limocitrin-3-*O*-β-D-glucopyranoside [a]	L
64	5.83	$C_{27}H_{30}O_{14}$	578.1637	578.1636	0.24	−H	**269.0475**, 227.0364, 177.0203, 151.0050, 119.0513	Apigenin-7-*O*-β-D-rutinoside	D
65	5.84	$C_{21}H_{20}O_{11}$	448.1016	448.1006	2.36	−H	295.0843, **284.0340**, 179.0362, 151.0411, 123.0102	Quercetin-3-*O*-α-L-rhamnoside	S
66	5.86	$C_{14}H_{18}O_3$	234.1243	234.1256	−4.58	+H	175.0746, **163.0746**, 133.0647, 119.0860, 111.0811	Lobetyol	R
67	6.02	$C_{26}H_{38}O_{13}$	558.2326	558.2312	2.37	+Na	**217.1197**, 199.1096, 145.0642, 128.0613, 115.0541	Lobetyolinin	R
68	6.12	$C_{21}H_{20}O_{10}$	432.1040	432.1056	−3.82	−H	**268.0367**, 227.0341, 177.0181, 151.0037, 124.0168	Cosmosiin	D
69	6.17	$C_{15}H_{12}O_6$	288.0643	288.0634	3.23	−H	271.0623, 177.0181, **151.0037**, 133.0297, 125.0254, 107.0143	Dihydrokaempferol	D
70	6.32	$C_9H_{10}O_4$	182.0584	182.0579	2.65	−H	166.0263, 151.0040, **135.0452**, 108.0226	2,6-Dimethoxy benzoic acid	D
71	6.59	$C_{21}H_{24}O_7$	388.1509	388.1522	−2.96	+HCOO	358.1066, 301.0369, 243.0306, **231.0308**, 151.0047	β-Hydroxyisovalerylshikonin [a]	D
72	6.61	$C_{20}H_{18}O_{10}$	418.0892	418.0900	−1.74	+HCOO	358.1066, 243.0306, **231.0308**, 178.9997, 151.0047, 121.0304	Cimicifugic acid D [a]	D
73	6.70	$C_{21}H_{24}O_{10}$	436.1373	436.1369	0.76	−H	**273.0781**, 255.0666, 179.0358, 149.0248, 123.0457	Epiafzelechin-3-*O*-β-D-allopyranoside [a]	D
74	6.75	$C_{42}H_{68}O_{16}$	828.4491	828.4507	−1.99	+H	667.4052, 651.4104, 505.3529, **487.3428**, 469.3321, 421.3113	Platycosaponin A	R
75	6.79	$C_{22}H_{22}O_{10}$	446.1231	446.1213	3.66	+HCOO	**285.0424**, 187.0053, 163.0414, 124.0179	Rhamnocitrin-3-*O*-rhamnoside [a]	S
76	6.81	$C_{20}H_{28}O_8$	396.1793	396.1784	2.03	+HCOO	**215.1094**, 185.0984, 159.0826, 143.0724, 125.0616	Lobetyolin	R
77 *	6.85	$C_{64}H_{104}O_{34}$	1416.6388	1416.6409	−1.49	+H	811.4487, **763.42581**, 647.37911, 485.3261	Deapio platycoside E	R
78	6.93	$C_{35}H_{58}O_6$	574.4227	574.4233	−1.03	+H	472.3166, **463.3096**, 378.2044, 302.1716	α-Spinasterol glucoside	R
79 *	6.98	$C_{69}H_{112}O_{38}$	1548.6799	1548.6832	−2.13	+H	1007.5104, 845.4571, **683.4034**, 521.3493, 485.3282	Platycoside E	R
80	6.99	$C_{23}H_{24}O_{11}$	476.1314	476.1319	−0.84	+HCOO	433.1097, **345.0819**, 313.0554, 183.0309, 151.0041	5-Hydroxy-6,4′-dimethoxy-flavone-7-*O*-β-D-gluco-pyranoside	S
81	7.35	$C_{29}H_{46}O_4$	458.3396	458.3396	−0.05	+H	**341.2455**, 217.1953, 149.1333, 121.1027	Neotigogenin acetate [a]	R
82	7.57	$C_{58}H_{94}O_{29}$	1254.5905	1254.5881	1.95	+H	931.4894, 845.4518, **799.4485**, 295.1007	Deapioplatycodin D_3	R
83 *	7.68	$C_{63}H_{102}O_{33}$	1386.6326	1386.6303	1.65	+H	1255.5937, 931.4894, 845.4518, **799.4484**, 665.3879, 441.1585	Platycodin D_3	R
84	7.69	$C_{15}H_{12}O_6$	288.0629	288.0634	−1.59	+H	255.0652, 179.0353, 163.0400, **153.0196**, 145.0295	3-Hydroxynaringenin [a]	D
85 *	7.77	$C_{15}H_{10}O_7$	302.0422	302.0427	−1.40	+H	243.0319, **151.0055,** 125.0260, 107.0157	Quercetin	S, L
86	7.86	$C_{15}H_{10}O_6$	286.0488	286.0477	3.61	+H	269.0460, 257.0450, **241.0490**, 161.0239, 135.0453	6-Hydroxyaloeemodin [a]	D
87	7.91	$C_{30}H_{26}O_{13}$	594.1373	594.1373	−0.14	−H	447.0966, 429.0832, **285.0440**, 145.0316, 119.0513	Buddlenoid A [a]	S, L
88	7.92	$C_{47}H_{76}O_{20}$	960.4934	960.4930	0.39	+HCOO	869.4537, **715.3371**, 529.2698, 295.2034	Platycoside F	R
89 *	7.94	$C_{63}H_{102}O_{32}$	1370.6373	1370.6354	1.40	+H	827.4398, 783.4476, 637.3944, 459.3430, 409.3090, **325.1130**	Platycoside G_3	R
90	8.33	$C_{57}H_{90}O_{29}$	1238.5577	1238.5568	0.71	+H	1107.5237, 957.4692, 895.4676, 811.4125, **697.3760**, 661.3582, 485.3245, 409.3094	Platyconic acid A	D

