Chinese Medicine

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Rapid discovery of chemical constituents and absorbed components in rat serum after oral administration of Fuzi-Lizhong pill based on high-throughput HPLC-Q-TOF/MS analysis

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Abstract

Background: Fuzi-Lizhong pill (FZLZP), which was first recorded in the Classic–"Taiping Huimin Heji Ju Fang" of the Song Dynasty, has been widely used to treat gastrointestinal disease in clinic for thousands of years in China. However, an in-depth understanding of the chemical constituents of FZLZP and its potential bioactive constituents is lacking.

Methods: A simple, sensitive and selective method of high-performance liquid chromatography coupled with quadrupole-time-of-flight high-definition mass spectrometry (HPLC-Q-TOF/MS) and automated data analysis (Agilent MassHunter Qualitative Analysis B.06.00 Workstation Software) was developed to simultaneously identify the chemical constituents of FZLZP and the absorbed prototypes as well as the metabolites in rat serum after the oral administration of FZLZP.

Results: Sixty-seven compounds, including alkaloids, flavonoids, triterpenes, gingerols, phenylpropanoids and volatile oil, in the FZLZP extract were tentatively characterized by comparing the retention time and mass spectrometry data and retrieving the reference literatures. Additionally, 23 prototype compounds and 3 metabolites in the rat serum samples were identified after oral administration of FZLZP, which might be the potential active components in vivo. In addition, the absorption of alkaloids decreased when *Aconitum carmichaeli* Debx. was in the form of combined application as a prescription compared to when it was in the form of herb powder.

Conclusions: Herein, the chemical constituent in vitro and the absorbed compounds in the serum of a traditional Chinese formula, Fuzi-Lizhong pill, were fully characterized using a rapid and comprehensive analysis approach based on high-performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry coupled to MassHunter Qualitative Analysis software data processing approach. The results provide helpful chemical information on FZLZP for further pharmacology and active mechanism research. In view of the bioactive constitutes that basically were derived from these absorbed compounds in vivo, this work could provide a useful strategy to explore the bioactive substances of traditional Chinese medicine.

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Keywords: Fuzi-Lizhong pill, Chemical constituents, Bioactive compounds, Metabolites, Traditional Chinese herbal medicine, High-performance liquid chromatography–electrospray ionization/quadrupole-time-of-flight high definition mass spectrometry

Background

Fuzi-Lizhong pill (FZLZP) is a popular Traditional Chinese medicine pill that was originally described in the Classic "Taiping Huimin Heji Ju F/ang" of the Song Dynasty (year 1102 by the Western calendar). It is composed of five herbal medicines, including *Aconitum carmichaeli* Debx. (Fuzi), *Codonopsis pilosula* (Franch.) Nannf. (Dangshen), *Atractylodes macrocephala* Koidz. (Baizhu), *Glycyrrhiza uralensis* Fisch. (Gancao) and *Zingiber officinale* Rosc. (Ganjiang). FZLZP is famous for warming the middle-jiao and tonifying the spleen and is used to treat spleen yang deficiency syndrome including enteritis, chronic diarrhoea, irritable bowel syndrome, abdominal pain, vomiting and spasm, peripheral chill, etc. [1–7]. Modern pharmacological research shows that FZLZP possesses a variety of pharmacological activities, including an increase in adaptive thermogenesis, pain relief, anti-inflammation, and spasmolytic benefits [8–15]. Although pharmacological activities of FZLZP have been extensively studied, very little is known about its systematic chemical constituents, and the bioactive compounds that account for its therapeutic effects remain unclear.

In our previous research, we focused on the dissolution behaviour of FZLZP in vitro and the results showed that some constituents in *Aconitum carmichaeli Debx.* and *Glycyrrhiza uralensis* Fisch., such as benzoylaconine, liquiritin and glycyrrhizic acid, were dissolved well in vitro [16–18]. While FZLZP has the so-called active ingredients, there are no empirical data to prove their effectiveness as bioactive compounds. According to the theory of serum pharmacochemistry, while there are multiple components in herbs, only compounds that are absorbed into the blood have the possibility of showing pharmacological bioactivities [19–24]. Therefore, simultaneous identification of systematic chemical constituents in vitro and potential active components in the blood of FZLZP are indispensable.

It was reported that the main components in *Aconitum carmichaeli Debx.* are monoester diterpenoid alkaloids (MDAs) and diester diterpenoid alkaloids (DDAs), which are toxicity and efficacy compounds and should be highly concerned [21]. Due to the toxicity, Fuzi is usually used in combination with other herbs as a prescription. Some researcher considered the combination to cause the reduction of the absorption of toxic compounds [21, 25]. As a typical combination, however, there are no detailed studies of this mechanism and the compound variations of FZLZP. The strategy of serum thermochemistry can provide us the accurate qualitative and the preliminary quantitative information for exploring the quantitative change of alkaloids and toxicity reducing mechanism.

Currently, LC–MS is widely applied for the analysis of herbal constituents in vitro and in vivo because of its superior sensitivity, selectivity and ability to conclusively identify the compounds [26–29]. In this study, an approach of high-performance liquid chromatography (HPLC) quadrupole time-of-flight mass spectrometry (QTOF-MS) based on serum pharmacochemistry was developed to identify the phytochemical constituents of FZLZP and multiple absorbed components in rat serum.

Methods

The Minimum Standards of Reporting Checklist contains details of the experimental design, and statistics, and resources used in this study (Additional file 1).

Chemicals and materials

Nine reference compounds were obtained from Sichuan Victor Biological Technology Co. Ltd. (Chengdu China). HPLC grade Ethanol, formic acid and methanol were obtained from Fisher (ThermoFisher Scientific Inc, Waltham, MA, USA). Deionised water (18 MΩ) was prepared by distilled water through a Milli-Q system (Millipore, Milford, MA, USA). Fuzi (No. 1703003), Dangshen (No. 1705003), Baizhu (No. 1704088), Ganjiang (No. 1703060) and Gancao (No. 1703034) were purchased from Sichuan Neautus Traditional Chinese Medicine Co., Ltd. (Chengdu China) and were authenticated by Prof. Jin Pei, Department of Pharmacognosy of Chengdu University of Chinese Medicine.

Preparation of FZLZP

Fuzi, Ganjiang, Dangshen, Baizhu and Gancao were ground into fine powers and weighed according to the instructions recorded in Chinese Pharmacopoeia (2015 edition) and mixed well. Honey was heated at 116–118 °C until bright yellow uniform bubbles appeared on the surface and the honey became sticky. Mixed power and thermal refined honey were mixed at a ratio of 1:0.8 and were made into FZLZP (there is 0.153 kg crude aconite for every 1 kg FZLZP).

Preparation of FZLZP extract samples for LC/MS analysis

FZLZP (1.5 g) was weighed and reflux-extracted with 50 mL 70% ethanol for 1 h. Then, the filtered supernatant sample was rotary evaporated at 40 °C to a concentration of 15 mL, and was centrifuged at 5000 revolutions/min (rpm) for 5 min. The solution was filtered through a 0.22-μm membrane for further analysis.

Animal handling and serum sample preparation

Eighteen male Sprague–Dawley rats (200 ± 20 g) were obtained from the Sichuan Dashuo Biotechnology Co., Ltd. and were randomly divided into three groups of 6 rats each (group A, FZLZP group for dosed rat serum; group B, Fuzi powder (FZP) group for dosed rat serum; group C, control group for blank rat serum). The animal facilities and protocols conformed to the Care and Use of Laboratory Animals published by the National Institutes of Health. The experiment was approved by the ethical committee of Chengdu University of TCM (No.20161105). The rats were housed in an animal room with a controlled environment (20–25 °C, 65–69% relative humidity, 12 h dark–light cycle), and were given water and fed normal food for 1 week before the experiment. All animals were fasted overnight before the experiments and had free access to water.

The FZLZP was dissolved in 0.5% CMC-Na and were grinded to prepare the FZLZP suspension (150 mg crude drug/mL). Fuzi powder was dissolved in 0.5% CMC-Na to prepare the FZPsuspension (23 mg crude drug/mL, the concentration of FuZi was calculated by the ratio in FZLZP). Group A was intragastric administration 1.5 g/kg body weight of FZLZP suspension for 3 days. Group B was intragastric administration 0.23 g/kg body weight of FZP suspension for 3 days. Group C was intragastric administration with an equivalent volume of 0.5% CMC-Na. Blood samples were collected from the abdominal aorta 45 min after oral administration on the 3rd day and were placed at room temperature for 1 h until solidification. Then, samples were centrifuged at 3000 rpm for 10 min at 4 °C. All samples were stored at − 80 °C until analysis. Three times methanol was added to the 2 mL serum samples, vortexed and then, centrifuged at 12,000 rpm for 20 min. The supernatant was dried with nitrogen gas. The residue was redissolved in 50 μL methanol, vortexed and then, centrifuged at 12,000 rpm for 20 min, and the filtrate was used as the LC/MS sample. 10 μL aliquot was injected for HPLC/MS analysis.

HPLC-QTOF-MS analysis condition

Chromatographic analysis was performed in an Agilent 1290 HPLC system controlled with MassHunter Workstation Software (V B.05.00, Agilent Technologies Inc, Santa Clara, CA, USA). Samples were separated on an Agilent HC-C_{18} column (4.6 × 250 mm, 5.0 μm, Agilent Technologies Inc.) held at 35 °C and the flow rate was 1.0 mL/min with the injection volume of 10 μL. The mobile phase consisted of 0.1% formic acid–water (v/v, A) and methanol (B). The optimal gradient elution programme was as follows: 0–15 min, 95–70% A; 15–30 min, 70–48% A; 30–45 min, 48–25% A; 45–48 min, 25–15% A; 48–55 min, 15–2% A; and 55–65 min, 2–2% A.

