Comprehensive profiling and characterization of the absorbed components and metabolites in mice serum and tissues following oral administration of Qing-Fei-Pai-Du decoction by UHPLC-Q-Exactive-Orbitrap HRMS

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[ABSTRACT] Qing-Fei-Pai-Du decoction (QFPDD) is a Chinese medicine compound formula recommended for combating corona virus disease 2019 (COVID-19) by National Health Commission of the People’s Republic of China. The latest clinical study showed that early treatment with QFPDD was associated with favorable outcomes for patient recovery, viral shedding, hospital stay, and course of the disease. However, the effective constituents of QFPDD remain unclear. In this study, an UHPLC-Q-Orbitrap HRMS based method was developed to identify the chemical constituents in QFPDD and the absorbed prototypes as well as the metabolites in mice serum and tissues following oral administration of QFPDD. A total of 405 chemicals, including 40 kinds of alkaloids, 162 kinds of flavonoids, 44 kinds of organic acids, 71 kinds of triterpene saponins and 88 kinds of other compounds in the water extract of QFPDD were tentatively identified via comparison with the retention times and MS/MS spectra of the standards or refereed by literature. With the help of the standards and in vitro metabolites, 195 chemical components (including 104 prototypes and 91 metabolites) were identified in mice serum after oral administration of QFPDD. In addition, 165, 177, 112, 120, 44, 53 constituents were identified in the lung, liver, heart, kidney, brain, and spleen of QFPDD-treated mice, respectively. These findings provided key information and guidance for further investigation on the pharmacologically active substances and clinical applications of QFPDD.

[KEY WORDS] Qing-Fei-Pai-Du decoction (QFPDD); Corona virus disease 2019 (COVID-19); Chemical profiling; Absorbed components; High-Resolution Mass Spectrometry (HRMS)


Introduction

Corona Virus Disease 2019 (COVID-19), a newly emerged infective disease, has spread all over the world, with a long incubation period, high infectivity and general susceptibility to all types of people [1-3]. The COVID-19 global pandemic has brought significantly negative effects for human health, economic and social stability. Researchers are now trying to find effective medications (including western therapeutics and herbal medicines) for combating COVID-19, while some therapeutics and herbal medicines have been used...
for treating COVID-19 in clinical settings \textsuperscript{[4-7].} In China, several Chinese medicine compound formulas, such as Qing-Fei-Pai-Du decoction (QFPDD), Jingyin granules and Lianhua Qingwen capsule, have been validated playing an active role in combating this epidemic, especially alleviating the symptoms of some moderate and mild patients \textsuperscript{[8,10].} Among all, we recommend Chinese medicines for combating COVID-19 in China, QFPDD has drawn much attention owing to its effectiveness of COVID-19. As the first Chinese medicine compound formula recommended by National Health Commission of the People’s Republic of China for combating COVID-19, QFPDD has been used to treat thousands of COVID-19 patients with the total effective rate of 97\% \textsuperscript{[9,12].} Following oral administration of QFPDD, the major symptoms and imaging manifestations of more than 60\% patients were significantly improved, while the symptoms of 30\% patients were stable and did not aggravate. Early treatment with QFPDD is associated with favorable outcomes for patient recovery, viral shedding, hospital stay, and course of the disease. And early treatment with QFPDD may serve as an effective strategy in controlling the epidemic \textsuperscript{[14]}. Unlike Western therapeutics, Chinese medicines are always the mixtures of a wide range of ingredients, these ingredients as well as their in vivo metabolites may interact with a wide range of targets in the human body in an extremely complex way \textsuperscript{[15,16].} To better understand the material basis for efficacy of QFPDD, the chemical constituents in this anti-COVID-19 Chinese medicine and the key components (including prototypes and metabolites) occurring in serum and target organs (such as the lung and the liver) following QFPDD treatment should be carefully investigated. Notably, QFPDD is deriving from four classic Chinese medicine prescriptions (including Maxing Shigan decoction, Shegan Mahuang decoction, Xiaoachaizu decoction, and Wuling powder) that are used for treating epidemic diseases and the related inflammatory symptoms for thousands of years \textsuperscript{[13,20].} As a super combination of 20 herbs and a mineral drug (Gypsum Fibrosum), QFPDD is composed by hundreds of ingredients and parts of them may be absorbed in circulation system and then exert their bioactivities, such as anti-inflammatory and anticoagulation effects \textsuperscript{[21,22].} However, the chemical constituents in QFPDD and the absorbed components of this Chinese medicine have not been well-investigated yet, which strongly block further investigations on the pharmacological and toxicological studies, the quality control and clinical applications of this anti-COVID-19 Chinese medicine.