Table 1. *Cont.*

No.	t_R (min)	Formula	Experimental (Da)	Theoretical (Da)	Mass Error (ppm)	Adducts	MS^E Fragmentation	Component Name	Source
91 *	8.46	$C_{52}H_{84}O_{24}$	1092.5397	1092.5353	4.07	−H	959.4846, 941.4753, **681.3871**, 663.3768, 649.3607, 503.3364, 485.3366, 295.1038, 277.0942	Deapioplatycodin D	R
92	8.48	$C_{59}H_{92}O_{30}$	1280.5649	1280.5673	−1.90	+H	1017.4875, 999.4760, 931.4860, 829.4192, **697.3796**, 679.3651, 651.3761, 519.3316, 503.3334, 487.3377	Platycodin L	R
93 *	8.51	$C_{58}H_{94}O_{29}$	1254.5847	1254.5881	−2.65	+H	931.4894, 845.4518, **799.4485**, 483.3065, 457.1533, 427.1433, 325.1116, 295.1007	Deapioplatycodin D_2	R
94 *	8.62	$C_{63}H_{102}O_{33}$	1386.6300	1386.6303	−0.26	+H	977.4981, 845.4558, 829.4604, 683.4031, 667.4073, 653.3919, **521.3488**, 485.3273	Platycodin D_2	R
95 *	8.68	$C_{57}H_{92}O_{28}$	1224.5778	1224.5775	0.23	+H	799.4485, 683.3961, 667.4052, **521.3444**, 503.3364, 485.3257	Platycodin D	R, D
96 *	8.73	$C_{65}H_{104}O_{34}$	1428.6407	1428.6409	−0.15	+H	1297.6065, 1165.5621, 845.4520, 841.4580, 681.3837, 665.3903, **653.3884**, 617.3663, 519.3298, 485.3243	2′-O-Acetylplatycodin D_2	R, D
97	8.78	$C_{59}H_{94}O_{29}$	1266.5869	1266.5881	−0.93	+H	1003.5108, 841.4569, 823.4458, **683.3979**, 189.0749, 171.0641	Platycodin A	R, D
98	8.80	$C_{65}H_{104}O_{33}$	1412.6458	1412.6460	−0.16	+H	985.4990, 823.4461, 635.3794, 617.3695, **503.3369**, 453.1605, 321.1182, 303.1076, 189.5707	3′′-*O*-Acetylpolygalacin D_2	R
99	8.86	$C_{15}H_{10}O_5$	270.0539	270.0528	4.15	−H	**151.0043**, 123.0099, 117.0359, 107.0154	Apigenol	D
100	8.87	$C_{52}H_{82}O_{25}$	1106.5163	1106.5145	1.57	+H	975.4806, 931.4908, 829.4243, 811.4113, **697.3814**, 679.3695, 517.3151, 503.3373, 455.3161	Platyconic acid C	R
101	8.94	$C_{59}H_{92}O_{30}$	1280.5705	1280.5673	2.47	+H	1017.4875, **829.4192**, 697.3796, 637.3939, 519.3316, 321.1178	Platycodin K	R, D
102	9.04	$C_{54}H_{86}O_{25}$	1134.5444	1134.5458	−1.23	+H	1003.5108, 841.4569, 823.4458, **683.3979**, 321.1160, 189.0749	Platycoside B	R
103 *	9.10	$C_{65}H_{104}O_{34}$	1428.6370	1428.6409	−2.71	+H	1297.6065, 955.4894, 841.4580, 813.4279, 797.4332, 681.3837, 665.3903, **653.3884**, 635.3780	3′-*O*-acetyl-platycodin D_2	R
104	9.11	$C_{15}H_{10}O_6$	286.0483	286.0477	1.85	+H	231.0662, 229.0504, 195.0289, **153.0187**,	Kaempferol	L
105	9.14	$C_{20}H_{24}O_{11}$	440.1314	440.1319	−1.01	−H	393.0860, 303.0523, 257.0104, 231.0303, 177.0204	(-)-Chebulic acid triethyl ester [a]	S, L
106	9.18	$C_{65}H_{104}O_{33}$	1412.6430	1412.6460	−2.14	+H	823.4461, 503.3369, **485.3255**, 455.3156, 321.1182, 189.0757	2′′-*O*-acetylpolygalacin D_2	R, D
