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•Research article•

Characterization of the chemical constituents of Jie-Geng-Tang and the metabolites in the serums and lungs of mice after oral administration by LC-Q-TOF-MS

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[ABSTRACT] Jie-Geng-Tang (JGT), a traditional formula, is employed in the treatment of sore throat and cough and comprises Platycodonis Radix and Glycyrrhizae Radix et Rhizoma in the ratio 1 : 2. Our previous study demonstrated that JGT protected mice from *S. aureus*-induced acute lung injury (ALI). Five constituents of JGT showed antibacterial activities against *S. aureus in vitro*. However, the potential effective constituents of JGT *in vivo* were still unclear. In this study, the chemical constituents of JGT were identified by liquid chromatography with quadrupole time-of-flight spectrometry (LC-Q-TOF-MS). A total of 96 constituents were identified or assumed, including seven organic acids, 45 flavonoids, 36 triterpene saponins, and eight compounds of other types. The structures of 31 of the constituents were confirmed by comparing them with corresponding authentic standards. Moreover, 15 prototypes and 49 metabolites were deduced in the serums of mice, 24 prototypes and 47 metabolites were deduced in the lungs of mice after the oral administration of JGT. Three types of constituents, namely organic acids, flavonoids, and triterpene saponins, could be absorbed into the blood. Moreover, flavonoids and triterpene saponins were more likely distributed in the lung than in the blood. To the best of our knowledge, this is the first report on the systematic metabolites profile of JGT *in vivo*. The results reported were beneficial to the elucidation of the effective material basis of JGT.

[KEY WORDS] Jie-Geng-Tang; LC-Q-TOF-MS; Constituents; Metabolite; Serum; Lung
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Introduction

Jie-Geng-Tang (JGT) is a traditional Chinese medicine (TCM) formula employed in the treatment of sore throat and cough. This TCM formula consists of two crude drugs, Platycodonis Radix and Glycyrrhizae Radix et Rhizoma in the ratio 1 : 2. It has the functions of "clearing heat and detoxification" and is often employed to treat sore throat and lung diseases, such as lung abscess, lobar pneumonia and chronic bronchitis [1, 2]. Several studies have demonstrated that JGT exhibits a protective effect against an endotoxin-induced acute lung injury (ALI). Tao *et al*. [3] observed that JGT could improve the pathological morphology of the lungs of mice suffering from ALI by blocking the activation of NF-*κ*B signaling pathway. Zheng *et al*. [4] confirmed that Qingrejiedu formulas combined with JGT could inhibit MPO activity, decrease TLR4 mRNA gene expression, and also enhance detoxification and anti-inflammation in rats suffering from lipopolysaccharide (LPS)-induced ALI.

JGT is a traditional formula to treat lung diseases. Our previous study [5] demonstrated the protective effects of JGT against *S. aureus*-induced ALI in mice. Five constituents, namely licochalcone A, licoisoflavone B, glyasperin A, isoliquiritigenin, and licochalcone B, exhibited good antibacterial activities against *S. aureus in vitro*. Tao *et al*. [3] reported 11 constituents, with potential anti-inflammatory activities, in JGT by auto-docking analysis. However, the activities were not confirmed *in vitro* or *in vivo*. Therefore, the constituents that could exert efficacy *in vivo* remained unclear.

It is well-known that the prototypes and metabolites of some constituents that were absorbed into the blood stream and target organs could exert therapeutic effect [6]. Zou [7] identified 80 compounds in JGT by linear trap quadrupole Orbitrap mass spectrometry (LC-LTQ-MS). Two researches on the pharmacokinetics of nine main constituents in rat plasma after the oral administration of JGT [7, 8] and the effect of *Platycodonis Radix* on the bioavailability of six main con-

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stituents [8] were found. However, up to now there has been no reports on the systematic analysis of the prototypes or metabolites of JGT absorbed into blood and lung, the target organ.

To investigate the effective constituents, which exerted protection against *S. aureus*-induced ALI, it was important to investigate the constituents absorbed into the blood and lung after oral administration. In this study, liquid chromatography with quadrupole time-of-flight spectrometry (LC-Q-TOF-MS) method was employed for the comprehensive investigation of the chemical profile of JGT.

Material and Methods

Chemicals and materials

Formic acid (HPLC grade) was purchased from ROE Scientific Inc. (Newark, USA). Acetonitrile, methanol (both HPLC grades) and pentobarbital sodium were purchased from Merck (Darmstadt, Germany).

Platycodonis Radix and Glycyrrhizae Radix et Rhizoma were purchased from Zhejiang Chinese Medical University Medical Pieces Co., Ltd. (Hangzhou, China).

Licoisoflavone A, glyasperin A, and isoliquiritin apioside were purchased from Shyuanye Biotechnology Co., Ltd. (Shanghai, China); glycyrrhizic acid, liquiritigenin, liquiritin apioside, liquiritin, isoliquiritigenin, and isoliquiritin were purchased from Shanghai Winherb Medical Technology Co., Ltd. (Shanghai, China); ononin and licochalcone A were purchased from Zhongxin Pharmaceuticals Group Co., Ltd. (Tianjin, China); isoschaftoside, schaftoside, quinic acid, succinic acid, malic acid, neoisoliquiritin, neoliquiritin, and citric acid were purchased from Pure-one Bio Technology Co., Ltd. (Shanghai, China); licochalcone B, deapio-platycodin D, glycyrol, platycodin D_3, and platycodin D were purchased from Desite Bio Technology Co., Ltd. (Chendu, China); licorice-saponin G_2, glyasperin F, licoisoflavone B, glabrone, and licoflavonol were isolated from Glycyrrhizae radix et rhizoma in our laboratory. Their structures were identified by UV, MS, and NMR analyses.

Preparation of Jie-Geng-Tang solution for LC-MS analysis

Presoaking is helpful for the extraction of chemical constituents of the plant [9, 10]. JGT comprises Platycodonis Radix and Glycyrrhizae Radix et Rhizoma in the ratio 1 : 2. The crude drugs were immersed in deionized water (six times their total weight) overnight. Firstly, they were decocted for 1 h. Thereafter, deionized water (four times their weight) was added to the filtered residue for another 1 h. Finally, the two extracts were mixed and lyophilized. The lyophilized JGT powder (1 mg) was dissolved with 1 mL of (50%, *V*/*V*) methanol. The solution was centrifuged at 12 000 r·min^{-1} for 15 min before the LC-MS analysis. The contents of main constituents in the extract were determined by LC-Q-TOF-MS as follows: neoliquiritin 0.288 μg·mg^{-1}, liquiritin 2.14 μg·mg^{-1}, platycodin D_3 0.496 μg·mg^{-1}, isoliquiritin 0.782 μg·mg^{-1}, neoisoliquiritin 0.0565 μg·mg^{-1}, deapio-platycodin D 0.234 μg·mg^{-1}, platycodin D 3.64 μg·mg^{-1}, licochalcone B 2.21 μg·mg^{-1}, liquiritigenin 0.223 μg·mg^{-1}, licorice-saponin G_2 2.45 μg·mg^{-1}, glycyrrhizic acid 9.82 μg·mg^{-1}, isoliquirtigenin 0.0200 μg·mg^{-1}, and licochalcone A 1.67 μg·mg^{-1}.

Animal experiments

Female C57BL/6 mice (pathogen-free) of six to eight weeks-old were purchased from Zhejiang Academy of Medical Sciences (Hangzhou, China). All the animal experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" approved by the experimental animal welfare ethics committee at Zhejiang University on March 1st, 2017 with agreement number ZJU-20170549.