This study aims to investigate the main chemical constituents in QFPDD and the absorbed components (including prototypes and metabolites) in serum and tissues after oral administration of QFPDD to mice. To this end, an UHPLC-Q-Orbitrap HRMS based method was well-established for systematic profiling the constituents in QFPDD, while the absorbed components (including the prototypes and the metabolites) occurring in mouse serum and tissues were also identified via profiling the serum and tissue samples from QFPDD-treated mice. The prototype components in both QFPDD and mouse serum were tentatively identified via comparison with the retention times and MS/MS spectra of the standards or refereed by literature, while the in vivo metabolites were tentatively identified via comparison with the retention times and MS/MS spectra of the in vitro metabolites generated in mice liver microsomes (MLMs). Finally, a total of 405 chemicals (including 40 kinds of alkaloids, 162 kinds of flavonoids, 44 kinds of organic acids, 71 kinds of triterpene saponins and 88 kinds of other compounds) in the water extract of QFPDD were identified, while 195, 165, 177, 112, 120, 44, 53 constituents were identified in the serum, lung, liver, heart, kidney, brain, and spleen of QFPDD-treated mice, respectively. All these findings are very helpful for deep understanding the fates of the constituents in QFPDD, as well as the key information of the tissue distribution of the absorbed components of this anti-COVID-19 Chinese medicine.

\textbf{Materials and Methods}

\textbf{Chemicals and materials}

\textit{Herba Ephedrae} (Mahuang, No. 191119-1), \textit{Ramulus Cinnamomi} (Guizhi, No. 190802-1), \textit{Rhizoma Alismatis} (Zixie, No. 191031-1), \textit{Polypropus} (Zhuling, No. 191109-1), \textit{Flos Farfarae} (Kuandonghua, No. 191207-1), \textit{Herba Asari} (Xixin, No. 191220-1) and \textit{Rhizoma Dioscoreae} (Shanyao, No. 191023-1) were purchased from Shanghai Wanshicheng Pharmaceutical Co., Ltd. (Shanghai, China). \textit{Gypsum Fibrosum} (Shengshigao, No. 190801), \textit{Poria} (Fuling, No. 191218), \textit{Radix Bupleuri} (Chaihu, No. 200103), \textit{Rhizoma Pinelliae} prepared with ginger juice (Jiangbanxia, No. 191009) and \textit{Herba Agastachis} (Huoxiang, No. 190814) were purchased from Shanghai Hongqiao Traditional Chinese Medicine Decoction Pieceess Co., Ltd. (Shanghai, China). \textit{Radix Glycyrrhizae Praeparata} (Zhigancao, No. 191213), \textit{Rhizoma Belamcandae} (Shigan, No. 191022), \textit{Fructus Auranti Immaturus} (Zhishi, No. 200119), \textit{Semen Armeniaceae Amarum} (Xingren, No. 200108) were purchased from Shanghai Kangqiao Traditional Chinese Medicine Decoction Pieceess Co., Ltd. (Shanghai, China). \textit{Radix Scutellariae} (Huangqin, No. 19112307), \textit{Radix Asteris} (Ziwan, No. 1909247) and \textit{Pericarpium Citri Reticulatae} (Chenpi, No. 191119) were purchased from Shanghai Yanghetang Traditional Chinese Medicine Decoction Pieceess Co., Ltd. (Shanghai, China). \textit{Rhizoma Atractylodis Macrocephalae} (Shengshigao, No. 190801), \textit{Fructus Armeniacae Amarum} (Xingren, No. 200108) were purchased from Shanghai Kangqiao Traditional Chinese Medicine Decoction Pieceess Co., Ltd. (Shanghai, China). Fresh \textit{Rhizoma Zingiberis Recens} (Shengjiang) was purchased from the supermarket. All of the herbs were authenticated by prof. WANG Chang-Hong, from the Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine.