107	9.23	$C_{59}H_{94}O_{28}$	1250.5904	1250.5932	−2.23	−H	**1208.5857**, 1159.5571, 635.3812, 499.3046, 131.0337	2′-*O*-acetyl Polygalacin D	R
108	9.32	$C_{20}H_{22}O_{11}$	438.1170	438.1162	1.78	−H	**419.0956**, 235.0654, 163.0050	6′-*O*-Galloyl-homoarbutin [a]	S, L
109	9.37	$C_{54}H_{84}O_{26}$	1148.5293	1148.5251	3.63	+H	1017.4908, 999.4786, 535.3279, 631.3477, **517.3170**, 499.3050, 453.3001, 321.1190, 189.0764	Platyconic acid D	R
110	9.45	$C_{35}H_{54}O_{11}$	650.3666	650.3666	0.04	+HCOO	451.2830, 441.2997, 197.1183, **149.0465**, 131.0354	15α-Hydroxy-ximicifugoside H_2 [a]	R
111	9.59	$C_{37}H_{60}O_{12}$	696.4087	696.4085	0.28	−H	487.3424, 469.3302, 425.3438	3-*O*-D-glucopyranosyl platycodigenin methyl ester	S
112	9.80	$C_{30}H_{42}O_7$	514.2938	514.2931	1.41	−H	436.2610, 319.1910, **301.1814**, 265.1468	Marstenacigenin A	R
113	9.91	$C_{36}H_{58}O_{12}$	682.3893	682.3928	−4.81	+HCOO	**635.3797**, 449.3263, 407.2948, 179.0565	3-*O*-D-glucopyranosyl platycodigenin	R
114	9.94	$C_{19}H_{16}O_7$	356.0886	356.0896	−2.55	+HCOO	401.0868, 313.0718, **121.0297**	6-Formyl-isoophiopogonanone A [a]	R
115	10.17	$C_{15}H_{18}O_3$	246.1258	246.1256	0.84	+H	**229.1220**, 163.0756, 149.0598, 119.0865, 105.0713	Curcolone [a]	S, L
116	10.25	$C_{18}H_{34}O_5$	330.2418	330.2406	3.57	−H	**311.2224**, 293.2140, 211.1348, 185.1189, 129.0928	Sanleng acid [a]	R, S, D
117	10.91	$C_{15}H_{14}O_4$	258.0901	258.0892	3.45	−H	239.0705, 163.0397, **151.0421**, 133.0313, 121.0296	Benzyl-2-hydroxy-6-methoxybenzoate	D
118 *	10.95	$C_{15}H_{20}O_3$	248.1413	248.1412	0.27	+H	231.1379, 219.1381, **203.1425**, 119.0864, 107.0867	Atractylenolide III	L
119	11.13	$C_{15}H_{20}O_2$	232.1464	232.1463	0.24	+H	215.1424, 187.1486, **159.1172**, 135.1174, 107.0867	Atractylenolide II	S, L
120	12.19	$C_{16}H_{12}O_6$	300.0637	300.0634	1.18	+H	**285.0761**, 242.0571, 167.0340, 136.0162, 108.0215	5-Methyl kaempferol	S, L
121	12.26	$C_{17}H_{14}O_6$	314.0794	314.0790	1.05	+H	299.0552, **275.0673**, 257.0445, 161.0597, 139.0397	3′,5-Dihydroxy-7,4′-dimethoxy flavone	S
122	12.94	$C_{17}H_{26}O_4$	294.1833	294.1831	0.56	−H	235.1341, 141.0919, **129.0924**	6-Gingerol [a]	R
123	13.46	$C_{36}H_{58}O_{12}$	682.3905	682.3928	−3.36	−H	**635.3787**, 473.3258, 443.3119, 425.3020, 179.0553	Trachelosperoside B-1 [a]	D
124	13.68	$C_{30}H_{48}O_5$	488.3514	488.3502	2.47	−H	455.3548, 439.3599, **281.2503**, 293.2127, 171.1035	2α,19α-Dihydroxyursolic acid	L
125	13.91	$C_{18}H_{16}O_6$	328.0949	328.0947	0.72	+H	314.0777, **296.0677**, 184.0737, 136.0166	4′,7-Dimethyltectorigenin [a]	S, L