Mass spectrometry conditions

Mass spectrometry was performed using an Agilent 6540 QTOF–MS (Agilent Corp., USA) equipped with a Dual AJS electrospray ionization (ESI) source, and the following operating parameters were used: positive mode, drying gas (nitrogen, N_2); flow rate, 8.0 L/min; gas temperature, 325 °C; nebulizer, 40 psig; sheath gas temperature, 350 °C; sheath gas flow, 11 L/min; capillary voltage, 4000 V; skimmer, 65 V; OCT 1 RF Vpp, 750 V; fragmentor, 110 V. The sample collision energy was set at 10, 20, 30 and 40 V. All the operations, acquisition, and analyses of data were controlled by Agilent LCMS-QTOF Mass Hunter Acquisition Software Ver. B.06.00 (Agilent Technologies Inc.) and operated under Mass Hunter Workstation Software Version B.06.00 (Agilent Technologies Inc.).

Establishment of FZLZP database

By searching databases, such as PubMed of the US National Library Medicine and the National Institutes of Health, SciFinder Scholar of American Chemical Society and the Chinese National Knowledge Infrastructure (CNKI) of Tsinghua University, all components reported in the literature on *Aconitum carmichaeli* Debx., *Codonopsis pilosula* (Franch.) Nannf., *Atractylodes macrocephala* Koidz., *Glycyrrhiza uralensis* Fisch. and *Zingiber officinale* Rosc. were summarized in an Agilent PCDL software Ver. B.06.00 (Agilent Technologies Inc.) to establish a database, which includes the name, molecular formula, chemical structure and literatures of each published known compound.

Results

Characterization of chemical constituents from FZLZP

Using the optimal conditions described above, all information on the MS data that was obtained from the robust HPLC-TOF-MS analysis, indicated the retention time and precise molecular mass and provided the MS/MS data. The protonated molecular weights of all target compounds were calculated within an error of 5 ppm. The base peak chromatogram (BPC) of the FZLZP extract sample in positive and negative ion modes are shown in Fig. 1A, and the data were processed by

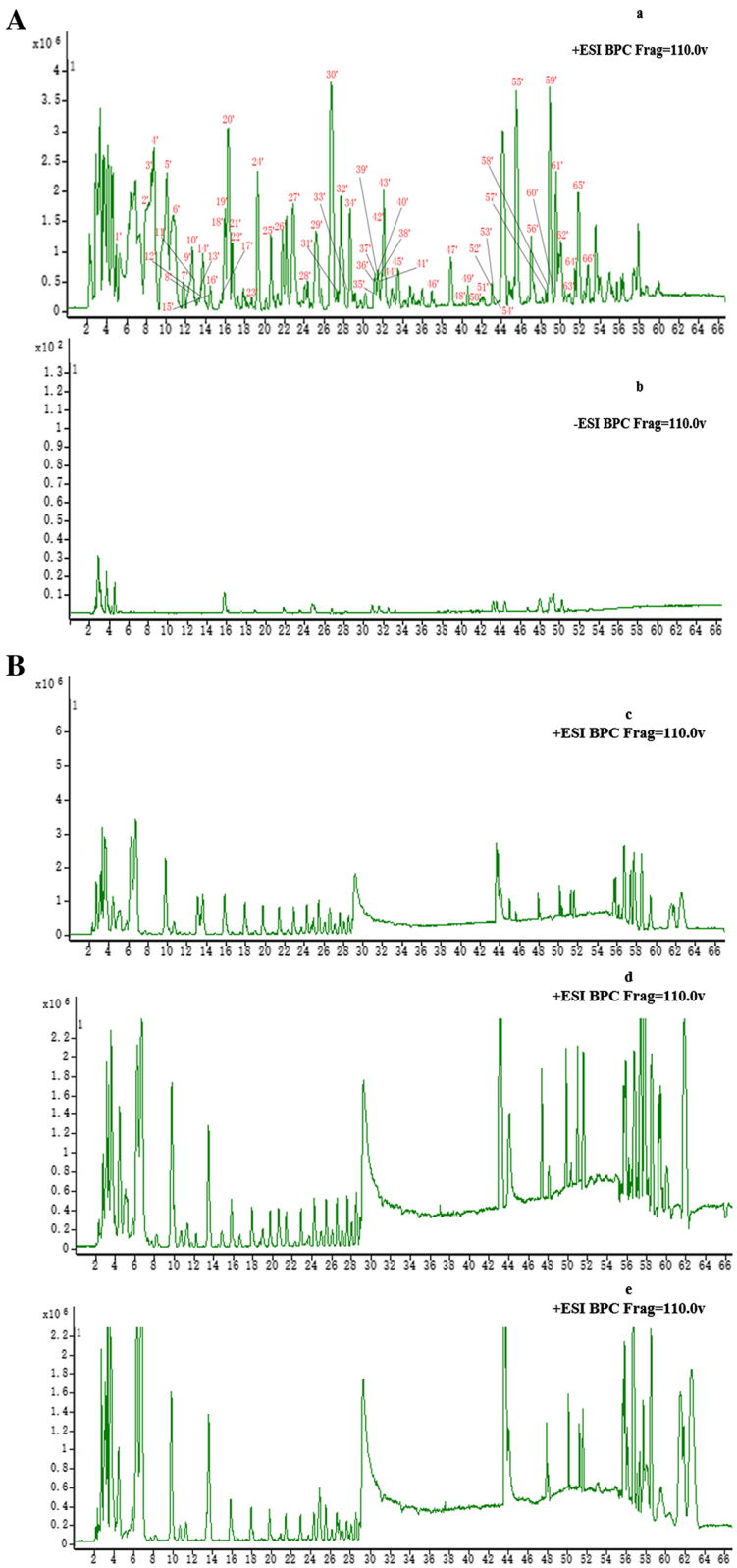

Fig. 1 The HPLC-ESI/QTOF/MS BPC chromatograms (**A** FZLZP extract samples: **a** in positive mode, **b** in negative mode; **B** Serum samples: **c** controlled serum in positive mode, **d** dosed FZLZP serum in positive mode, **e** dosed FZP serum in positive mode.)

the Agilent MassHunter Qualitative Analysis B.06.00 Workstation Software with the "find compounds by molecular formula" tool. A total of 73 peaks were obtained, and 67 compounds were identified or tentatively characterized by comparing the t_R values and the MS fragment characteristics of the compounds.

The reference standards are summarized in Table 1 and their fragmentation mechanism are proposed in Fig. 2. The compounds in FZLZP which are identified by the reference standards are summarized and marked in Table 2. For example, reference standards (RS) 1 liquiritigenin in Table 1 were detected in the positive ion mode at the Rt in 24.843 min with the *m/z* of 257.0809 ($C_{15}H_{13}O_4$). Its

Table 1 Retention time, m/z values of ions of reference standards

Peak no.	Rt (min)	Systematic name	Molecular formula	$[M+H]^+$		$[M+Na]^+$		Fragmentations (m/z)
				Measured mass (m/z)	Error (ppm)	Measured mass (m/z)	Error (ppm)	
1	24.843	Liquiritigenin	$C_{15}H_{12}O_4$	257.0809	0.3890			257.0809$[M+H]^+$, 239.0698$[M+H–H_2O]^+$, 137.0234 $[C_7H_4O_3+H]^+$, 121.0293 $[C_8H_8O+H]^+$, 120.0721 $[C_7H_4O_3+H–OH]^+$
2	27.507	Benzoylmesaconine	$C_{31}H_{43}NO_{10}$	590.2952	− 1.3553	–	–	590.2952$[M+H]^+$, 572.2832$[M+H–H_2O]^+$, 558.2683$[M+H\text{-}CH_3OH]^+$, 540.2580$[M+H–CH_3OH–H_2O]^+$
3	28.228	Benzoylaconine	$C_{32}H_{45}NO_{10}$	604.3130	2.3167	–	–	604.3130$[M+H]^+$, 586.2995$[M+H–H_2O]^+$, 572.2852$[M+H–CH_3OH]^+$ 554.2735$[M+H–2H_2O]^+$, 540.2577$[M+H–CH_3OH]^+$, 522.2475$[M+H–2CH_3OH–H_2O]^+$
4	29.152	Benzoylhypaconine	$C_{31}H_{43}NO_9$	574.3003	− 1.3930	–	–	574.3003$[M+H]+$, 542.2741$[M+H–CH_3OH]^+$, 524.2615$[M+H–CH_3OH–H_2O]^+$, 510.2477$[M+H–2CH_3OH]^+$
5	31.663	Mesaconitine	$C_{33}H_{45}NO_{11}$	632.3064	− 0.1582	–	–	632.3064$[M+H]^+$, 600.2787$[M+H–CH_3OH]^+$, 572.2853$[M+H–AcOH]^+$, 540.2594$[M+H–AcOH–CH_3OH]^+$, 512.2637$[M+H–AcOH–CH_3OH–CO]^+$,
6	39.648	Isoliquiritigenin	$C_{15}H_{12}O_4$	257.0809	0.3890			257.0809$[M+H]^+$, 239.0692$[M+H–H_2O]^+$, 137.0235$[C_7H_4O_3+H]^+$, 121.0287 $[C_8H_8O+H]^+$, 120.0514 $[C_7H_4O_3+H–OH]^+$
7	48.854	Atractylenolide II	$C_{15}H_{20}O_2$	233.1538	0.8578			233.1538$[M+Na]^+$, 215.1440$[M+Na–H_2O]^+$, 187.1484$[M+Na–CH_2O_2]^+$, 159.1165$[M+Na–CH_2O_2–C_2H_4]^+$, 145.101 $[M+Na–CH_2O_2–C_3H_6]^+$ 131.0856$[M+Na–CH_2O_2–C_4H_8]^+$, 105.0702$[M+Na–CH_2O_2–C_4H_8–C_2H_2]^+$,
8	49.134	Glycyrrhizic acid	$C_{42}H_{62}O_{16}$			845.3947	2.0109	845.3947$[M+Na]^+$, 669.3614$[M+Na–(GluA–H_2O)]^+$
9	55.125	Glycyrrhetinic acid	$C_{30}H_{46}O_4$	471.3458	− 2.3337			471.3458$[M+H]^+$, 453.3349$[M+H–H_2O]^+$, 435.3244$[M+H\text{-}2H_2O]^+$