The mice were randomly divided into four groups of six mice each: the control group and three JGT groups (0.5, 1, and 2 h). The mice were denied food but were free to access water 12 h before the drug administration.

The mice in the JGT groups were intragastrically administered 2.7 g·kg^{-1} of their body weights of the JGT solution. The mice in the control group were intragastrically administered the same volume of deionized water. After 0.5, 1, and 2 h of administrations, the mice were anaesthetized by the intraperitoneal (IP) injection of 1.5% pentobarbital sodium.

Preparation of serum samples

Blood samples were collected 0.5, 1, and 2 h after the oral administration, allowed to stand at room temperature for 2 h, and then centrifuged at 3000 r·min^{-1} for 15 min at the temperature of 4 °C. The serums were separated immediately. Methanol (4 mL) was added to 1 mL of the serum to precipitate protein. Thereafter, the serum was centrifuged at 12 000 r·min^{-1} for 15 min. The supernatants were collected and evaporated to dryness. The residues were redissolved in 200 μL (50%, *V*/*V*) methanol and centrifuged at 12 000 r·min^{-1} for 15 min before the LC-MS analysis.

Preparation of lung samples

After collecting the blood samples, the whole lung was harvested and homogenized with 1 mL (80%, *V*/*V*) methanol. The lung homogenates were centrifuged at 12 000 r·min^{-1} for 15 min. Thereafter, the supernatants were collected and evaporated to dryness. The residues were redissolved in 200 μL (50%, *V*/*V*) methanol and centrifuged at 12 000 r·min^{-1} for 15 min before the LC-MS analysis.

LC-Q-TOF-MS analysis

LC-Q-TOF-MS analysis was performed on a Triple TOF 5600+ mass spectrometer (AB SCIEX, Framingham, MA, USA) with an Acquity^{TM} ultra performance LC system (Waters Corp., Milford, MA, USA).

The chromatographic separation was manipulated in an Agilent Zorbax SB-C_{18} column (4.6 mm × 250 mm, 5 μm) at 30 ºC and at a flow rate of 0.6 mL·min^{-1}. Mobile phases A and B were 0.05% formic acid and acetonitrile. The injection volume was 20 μL. The elution gradient was as follows: 0 min, 6% B; 35 min, 40% B; 60 min, 100% B; 70 min, 100% B.

The samples were analyzed with ESI source in the negative ion mode. For the full-scan acquisition mode at *m*/*z* 100−1500, the parameters of TOF were as follows: curtain gas was set at 30 psi, ion source GS1 was set at 50 psi, ion source GS2 was set at 50 psi, the temperature was set at 550 ºC, and collision energy was set at ± 10 V. For the MS/MS acquisition mode, the collision energy was set at 35 ± 10 V,

the ion release width was set at 25, and the ion release delay was set at 67.

The data from LC-MS was analyzed on the PeakView® software (version 1.2, AB SCIEX, USA). The analyses of the prototypes and metabolites were performed on the MetabolitePilot™ software (version 1.5, AB SCIEX, USA).

Results and Discussion

Chemical profiling of JGT

The chemical constituents of JGT were analyzed by LC-Q-TOF-MS in the negative ion mode (Fig. 1). A total of 96 compounds, including seven organic acids, 45 flavonoids, 36 triterpene saponins, and eight other types of compounds, were identified or assumed (Supplementary materials, Table S1), and their structures are shown in Fig. 2. Among the compounds, 31 were confirmed by comparing their retention times (RTs) and MS/MS spectra with corresponding authentic standards.

Characterization of the organic acids in JGT

Seven compounds, namely compounds **1**, **2**, **3**, **4**, **5**, **6**, and **9**, were characterized as organic acids. Compounds **1**, **2**, **3**, and **5** were confirmed to be quinic acid, malic acid, citric acid, and succinic acid by comparing them with authentic standards. In MS/MS spectra, organic acids are usually detected by the loss of CO_2. For example, compound **6** exhibited a deprotonated ion at *m/z* 285 $[M-H]^-$ and possessed the molecular formula, $C_{12}H_{14}O_8$. It displayed a base peak ion at *m/z* 108 $[M-Xyl-CO_2-H-H]^{\cdot-}$ in MS/MS spectrum. According to the literature [11], it was identified as uralenneoside.

Characterization of the flavonoids in JGT

A total of 45 flavonoids, which were classsified into eight flavones, three flavonols, nine isoflavones, 12 flavonones, and 13 chalcones according to their structural characteristics, were identified in JGT. Based on the MS/MS spectra information, the cleavage characteristics of some of the flavonoids are summarized in Fig. 3.

A total of eight compounds were characterized as flavones by the successive losses of their glycosyl segments in the fragment pathway. For example, flavone C-glucosides exhibited the typical losses of $C_3H_6O_3$ (90 Da) and $C_4H_8O_4$ (120 Da), while flavone C-rhamnosides exhibited the typical losses of $C_3H_6O_2$ (74 Da) and $C_4H_8O_3$ (104 Da) from the precursor ions [12, 13] like compounds **10**, **11**, **12**, **14**, and **17**. The compounds with a 3-hydroxyl-3-methylglutaroyl glucoside could generate base peaks at *m/z* $[M-C_6H_8O_4$ (144 Da) $-C_4H_8O_4$ (120 Da) $-H]^-$ like compounds **20**, **23**, and **25**. Among the eight flavones, compounds **10, 11,** and **14** were confirmed to be vicenin-2, schaftoside, and isoschaftoside by comparison with authentic standards.

A total of 12 compounds were identified as flavanones. Compounds **21**, **22**, **24**, and **49** were confirmed to be liquiritin apioside, neoliquiritin, liquiritin, and liquiritigenin, by comparing them with authentic standards. Some flavanones formed typical ions at *m/z* 255 *via* the elimination of the saccharide groups and yielded fragment ions at *m/z* 135 and 119 *via* the RDA reaction like compounds **13, 16**, **19**, **21, 22, 24**, **34**, **36**, and **49**. Some afforded typical ions at *m/z* 271, after which the RDA reaction occurred, thereby affording ions at *m/z* 151 and 119, like compound **27**.

Further, 13 compounds were identified as chalcones. Among them, compounds **30**, **32**, **35**, **44**, **68**, and **80** were confirmed to be isoliquiritin apioside, isoliquiritin, neoisoliquiritin, licochalcone B, isoliquirtigenin, and licochalcone A by comparison with authentic standards. Chalcones with *C*-2'-OH skeletons, like compounds **7**, **15**, **18**, **26**, **30**, **31**, **32**, **35**, and **68**, could be converted into their corresponding flavanone isomers by RDA reaction, after which they produced similar fragment ions with their corresponding flavanone isomers [14].

Characterization of triterpene saponins in JGT

In our study, 36 triterpene saponins were identified or assumed. Among them, 28 were from Glycyrrhizae Radix et Rhizoma and eight were from Platycodonis Radix.