Table 1. *Cont.*

No.	t_R (min)	Formula	Experimental (Da)	Theoretical (Da)	Mass Error (ppm)	Adducts	MS^E Fragmentation	Component Name	Source
126 *	14.58	C18H34O4	314.2466	314.2457	2.86	−H	201.1140, 199.0980, 155.1082, 127.1135	Dibutyl sebacate	R
127	14.85	$C_{19}H_{18}O_7$	358.1051	358.1053	−0.47	+H	**343.0809**, 326.0778, 301.0705, 283.0599	3,4-Dihydro-6,8-dihydroxyl-3-(2′-acetyl-3′-hydroxyl-5′-methoxyphenyl)methyl-1*H*-[2] benzoplyran-1-one [a]	S, L
128	14.86	$C_{17}H_{30}O_2$	266.2258	266.2246	3.76	+HCOO	311.2240, **155.1083**, 139.1137	Methyl 7, 10-hexadecadienoate	R
129	15.36	$C_{30}H_{48}O_7$	520.3385	520.3400	−2.93	−H	476.2774, **473.3256**, 443.3168, 425.3093, 407.2940, 395.2941	Platycodigenin	D
130	15.39	$C_{17}H_{14}O_5$	298.0843	298.0841	0.51	+H	284.0679, **256.0730**, 241.0495, 167.0339, 133.0648	5-Hydroxy-7, 4′-dimethoxyflavanone	S, L
131	15.57	$C_{26}H_{40}O_6$	448.2818	448.2825	−1.59	+H	393.2636, **350.1875**, 242.1877	Tenasogenin [a]	R
132	15.89	$C_{14}H_{20}O$	204.1513	204.1514	−0.51	+H	163.1118, 159.1169, 149.0956, 119.0863, **107.0502**	2-(*p*-Anisyl)-5-methyl-1-hexen	L
133	16.28	$C_{18}H_{16}O_6$	328.0957	328.0947	2.95	+H	**314.0790**, 299.0550, 286.0830, 271.0604, 150.0314	5-Hydro-7, 8, 2′-trimethoxyflavanone	S, L
134	16.57	$C_{32}H_{44}O_9$	572.2965	572.2985	−3.51	−H	**481.2572**, 429.2997, 227.0350, 183.1043	Ganoderic acid H [a]	L
135	17.23	$C_{30}H_{48}O_4$	472.3550	472.3553	−0.49	−H	471.3448, 437.3061, 419.2937, 339.2705, 253.2187	2α-Hydroxybetulinic acid	S, L
136	17.62	$C_{16}H_{30}O_2$	254.2252	254.2246	2.21	+Na	207.1743, 165.1274, **143.1067**, 125.0961	Palmitoleic acid	R
137	17.78	$C_{18}H_{34}O_3$	298.2505	298.2508	−1.05	−H	217.1615, 195.1391, **183.1401**, 113.0984	Ricinoleic acid	D
138	18.00	$C_{18}H_{30}O_3$	294.2203	294.2195	2.51	+Na	**277.2177**, 165.1284, 151.1127, 109.1035	(*E*,*E*)-9-Oxooctadeca-10,12-dienoic acid [a]	R
139	18.01	$C_{18}H_{28}O_2$	276.2100	276.2089	3.85	+H	**179.1424**, 135.1180, 119.0862	Stearidonic acid	R
140	18.26	$C_{28}H_{42}N_4O_6$	530.3100	530.3104	−0.77	−H	529.3027, **511.2928**, 293.2163	Kukoamine A [a]	R
141	19.02	$C_{18}H_{32}O_3$	296.2358	296.2351	2.19	+Na	279.2312, 161.1323, **147.1165**, 133.1018, 121.1023	Coronaric acid	R
142	19.23	$C_{28}H_{40}O_5$	456.2878	456.2876	0.46	−H	409.2359, 343.1925, **339.2004**, 275.2022	Siraitic acid D [a]	R
143	20.35	$C_{32}H_{50}O_5$	514.3662	514.3658	0.81	−H	**495.3495**, 469.3702, 451.3596, 449.3449	19α-Hydroxy-3-acetyl-ursolic acid	S
144	20.39	$C_{30}H_{46}O_3$	454.3452	454.3447	1.03	+H	437.3422, **409.3470**, 247.1695, 203.1796, 189.1642	Oleanonic acid	S
145	20.77	$C_{30}H_{48}O_3$	456.3604	456.3603	0.13	−H	455.3531, 443.3528, **233.1561**	3-Epioleanolic acid	S
146	20.78	$C_{33}H_{36}N_4O_6$	584.2660	584.2635	4.08	+Na	**567.2589**, 535.2340, 501.2257, 467.20432, 417.1830	Bilirubin [a]	L
147	21.49	$C_{15}H_{30}O$	226.2309	226.2297	4.48	+HCOO	**271.2302**, **197.1911**, 195.1754	n-Pentadecanal	S
148	22.20	$C_{30}H_{50}O_2$	442.3803	442.3811	−1.76	+H	425.3776, **407.3666**, 217.1950, 203.1791, 189.1641	Betulin	R
149 *	22.93	$C_{18}H_{30}O_2$	280.2402	280.2400	−0.25	−H	**149.0972**	Linolenic acid	R
150 *	22.95	$C_{19}H_{38}O_4$	330.2774	330.2770	1.00	+Na	313.2738, 239.2368	1-Monopalmitin	S
151	22.98	$C_{16}H_{32}O$	240.2452	240.2453	−0.47	+Na	263.2344, 125.1317, **111.1175**	*n*-Hexadecanal	D
152	24.06	$C_{21}H_{42}O$	310.3240	310.3236	1.28	+HCOO	355.3214, **125.0972**	*n*-Henicosanal	S
153	24.40	$C_{16}H_{32}O_2$	256.2401	256.2402	−0.49	−H	**241.2176**, 237.226, 227.2019, 125.0976	Palmitic acid	S
154	24.74	$C_{18}H_{34}O_2$	282.2569	282.2559	3.70	−H	253.2185, 163.1132, **125.0982**, 111.0825	Ethyl palmitate	R
155	25.73	$C_{29}H_{46}O$	410.3565	410.3549	4.03	+H	**395.3680**, 203.1799, 145.1021, 133.1019	Δ7-stigmasterol	R
156	26.87	$C_{24}H_{38}O_4$	390.2771	390.2770	0.21	+H	301.1413, 189.0156, 165.0905, **149.0235**	Bis(2-ethylhexyl)phthalate	R
157	27.09	$C_{22}H_{43}NO$	337.3356	337.3345	3.47	+H	321.3149, **212.2014,** 198.1857, 153.1275	Erucic amide [a]	R
158	27.63	$C_{20}H_{40}O$	296.3093	296.3079	4.10	+HCOO	251.2393, **179.1459**, 113.0987	Phytol	S
159 *	28.49	$C_{29}H_{48}O$	412.3695	412.3705	−2.48	+H	**135.1178**, 109.1025	Stigmasterol	R

* Identified with a reference standard. [a] Tentatively new identifications in *Campanulaceae*. The fragment ion mass highlighted as bold font is the characteristic MS fragmentation for each compound.