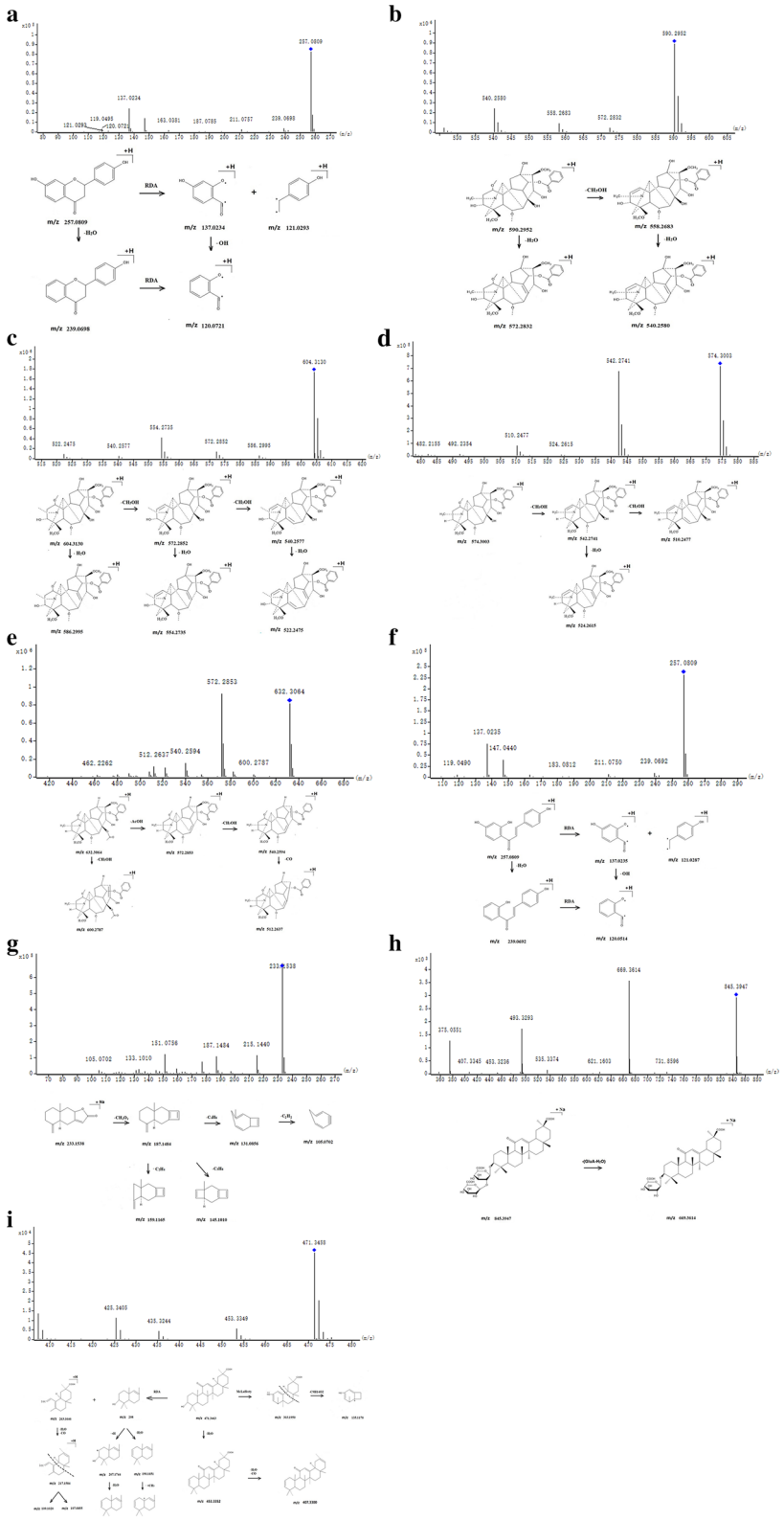

Fig. 2 The mass fragment and fragmentation pathway of **a** Liquiritigenin, **b** Benzoylmesaconine, **c** Benzoylaconine, **d** Benzoylhypaconine, **e** mesaconitine, **f** Isoliquiritigenin, **g** Atractylenolide II, **h** Glycyrrhizic acid, **i** Glycyrrhetinic acid

Table 2 Identification information of constituents in vitro of FZLZP by HPLC-ESI/QTOF/MS

Peak no.	Rt (min)	Systematic name	Molecular formula	Molecular weight	$[M+H]^+$		$[M+Na]^+$		Fragmentations (m/z)	Source
					Measured mass (m/z)	Error (ppm)	Measured mass (m/z)	Error (ppm)		
1	5.091	L-Pyroglutamic acid	$C_5H_7NO_3$	129.0426	130.0505	4.6136			130.0505$[M+H]^+$, 112.0123$[M+H–H_2O]^+$, 84.0449$[M+H–HCOOH]^+$	Dangshen
2	8.051	Codonopsine	$C_{14}H_{21}NO_4$	267.1471	268.1543	0			268.1543$[M+H]^+$, 250.1451$[M+H–H_2O]^+$, 205.0863$[M+H–2CH_3OH]^+$	Dangshen
3	9.229	5-hydroxymethyfurfural	$C_6H_6O_3$	126.0317	127.0394	3.1486			127.0394$[M+H]^+$, 109.0291$[M+H–H_2O]^+$	Dangshen
4	9.398	Karakolidine	$C_{22}H_{35}NO_5$	393.2515	394.2590	0.5072			394.2590$[M+H]^+$, 376.2489$[M+H–H_2O]^+$, 358.2371$[M+H–2H_2O]^+$	Fuzi
5	10.142	Phenylalanine	$C_9H_{11}NO_2$	165.0790	166.0872	5.4188			166.0872$[M+H]^+$, 120.0817$[M+H–HCOOH]^+$	Dangshen
6	11.288	Senbusine A	$C_{23}H_{37}NO_6$	423.2621	424.2696	0.4713			424.2696$[M+H]^+$, 406.2579 $[M+H–H_2O]^+$	Fuzi
7	11.407	9-OH-senbusine A	$C_{23}H_{37}NO_7$	439.2570	440.2635	−1.8170			440.2635$[M+H]^+$, 422.2531$[M+H–H_2O]^+$, 408.2318$[M+H–CH_3OH]^+$	Fuzi
8	12.042	16-β-hydroxycardiopetaline	$C_{21}H_{33}NO_4$	363.2410	364.2480	−0.5490			364.2480$[M+H]^+$, 346.2372$[M+H–H_2O]^+$, 328.2273$[M+H–2H_2O]^+$	Fuzi
9	12.389	Mesaconine	$C_{24}H_{39}NO_9$	485.2625	486.2697	−0.2056			486.2697 $M+H]^+$, 468.2573$[M+H–H_2O]^+$, 436.2323$[M+H–H_2O–CH_3OH]$	Fuzi
10	12.578	Songorine	$C_{22}H_{31}NO_3$	357.2304	358.2382	1.3957			358.2382$[M+H]^+$, 340.2267$[M+H–H_2O]^+$	Fuzi
11	12.908	Karakoline	$C_{22}H_{35}NO_4$	377.2566	378.2639	0			378.2639$[M+H]^+$, 360.2533$[M+H–H_2O]^+$	Fuzi
12	13.081	Isotalatizidine	$C_{23}H_{37}NO_5$	407.2672	408.2743	−0.2449			408.2743$[M+H]^+$, 390.2630$[M+H–H_2O]^+$, 372.2517$[M+H–2H_2O]^+$, 358.2374$[M+H–H_2O–CH_3OH]^+$	Fuzi
13	13.109	Senbusine B	$C_{23}H_{37}NO_6$	423.2621	424.2707	3.0640			424.2707$[M+H]^+$, 406.2584 $[M+H–H_2O]^+$	Fuzi
14	13.937	14-Acetylkarakoline	$C_{24}H_{37}NO_5$	419.2672	420.2750	1.4276			420.2750$[M+H]^+$, 402.1695$[M+H–H_2O]^+$, 356.1122$[M+H–H_2O–2CH_3OH]^+$,	Fuzi

Table 2 (continued)