Eight triterpene saponins isolated from *Platycodon* were all oleanane-type pentacyclic triterpene saponins (Fig. 4). They primarily prossessed C-28 glycosyl groups, like glucose, arabinose, rhamnose, and xylose. For example, compounds **28**, **38**, **39**, and **41** exhibited fragment ions at *m/z* $[M-Api-Xyl-Rha-Ara]^-$; compound **37** exhibited a fragment ion at *m/z* $[M-Xyl-Rha-Ara]^-$; and compounds

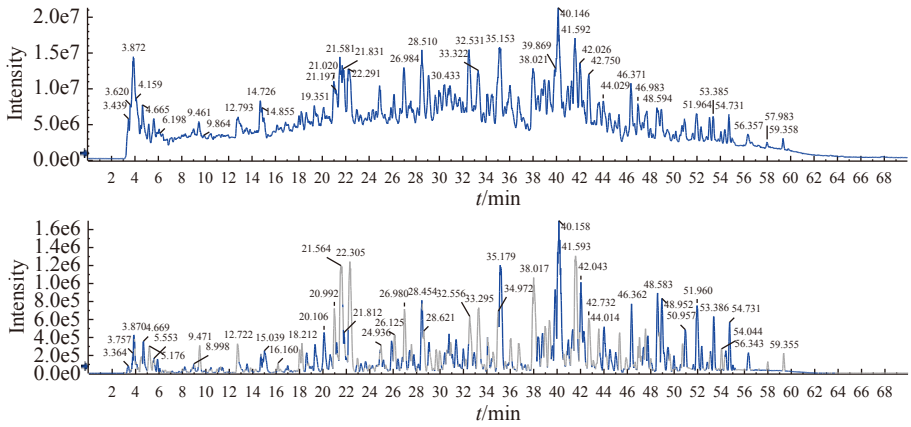

Fig. 1 LC-Q-TOF-MS chromatograms of JGT. (a) Total ion chromatogram-negative mode; (b) Base peak chromatogram-negative mode

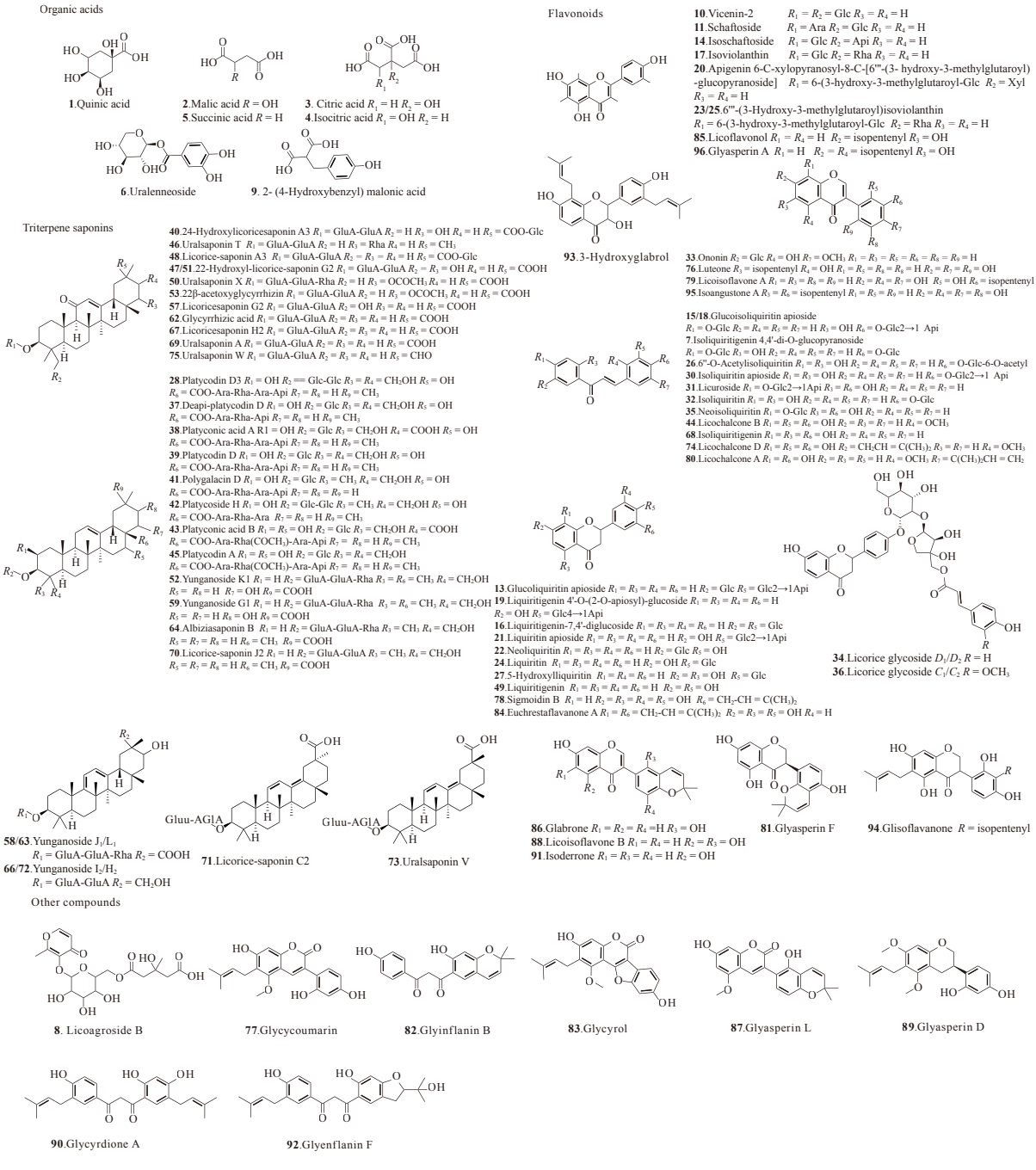

Fig. 2 Chemical structures of compounds in JGT

43 and **45** exhibited fragment ion at *m/z* [M − Api − Xyl − (CH_3CO)Rha − Ara]$^-$. Further, compounds **28**, **37**, and **39** were confirmed to be platycodin D_3, deapio-platycodin D, and platycodin D by comparison with authentic standards. According to the literature [15], compounds **38, 41**, **42**, **43**, and **45** were assumed to be platyconic acid A, polygalacin D, platycoside H, platyconic acid B, and platycodin A, respectively.

Characterization of other compounds in JGT

A total of eight other types of compounds were characterized in JGT. For example, compound **8** exhibited the deprotonated molecular ion at *m/z* 431 [M − H]$^-$ and possessed the molecular formula, $C_{18}H_{24}O_{12}$. In MS/MS spectrum, it generated the base peak at *m/z* 125 [M − $C_{12}H_{18}O_9$ − H]$^-$ and other fragment ions at *m/z* 161 [M − $C_{12}H_{14}O_7$ − H]$^-$ and *m/z*101 [M − $C_{12}H_{14}O_7$ − $C_2H_4O_2$ − H]$^-$. According to the literature [12], compound **8** was assumed to be licoagroside B.

Characterization of the prototype compounds in the mouse serums

Drug-containing mouse serums, at different sampling times (0.5, 1, and 2 h) were all analyzed by LC-Q-TOF-MS. It was observed that the 0.5 h serum sample exhibited the most peaks than the others. Thus, the serum at 0.5 h were obtained for the analysis to detect more prototypes and metabol-