Their identical MS fragment pattern were similar. But according to the literature, the C3-glucoside was eluted earlier than the C2-glucoside [26–28] in the ESI-BPI chromatogram, so the compound with the earlier RT was identified as the C3-glucoside, 3″-*O*-acetylpolygalacin D2, and the other one with the later RT was identified as the C2-glucoside, 2″-*O*-acetylpolygalacin D2.

2.2. Biomarker Discovery for Differentiating Four Parts of PG

The PCA 2D plots of the samples from the root, stem, leaf and seed groups were classified in four clusters according to their common spectral characteristics (Figure 3). That means the four parts of PG could be easily differentiated.

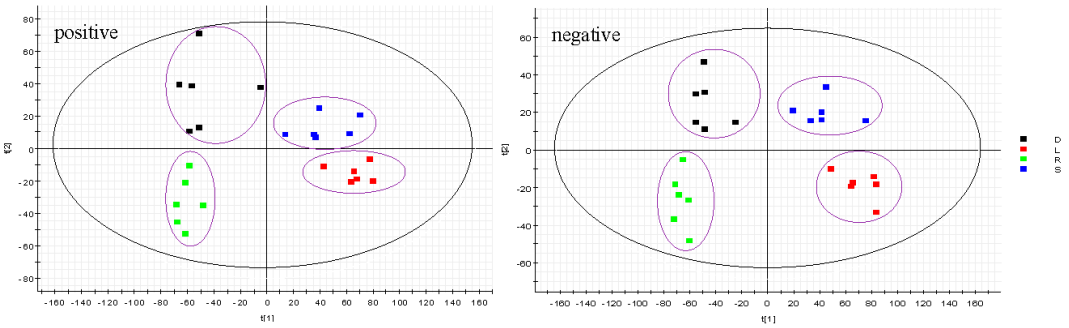

Figure 3. PCA of root (R), stem (S), leaf (L) and seed (D) of PG in positive mode and negative mode.

In order to differentiate one part from other three parts, the OPLS-DA models were built in both positive and negative modes. Then, OPLS-DA score plot, S-plot, variable trend and VIP (variable importance in the projection) values were obtained to understand which variables are the responsible for this sample separation [29]. Based on VIP values (VIP > 4) (Figure 4) and p values ($p < 0.05$) [30] from univariate statistical analysis, 38 robust known biomarkers enabling the differentiation among root, stem, leaf and seed, were discovered and marked in S-plots (Figure 5). In order to systematically evaluate the biomarkers, a heatmap was generated from these biomarkers (shown in Figure 6), which shows distinct segregation among the four parts.

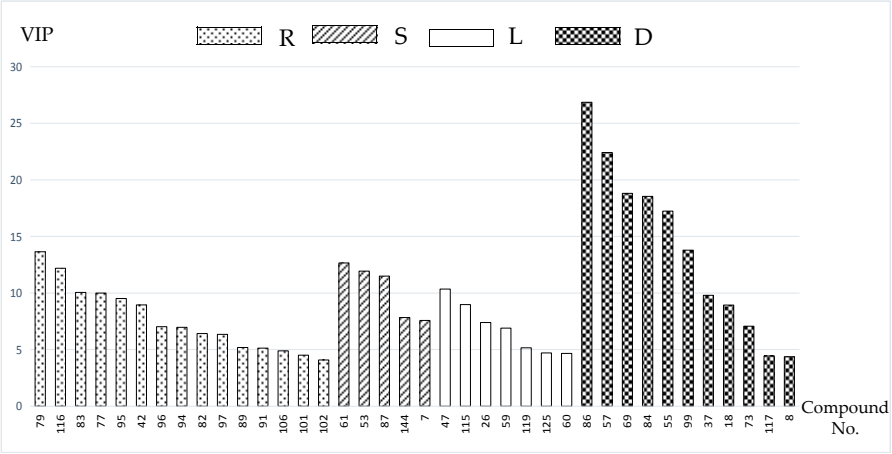

Figure 4. VIP value obtained from OPLS-DA model of the potential markers in root (R), stem (S), leaf (L) and seed (D) of PG.