Peak no.	Rt (min)	Systematic name	Molecular formula	Molecular weight	$[M+H]^+$		$[M+Na]^+$		Fragmentations (m/z)	Source
					Measured mass (m/z)	Error (ppm)	Measured mass (m/z)	Error (ppm)		
15	14.091	Aconine	$C_{25}H_{41}NO_9$	499.2781	500.2850	−0.7995			500.2850$[M+H]^+$, 482.2741$[M+H-H_2O]^+$, 468.2564$[M+H-CH_3OH]^+$, 450.2478$[M+H-H_2O-CH_3OH]^+$, 436.2309$[M+H-2CH_3OH]^+$, 418.2209$[M+H-H_2O-2CH_3OH]^+$	Fuzi
16	14.380	Hetisine	$C_{20}H_{27}NO_3$	329.1991	330.2064	0			330.2064$[M+H]^+$, 312.1951$[M+H-H_2O]^+$	Fuzi
17	15.319	Hypaconine	$C_{24}H_{39}NO_8$	469.2676	470.2744	−0.8506			470.2744$[M+H]^+$, 453.2301$[M+H-OH]^+$, 438.2474$[M+H-CH_3OH]^+$, 406.2212$[M+H-2CH_3OH]^+$, 374.1941$[M+H-3CH_3OH]^+$	Fuzi
18	15.810	Fuzitine	$C_{20}H_{23}NO_4$	341.1627	342.1697	−0.8767			342.1697$[M+H]^+$, 324.1026$[M+H-H_2O]^+$	Fuzi
19	16.070	Fuziline	$C_{24}H_{39}NO_7$	453.2727	454.2800	0.2201			454.2800$[M+H]^+$, 436.2677$[M+H-H_2O]^+$, 418.2583$[M+H-2H_2O]^+$, 404.2443$[M+H-H_2O-CH_3OH]^+$, 386.2295$[M+H-2H_2O-CH_3OH]^+$, 354.2069$[M+H-2H_2O-2CH_3OH]^+$	Fuzi
20	16.248	Tau-cadinol	$C_{15}H_{26}O$	222.1984	245.1852	−9.7884			245.1852$[M+H]^+$, 213.0195$[M+H-CH_3OH]^+$, 199.1252$[M+H-CH_3OH-CH_3]^+$, 184.9885$[M+H-CH_3OH-2CH_3]^+$, 169.0055$[M+H-CH_3OH-3CH_3]^+$,	Ganjiang
21	16.573	Neoline	$C_{24}H_{39}NO_6$	437.2777	438.2848	−0.4563			438.2848 $M+H]^+$, 420.2756$[M+H-H_2O]^+$, 388.2478$[M+H-H_2O-CH_3OH]^+$, 370.2365$[M+H-2H_2O-CH_3OH]^+$, 356.2213$[M+H-H_2O-2CH_3OH]^+$	Fuzi

Table 2 (continued)

Peak no.	Rt (min)	Systematic name	Molecular formula	Molecular weight	$[M+H]^+$		$[M+Na]^+$		Fragmentations (m/z)	Source
					Measured mass (m/z)	Error (ppm)	Measured mass (m/z)	Error (ppm)		
22	16.743	Talatisamine	$C_{24}H_{39}NO_5$	421.2828	422.2899	0.4736			422.2899$[M+H]^+$, 390.2621$[M+H–CH_3OH]^+$, 358.2349$[M+H–2CH_3OH]^+$	Fuzi
23	18.651	Chasmanine	$C_{25}H_{41}NO_6$	451.2934	452.3008	02210			452.3008$[M+H]^+$, 420.2737$[M+H–CH_3OH]^+$	Fuzi
24	19.739	Geranial	$C_{10}H_{16}O$	152.1201	153.1275	0.6530			153.1275$[M+H]^+$, 135.1162$[M+H–H_2O]^+$, 125.0940$[M+H–CO]^+$	Ganjiang
25	20.390	14-Acetyltalatizamine	$C_{26}H_{41}NO_6$	463.2934	464.3014	1.5076			464.3014$[M+H]^+$, 432.2753$[M+H–CH_3OH]^+$, 414.2645$[M+H–CH_3OH–H_2O]^+$, 400.2486$[M+H–2CH_3OH]^+$, 372.2522$[M+H–CH_3OH–AcOH]^+$	Fuzi
26	21.828	7-hydroxycoumarin	$C_9H_6O_3$	162.0317	163.0395	3.0667			163.0395$[M+H]^+$, 145.0627$[M+H–H_2O]^+$	Baizhu
27	23.891	Schaftoside	$C_{26}H_{28}O_{14}$	564.1479	565.1542	−1.7694			565.1542$[M+H]^+$, 547.1434$[M+H–H_2O]^+$, 529.1303$[M+H–2H_2O]^+$, 511.1220$[M+H–3H_2O]^+$	Gancao
28	24.041	Scopoletin	$C_{10}H_8O_4$	192.0423	193.0500	2.5900			193.0500$[M+H]^+$, 161.0603$[M+H–CH_3OH]^+$	Baizhu
29#	24.785	Liquiritigenin	$C_{15}H_{12}O_4$	256.0736	257.0819	4.2788			257.0819$[M+H]^+$, 239.0707$[M+H–H_2O]^+$, 137.0235$[C_7H_4O_3+H]^+$, 121.0280$[C_8H_8O+H]^+$, 120.0525 $[C_7H_4O_3+H–OH]^+$	Gancao
30#	27.065	Benzoylmesaconine	$C_{31}H_{43}NO_{10}$	589.2887	590.2959	−0.1694	–	–	590.2959$[M+H]^+$, 572.2826$[M+H–H_2O]^+$, 558.2663$[M+H–CH_3OH]^+$ 540.2573$[M+H–CH_3OH–H_2O]^+$	Fuzi
31	27.325	Isoviolanthin	$C_{27}H_{30}O_{14}$	578.1636	579.1700	−1.3812			579.1700$[M+H]^+$, 561.1588$[M+H–H_2O]^+$, 543.1485$[M+H–2H_2O]^+$, 525.1382$[M+H–3H_2O]^+$	Gancao
32#	27.614	Benzoylaconine	$C_{32}H_{45}NO_{10}$	603.3043	604.3114	−0.3309			604.3114$[M+H]^+$, 587.2801$[M+H–OH]^+$, 554.2711$[M+H–2CH_3OH]^+$	Fuzi

Table 2 (continued)

Peak no.	Rt (min)	Systematic name	Molecular formula	Molecular weight	$[M+H]^+$		$[M+Na]^+$		Fragmentations (m/z)	Source
					Measured mass (m/z)	Error (ppm)	Measured mass (m/z)	Error (ppm)		
33#	28.595	Benzoylhypaconine	$C_{31}H_{43}NO_9$	573.2938	574.3011	0			574.3011$[M+H]^+$, 542.2745$[M+H-CH_3OH]^+$,,510.2457$[M+H-2CH_3OH]^+$	Fuzi
34	28.748	Lobetyolinin	$C_{26}H_{38}O_{13}$	558.2312			581.2203	−0.3441	581.2203$[M+Na]^+$, 419.1709$[M+Na-C_6H_{10}O_5]^+$	Dangshen
35	31.019	Liquiritin apioside or Isoliquiritin apioside	$C_{26}H_{30}O_{13}$	550.1686	551.1751	−1.4514			551.1751$[M+H]^+$, 419.1333$[M+H-(Apiose-H_2O)]^+$, 257.0830$[M+H-(Apiose-H_2O)-(Glc-H_2O)]^+$	Gancao
36#	31.163	Mesaconitine	$C_{33}H_{45}NO_{11}$	631.2993	632.3067	0.3163	–	–	632.3067$[M+H]^+$, 614.1110$[M+H-H_2O]^+$, 600.2748$[M+H-CH_3OH]^+$, 572.2834$[M+H-AcOH]^+$	Fuzi
37	31.423	7-methoxy-liquiritin	$C_{22}H_{22}O_9$	430.1264	431.1332	−1.1597			431.1332$[M+H]^+$, 269.0811$[M+H-(Glc-H_2O)]^+$	Gancao
38	31.646	14-Benzoylneoline	$C_{31}H_{43}NO_7$	541.3040	542.3135	4.2411			542.3135$[M+H]^+$, 524.3010$[M+H-H_2O]^+$, 510.2731$[M+H-CH_3OH]^+$, 492.2733$[M+H-H_2O-CH_3OH]^+$	Fuzi
39	31.659	Dehydrated benzoylhypaconine	$C_{31}H_{41}NO_8$	555.2832	556.2906	0.1798			556.2906$[M+H]^+$, 524.2647$[M+H-CH_3OH]^+$, 492.2381$[M+H-2CH_3OH]^+$	Fuzi
40	31.683	Liquiritin or Isoliquiritin	$C_{21}H_{22}O_9$	418.1264	419.1335	0.4771			419.1335$[M+H]^+$, 257.0811$[M+H-(Glc-H_2O)]^+$	Gancao
41	31.921	Aconifine	$C_{34}H_{47}NO_{12}$	661.3098	662.3172	0.1509			662.3172$[M+H]^+$, 644.3095$[M+H-H_2O]^+$, 626.1346 $[M+H-2H_2O]^+$	Fuzi
42	32.100	Hypaconitine	$C_{33}H_{45}NO_{10}$	615.3043	616.3116	0			616.3116$[M+H]^+$, 584.2843$[M+H-CH_3OH]^+$ 556.2899$[M+H-C_2H_4O_2]^+$, 524.2533$[M+H-C_2H_4O_2-CH_3OH]^+$, 496.2678$[M+H-C_2H_4O_2-CH_3OH-CO]^+$	Fuzi

Table 2 (continued)