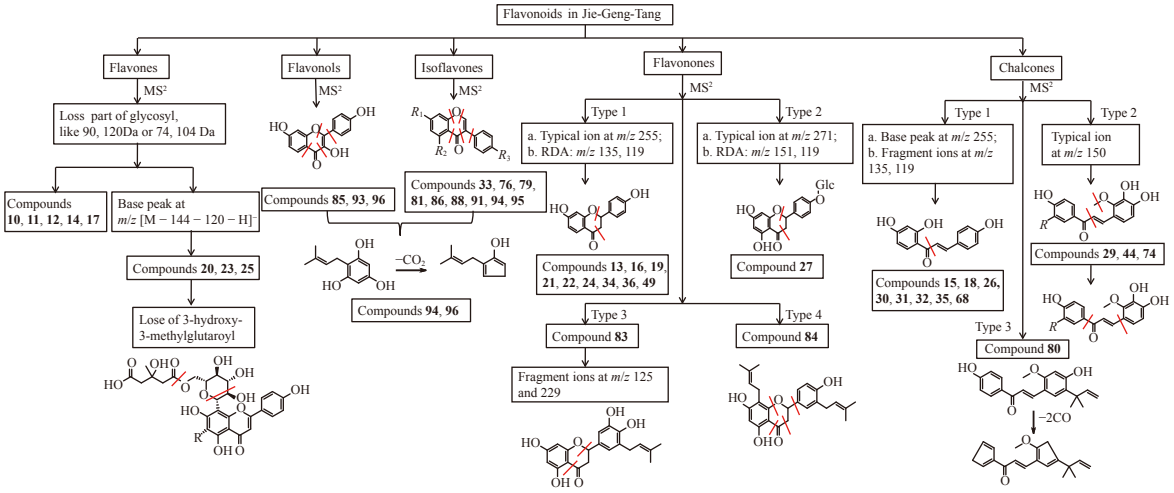

Fig. 3 Cleavage characteristics of flavonoids in JGT

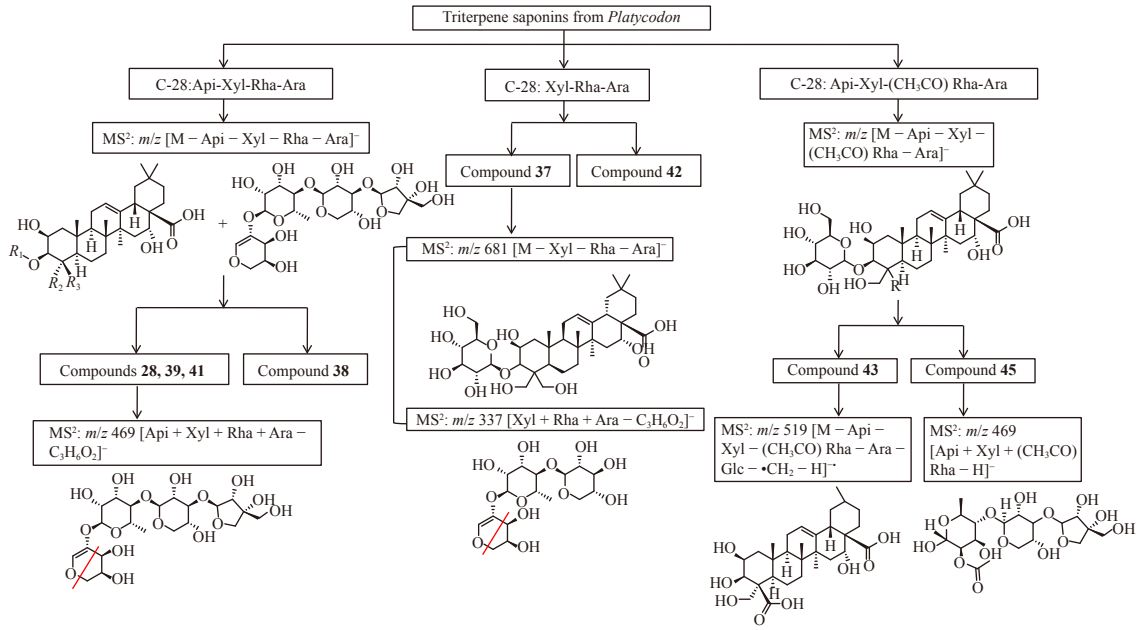

Fig. 4 Cleavage characteristics of triterpene saponins from *Platycodon* in JGT

ites.

A total of 15 prototype compounds, including 2 organic acids, 10 flavonoids, and 3 triterpene saponins, were detected, as listed in Table 1.

Two prototypes of organic acids, namely compounds **p-6** and **p-9**, were absorbed into the blood. Using compound **p-6** for example, it exhibited the same deprotonated molecular ion at *m/z* 285 $[M-H]^-$ and similar fragmentation behavior with compound **6** in the MS/MS spectra at *m/z* 152 $[M-Xyl-H-H]^{-\cdot}$ and *m/z* 108 $[M-Xyl-CO_2-H-H]^{-\cdot}$. Thus, compound **p-6** was also identified as uralenneoside.

Flavonoids were mainly regarded as the active constituents of Glycyrrhizae Radix et Rhizoma. For example, compound **p-11** generated a deprotonated molecular ion at *m/z* 563 $[M-H]^-$ along with characteristic fragment ions at *m/z* 473 $[M-90-H]^-$, 443 $[M-120-H]^-$, 383 $[M-90-90-H]^-$, and 353 $[M-90-120-H]^-$ by the elimination of the glycosyl segments, which corresponded to compound **11** (schaftoside) *in vitro*.

Triterpene saponins were the major constituents of Platycodonis Radix and Glycyrrhizae Radix et Rhizoma. Three triterpene saponins were detected in the mouse serums. For example compound **p-62**, it generated a deprotonated molecular ion at *m/z* 821 $[M-H]^-$ along with fragment ions at *m/z* 351 $[2GluA-H]^-$ and *m/z* 193 $[GluA+H_2O-H]^-$ in MS/MS spectrum. Additionally, the RT of compound **p-62** corresponded to that of compound **62** (glycyrrhizic acid).

Characterization of the metabolites in the mouse serums

The chromatogram of the drug-containing serums were compared with that of blank serums. The MetabolitePilot™ software was utilized to analyze the data of LC-Q-TOF-MS and afforded information on the metabolic reactions from the prototypes to the metabolites. Consequently, 49 metabolites were presumed, as listed in Table 2. Some prototypes under-

Table 1 Characterization of the prototype compounds of JGT in the serums and lungs of mice by LC-Q-TOF-MS in negative ion mode