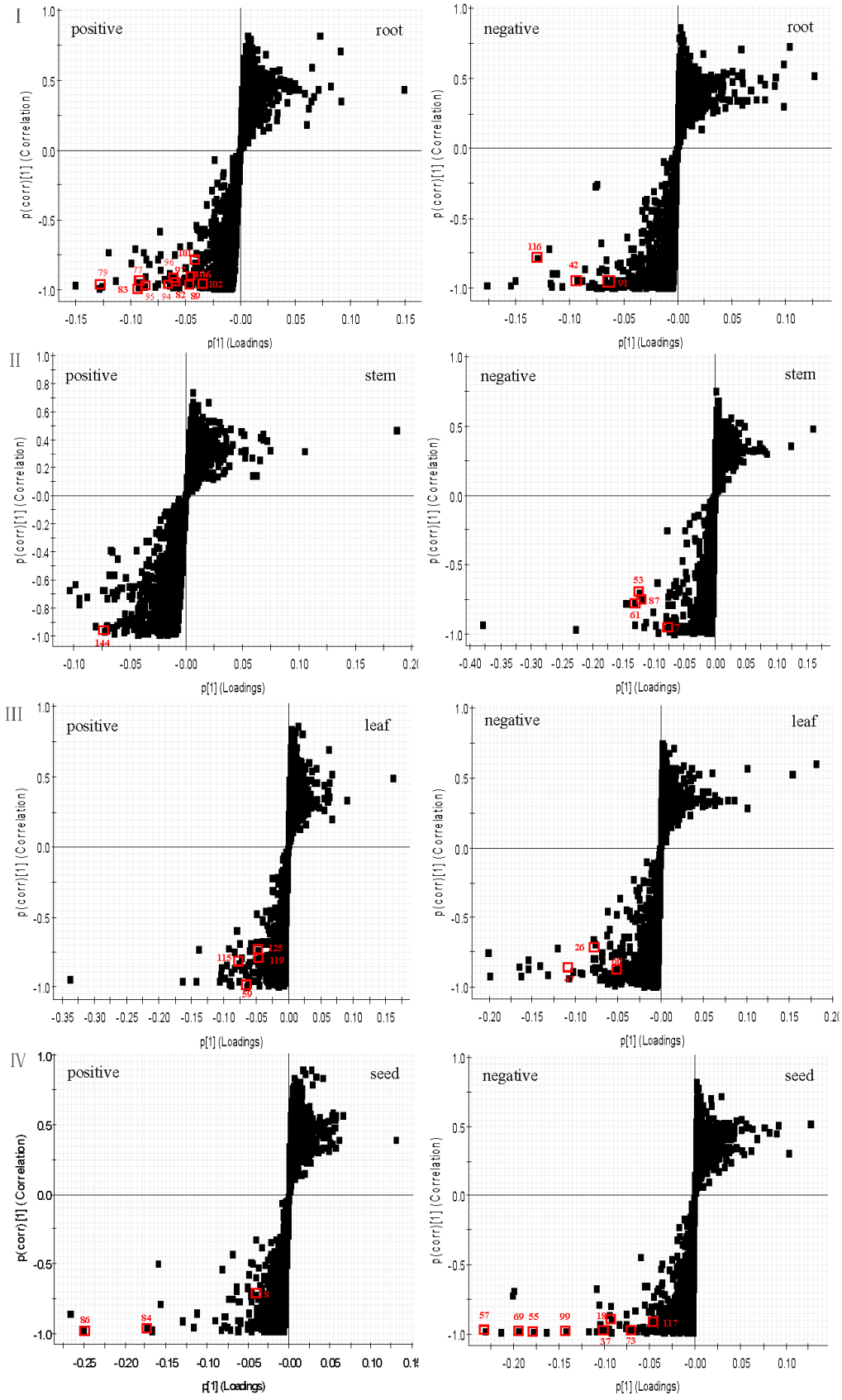

Figure 5. The OPLS-DA/S-plots of root (I), stem (II), leaf (III) and seed (IV) of PG in positive mode and negative mode.

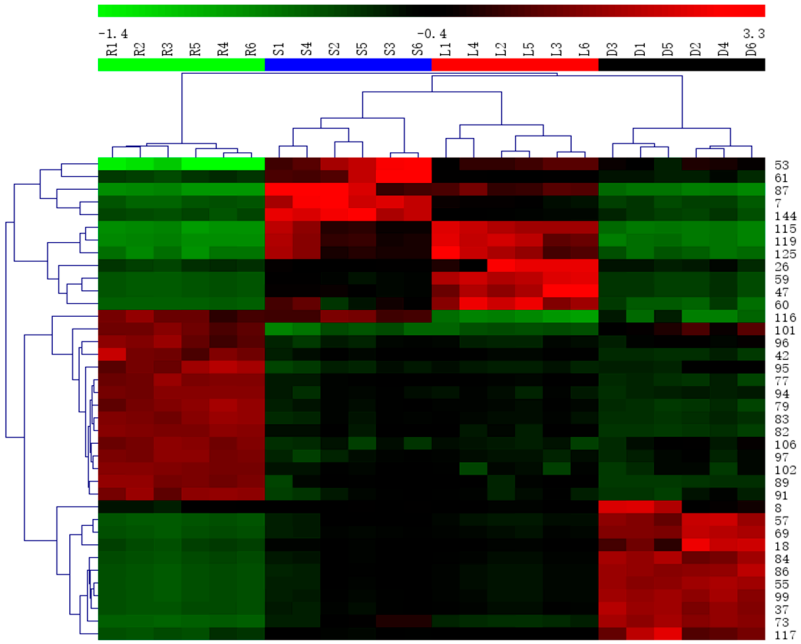

Figure 6. Heatmap visualizing the intensities of potential biomarkers.

3. Discussion

There are 73, 42, 35, 44 compounds that were characterized from the root, stem, leaf and seed, respectively. As the results show, 95 compounds were identified in ESI(−) mode and 64 compounds were identified in ESI(+) mode. According to the BPI chromatograms of the four parts of PG, it seems that ESI(−) ionization mode is better than ESI(+) based on the quantity and the responses of the identified compounds, but it is still necessary to run the ESI(+) mode because some compounds showed better respond than in ESI(−) mode.