Peak no.	Rt (min)	Systematic name	Molecular formula	Molecular weight	$[M+H]^+$		$[M+Na]^+$		Fragmentations (m/z)	Source
					Measured mass (m/z)	Error (ppm)	Measured mass (m/z)	Error (ppm)		
43	32.245	Formononetin	$C_{16}H_{12}O_4$	268.0736	269.0814	2.2298			269.0814$[M+H]^+$, 254.0580$[M+H–CH_3]^+$, 237.0536$[M+H–CH_3OH]^+$, 225.0554$[M+H–CH_3–CO]^+$, 213.0908$[M+H–C_2O_2]^+$, 181.0666$[M+H–C_2O_2–CH_3OH]^+$	Gancao
44	32.528	Aconitine	$C_{34}H_{47}NO_{11}$	645.3149	646.3216	−0.9283			646.3216$[M+H]^+$, 628.3140$[M+H–H_2O]^+$, 596.2849$[M+H–H_2O–CH_3OH]^+$	Fuzi
45	33.241	Deoxyaconitine	$C_{34}H_{47}NO_{10}$	629.3200	630.3273	0			630.3273$[M+H]^+$, 598.3070$[M+H–CH_3OH]^+$	Fuzi
46	36.853	Echinatin	$C_{16}H_{14}O_4$	270.0892	271.0963	−0.7377			271.0963$[M+H]^+$, 253.0850$[M+H–H_2O]^+$	Gancao
47	38.085	Benzoic acid	$C_7H_6O_2$	122.0368	123.0447	4.8763			123.0447$[M+H]^+$, 77.0379$[M+H–HCOOH]^+$	Baizhu
48[#]	39.763	Isoliquiritigenin	$C_{15}H_{12}O_4$	256.0736	257.0814	2.334			257.0814$[M+H]^+$, 239.0704$[M+H–H_2O]^+$, 137.0235$[C_7H_4O_3+H]^+$, 121.0277$[C_8H_8O+H]^+$, 120.0527 $[C_7H_4O_3+H–OH]^+$	Gancao
49	40.720	Glycycoumarin	$C_{21}H_{20}O_6$	368.1260	369.1345	3.2508			369.1345$[M+H]^+$, 333.2235$[M+H–2H_2O]^+$, 313.1057 $[M+H–C_4H_8]^+$,	Gancao
50	41.513	6-gingerdione	$C_{17}H_{24}O_4$	292.1675	293.1736	−2.7520			293.1736$[M+H]^+$, 275.1650$[M+H–H_2O]^+$ 257.1517$[M+H–2H_2O]^+$	Ganjiang
51	42.593	Kumatakenin	$C_{17}H_{14}O_6$	314.0790	315.0859	−1.2694			315.0859$[M+H]^+$, 298.2146$[M+H–OH]^+$, 279.0782$[M+H–2H_2O]^+$	Ganjiang
52	43.486	6-gingerol	$C_{17}H_{26}O_4$	294.1831			317.1737	4.4140	317.1771$[M+Na]^+$, 299.2546$[M+Na\text{-}H_2O]^+$	Ganjiang
53	43.507	Gingerenone-A	$C_{21}H_{24}O_5$	356.1624	357.1710	3.6397			357.1710$[M+H]^+$, 339.2718$[M+H–H_2O]^+$, 321.2612$[M+H–2H_2O]^+$	Ganjiang
54	43.544	6-shogaol	$C_{17}H_{24}O_3$	276.1725	277.1795	1.0823			277.1795$[M+H]^+$, 259.1694$[M+Na–H_2O]^+$,	Ganjiang
55	45.779	lupiwighteone	$C_{20}H_{18}O_5$	338.1154	339.1239	3.5385			339.1239$[M+H]^+$, 321.2818$[M+H–H_2O]^+$	Gancao

Table 2 (continued)

Peak no.	Rt (min)	Systematic name	Molecular formula	Molecular weight	$[M+H]^+$		$[M+Na]^+$		Fragmentations (m/z)	Source
					Measured mass (m/z)	Error (ppm)	Measured mass (m/z)	Error (ppm)		
56	46.339	Atractylenolide III	$C_{15}H_{20}O_3$	248.1412	245.1485	0			249.1485$[M+H]^+$, 231.1389$[M+H-H_2O]^+$, 175.0751$[M+H-H_2O-2CO]^+$, 163.0756$[M+H-H_2O-C_5H_8]^+$	Baizhu
57	48.364	Gancaonin L	$C_{20}H_{18}O_6$	354.1103	355.1189	3.6607			355.1189$[M+H]^+$, 337.2536$[M+H-H_2O]^+$	Gancao
58	48.398	Licoricesaponin G2	$C_{42}H_{62}O_{17}$	838.3987	839.4076	1.9061			839.4076$[M+H]^+$, 663.3722$[M+H-(GluA-H_2O)]]^+$, 469.3308$[M+H-2\ (GluA-H_2O)-H_2O]^+$	Gancao
59#	48.887	Atractylenolide II	$C_{15}H_{20}O_2$	232.1463	233.1541	2.1445			233.1541$[M+Na]^+$, 187.1485$[M+Na-CH_2O_2]^+$, 159.0806$[M+Na-CH_2O_2-C_2H_4]^+$, 145.1013 $[M+Na-CH_2O_2-C_3H_6]^+$, 131.0857$[M+Na-CH_2O_2-C_4H_8]^+$, 105.0703$[M+Na-CH_2O_2-C_4H_8-C_2H_2]^+$	Baizhu
60#	49.296	Glycyrrhizic acid	$C_{42}H_{62}O_{16}$	822.4038	823.4130	2.3075			823.4130 $[M+H]^+$, 647.3793$[M+H-(GluA-H_2O)]]^+$	Gancao
61	49.667	Farnesal	$C_{15}H_{24}O$	220.1827	221.1907	3.1647			221.1907 $M+H]^+$, 192.9740$[M+H-CO]^+$	Ganjiang
62#	49.841	Glycyrrhetinic acid	$C_{30}H_{46}O_4$	470.3396	471.3488	4.031			471.3488$[M+H]^+$, 453.3354$[M+H-H_2O]^+$, 435.3224$[M+H-2H_2O]^+$, 425.3378$[M+H-HCOOH]^+$	Gancao
63	50.671	Licorice saponin B2	$C_{42}H_{64}O_{15}$	808.4245			831.4151	1.6838	831.4151 $[M+Na]^+$, 655.3825$[M+Na-(GluA-H_2O)]]^+$, 479.3547$[M+Na-2\ (GluA-H_2O)]]^+$	Gancao
64	51.232	Licoricone	$C_{22}H_{22}O_6$	382.1416	383.1502	3.3929			383.1502$[M+H]^+$, 355.1587$[M+H-C_2H_4]^+$	Gancao
65	51.390	Atractylenolide I	$C_{15}H_{18}O_2$	230.1307	231.1383	1.2979			231.1383$[M+H]^+$, 185.1326$[M+H-HCOOH]^+$, 157.1012$[M+H-HCOOH-C_2H_4]^+$, 105.0701 $[M+H-HCOOH-2C_2H_4-2C]^+$	Baizhu

Table 2 (continued)

Peak no.	Rt (min)	Systematic name	Molecular formula	Molecular weight	$[M+H]^+$		$[M+Na]^+$		Fragmentations (m/z)	Source
					Measured mass (m/z)	Error (ppm)	Measured mass (m/z)	Error (ppm)		
66	52.950	Neoglycyrol	$C_{21}H_{18}O_6$	366.1103	367.1165	−0.5447			367.1165$[M+H]^+$, 349.2239$[M+H–H_2O]^+$, 335.2389$[M+H–CH_3OH]^+$, 317.2283$[M+H–2H_2O–CH_3OH]^+$	Gancao
67	54.310	Licorice-saponin J2	$C_{42}H_{64}O_{16}$	824.4194	825.4286	2.3018			825.4286$[M+H]^+$, 649.3906 $[M+H–(GluA–H_2O)]^+$, 455.3537$[M+H$-2 $(GluA–H_2O)–H_2O]^+$, 437.3435 $[M+H$-2 $(GluA–H_2O)–2H_2O]^+$	Gancao

[#] Indicates compounds identified by comparing with the reference standards

MS/MS data were shown as *m/z* of 239.0698[M + H–H_2O]$^+$, 137.0234 [$C_7H_4O_3$ + H]$^+$, 121.0293[C_8H_8O + H]$^+$ and 120.0721 [$C_7H_4O_3$ + H–OH]$^+$. And the compound 29 in Table 2 were detected in the positive ion mode at the Rt in 24.785 min with the *m/z* of 257.0819 ($C_{15}H_{13}O_4$), 239.0707[M + H–H_2O]$^+$ and 137.0235 [$C_7H_4O_3$ + H]$^+$. Then compound 29 were characterized as liquiritigenin. Similar to the identification process above, among 67 compounds, 9 compounds were identified as benzoylaconine, benzoylmesaconine, benzoylhypaconine, mesaconitine, liquiritigenin, isoliquiritigenin, glycyrrhizic acid, glycyrrhetinic acid and atractylenolide II. The MS data of the (+) ESI–MS spectra are shown in Table 2.

The remaining 58 compounds were tentatively characterized based on their chromatographic and spectrometric data, referring to previous literature [25, 30–33]. For example, MS^2 spectra of compound 4 (molecular ion at *m/z* [M + H]$^+$ 394.2590) in Table 2 gave characteristic fragment ions of [M + H–H_2O]$^+$ at *m/z* 376.2489 and [M + H–2H_2O]$^+$ at *m/z* 358.2371. Thus, it corresponded to Karakolidine by comparison with literature data [30]. Moreover, MS^2 spectra of compound 12 (molecular ion at *m/z* [M + H]$^+$ 408.2743) in Table 2 gave characteristic fragment ions of [M + H–H_2O]$^+$ at *m/z* 390.2630, 372.2517[M + H–2H_2O]$^+$ and [M + H–CH_3OH]$^+$ at *m/z* 358.2374. Then it was identified as Isotalatizidine. All the MS data of the (+) ESI–MS spectra are shown in Table 2. Besides, all the structures of the compounds identified are shown in Figs. 3 and 4. The deriving herb for each compound was also assigned. The majority of constituents are identified as alkaloids, flavonoids, triterpenes, gingerols, phenylpropanoids and volatile oil.