No.	Source	RT (min)	$[M-H]^-$ ions (*m/z*)	Prototype Compound	Formula	Error (ppm)	Fragment ions in MS/MSspectra
P-6	Serum	12.59	285.0614	Uralenneoside	$C_{12}H_{14}O_8$	−0.8	153.0185 152.0109, 108.0222
	Lung	12.55	285.0609			−2.5	152.0158, 108.0227
P-9	Serum	14.99	209.0455	2-(4-Hydroxybenzyl)malonic acid	$C_{10}H_{10}O_5$	−0.2	121.0676, 119.0490, 93.0372, 91.0154, 71.0186
P-11	Serum	18.11	563.1381	Schaftoside	$C_{26}H_{28}O_{14}$	−4.4	473.1073, 443.0960, 383.0750, 353.0642
	Lung	18.13	563.1404			−0.5	473.1231, 443.1014, 383.0759, 353.0669, 325.0699, 297.0763
P-13	Lung	18.75	711.2154	Glucoliquiritin apioside	$C_{32}H_{40}O_{18}$	1.7	255.0658
P-15	Lung	19.18	711.2164	Glucoisoliquiritin apioside	$C_{32}H_{40}O_{18}$	3.0	549.1710, 255.0668
P-17	Serum	20.20	577.1551	Isoviolanthin	$C_{27}H_{30}O_{14}$	−2.0	457.1143, 383.0754, 353.0661, 297.0913
	Lung	20.19	577.1560			−0.5	383.0755, 353.0660, 297.0768
P-20	Lung	21.27	707.1836	Apigenin-6-C-*β*-xylopyranosyl-8-C-[6‴-*O*-(3-hydroxy-3-methylglutaroyl)-*β*-glucopyranoside	$C_{32}H_{36}O_{18}$	1.1	443.0996, 383.0772, 353.0666, 297.0782
P-21	Serum	21.68	549.1607	Liquiritin apioside	$C_{26}H_{30}O_{13}$	−1.1	417.1192, 255.0657, 135.0083, 119.0505, 91.0222
	Lung	21.68	549.1612			−0.3	417.1191, 255.0657, 135.0085, 119.0506, 91.0205
P-22	Lung	21.95	417.1189	Neoliquiritin	$C_{21}H_{22}O_9$	−0.5	255.0650, 135.0091, 119.0497, 91.0187
P-24	Serum	22.42	417.1182	Liquiritin	$C_{21}H_{22}O_9$	−2.2	255.0654, 135.0087, 119.0509, 91.0203
	Lung	22.43	417.1185			−1.5	255.0655, 135.0282, 135.0085, 119.0505, 91.0194
P-30	Serum	27.09	549.1608	Isoliquiritin apioside	$C_{26}H_{30}O_{13}$	−1.1	417.1213, 255.0650, 135.0076, 119.0512
	Lung	27.10	549.1612			−0.4	255.0657, 135.0078, 119.0504, 91.0193
P-32	Serum	28.67	417.1185	Isoliquiritin	$C_{21}H_{22}O_9$	−1.6	255.0662, 148.0159, 135.0502, 119.0502, 91.0220
	Lung	28.56	417.1189			−0.6	255.0660, 148.0160, 135.0084, 119.0501, 91.0220
P-38	Lung	29.77	1237.5534	Platyconic acid A	$C_{57}H_{90}O_{29}$	3.2	695.3695, 469.1521
P-39	Lung	30.10	1223.5737	Platycodin D	$C_{57}H_{92}O_{28}$	2.8	681.3913, 469.1586
P-40	Lung	30.41	999.4473	24-Hydroxy licorice saponin A_3	$C_{48}H_{72}O_{22}$	3.0	837.4000, 351.0584
P-44	Lung	31.42	285.0769	Licochalcone B	$C_{16}H_{14}O_5$	0.0	150.0324
P-47	Lung	32.52	853.3891	22-Hydroxyl-licorice saponin G_2	$C_{42}H_{62}O_{18}$	3.2	351.0576, 193.0344
P-48	Serum	32.50	983.4518	Licorice-saponin A_3	$C_{48}H_{72}O_{21}$	2.5	821.4078, 351.0554
	Lung	32.52	983.4512			1.9	821.4046, 803.3912, 351.0563, 193.0316
P-49	Serum	33.41	255.0658	Liquiritigenin	$C_{15}H_{12}O_4$	−1.7	135.0064, 119.0507, 91.0189
	Lung	33.37	255.0663			0.0	135.0074, 119.0505, 91.0203
P-57	Serum	37.92	837.3933	Licorice-saponin G_2	$C_{42}H_{62}O_{17}$	2.2	351.0565, 193.0342
	Lung	37.91	837.3938			2.9	351.0568, 193.0341
P-62	Serum	40.11	821.3979	Glycyrrhizic acid	$C_{42}H_{62}O_{16}$	1.7	351.0565, 193.0346
	Lung	40.08	821.3983			2.1	351.0573, 193.0347
P-67	Lung	41.60	821.3976	Licorice-saponin H_2	$C_{42}H_{62}O_{16}$	1.4	351.0571, 193.0345
P-68	Serum	42.06	255.0659	Isoliquirtigenin	$C_{15}H_{12}O_4$	−1.5	135.0072, 119.0459, 91.0211
	Lung	42.08	255.0664			0.5	135.0103, 119.0504, 91.0194
P-86	Serum	51.09	335.0922	Glabrone	$C_{20}H_{16}O_5$	−0.8	319.0602, 291.1015, 135.0082, 91.0195
	Lung	51.08	335.0926			0.2	291.1017, 199.0750, 135.0078, 91.0196
P-88	Serum	52.11	351.0867 $[M-H]^-$	Licoisoflavone B	$C_{20}H_{16}O_6$	−2.0	283.0965, 199.0760
	Lung	52.11	351.0867 $[M-H]^-$			−2.1	283.0965, 265.0872, 241.0873, 199.0754

Table 2 Characterization of the metabolites of JGT in the serums and lungs of mice by LC-Q-TOF-MS in negative ion mode

No.	Source	t_R/min	$[M-H]^-$		Fragment ions in MS/MS spectra	Metabolic pathway
			Ions (*m*/*z*)	Error (ppm)		
M1	Serum	7.82	329.0511	−1.1	153.0180, 135.0075, 109.0285	P6-Loss of $C_5H_8O_4$ + Glucuronide Conjugation
M2	Serum	8.14	315.0719	−0.9	153.0177, 135.0091, 109.0294	P6-Loss of $C_5H_8O_4$ + Glucose Conjugation
M3	Serum	8.76	315.0719	−1.3	153.0180, 152.0106, 108.0224	P6-Loss of $C_5H_8O_4$ + Glucose Conjugation
M4	Lung	9.07	315.0720	−0.4	152.0103, 109.0295, 108.0217, 80.9679	P6-Loss of $C_5H_8O_4$ + Glucose Conjugation
M5	Serum	9.37	315.0720	−0.6	153.0185, 152.0103, 108.0219	P6-Loss of $C_5H_8O_4$ + Glucose Conjugation
M6	Serum	10.68	232.9757	−1.8	153.0182, 109.0302	P6-Loss of $C_5H_8O_4$ + Sulfate Conjugation
M7	Serum	11.16	232.9754	−3.4	153.0182, 123.0447, 109.0320, 108.0195	P6-Loss of $C_5H_8O_4$ + Sulfate Conjugation
	Lung	11.14	232.9760	−0.5	153.0185, 123.0449, 109.0298	
M8	Serum	13.99	223.0612	−0.2	208.0470, 193.0131, 163.0420, 149.0262, 121.0315	P9-Methylation
M9	Lung	14.78	417.1177	−3.3	373.1581, 331.1368, 255.0644, 135.0087	P13/P15-Loss of $C_{11}H_{18}O_9$P21/P30- Loss of $C_5H_8O_4$P49/P68-Glucose Conjugation
M10	Serum	14.91	329.0510	−1.3	153.0183, 135.0085, 109.0317, 91.0210	P6-Loss of $C_5H_8O_4$ + Glucuronide Conjugation
M11	Lung	14.97	549.1600	−2.6	531.0872, 417.1309, 295.1579, 267.7634, 255.0655	P13/P15-Loss of $C_6H_{10}O_5$
M12	Serum	15.10	593.1509	−0.4	417.1199, 255.0658, 135.0083, 119.0500	P21/P30-Loss of $C_5H_8O_4$ + Glucuronide ConjugationP24/P32-Glucuronide Conjugation
	Lung	15.08	593.1506	−1.0	255.0655	P13/P15-Loss of $C_{11}H_{18}O_9$ + Glucuronide ConjugationP21/P30-Loss of $C_5H_8O_4$ + Glucuronide ConjugationP22/P24/P32- Glucuronide Conjugation
M13	Serum	15.23	725.1948	1.8	549.1639, 417.1138, 255.0654, 175.0240	P21/P30-Glucuronide Conjugation
M14	Serum	15.53	329.0512	−0.7	153.0186, 135.0077, 91.0222	P6-Loss of $C_5H_8O_4$ + Glucuronide Conjugation
M15	Serum	17.50	593.1511	−0.2	417.1202, 255.0659, 135.0091, 119.0518	P21/P30-Loss of $C_5H_8O_4$ + Glucuronide ConjugationP24/P32-Glucuronide Conjugation
M16	Serum	17.52	511.0545	−1.4	431.1005, 335.0229, 255.0654	P21/P30-Loss of $C_{11}H_{18}O_9$ + Sulfate and Glucuronide ConjugationP24/P32-Loss of $C_6H_{10}O_5$ + Sulfate and Glucuronide ConjugationP49/P68- Sulfate and Glucuronide Conjugation
M17	Serum	18.03	629.1164	−2.9	549.1631, 417.1201, 255.0648, 135.0084	P21/P30-Sulfate Conjugation
M18	Lung	18.13	563.1404	−0.5	473.1076, 443.1014, 383.0759, 353.0669, 297.0763	P20-Loss of $C_6H_8O_4$
M19	Serum	18.27	497.0747	−2.5	417.1199, 255.0655, 135.0078, 119.0512	P21/P30-Loss of $C_5H_8O_4$ + Sulfate ConjugationP24/P32-Sulfate Conjugation
M20	Lung	18.33	563.1407	0.2	503.1176, 473.1072, 353.0657, 311.0488	P17-Demethylation; P20-Loss of $C_6H_8O_4$
M21	Serum	18.36	607.1292	−2.0	431.0968, 255.0648, 135.0084	P21/P30-Loss of $C_{11}H_{18}O_9$ + Bis-Glucuronide ConjugationP24/P32-Loss of $C_6H_{10}O_5$ + Bis-Glucuronide ConjugationP49/P68-Bis-Glucuronide Conjugation
M22	Serum	18.64	511.0543	−1.7	431.1018, 335.0240, 255.0650, 135.0074, 119.0500	P30-Loss of $C_6H_{10}O_5$ + Sulfate and Glucuronide ConjugationP24/P32- Loss of $C_6H_{10}O_5$
M23	Lung	18.75	711.2154	1.7	255.0658, 135.0091	P21/P30-Glucose Conjugation
M24	Serum	19.33	725.1936	0.2	549.1619, 255.0646	P21/P30-Glucuronide Conjugation
M25	Lung	19.48	579.1717	−0.4	255.0654, 135.0084	P13/P15-Loss of $C_5H_8O_4$P21/P30- Loss of $C_5H_8O_4$ + Glucose ConjugationP22/P24/P32-Glucose Conjugation
M26	Serum	19.87	417.1182	−2.2	148.0140, 135.0062, 119.0487	P21/P30-Loss of $C_5H_8O_4$P49/P68-Glucose Conjugation