Arginine, uridine, uracil, tyrosine, leucine, isoleucine, adenosine, 6-hydroxypurine, guanine, guanosine, adenosine, xanthosine, L-phenylalanine, tryptophan were purchased from Dalian Meilun Biotechnology Co. Ltd. (Dalian, China). Neochlorogenic acid, caffeic acid, chlorogenic acid, crypto-
cholorogenic acid, L-amygdalin, prunasin, 1,3-O-dicaffeoylquinic acid, ferulic acid, isochlorogenic acid B, isochlorogenic acid A, resveratrol, isochlorogenic acid C, tiliroside, 6-Gingerol, 8-gingerol, 10-gingerol, alisol B-23-acetate and pachymic acid were purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, China). Quinic acid, stachydrine, citric acid, succinic acid, gallic acid, syneprine, phenylpropanolamine, D-demethyl pseudoephedrine, vanillyl acid, ephedrine, pseudoephedrine, methylephedrine, catechin, epicatechin, neoliquiritin, 3, 4-dihydroxylbenzaldehyde, luteolin-7-O-β-D-glucoside, liquiritin, vitexin, tectoridin, isovitexin, hyperin, scutellaria, quercetin, isoquercitrin, verbascoside, hesperetin, isoacetoside, hesperidin, astragalin, neohesperidin, cinnamaldehyde, liquiritigenin, isoliquiritin, ononin, naringin, luteolin, baicalin, cinnamic acid, naringenin, licochalcone A, licochalcone B, wogonoside, kaempferol, rosmarinic acid, isoliquiritigenin, baicalin, formononetin, sinenestin, irisinflorentin, oroxylin A, nobiletin, atracylenolide-I, tangeretin, asarinin, glycyrrhizic acid, tussilagone and alisol A were provided by Prof. LIANG Xin-Miao from Dalian Institute of Chemical Physics, Chinese Academy of Sciences (Dalian, China). The purities of those reference substances were more than 98%.

Glucose 6-phosphate (G-6-P), glucose-6-phosphate dehydrogenase (G-6-PDH), nicotinamide adenine dinucleotide phosphate disodium salt (β-NADP), uridine di-phosphate glucuronic acid (UDPGLA), malateminic, S-adenosyl-L-methionine (SAM) and 3-phosphoadenosine 5-phosphosulfate (PAPS) were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Tris base and MgCl₂ were purchased from Majorbio Biotech Corp., Ltd. (Shanghai, China). Pooled liver microsomes from male ICR/CD-1 mouse (MLM, Lot no. STOM) was ordered from Research Institute for Liver Diseases (RILD, Shanghai, China). Acetoniitrile, methanol and formic acid of HPLC grade were purchased from Fisher Scientific Co. (Santa Clara, USA). Deionized water was produced with a Milli-Q Academic System (Millipore, Billerica, MA). All other reagents and solvents were either analytical or HPLC grade.

Preparation of the Chinese multi-herbal formula QFPDD

The Chinese multi-herbal formula Qing-Fei-Pai-Du decoction (QFPDD) was prepared according to the following procedure, pieces of Herba Ephedrae (Mahuang) 45 g, Radix Glycerrhiza Praeparata (Zhiangcao) 30 g, Semen Armeniacae Amarum (Xingren) 45 g, Gypsum Fibrosum (Shengshigao) 75 g (fried first), Ramulus Cinnamomi (Guizhi) 45 g, Rhizoma Alismatis (Zixie) 45 g, Polyergus (Zhuling) 45 g, Rhizoma Atractylodis Macrocephalae (Baizhu) 45 g, Poria (Fuling) 75 g, Radix Bupleuri (Chaihu) 80 g, Radix Scutellariae (Huangjin) 30 g, Rhizoma Pinelliae prepared with ginger juice (Jiangbanxia) 45 g, Rhizoma Zingiberis Recens (Shengjiang) 45 g, Radix Asteris (Ziwan) 45 g, Flos Farfarae (Kuandonghua) 45 g, Rhizoma Belamcandae (Shegan) 45 g, Herba Asari (Xixin) 30 g, Rhizoma Dioscoreae (Shanyao) 75 g, Fructus Aurantii Immaturus (Zhishi) 30 g, Pericarpium Citri Reticulatae (Chenpi) 30 g and Herba Agastachis (Huoxi-ang) 45 g, were mixed together and macerated in 12 L deionized water for 40 min before being decocted. After then, 980 g of the herbal mixture was decocted for 45 min. A total of 100 batches of QFPDD were decocted repeatedly and then combined. After filtration, the decoction (about 180 L) was further concentrated to 20 L by rotary evaporation at 40–50 °C in vacuum with a 93.98% concentrated yield. Finally, 6.52 kg powder was obtained by spray-drying with a 78.55% spray-dried yield.