Characterization of the absorbed chemical constituents in rat serum

Identification of the bioactive chemical prototype constituents in rat serum

As the results of constituents in rat serum show in Table 3, by comparing the t_R values and MS fragment characteristics between compounds in serum and compounds in FZLZP extract, 10 alkaloid components sourced from *Aconitum carmichaeli Debx.* were identified, including benzoylaconine, benzoylmesaconine, benzoylhypaconine, mesaconitine, Hypaconitine, fuziline, neoline, talatisamine, chasmanine, and 14-acetyltalatizamine. These constituents have been reported as parts of the main constituents with significant effects of analgesia, anti-inflammation, thermogenesis and increasing blood oxygen in Fuzi [34, 35]. The MS data of the (+) ESI–MS spectra are shown in Table 3. For example, MS^2 spectra of compound 19 in Table 2 was detected at the Rt in 16.070 min with the molecular ion at *m/z* 454.2800[M + H]$^+$ and gave characteristic fragment ions of [M + H–H_2O]$^+$ at *m/z* 436.2677. Similarly, MS^2 spectra of compound 2 in Table 3 was detected at the Rt in 16.615 min with the molecular ion at *m/z* 454.2808[M + H]$^+$ and gave characteristic fragment ions of [M + H–H_2O]$^+$ at *m/z* 436.0243. Thus, compound 2 in Table 3 was identified as the absorbed prototype of Fuziline in rat serum. The other alkaloid components were identified in a similar way.

Six compounds sourced from *Glycyrrhiza uralensis* Fisch. were identified, including 3 flavonoids, namely, liquiritigenin, isoliquiritigenin, and formononetin and 2 triterpenes, namely, glycyrrhetinic acid and glycyrrhizic acid. The MS data of the (+)ESI–MS spectra are shown in Table 3. For example, MS^2 spectra of compound 48 in Table 2 was detected at the Rt in 39.763 min with the molecular ion at *m/z* 257.0814[M + H]$^+$ and gave characteristic fragment ions of 239.0704[M + H–H_2O]$^+$, 137.0235[$C_7H_4O_3$ + H]$^+$, 121.0277[C_8H_8O + H]$^+$, 120.0527 [$C_7H_4O_3$ + H–OH]$^+$. Similarly, MS^2 spectra of compound 14 in Table 3 was detected at the Rt in 40.710 min with the molecular ion at *m/z* 257.0807[M + H]$^+$ and gave characteristic fragment ions of [M + H–H_2O]$^+$ at *m/z* 239.1624. Thus, compound 14 in Table 3 was identified as the absorbed prototype of Isoliquiritigenin in rat serum. Furthermore, liquiritin or isoliquiritin may also have been found, but further comparison with reference compounds is needed to identify these isomers. The flavonoids and triterpenes in *Glycyrrhiza uralensis* Fisch. have been reported as having significant anti-inflammatory, abirritation and immunoregulation effects [36–38].

7-Hydroxycoumarin, atractylenolide I and atractylenolide II have been identified as bioactive chemical constituents sourced form *Atractylodes macrocephala* Koidz. (Baizhu) and were found as the main institutes with the effect of anti-inflammatory, antitumor and gastrointestinal regulation in Baizhu [39–42]. The MS data of the (+) ESI–MS spectra are shown in Table 3. For example, MS^2 spectra of compound 26 in Table 2 was detected with the molecular ion at *m/z* 163.0395 [M + H]$^+$ and gave characteristic fragment ions of 145.0627[M + H–H_2O]$^+$. Similarly, MS^2 spectra of compound 25 in Table 3 was detected with the molecular ion at *m/z* 163.0396[M + H]$^+$ and gave characteristic fragment ions of [M + H–H_2O]$^+$ at *m/z* 145.5012. Thus, compound 25 in Table 3 was identified as the absorbed prototype of 7-hydroxycoumarin in rat serum.

6-Gingerdione, 6-gingerol and 6-shogaol sourced from *Zingiber officinale* Rosc (Ganjiang) were identified and were reported as having obvious antioxidant, anti-inflammatory, gastrointestinal protective and antitumor effects [43, 44]. The MS data of the (+) ESI–MS spectra are shown in Table 3. For example, MS^2 spectra of compound