Continued

No.	Source	t_R/min	$[M-H]^-$		Fragment ions in MS/MS spectra	Metabolic pathway
			Ions (*m*/*z*)	Error (ppm)		
M27	Serum	19.87	593.1499	−2.3	417.1194, 255.0654, 148.0159, 135.0085, 119.0506	P21/P30-Loss of $C_5H_8O_4$ + Glucuronide Conjugation
	Lung	19.87	593.1509	−0.5	417.1239, 255.0655, 148.0172	P13/P15-Loss of $C_{11}H_{18}O_9$ + Glucuronide ConjugationP21/P30-Loss of $C_6H_8O_4$ + Glucuronide ConjugationP22/P32-Glucuronide Conjugation
M28	Lung	20.19	577.1560	−0.5	473.1101, 413.0893, 383.0755, 353.0650, 297.0768	P11- Methylation; P20-Loss of $C_6H_8O_4$ + Methylation
M29	Lung	21.13	549.1609	−0.9	429.1054, 255.0653	P13/P15-Loss of $C_6H_{10}O_5$
M30	Lung	21.35	579.1722	0.4	323.1035, 255.0650, 1350072	P13/P15-Loss of $C_5H_8O_4$P22/P24/P32- Glucose ConjugationP30-Loss of $C_5H_8O_4$ + Glucose Conjugation
M31	Lung	21.68	549.1612	−0.3	417.1191, 255.0657	P13/P15-Loss of $C_6H_{10}O_5$
M32	Lung	21.93	579.1719	−0.1	285.0755, 255.0648, 135.0098	P13/P15-Loss of $C_5H_8O_4$P21-Loss of $C_5H_8O_4$ + Glucose ConjugationP22/P24/P32-Glucose ConjugationP30-Loss of $C_5H_8O_4$ + Glucose Conjugation
M33	Lung	21.95	417.1189	−0.5	255.0650, 135.0091, 119.0497	P13/P15-Loss of $C_{11}H_{18}O_9$; P21/P30- Loss of $C_5H_8O_4$P49/P68- Glucose Conjugation
M34	Serum	22.42	417.1182	−2.2	255.0654, 135.0087, 119.0509, 91.0203	P21/P30-Loss of $C_5H_8O_4$; P49/P68-Glucose Conjugation
	Lung	22.43	417.1185	−1.5	255.0655, 135.0085, 119.0505	P13/p15-Loss of $C_{11}H_{18}O_9$; P21/P30-Loss of $C_5H_8O_4$P49/P68- Glucose Conjugation
M35	Serum	22.46	255.0663	0.0	149.0264, 135.0125, 119.0509, 93.0381	P21/P30-Loss of $C_{11}H_{18}O_9$
	Lung	22.48	255.0662	−0.2	135.0075, 119.0527, 91.0198	P21/P30-Loss of $C_{11}H_{18}O_9$; P22/P24/P32-Loss of $C_6H_{10}O_5$
M36	Serum	22.48	431.0977	−1.6	255.0664, 135.0088, 119.0507, 91.0212	P21-Loss of $C_{11}H_{18}O_9$ + Glucuronide ConjugationP49/P68- Glucuronide Conjugation
	Lung	22.48	431.0977	−1.6	255.0655, 135.0089, 119.0508	P21/P30-Loss of $C_{11}H_{18}O_9$ + Glucuronide ConjugationP22/P24/P32- Loss of $C_6H_{10}O_5$ + Glucuronide ConjugationP49/P68-Glucuronide Conjugation
M37	Serum	22.97	431.0973	−2.5	255.0653, 135.0085, 119.0507, 91.0203	P21/P30-Loss of $C_{11}H_{18}O_9$ + Glucuronide ConjugationP24/P32-Loss of $C_6H_{10}O_5$ + Glucuronide ConjugationP49/P68-Glucuronide Conjugation
	Lung	22.98	431.0978	−1.4	369.0985, 255.0655, 175.0236, 135.0080, 119.0502	P21/P30-Loss of $C_{11}H_{18}O_9$ + Glucuronide ConjugationP22/P24/P32- Loss of $C_6H_{10}O_5$ + Glucuronide ConjugationP49/P68-Glucuronide Conjugation
M38	Lung	23.04	721.1991	0.7	577.1573, 457.1149, 383.0776, 353.0656, 297.0753	P20-Methylation
M39	Serum	23.10	353.0994	1.1	165.0568, 147.0449, 119.0522, 103.0576	P9-Oxidation and Glutamine Conjugation
M40	Serum	23.28	497.0749	−2.0	417.1161, 255.0653, 148.0141	P21/P30-Loss of $C_5H_8O_4$ + Sulfate ConjugationP24/P32- Sulfate Conjugation
M41	Serum	23.32	511.0545	−1.2	255.0658, 135.0080, 119.0521	P21/P30-Loss of $C_{11}H_{18}O_9$ + Sulfate and Glucuronide ConjugationP24-Loss of $C_6H_{10}O_5$ + Sulfate and Glucuronide Conjugation
M42	Serum	25.20	255.0663	−1.0	135.0152, 119.0506, 91.0296	P21/P30-Loss of $C_{11}H_{18}O_9$; P32-Loss of $C_6H_{10}O_5$
M43	Serum	25.21	335.0228	−1.0	255.0648, 135.0077, 119.0502, 91.0192	P21/P30-Loss of $C_{11}H_{18}O_9$ + Sulfate ConjugationP24/P32-Loss of $C_6H_{10}O_5$ + Sulfate ConjugationP49/P68-Sulfate Conjugation
	Lung	25.22	335.0229	−0.7	255.0651, 135.0084, 119.0500, 91.0217	P21/P30-Loss of $C_{11}H_{18}O_9$ + Sulfate ConjugationP22/P24/P32-Loss of $C_6H_{10}O_5$ + Sulfate ConjugationP49/p68-Sulfate Conjugation