Preparation of QFPDD extract

QFPDD (1.0 g) was weighed and dissolved in 125 mL deionized water by ultrasonics (250 W, 50 HZ) for 1 h. Then, the sample solution was centrifuged at 10 000 × g for 15 min. The supernatant solution was filtered through a 0.22 μm membrane for further analysis.

Animal handling and sampling

Twelve male C57BL/6J mice (SPF grade, weight of 20 ± 2 g) were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China) and were fed in Experimental Animal Center of Shanghai University of Traditional Chinese Medicine. All of animals were housed in an environment-controlled breeding room at 25 °C with a 12 hours light/dark cycle. All experiments performed in mice were in accordance with the State Committee of Science and Technology of P.R. of China, and the protocol for this study was approved and monitored by the Animal Ethics Committee of Shanghai University of Traditional Chinese Medicine (PZSHUTCM-200717018).

After seven days of acclimatization, twelve male mice were randomly divided into two groups (6 mice for each group). QFPDD group was oral administered QFPDD (18 g dried material/kg); control group was oral administered deionized water. All animals were fasted overnight before the experiments and had free access to water. One hour after the once oral administration, all mice were anesthetized, blood samples were collected from the abdominal aorta, and their various tissues (lung, liver, heart, kidney, brain, spleen) were removed rapidly. Blood samples were placed at room temperature for 1 h until solidification. Then, serum samples were obtained by centrifuged at 5000 r·min⁻¹ for 15 min at 4 °C. All samples were stored at −80 °C until analysis. The serum samples (200 μL) were added five times methanol (1 mL), vortexed and then, centrifuged at 10 000 × g for 15 min. The supernatant (960 μL) was dried with nitrogen gas. The residue was dissolved in 80 μL of methanol, vortexed and then, centrifuged at 10 000 × g for 15 min, and the filtrate was used as the LC/MS sample. The 100 mg various tissues (lung, liver, heart, kidney, brain, spleen) were homogenized in 1 mL methanol on ice, the homogenate of them was centrifuged at 4 °C at 10 000 × g for 15 min. The supernatant (800 μL) was dried with nitrogen gas. The residue was redissolved in 80 μL of methanol, vortexed and then, centrifuged at 10 000 × g for 15 min, and the filtrate was used as the LC/MS sample. The supernatant (2 μL) was used in the
Microsomal incubation with NADPH: The incubation was performed in a 50 mmol L\(^{-1}\) Tris–HCl buffer (pH 7.4) containing MLMs (1.0 mg protein/mL), the NADPH-generating system contained 10 mmol L\(^{-1}\) G-6-P, 1 mmol L\(^{-1}\) NADP\(^{\prime}\), 4 mmol L\(^{-1}\) MgCl\(_2\), and 1 unit mL\(^{-1}\) G-6-PDH, and 200 μmol L\(^{-1}\) analyte (nobiletin) which was previously dissolved in methanol with concentration 200 μmol L\(^{-1}\) in a total volume of 200 μL with concentration 200 μmol L\(^{-1}\) in a total volume of 200 μL. The final concentration of methanol in the incubation system was less than 1% (V/V). The reactions were terminated with 600 μL ice-cold acetonitrile after 60 min incubation. The mixture was centrifuged at 10,000 × g for 15 min, and then the supernatant (720 μL) was evaporated to dryness by a gentle stream of nitrogen (37 °C). The residue was dissolved by 90 μL of 5% methanol and centrifuged at 10,000 × g for 10 min. 2 μL of the supernatant was injected for UHPLC-Q-Exactive Orbitrap HRMS system.

Control samples containing no NADPH or substrates were prepared. Incubation was performed in duplicate.