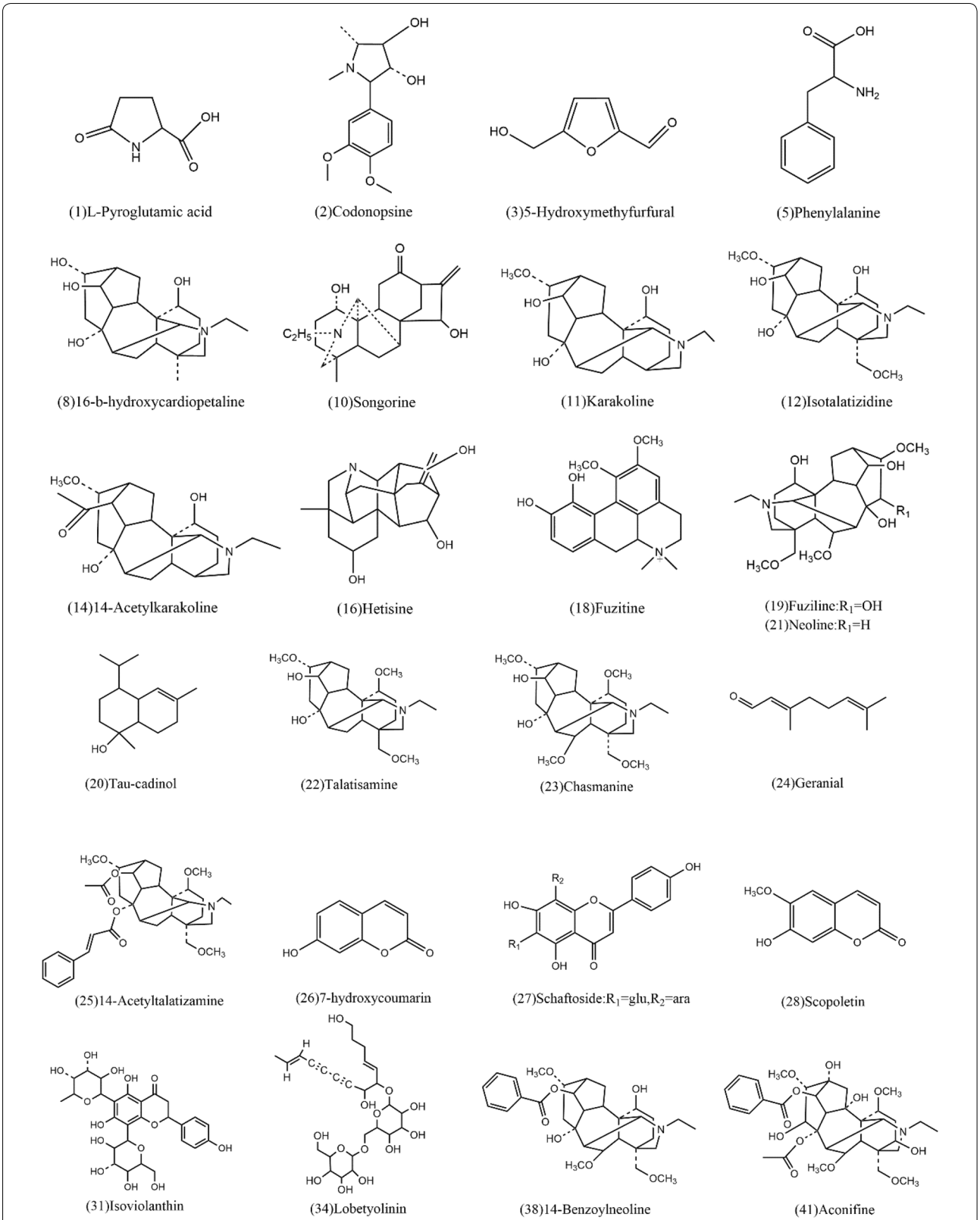

Fig. 3 Structures of compounds identified in the extract of Fuzi Lizhong Pill

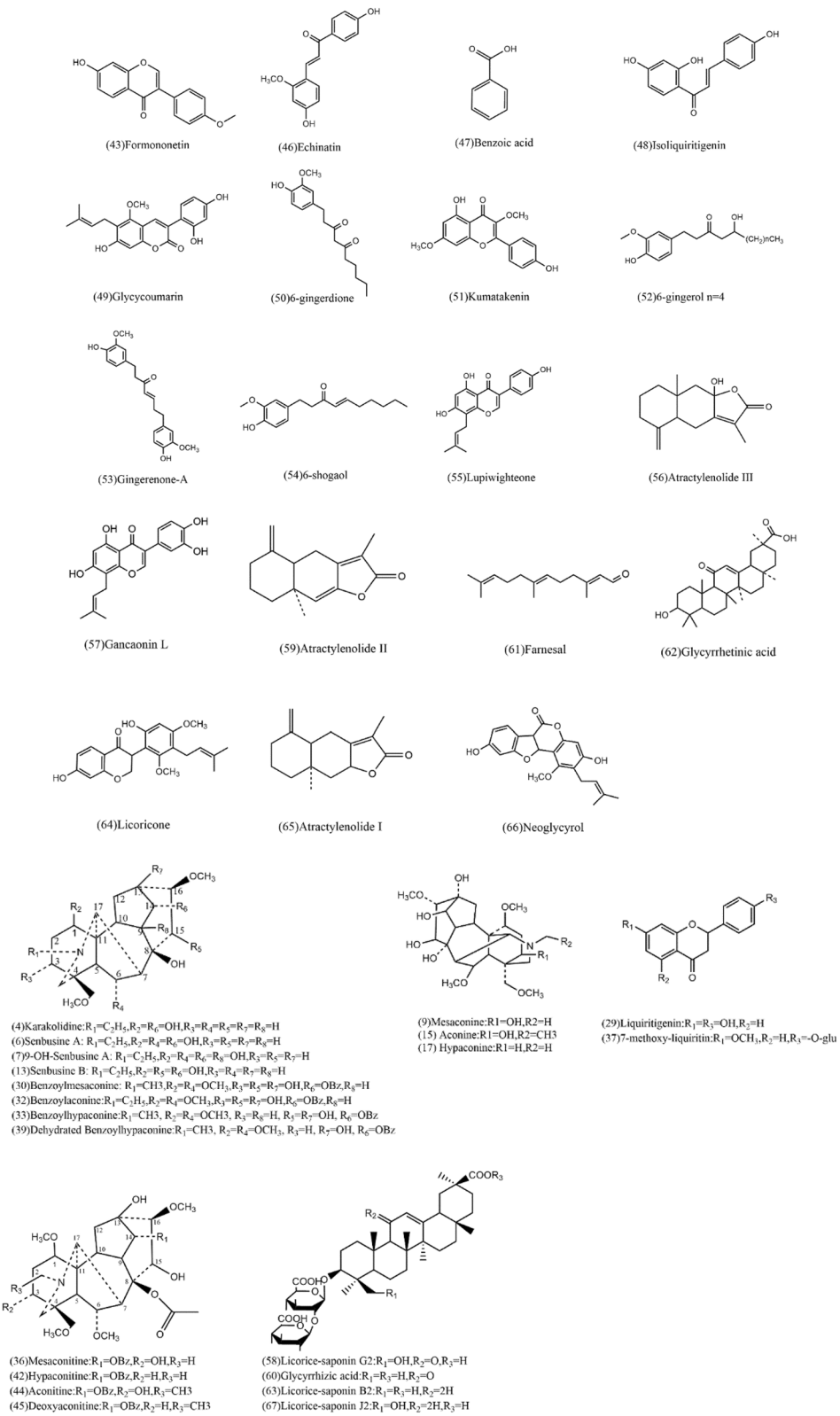

Fig. 4 Structures of compounds identified in the extract of Fuzi Lizhong Pill

Table 3 Characterization of chemical constituents in vivo and metabolites of FZLZP by HPLC-ESI/QTOF/MS

Peak no.	Rt (min)	Systematic name	Molecular formula	Molecular weight (Da)	$[M+H]^+$		$[M+Na]^+$		Fragmentations (m/z)	Source/ prototype
					Measured value (Da)	Error (ppm)	Measured value (Da)	Error (ppm)		
1	4.841	L-Pyroglutamic acid	$C_5H_7NO_3$	129.0426	130.0498	−0.7689			130.0498$[M+H]^+$, 112.9741$[M+H-H_2O]^+$	Dangshen
2	16.615	Fuziline	$C_{24}H_{39}NO_7$	453.2727	454.2808	1.981			454.2808$[M+H]^+$, 436.0243$[M+H-H_2O]^+$	Fuzi
3	17.021	Talatisamine	$C_{24}H_{39}NO_5$	421.2828	422.2905	0.9472			422.2905$[M+H]^+$, 390.2651$[M+H-CH_3OH]^+$, 359.3263$[M+H-CH_2OH-CH_3OH]^+$	Fuzi
4*	24.357	Glucuronide conjugation metabolite	$C_{21}H_{20}O_{10}$	432.1056	433.1132	0.6927			433.1132$[M+H]^+$, 257.0843$[M+H-(GluA-H_2O)]^+$	Liquiritigenin
5	25.811	Liquiritigenin	$C_{15}H_{12}O_4$	256.0736	257.0819	4.2788			257.0819 $[M+H]^+$, 239.0713$[M+H-H_2O]^+$, 137.0237$[C_7H_4O_3+H]^+$	Gancao
6	27.236	Benzoylmesaconine	$C_{31}H_{43}NO_{10}$	589.2887	590.2948	−2.033			590.2948$[M+H]^+$, 558.2657$[M+H-CH_3OH]^+$ 540.2537$[M+H-CH_3OH-H_2O]^+$, 508.2218$[M+H-2CH_3OH-H_2O]^+$	Fuzi
7	27.520	Benzoylaconine	$C_{32}H_{45}NO_{10}$	603.3043	604.3134	2.97			604.3134$[M+H]^+$, 540.6158$[M+H-2CH_3OH]^+$, 508.8095$[M+H-3CH_3OH]^+$	Fuzi
8	28.379	Liquiritin or Isoliquiritin	$C_{21}H_{22}O_9$	418.1264			441.1144	−2.72	441.1144 $[M+Na]^+$, 424.0979 $[M+Na-OH]^+$, 350.8191$[M+Na-C_6H_3O]^+$	Gancao
9	28.595	Benzoylhypaconine	$C_{31}H_{43}NO_9$	573.2938	574.3025	0	–	–	574.3025$[M+H]^+$, 443.8613$[M+H-3CH_3OH-H_2O-HO^-]^+$	Fuzi
10	31.405	Mesaconitine	$C_{33}H_{45}NO_{11}$	631.2993	632.3079	2.2141			632.3079$[M+H]^+$, 599.9372$[M+H-CH_3OH]^+$, 540.2653$[M+H-AcOH-CH_3OH]^+$	Fuzi
11	32.453	Hypaconitine	$C_{33}H_{45}NO_{10}$	615.3043	616.3089	−4.381			616.3089$[M+H]^+$, 597.8211 $[M+H-H_2O]^+$, 556.2792$[M+H-C_2H_4O_2]^+$	Fuzi
12*	33.299	Glucuronide conjugation metabolite	C30H47NO13	629.3047	630.3295	27.7640			630.3295 $[M+H]^+$, 454.8397$[M+H-(GluA-H_2O)]^+$	Fuziline
13*	33.165	Glucuronide conjugation metabolite	$C_{21}H_{20}O_{10}$	432.1056	433.1145	3.6942			433.1145$[M+H]^+$, 257.0829$[M+H-(GluA-H_2O)]^+$	Isoliquiritigenin
14	40.710	Isoliquiritigenin	$C_{15}H_{12}O_4$	256.0736	257.0807	−0.3889			257.0807$[M+H]^+$, 239.1624$[M+H-H_2O]^+$	Gancao
15	42.275	6-gingerdione	$C_{17}H_{24}O_4$	292.1675	293.1734	−4.4342			293.1734$[M+H]^+$, 275.1586$[M+H-H_2O]^+$	Ganjiang

Table 3 (continued)

Peak no.	Rt (min)	Systematic name	Molecular formula	Molecular weight (Da)	$[M+H]^+$		$[M+Na]^+$		Fragmentations (m/z)	Source/ prototype
					Measured value (Da)	Error (ppm)	Measured value (Da)	Error (ppm)		
16	42.514	Formononetin	$C_{16}H_{12}O_4$	268.0736	269.0799	−3.3447			269.0799$[M+H]^+$, 181.0511$[M+H–C_2O_2–CH_3OH]^+$	Gancao
17	44.584	14-Acetyltalatizamine	$C_{26}H_{41}NO_6$	463.2934	464.3015	1.7230			464.3015$[M+H]^+$, 446.2652$[M+H–H_2O]^+$, 432.6414$[M+H–CH_3OH]^+$	Fuzi
18	46.555	6-gingerol	$C_{17}H_{26}O_4$	294.1831	295.1905	0.3388			295.1905$[M+H]^+$, 263.1618$[M+H–CH_3OH]^+$, 179.1028$[M+H–C_7H_{15}O]^+$	Ganjiang
19	46.980	6-shogaol	$C_{17}H_{24}O_3$	276.1725	277.1781	−6.1332			277.1794$[M+H]^+$, 260.1816$[M+Na–OH]^+$, 245.1533$[M+H–CH_3OH]^+$	Ganjiang
20	47.690	Atractylenolide II	$C_{15}H_{20}O_2$	232.1463	233.1533	−1.2867			233.1533$[M+Na]^+$, 187.1487$[M+Na–CH_2O_2]^+$, 159.1179$[M+Na–CH_2O_2–C_2H_4]^+$, 145.1005$[M+Na–CH_2O_2–C_3H_6]^+$	Baizhu
21	48.102	Chasmanine	$C_{25}H_{41}NO_6$	451.2934			474.2841	3.1627	474.2841$[M+H]^+$, 442.0836$[M+H–CH_3OH]^+$	Fuzi
22	49.895	Glycyrrhizic acid	$C_{42}H_{62}O_{16}$	822.4038	823.4094	−2.0646			823.4094$[M+H]^+$, 647.3792$[M+H–(GluA–H_2O)]^+$	Gancao
23	50.826	Atractylenolide I	$C_{15}H_{18}O_2$	230.1307	231.1382	0.8653			231.1382$[M+H]^+$, 105.9823$[M+H–HCOOH–2C_2H_4–2C]^+$	Baizhu
24	51.095	Neoline	$C_{24}H_{39}NO_6$	437.2777			460.2669	−0.2173	460.2669 $[M+Na]^+$, 442.2666$[M+Na–H_2O]^+$	Fuzi
25	54.144	7-hydroxycoumarin	$C_9H_6O_3$	162.0317	163.0396	3.6801			163.0396$[M+H]^+$, 145.5012$[M+H–H_2O]^+$	Baizhu
26	56.004	Glycyrrhetinic acid	$C_{30}H_{46}O_4$	470.3396	471.3479	−2.122			471.3479$[M+H]^+$, 453.4285$[M+H–H_2O]^+$	Gancao

* Indicates metabolites

50 in Table 2 was detected with the molecular ion at *m/z* 293.1736$[M+H]^+$ and gave characteristic fragment ions of 275.1650$[M+H-H_2O]^+$, 257.1517$[M+H-2H_2O]^+$. Similarly, MS^2 spectra of compound 15 in Table 3 was detected with the molecular ion at *m/z* 293.1734$[M+H]^+$ and gave characteristic fragment ions of $[M+H-H_2O]^+$ at *m/z* 257.1586. Thus, compound 15 in Table 3 was identified as the absorbed prototype of 6-gingerdione in rat serum.