Compared with the results from previous studies [2,8,16,31,32], 56 chemical components were identified for the first time in Campanulaceae. The stem, leaf and seed contain more flavonoids but few saponins that can be easily discriminated from the root. In previous study, various metabolites in Korean *Platycodon grandiflorum* were profiled by UPLC-QTOF/MS [16]. Compared with the root of PG in Korea, there were only nine constituents (compounds **5**, **31**, **76**, **79**, **83**, **91**, **94**, **95**, **97**) in common. Meanwhile, the stems and leaves of PG in Korea and in China are both rich in natural components with various structural patterns, including triterpenoid saponins, flavonoids, organic acids, phenols, alcohols, amino acids, coumarins and amino acids, etc., but there are only two similar chemical components (compounds **99**, **104**). It is also interesting that there are eleven components (compounds **5**, **14**, **17**, **21**, **23**, **31**, **52**, **83**, **94**, **95**, **97**) reported in stems and leaves of PG in Korea that were found in the root of PG in China. The reason for this phenomenon may be the different analytical methods and the different growing locations.

In this paper, 38 robust known biomarkers enabling the differentiation among root, stem, leaf and seed, were discovered. For the root part, there are 15 potential biomarkers including triterpenoid saponins (**77**, **79**, **82**, **83**, **89**, **91**, **94**, **95**, **96**, **97**, **101**, **102**, **106**), an organic acid (**116**) and a phenyl-propanoid (**42**). For stem part, there are five potential biomarkers including flavonoids (**53**, **61**, **87**), a tannin (**7**) and a triterpenoid saponin (**144**). For leaf part, there are seven potential biomarkers including flavonoids (**47**, **59**, **125**), sesquiterpenoids (**115**, **119**) and tannins (**26**, **60**). For seed part, there are 11 potential biomarkers including flavonoids (**8**, **18**, **37**, **57**, **69**, **73**, **84**, **99**), quinones (**55**, **86**) and an organic acid (**117**). These robust biomarkers enabling the differentiation among root, stem, leaf and seed can be used for rapid identification of four different parts of PG grown in northeast China.

Even so, there are still some unresolved issues. Firstly, pharmaceutical effects associated with these robust biomarkers or these identified compounds should be screened in the future. Additionally, as shown in BPI chromatograms, though 159 compounds were identified there are still many unidentified components. Further research should be carried on based on the formula of these unknown compounds [13]. Most importantly, the stems and leaves of PG should be developed and utilized due to the presence of so many different components from the root. This comprehensive and unique phytochemical profile study revealed the structural diversity of secondary metabolites and the different patterns in various parts of PG. The method developed in this study can be used as a standard protocol for discriminating and predicting parts of PG directly.

4. Experimental Section

4.1. Materials and Reagents

All samples were harvested from Jilin Province, China, as listed in Table 2, and identified by Professor Ping-Ya Li (School of Pharmaceutical Sciences, Jilin University, Changchun, China). The voucher specimens (No. 2016121-2016144) had been deposited at the Research Center of Natural Drug, School of Pharmaceutical Sciences, Jilin University, Changchun, China. The cultivation ages of the roots are all 2 years, while the others are all 1 year old.

Table 2. Information of samples from Jilin Province, China.

Collection Region	Mark of Samples	Collection Date	Collection Region	Mark of Samples	Collection Date
Antu County	S1	2 October 2016	Fusong County	S4	4 October 2016
	L1	2 October 2016		L4	4 October 2016
	R1	26 October 2016		R4	30 October 2016
	D1	2 October 2016		D4	4 October 2016
Hunchun City	S2	1 October 2016	Tonghua City	S5	5 October 2016
	L2	1 October 2016		L5	5 October 2016
	R2	27 October 2016		R5	28 October 2016
	D2	1 October 2016		D5	5 October 2016
Changbai County	S3	30 September 2016	Jiaohe City	S6	3 October 2016
	L3	30 September 2016		L6	3 October 2016
	R3	29 October 2016		R6	25 October 2016
	D3	30 September 2016		D6	3 October 2016

S: stem, L: leaf, R: root; D: seed.

Acetonitrile and methanol suitable for UHPLC-MS purchased from Fisher Chemical Company (Geel, Belgium). Formic acid for UPLC was purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionized water was purified using a Millipore water purification system (Millipore, Billerica, MA, USA). All other chemicals were of analytical grade. Fourteen standard compounds including platycodin D (111851-201607), mannitol (100533-201304), citric acid (111679-201602), phenylalanine (140676-201405), tryptophan (140686-201303), chlorogenic acid (110753-201716), caffeic acid (110885-201102), dibutyl sebacate (190102-201501), linolenic acid (111631-201605), sucrose (111507-201303), adenosine (110879-201202), monopalmitin (190011-201302), rutin (100080-201610), quercetin (100081-201610), were purchased from the National Institutes for Food and Drug Control (Beijing, China). Seven standard compounds including gallocatechin (201512013), quinine acid (20150321), brusatol (20150410), stigmasterol (20150111), xanthotoxol (20109376), delphinidin (20159567), and atractylenolide III (2014712) were purchased from Beijing Putian Genesis Biotechnology Co., Ltd. (Beijing, China). Nine standard compounds including deapioplatycoside E (160712), deapioplatycodin D (160518), -D_2 (160407), platycoside E (160112), platycodin D_2 (160721), -D_3 (160909), platycoside G_3 (160921), 2′-*O*-acetyl-platycodin D_2 (160112), 3′-*O*-acetylplatycodin D_2 (160923) were provided by Institute of Frontier Medical Science of Jilin University (Changchun, China).

4.2. Sample Preparation and Extraction

The roots, stems, leaves and seeds of PG from the different sites were respectively air dried, ground and sieved (40 mesh) to give a homogeneous powder. Then 200 mg of the powder was respectively extracted thrice with 80% methanol at 80 °C for 3 h each time. After filtering, the extracts were combined, concentrated and evaporated to dryness. Finally, the desiccated extracts were dissolved and diluted with 80% methanol to 10.0 mL. The solution was filtered through a syringe filter (0.22 μm) and injected directly into the UPLC system. The volume injected was 2 μL for each run.