Microsomal incubation with NADPH and UDPGA: 200 μmol L\(^{-1}\) analyte (baicalein, scutellarin, wogonin, oroxylin A, formononetin, liquiritigenin, hesperetin, quercetin, oroxylin A and caffeic acid, each compound was incubated separately) was mixed with alamethicin (25 μg/mg protein) and the microsomes (1.0 mg protein/mL) in 100 mmol L\(^{-1}\) Tris-HCl buffer (50 mmol L\(^{-1}\), pH 7.4). After 5 min of pre-incubation at 37 °C, the incubation reaction was initiated by the addition of G-6-P (10 mmol L\(^{-1}\)), G-6-PDH (1 unit mL\(^{-1}\)), NADP\(^{\prime}\) (1.0 mmol L\(^{-1}\)), and UDPGA (5.0 mmol L\(^{-1}\)). The total incubation volume was 200 μL. The final concentration of methanol in the incubation system was less than 1% (V/V). The reactions were terminated with 600 μL ice-cold acetonitrile after 1 h of incubation. The mixture was centrifuged at 10,000 × g for 15 min, and then the supernatant (720 μL) was evaporated to dryness by a gentle stream of nitrogen at 37 °C. The residue was dissolved by 90 μL of 5% methanol and centrifuged at 10,000 × g for 15 min. 2 μL of the supernatant (2 μL) was then injected for UHPLC-Q-Exactive Orbitrap HRMS system. Control samples without NADPH and UDPGA or substrates were prepared. Incubation was performed in duplicate.

Microsomal incubation with NADPH and PAPS: The incubation was performed in a 50 mmol L\(^{-1}\) Tris–HCl buffer at pH 7.4 containing microsomes (1.0 mg protein/mL), the NADPH-generating system contained 10 mmol L\(^{-1}\) G-6-P, 1 mmol L\(^{-1}\) NADP\(^{\prime}\), 4 mmol L\(^{-1}\) MgCl\(_2\), and 1 unit mL\(^{-1}\) of G-6-PDH, 100 μmol L\(^{-1}\) PAPS, and 200 μmol L\(^{-1}\) analyte (baicalein, wogonin, formononetin, liquiritigenin, hesperetin and caffeic acid, each compound was incubated separately) in a total volume of 200 μL. The final concentration of methanol in the incubation system was less than 1% (V/V). The reactions were terminated with 600 μL ice-cold acetonitrile after 60 min incubation. The next process was as same as above and the control samples containing no NADPH, no PAPS or substrates were prepared. Each of the incubations was performed in duplicate.

Microsomal incubation with NADPH and SAM: The incubation was performed in a 50 mmol L\(^{-1}\) Tris–HCl buffer at pH 7.4 containing microsomes (1.0 mg protein/mL), the NADPH-generating system contained 10 mmol L\(^{-1}\) G-6-P, 1 mmol L\(^{-1}\) NADP\(^{\prime}\), 4 mmol L\(^{-1}\) MgCl\(_2\), and 1 unit mL\(^{-1}\) of G-6-PDH, 100 μmol L\(^{-1}\) SAM, and 200 μmol L\(^{-1}\) analyte (baicalein, scutellarin, chlorogenic acid and caffeic acid, each compound was incubated separately) in a total volume of 200 μL. The final concentration of methanol in the incubation system was less than 1% (V/V). The reactions were terminated with 600 μL ice-cold acetonitrile after 60 min incubation. The next process was as same as above and the control samples containing no NADPH, no SAM or substrates were prepared. Each of the incubations was performed in duplicate.

**Chromatography conditions for UHPLC-Q-Exactive Orbitrap HRMS**

Chromatographic separation was performed on a UHPLC-Q-Exactive Orbitrap system (Thermo Fisher Scientific Inc., Grand Island, NY, USA). The UHPLC system consisted of a Thermo Scientific Dionex Ultimate 3000 Series RS pump coupled with a Thermo Scientific Dionex Ultimate 3000 Series TCC-3000RS column compartments and WPS-3000 autosampler controlled by Chromeleon 7.2 Software. The cooling autosampler was set at 4 °C and protected from light, and the column heater was set at 40 °C. A Waters ACQUITY UPLC BEH C\(_18\) column (2.1 mm × 100 mm, 1.7 μm) was employed with the temperature set at 40 °C. The mobile phase consisted of A (methanol) and B (0.1% formic acid) at a flow rate of 0.3 mL min\(^{-1}\) and eluted with gradient elution: 0–4 min (4% A), 4–10 min (4%–12% A), 10–30 min (12%–70% A), 30–35 min (70% A), 35–38 min (70%–95% A), 38–42 min (95% A), 42–45 min (4% A). The injection volume was 2 μL.