One compound was sourced from *Codonopsis pilosula* (Franch.) Nannf. (Dangshen) and was identified as L-pyroglutamic acid. MS^2 spectra of compound 1 in Table 2 was detected with the molecular ion at *m/z* 130.0505$[M+H]^+$ and gave characteristic fragment ions of 112.0123$[M+H-H_2O]^+$, 84.0449$[M+H-HCOOH]^+$. Similarly, MS^2 spectra of compound 1 in Table 3 was detected with the molecular ion at *m/z* 130.0498$[M+H]^+$ and gave characteristic fragment ions of $[M+H-H_2O]^+$ at *m/z* 112.9741. Thus, compound 1 in Table 3 was identified as the absorbed prototype of L-pyroglutamic acid in rat serum.

Identification of the bioactive metabolites in rat serum

Based on a comparison of the information for ions, 8 peaks were detected only in dosed serum and were assigned to metabolites. Detailed information about the elemental compositions, retention times, and the characteristic fragment ions of metabolites are shown in Table 3. Alkaloid-, phenylpropanoids- and gingerols-related metabolites are the main metabolic constituents of FZPLP absorbed in vivo, and the main metabolic pathways in vivo were glucuronide conjugation and glucuronide. Identification of the corresponding fragment ions was obvious. For example, compound 4 (24.357 min) in Table 3 produced $[M+H]+$ at *m/z* 433 and MS^2 yielded a major ion at *m/z* 257 (−176, Da with the loss of $C_6H_8O_6$) in the positive ion mode, combined with the retention time of the reference standard 1 in Table 1 and compound 29 in Table 2. Therefore, the peak was identified tentatively as a glucuronide conjugation metabolite of liquiritigenin. Similarly, compound 13 (the t_R 33.165 min) in Table 3 has the similar retention time compared with the reference standard 6 in Table 1 and compound 48 in Table 2. And it produced $[M+H]+$ at *m/z* 433 and MS^2 yielded a major ion at *m/z* 257 (−176, Da with the loss of $C_6H_8O_6$) in the positive ion mode. Therefore, the peak was identified tentatively as a glucuronide conjugation metabolite of isoliquiritigenin. The possible structures of metabolites were elucidated as described above. All of the structures of metabolites were identified, and the MS data of the (+) ESI–MS spectra are shown in Table 3. This article reports these metabolites of FZLZP for the first time. The bioactivities are the subject of ongoing research.

Alkaloids difference between Group A and Group B

As the result shows in Fig. 5a, 10 kinds of alkaloids were detected in Group A. Most of them were trace amounts in vivo, which indicated the alkaloids' poor absorption in the prescription. Conversely, unlike Group A, the amount of the alkaloids in vivo increased obviously in Group B (Fig. 5b). The difference indicated that the absorption amount of alkaloids in the prescription can be decreased compared to the absorption amount of alkaloids in the herb powder.

Discussion

To obtain LC chromatograms of lower pressure, greater baseline stability, better resolution and higher ionization efficiency, methanol and acetonitrile and series of concentrations of aqueous formic acid solution were prepared for analysis. The best result was achieved when the mobile phase consisted of 0.1% formic acid aqueous solution and methanol. Both positive and negative modes were investigated, and the results showed that the positive ion mode was more sensitive and could provide more information for both extract samples and serum samples analyses.

FZLZP is a formula composed under the guidance of traditional Chinese medicine theory. According to TCM theory, *Aconitum carmichaeli Debx.* is the "monarch drug" and the main herb in FZLZP recipe to warm middle jiao and eliminate cold. This was confirmed in this research with 10 constituents among 23 prototype components sourced from *Aconitum carmichaeli* Debx., which maintains the maximum bioactive compounds. *Glycyrrhiza uralensis* Fisch. is frequently prescribed in combination with other herbs to decrease toxicity and to increase efficacy. In this recipe, it is the "envoy drug" and is considered to be the paramount assistant herb, which can detoxify the toxicity of aconitum. In this study, we found that *Glycyrrhiza uralensis* Fisch. was the second most-absorbed herb. The results that some compounds absorbed well in vivo derived from *Aconitum carmichaeli Debx.* and *Glycyrrhiza uralensis* Fisch. are consistent with our previous studies that they were dissolved very well in vitro [16].

Alkaloids in Fuzi herb are the toxicity as well as the efficacy compounds. The prescriptions which contains Fuzi herb should be highly concerned. In our study, the results on the differences in alkaloids between Group A and Group B show that the amount of absorption of bioactive constituents in Fuzi can be significantly reduced when this herb is used as part of a prescription rather than used alone. We think there are two reasons.

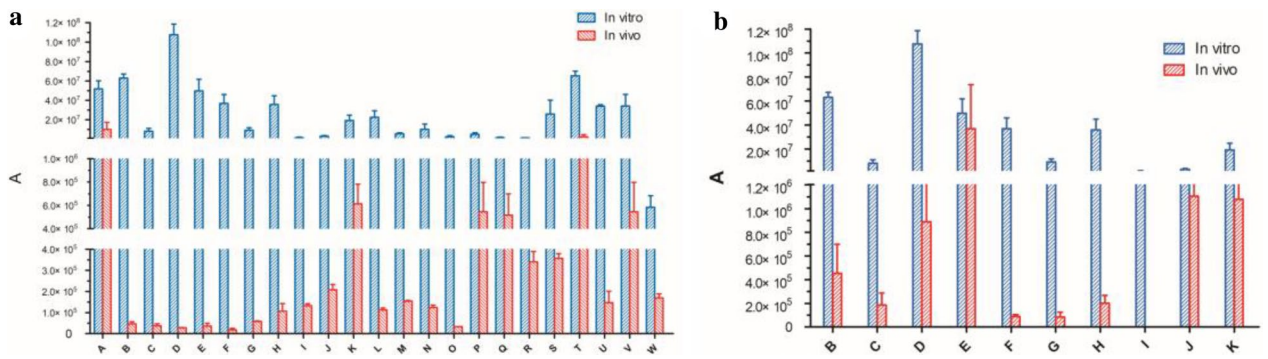

Fig. 5 The difference in the absorbed compounds in vitro and in vivo. (**a** The difference in the absorbed compounds in vitro and in vivo of Group A; **b** The difference in the absorbed alkaloids in vitro and in vivo of Group B.) (Columns: A, L-pyroglutamic acid; B, Fuziline; C, Talatisamine; D, Benzoylmesaconine; E, Benzoylaconine; F, Benzoylhypaconine; G, Mesaconitine; H, Hypaconitine; I, 14-Acetyltalatizamine; J, Chasmanine; K, Neoline; L, Liquiritigenin; M, Liquiritin or Isoliquiritin; N, Isoliquiritigenin; O, Formononetin; P, Glycyrrhizic acid; Q, Glycyrrhetinic acid; R, 6-gingerdione; S, 6-gingerol; T, 6-shogaol; U, Atractylenolide II; V, Atractylenolide I; W, 7-hydroxycoumarin)

Firstly, according to the TCM theory, the toxicity of Fuzi can be reduced in combination with Gancao [25]. This should be further confirmed by researching the relationship and differences in the chemistry constituents between Fuzi-Gancao herb pairs in FZLZP. Secondly, the pill form is the embryonic form of sustained-release preparations. As a TCM classic says: only pill among all dosage forms can reduce the toxicity of toxic drugs. The toxic herb was usually made into a pill form to reduce the toxicity in TCM [17]. And it can be further confirmed by researching differences in the chemistry constituents between FZLZP and the Fuzi pill that made from *Aconitum carmichaeli Debx*. powder.

Conclusions

This study describes a simple, sensitive and selective HPLC-QTOF-MS method for structural characterization of chemical constituents in FZLZP and bioactive components in rat serum following oral administration of FZLZP. As a result, in vitro, a total of 67 compounds were successfully identified, and 23 prototype compounds that were absorbed in vivo were identified for the first time. In addition, 3 metabolites of the bioactive compounds were tentatively identified. In this prescription, the majority of compounds absorbed in vivo derived from Fuzi and Gancao. The results provide helpful chemical information for FZLZP for further pharmacological and active mechanism research. In addition, it helped to classify the material basis responsible for the therapeutic effects of FZLZP. Furthermore, the HPLC-QTOF-MS was a potentially powerful strategy for simultaneously achieving screening and analysis of multiple bioactive compounds in FZLZP.

Additional file

Additional file 1. Minimum standards of reporting checklist.

Abbreviations

HPLC-QTOF-MS: high-performance liquid chromatography–electrospray ionization/quadrupole-time-of-flight high-definition mass spectrometry; FZLZP: Fuzi Lizhong Pill; FZP: Fuzi powder; Group A: FZLZP group for dosed rat serum; Group B: Fuzi powder group for dosed rat serum; Group C: control group for blank rat serum.

Authors' contributions

ZZ and MJ carried out the screening experiments, ZZ wrote the manuscript and analyzed the data, MJ, XW, SY, JS graphed the picture, GZ revised the manuscript, CF and LG conceived of the study, contributed to the design and interpretation of the research. All authors read and approved the final manuscript.

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Acknowledgements

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The dataset supporting the conclusions of this article is included within the article.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81803742), Key Project of Natural Science Fund of Sichuan Province (No.

18ZA0187), Pre-research National Natural Science Foundation of Chengdu University of Traditional Chinese Medicine (No. ZRYY1718).

Publisher's Note

Received: 7 November 2018 Accepted: 1 February 2019
Published online: 01 March 2019

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