Continued

No.	Source	t_R/min	$[M-H]^-$		Fragment ions in MS/MS spectra	Metabolic pathway
			Ions (*m*/*z*)	Error (ppm)		
M44	Serum	26.37	335.0228	−1.0	255.0653, 135.0082, 119.0506, 91.0201	P21/P30-Loss of $C_{11}H_{18}O_9$ + Sulfate ConjugationP24/P32-Loss of C6H10O5 + Sulfate ConjugationP49/P68-Sulfate Conjugation
	Lung	26.36	335.0231	−0.1	255.0658, 135.0082, 119.0504, 91.0204	P21/P30-Loss of $C_{11}H_{18}O_9$ + Sulfate ConjugationP22/P24/P32- Loss of $C_6H_{10}O_5$ + Sulfate ConjugationP49/P68-Sulfate Conjugation
M45	Lung	26.46	447.0926	−1.5	271.0611, 151.0022, 119.0502	P44-Loss of CH_2 + Glucuronide Conjugation
M46	Lung	27.10	549.1612	−0.4	255.0657, 135.0078	P13/P15-Loss of $C_6H_{10}O_5$
M47	Lung	28.56	417.1189	−0.6	255.0660	P13/P15-Loss of $C_{11}H_{18}O_9$; P21/P30- Loss of $C_5H_8O_4$P49/P68-Glucose Conjugation
M48	Serum	29.20	431.0975	−1.9	255.0646, 135.0082, 119.0500, 91.0228	P21-Loss of $C_{11}H_{18}O_9$ + Glucuronide Conjugation
M49	Serum	29.78	255.0664	0.3	135.0076, 119.0509, 91.0203	P21/P30-Loss of $C_{11}H_{18}O_9$; P32-Loss of $C_6H_{10}O_5$
M50	Serum	29.78	431.0975	−2.0	255.0656, 135.0087, 119.0507	P21/P30-Loss of $C_{11}H_{18}O_9$ + Glucuronide ConjugationP24/P32-Loss of $C_6H_{10}O_5$ + Glucuronide ConjugationP49/P68- Glucuronide Conjugation
	Lung	29.77	431.0982	−0.5	255.0659, 213.0525, 145.0253, 135.0075, 119.0501	P21/P30- Loss of $C_{11}H_{18}O_9$ + Glucuronide ConjugationP22/P24/P32- Loss of $C_6H_{10}O_5$ + Glucuronide ConjugationP49/P68- Glucuronide Conjugation
M51	Lung	30.41	999.4473	3.0	837.4000, 351.0584	P47- Loss of O + Glucose Conjugation; P48-OxidationP57-Glucose Conjugation
M52	Lung	30.45	823.4144	2.8	761.4078, 351.0587	P40-Loss of $C_6H_8O_6$; P48-Loss of $C_6H_8O_6$ + OxidationP57-Loss of $C_6H_8O_6$ + Glucose ConjugationP62- Hydrogenation; P67-Hydrogenation
M53	Lung	30.57	365.0329	−2.1	285.0769, 177.0172, 150.0310	P44-Sulfate Conjugation
M54	Lung	30.85	837.3941	3.2	351.0562	P40-Loss of $C_6H_{10}O_5$; P47- Loss of OP48- Loss of $C_6H_8O_6$ + Demethylation to Carboxylic AcidP62-Oxidation; P67-Oxidation
M55	Serum	31.19	703.1518	0.4	527.1204, 351.0864	P88-Bis-Glucuronide Conjugation
M56	Serum	32.46	335.0229	−0.7	255.0657, 135.0076, 119.0504, 91.0203	P21/P30-Loss of $C_{11}H_{18}O_9$ + Sulfate ConjugationP24/P32-Loss of $C_6H_{10}O_5$ + Sulfate ConjugationP49/P68-Sulfate Conjugation
	Lung	32.46	335.0230	−0.3	119.0476	P49/P68- Sulfate Conjugation
M57	Lung	32.52	853.3891	3.2	351.0576, 193.0344	P40-Loss of $C_6H_8O_6$ + Demethylation to Carboxylic AcidP48- Loss of $C_6H_{10}O_5$ + Di-OxidationP57-Oxidation; P62/P67-Di-Oxidation
M58	Serum	32.50	983.4518	2.5	821.0478, 351.0554	P62-Glucose Conjugation
	Lung	32.52	983.4512	1.9	821.4046, 803.3912, 351.0563, 193.0316	P40-Loss of O; P62- Glucose ConjugationP67-Glucose Conjugation
M59	Serum	33.41	255.0658	−1.7	135.0064, 119.0507, 91.0189	P21/P30-Loss of $C_{11}H_{18}O_9$; P24- Loss of $C_6H_{10}O_5$
	Lung	33.37	255.0663	0.0	135.0074, 119.0506, 91.0203	P21/P30-Loss of $C_{11}H_{18}O_9$; P22/P24/P32-Loss of $C_6H_{10}O_5$
M60	Serum	33.62	335.0228	−0.8	255.0657, 135.0084, 119.0507, 91.0205	P21/P30-Loss of $C_{11}H_{18}O_9$ + Sulfate ConjugationP24/P32-Loss of $C_6H_{10}O_5$ + Sulfate ConjugationP49/P68-Sulfate Conjugation
M61	Serum	33.64	255.0661	−0.7	135.0065, 119.0500, 91.0196	P21/P30-Loss of $C_{11}H_{18}O_9$; P32- Loss of $C_6H_{10}O_5$
M62	Serum	34.25	527.1189	−1.1	351.0865, 283.0962	P88-Glucuronide Conjugation
M63	Lung	35.18	837.3931	2.0	351.0570	P40-Loss of $C_6H_{10}O_5$; P47- Loss of OP48-Loss of $C_6H_8O_6$ + Demethylation to Carboxylic AcidP62-Oxidation; P67-Oxidation