The mass spectrometer Q-Exactive Orbitrap system was connected to the UHPLC system via heated electrospray ionization and controlled by Xcalibur 4.1 software that was used for data collection and analysis. The electrospray ionization source was operated and optimized in negative and positive ionization mode. The optimized parameters of mass spectrometry were: capillary temperature: 325 °C; sheath gas (N\(_2\)) flow rate: 45 arbitrary units; auxiliary gas (N\(_2\)) flow rate: 8 arbitrary units; sweep gas flow rate: 0 arbitrary units; spray voltage: 2.5 kV (negative), 3.5 kV (positive); S-lens RF level: 50V; auxiliary gas heater temperature, 300 °C; scan mode: Full MS/SIM and Full MS/dd-MS2 mode, which includes 1 first-level full scan (resolution 70000 FWHM) and 1 data-dependent secondary scan (resolution 17500 FWHM) 2 events, the scanning range is 80–1200 m/z, and the collision energy gradient is 20, 50, 100V.

**Establishment of QFPDD chemical database and chemical profiling**

By searching databases, such as PubMed of the US Na-
Results

Identification and characterization of chemical constituents from QFPDD

Under the optimal conditions described above, the constituents in the crude extract of QFPDD were comprehensively analyzed by UHPLC-Q-Exactive Orbitrap HRMS, with a running time of 45 min. The total ion chromatograms (TICs) of the extract of QFPDD under both positive and negative ion modes are shown in Fig. 1. The protonated molecular weights of all identified compounds were calculated within an error of 10 ppm. Following carefully comparison with the retention times and MS/MS spectra of the reference standards, reference literature, Chemical Book and self-built databases, a total of 405 chemicals were identified or tentatively characterized from QFPDD, including 40 kinds of alkaloids, 162 kinds of flavonoids, 44 kinds of organic acids, 71 kinds of triterpene saponins and 88 kinds of other compounds (Supplementary Table S1). The detailed information of the identified chemical constituents in QFPDD including retention time, precise molecular weight, MS/MS fragment ions and the source of each constituent, have been listed in Supplementary Table S1. Among all identified chemical constituents, ninety-one constituents are confirmed by the authentic standards.

It is well-accepted that deep understanding of the fragmentation pattern of each authentic standard or its analogous is very helpful for the identification of herbal constituents by using HRMS [17]. In this study, the fragmentation pathways of various constituents (ephedrine, amygdalin, nootkatone, liquiritin, gallic acid, chlorogenic acid, saikosaponin A and glycyrrhizic acid) in QFPDD were carefully studied and proposed in Fig. 2 and Fig. 3. For example, ephedrine ($t_b = 7.30$ min), an abundant alkaloid distributed in *Herba Ephedrae*, could be easily detected in the positive ion mode with a quasi-molecular ion [M + H]$^+$ at $m/z$ 166.1226 (C$_{16}$H$_{20}$NO). MS/MS fragmentation analysis demonstrated that this natural compound possessed a neutral loss of H$_2$O, subsequently by the cleavage of C-N bond moiety (Fig. 2A), evidenced by the product ions of $m/z$ 148.1119 [M + H − H$_2$O]$^+$, $m/z$ 133.0886 [M + H − H$_2$O − CH$_3$]$^+$, $m/z$ 117.0701 [M + H − H$_2$O − CH$_3$N]$^+$ and $m/z$ 91.0548 [M + H − H$_2$O − CH$_3$N − C$_2$H$_5$]$^+$. The compound 56 ($t_b = 7.30$ min) in Supplementary Table S1 were detected in the positive ion mode at the same retention time with the identical $m/z$ of 166.1225 (C$_{16}$H$_{20}$NO), thus was characterized as ephedrine. amygdalin ($t_b = 13.93$ min), an abundant alkaloid distributed in *Semen Armeniaceae Amaraeum*, could be easily detected in the negative ion mode with a quasi-molecular ion [M − HCOOH − H]$^−$ at $m/z$ 502.1567 (C$_{16}$H$_{22}$NO$_3$$_3$). It was inclined to lose the whole sugar moiety to generate base peak ions $m/z$ of 323.0972 [M − HCOOH − H − C$_6$H$_4$O$_2$]$^−$ and 179.0550 [C$_6$H$_4$O$_2$]$^−$ in MS$^2$ spectra (Fig. 2B). Subsequently by cleavage of the functional group on the second glucose, such as $m/z$ of 263.0772 [M + HCOOH − H − C$_6$H$_4$O$_6$ − C$_2$H$_4$O$_2$]$^−$ and 221.0661 [M + HCOOH − H − C$_6$H$_4$O$_6$ − C$_2$H$_4$N]$^−$. Simultaneously the fragmentation signal of glucose was detected, its MS/MS data were shown as $m/z$ of 161.0444 [C$_6$H$_4$O$_6$ − H$_2$O].119.0335[C$_6$H$_4$O$_6$ − H$_2$O − C$_2$H$_4$O].101.0229[C$_6$H$_4$O$_6$ − H$_2$O − C$_2$H$_4$O − H$_2$O] and 89.0229[C$_6$H$_4$O$_6$ − H$_2$O − C$_2$H$_4$O − CH$_3$O]. The compound 90 in Supplementary Table S1 were detected in the negative ion mode at 13.93 min with the identical m/z of 502.1568 (C$_{21}$H$_{32}$NO$_3$$_3$), this peak was characterized as amygdalin. Similar to the identification process above, a total of 40 alkaloids were identified from nootkatone, methylephedrine, ligustrazine, prunasin, *et al.* methylephedrine, ligustrazine, prunasin, *et al.*