4.3. UPLC-QTOF-MSE

The UPLC analysis was performed by a Waters ACQUITY UPLC System. The column used was an ACQUITY UPLC BEH C18 (100 mm × 2.1 mm, 1.7 μm) from Waters Corporation (Milford, MA, USA). The mobile phases consisted of eluent A (0.1% formic acid in water, v/v) and eluent B (0.1% formic acid in acetonitrile, v/v) with flow rate of 0.4 mL/min with a liner gradient program: 10% B from 0 to 2 min, 10–90% B from 2 to 26 min, 90% B from 26 to 28 min, 90–10% B from 28 to 28.1 min, 10% B from 28.1 to 30 min. The temperature of the UPLC column and autosampler were set at 30 °C and 15 °C. Mixtures of 10/90 and 90/10 water/acetonitrile were used as the strong wash and the weak wash solvent respectively.

The MS experiments were performed on a Waters Xevo G2-S QTOF mass spectrometer (Waters Co., Milford, MA, USA.) connected to the UPLC system through an electrospray ionization (ESI) interface. The optimized instrumental parameters were as follows: capillary voltage floating at 2.6 kV (ESI+) or 2.2 kV (ESI−); cone voltage at 40 V; source temperature at 120 °C, desolvation temperature at 300 °C and cone gas flow was 50 L/h, desolvation gas flow was 800 L/h. In MSE mode, collision energy of low energy function was set at 6 V, while ramp collision energy of high energy function was set at 20–40 V. To ensure mass accuracy and reproducibility, the mass spectrometer was calibrated over a range of 100–1600 Da with sodium formate. Leucine-enkephalin (m/z 556.2771 in positive ion mode; m/z 554.2615 in negtive ion mode) was used as the lockmass at a concentration of 200 ng/mL and flow rate of 20 μL/min. Data were collected in continuum mode, all the acquisition of data were controlled by the Waters MassLynx v.4.1 software (waters, Milford, MA, USA).

4.4. Data Analysis

For the screening analysis, the raw data were processed using the streamlined workflow of UNIFI 1.7.0 software (Waters, Manchester, UK) to quickly identify the chemical components [15]. Besides the Waters Traditional Medicine Library in the UNIFI software, a self-built database was created including the information of chemical components from PG based on the literature and on-line databases such as China Full-text Journals Database (CNKI), PubMed, Medline, Web of Science and ChemSpider. Minimum peak area of 200 was set for 2D peak detection.The peak intensity of high energy over 200 counts and over 1000 counts for low energy were the selected parameters in 3D peak detection. A margin of error up to 5 ppm for identified compounds was allowed. Positive adducts containing +H, +Na, and negative adducts including +COOH and −H were selected. The verification of compounds was carried out by comparison with retention time of reference standards and characteristic MS fragmentation patterns reported in literature.

For metabonomics analysis, the raw data were processed by MarkerLynx XS V4.1 software for alignment, deconvolution, data reduction, etc. [33]. As a result, the list of mass and retention time pairs with corresponding intensities for all the detected peaks from each data file. The main parameters were as follows: retention time range 0–28 min, mass range 100–1600 Da, mass tolerance 0.10, minimum intensity 5%, marker intensity threshold 2000 counts, mass window 0.10, retention time window 0.20, and noise elimination level 6. The resulting data were analyzed by principle component analysis (PCA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA). S-plots and VIP-plots

were obtained via OPLS-DA analysis to find potential biomarkers that significantly contributed to the difference among the groups.

5. Conclusions

In the present study, UPLC-QTOF-MSE coupled with UNIFI platform and precise multivariate statistical analyses was used to profile the four parts of PG. For the constituent screening under the optimized conditions, a total of 159 chemical compounds (73, 42, 35, 44 compounds characterized from root, stem, leaf and seed, respectively) were identified from PG. The results showed various structural patterns including triterpenoid saponins, organic acids, steroids, phenols, flavonoids, alcohols, amino acids, coumarins, terpenoids, alkaloids and amides. The stem, leaf and seed contain more flavonoids but few saponins that can be easily discriminated from the root.

For the metabolomic analysis, four parts of PG were successfully discriminated into four different clusters. A total of 38 robust biomarkers were discovered. That is to say, 15, 5, 7, and 11 robust biomarkers enabling the differentiation among root, stem, leaf and seed, were characterized. These biomarkers can be suitable for the simultaneous differentiation of four different parts of PG, which is reported for the first time. In a word, these results provided the reliable characterization profiles and the differentiate components among root, leaf, stem and seed of PG grown in northeast China. The method developed in this study can be used as a standard protocol for discriminating and predicting the different parts of PG directly.

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Author Contributions: Pingya Li and Jinping Liu conceived and designed the experiments; Cuizhu Wang, Nanqi Zhang and Zhenzhou Wang performed the experiments; Cuizhu Wang, Zeng Qi, Hailin Zhu and Bingzhen Zheng were responsible for data analysis. Cuizhu Wang wrote the paper. Jinping Liu and Pingya Li assisted paper revision.

Conflicts of Interest: The authors declare that they have no conflicts of interest concerning this article.

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Sample Availability: Samples of the compounds are available from the authors.