Continued

No.	Source	t_R/min	$[M-H]^-$		Fragment ions in MS/MS spectra	Metabolic pathway
			Ions (*m*/*z*)	Error (ppm)		
M64	Serum	37.92	837.3933	2.2	351.0565	P48-Loss of $C_6H_8O_6$ + Demethylation to Carboxylic AcidP62-Oxidation
	Lung	37.91	837.3938	2.9	351.0568	P40-Loss of $C_6H_{10}O_5$; P47- Loss of OP48- Loss of $C_6H_8O_6$ + Demethylation to Carboxylic AcidP62-Oxidation; P67-Oxidation
M65	Lung	38.76	819.3829	2.5	351.0556	P40-Loss of $C_6H_{10}O_5$ + Loss of WaterP47-Loss of O + Loss of WaterP48-Loss of $C_6H_{10}O_6$ + Ketone FormationP57- Loss of Water; P62-Desaturation; P67-Desaturation
M66	Serum	39.19	527.1183	−2.2	393.0987, 351.0874, 283.0970	P88-Glucuronide Conjugation
M67	Serum	39.30	837.3922	0.9	643.3413, 351.0548, 193.0343	P48-Loss of $C_6H_8O_6$ + Demethylation to Carboxylic Acid
	Lung	39.29	837.3937	2.8	351.0565	P40-Loss of $C_6H_{10}O_5$; P47- Loss of OP48- Loss of $C_6H_8O_6$ + Demethylation to Carboxylic AcidP62-Oxidation; P67-Oxidation
M68	Lung	39.87	837.3937	1.8	351.0569	P40-Loss of $C_6H_{10}O_5$; P62-Oxidation; P67-Oxidation
M69	Serum	40.11	821.3979	1.7	351.0568	P48-Loss of $C_6H_{10}O_5$
	Lung	40.08	821.3983	2.1	351.0573	P40-Loss of $C_6H_{10}O_6$; P48-Loss of $C_6H_{10}O_5$
M70	Lung	41.30	807.4175	0.3	351.0563, 193.0337	P40-Loss of $C_6H_8O_7$; P48-Loss of $C_6H_8O_6$P62-Loss of $C_6H_8O_6$ + Glucose ConjugationP67-Loss of $C_6H_8O_6$ + Glucose Conjugation
M71	Lung	41.60	821.3976	1.4	351.0571	P40-Loss of $C_6H_{10}O_6$P48-Loss of $C_6H_{10}O_5$
M72	Serum	42.06	255.0659	−1.5	135.0072, 119.0495, 91.0211	P21/P30-Loss of $C_{11}H_{18}O_9$P24/P32-Loss of $C_6H_{10}O_5$
	Lung	42.08	255.0664	0.5	135.0103, 119.0504, 91.0194	P21/P30-Loss of $C_{11}H_{18}O_9$P22/P24/P32-Loss of $C_6H_{10}O_5$
M73	Serum	42.08	527.1183	−2.3	351.0868, 336.0610, 283.0963, 241.0859	P88-Glucuronide Conjugation
M74	Lung	42.10	821.3974	1.1	759.3965, 351.0559, 193.0355	P40-Loss of $C_6H_{10}O_6$; P48-Loss of $C_6H_{10}O_5$
M75	Serum	42.61	823.4137	1.9	351.0551, 193.0343	P48- Loss of $C_6H_8O_6$ + Oxidation;P62-Hydrogenation
	Lung	42.61	823.4128	0.8	351.0563	P40-Loss of $C_6H_8O_6$; P48-Loss of $C_6H_8O_6$ + OxidationP57-Loss of $C_6H_8O_6$ + Glucose ConjugationP62/P67- Hydrogenation
M76	Lung	43.88	807.4182	1.1	351.0569, 193.0367	P40-Loss of $C_6H_8O_7$; P48- Loss of $C_6H_8O_6$P62-Loss of $C_6H_8O_6$ + Glucose ConjugationP67-Loss of $C_6H_8O_6$ + Glucose Conjugation
M77	Serum	44.24	415.0484	−2.2	335.0919, 305.0428, 291.1019, 135.0100	P86-Sulfate Conjugation
M78	Serum	45.60	835.4135	1.6	351.0573, 193.0348	P48- Loss of $C_6H_{10}O_5$ + Methylation; P62-Methylation

went phase I metabolic reactions, such as oxidation, methylation, hydrogenation, and hydrolyzation. Some favored phase II metabolic reactions, such as glucuronide conjugation, sulfate conjugation, glucose conjugation, and glutamine conjugation. The others underwent two or three-step metabolic reactions, including phase I and phase II metabolic reactions. Moreover, one prototype compound could undergo a series of metabolic reactions, and one metabolite could be produced by multiple prototypes [13].

Metabolite **M12**, for example, could be generated from compounds **p-21**, **p-24**, **p-30**, and **p-32**. Metabolite **M12** formed a deprotonated molecular ion at *m*/*z* 593 $[M-H]^-$ along with a base peak ion at *m*/*z* 255 $[M-GluA-Glc-H]^-$ and a fragment ion at *m*/*z* 417 $[M-Glc-H]^-$. Thereafter, the base peak ion formed fragment ions at *m*/*z* 135 and 119. Compounds **p-21**/**p-30** and **p-24**/**p-32** were the two pairs of isomers, and they all produced fragment ions at *m*/*z* 255, 135, and 119. Compounds **p-21** and **p-30** could generate metabolite **M12** by two-step metabolic reactions, including the loss of apiose and glucuronide conjugation. Compounds **p-24** and **p-32** could also generate metabolite **M12** by glucuronide conjugation.

Characterization of prototype compounds in mouse lungs

The drug-containing lung samples, at different sampling times (0.5, 1, and 2 h), were all analyzed by LC-Q-TOF-MS. More peaks were noticed in the 0.5 h lung sample. Thus, the

lung sample at 0.5 h was obtained for analysis to detect more prototypes and metabolites. A total of 24 prototype compounds were presumed, including one organic acid, 15 flavonoids, and eight triterpene saponins.

The prototype compound of the organic acid that was absorbed into the lung was uralenneoside, which was also detected in the serum. More flavonoids and triterpene saponins were detected in the lung, suggesting that these constituents were more easily distributed in the lung than in the blood and could be beneficial in the treatment of lung diseases.

Among the eight triterpene saponins that were absorbed into the lung, five, namely compounds **p-38**, **p-39**, **p-40**, **p-47**, and **p-67**, were not detected in the serums. Compound **p-39**, for example, possessed the molecular formula, $C_{57}H_{92}O_{28}$, and exhibited a deprotonated molecular ion at *m/z* 1223 $[M - H]^-$. It exhibited the same RT with compound **39** *in vitro*, as well as the typical fragment ions at *m/z* 681 $[M - Api - Xyl - Rha - Ara - H]^-$ and 469 $[Api + Xyl + Rha + Ara - C_3H_6O_2 - H]^-$. Thus, compound **p-39** was inferred to be platycodin D.

Characterization of the metabolites in the lungs of mice

In the lung samples, 47 metabolites were tentatively identified by LC-Q-TOF-MS. Among the metabolites, 18 were also detected in the mouse serums.

The prototype compounds, **p-13** and **p-15**, were a pair of isomers, they were identified in the lung samples but not detected in the serums. Morover, they could produce a variety of metabolites. For example, metabolites **M9**, **M33**, **M34**, and **M47** each formed a deprotonated molecular ion at *m/z* 417 $[M - H]^-$ along with a base peak ion at *m/z* 255 of the MS/MS spectra. They could be metabolized from compounds **p-13** and **p-15** by the loss of $C_{11}H_{18}O_9$ (glucose + apiose). Metabolites **M11, M29, M31,** and **M46** each formed a deprotonated molecular ion at *m/z* 549 $[M - H]^-$ along with a corresponding base peak ion at *m/z* 255 of the MS/MS spectra. They could be metabolized from compounds **p-13** and **p-15** by loss of glucose. Metabolites **M12** and **M27** formed deprotonated molecular ion at *m/z* 593 $[M - H]^-$, along with base peak ion at *m/z* 255 in MS/MS spectra. They could be metabolized from prototype compounds **p-13** and **p-15** by the loss of the $C_{11}H_{18}O_9$ (glucose + apiose) and glucuronide conjugation.

Conclusion

Here, the chemical constituents of JGT were systematically analyzed by LC-Q-TOF-MS. The structures of 96 constituents, including seven organic acids, 45 flavonoids, 36 triterpene saponins, and eight other type compounds, were identified or assumed,. Moreover, 15 prototypes and 49 metabolites were deduced in mice serum, 24 prototypes and 47 metabolites were deduced in the serums of the mice after the oral administration of JGT. Furthermore, it was observed that three types of constituents, namely organic acids, flavonoids, and triterpene saponins, could be absorbed into the blood. Moreover, flavonoids and triterpene saponins were more likely distributed in the lung than in the blood. To the best of our knowledge, this is the first report on the systematic metabolites profiling of JGT *in vivo*. The results were beneficial for revealing the effective constituents of JGT against ALI and could even aid further pharmacological studies.

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