Nootkatone ($t_b = 29.30$ min), a flavonoid distributed in *Fructus Aurantii Immaturus* and *Pericarpium Citri Reticulatae*, could be easily detected in the positive ion mode with a quasi-molecular ion [M + H]$^+$ at $m/z$ 403.1385 (C$_{21}$H$_{32}$O$_3$).
MS/MS fragmentation analysis demonstrated that this natural compound possessed demethylation, dehydration, and CO, evidenced by the product ions of $m/z$ 388.1151 [M + H – CH$_3$]$^+$, 373.0916 [M + H – CH$_3$ – CH$_2$]$,^+$, 355.0811 [M + H – 2CH$_3$ – H$_2$O]$^+$ and 327.0856 [M + H – 2CH$_3$ – H$_2$O – CO]$^+$ (Fig. 2C). The compound 321 listed in Supplementary Table S1 were detected in the positive ion mode at 29.30 min with the identical $m/z$ of 403.1382 (C$_{21}$H$_{22}$O$_5$), thus this peak was characterized as nobeltein. Liquiritin ($t_R$ = 18.59 min), a flavonoid glycoside distributed in Radix Glycyrrhizae Praeparata, which was detected in the negative ion mode with a quasi-molecular ion [M – H]$^-$ at $m/z$ of 417.1172 (C$_{21}$H$_{22}$O$_5$). Its MS/MS data were shown as $m/z$ of 255.0654 [M – H – C$_6$H$_5$O$_3$]$^-$, 153.0176 [M – H – C$_6$H$_5$O$_3$ – C$_8$H$_5$]$^-$, 135.0071 [M – H – C$_6$H$_5$O$_3$ – C$_8$H$_5$O]$^-$ and 119.0484 [M – H – C$_6$H$_5$O$_3$ – C$_7$H$_4$O$_3$] (Fig. 2D). The compound 135 listed in Supplementary Table S1 could be easily detected in the negative ion mode with the same $t_R$ (at 18.59 min) and the identical $m/z$ of 417.1195 (C$_{21}$H$_{22}$O$_5$), this peak was then characterized as liquiritin. Similar to the identification pro-

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**Fig. 2** The mass fragment and fragmentation pathway of ephedrine (A), amygdalin (B), nobeltein (C) and liquiritin (D)
cess above, a total of 162 flavonoids were identified from QFPDD, including isoorientin, neoorientin, kaempferol, luteolin, quercetin, rutin, hesperidin, neohesperidin, formononetin, isoflavins, formononetin et al.

Gallic acid ($t_R = 2.09$ min), an organic acid distributed in a variety of herbs, which was detected in the negative ion mode with a quasi-molecular ion [M − H]$^-$ at the $m/z$ of 169.0127 (C$_7$H$_6$O$_5$). MS/MS fragmentation analysis demonstrated that this natural compound possessed neutral loss of H$_2$O, CO$_2$ and/or CO (Fig. 3A), evidenced by the product ions of $m/z$ 151.0020 [M − H − H$_2$O]$^-$, 125.0226 [M − H − CO$_2$]$^-$, 97.0277 [M − H − CO$_2$ − CO]$^-$, 81.0327 [M − H − 2CO$_2$]$^-$. Compound 40 listed in Supplementary Table S1 could be detected in the negative ion mode with the same $t_R$ (2.09 min) and the identical $m/z$ of 169.0133 (C$_7$H$_6$O$_5$), this compound was then characterized as gallic acid. Chlorogenic acid ($t_R = 12.51$ min), an organic acid which was produced from caffeic acid and quinic acid, it was distributed in variety of herbs. It could be easily detected in the positive ion mode with a quasi-molecular ion [M − H]$^+$ at $m/z$ 353.0883 (C$_{36}$H$_{53}$O$_{10}$). MS/MS fragmentation analysis demonstrated that it was easily broken down into caffeic acid and quinic acid to generate base peak ions $m/z$ of 191.0549 [M − H − C$_6$H$_8$O$_6$]$^+$ and 179.0337 [M −

Fig. 3 The mass fragment and fragmentation pathway of gallic acid (A), chlorogenic acid (B), saikosaponin A (C) and glycyrrhizic acid (D)
H – C₂H₄O₂⁻ in MS² spectra (Fig. 3B). Subsequently by neutral loss of H₂O or CO₂, evidenced by the product ions of m/z 173.0442 [M − H – C₆H₄O₂ – H₂O]⁻, 161.0228 [M − H – C₆H₄O₂ – H₂O]⁻ and 155.0335 [M − H – C₆H₄O₂ – 2H₂O]⁻ and 135.0435 [M − H – C₆H₄O₂ – CO₂]⁻. Compound 81 listed in Supplementary Table S1 could be easily detected in the negative ion mode with the same Rt (at 12.51 min) and the identical m/z of 353.0880 (C₁₅H₂₁O₇), thus this compound was characterized as chlorogenic acid. Similar to the identification process above, a total of 44 organic acids were identified from QFPDD, including amino acids, nucleosides, alkaloids, 55 kinds of flavonoids, 21 kinds of organic acids, 8 kinds of triterpene saponins and 8 kinds of other compounds (Supplementary Table S2, Fig. 4 and Fig. 5). Among them, 44 ingredients are confirmed by comparison with the reference standards. Take compound p9 for example, this peak generated quasi-molecular ion [M + H]⁺ at m/z 166.1225, along with characteristic fragment ions at m/z 148.1117 [M+H-H₂O]⁺, 133.0879 [M + H – H₂O – CH₃]⁺, 117.0699 [M + H – H₂O – CH₃N]⁺ and 91.0545 [M + H – H₂O – CH₃N – C₂H₃]⁻, which was identical to the compound 62 in the extract of QFPDD (Supplementary Table S1). Compound p9 was thus identified as pseudoephedrine. Similarly, compound p21 generated deprotonated molecule [M − H]⁻ at m/z 294.0988, along with characteristic fragment ions at m/z 161.0445 [C₆H₄O₂]⁻, 101.0229 [C₆H₄O₂ – C₆H₄O₂]⁻, 85.0279 [C₆H₄O₂ – C₆H₄O₂]⁻, 71.0123 [C₆H₄O₂ – C₆H₄O₂ – CH₂O]⁻, which was identical to the product ions of compound 93 in QFPDD. Thus, compound p21 was characterized as prunasin.

Flavonoids were major constituents in several herbs for preparing QFPDD, it is necessary to identify the absorbed flavonoids in serum from QFPDD-treated mice. In this study, a total of 54 flavonoids (as the absorbed prototype constituents) were detected in mice serum. Take compound p87 for example, this peak generated a quasi-molecular ion [M + H]⁺ at m/z 387.1073, along with characteristic fragment ions at
Fig. 4 Structures of the absorbed prototype compounds (alkaloids and flavonoids) in mice serum after oral administration of Qing-Fei-Pai-Du decoction

\[ m/z = 372.0831 \ [M + H - CH_3]^+, 357.0619 \ [M + H - CH_3 - CH_2]^+, 341.0747 \ [M + H - CH_3 - CH_2O]^+, 326.0788 \ [M + H - CH_3 - CH_2O_2]^+ \], which was highly consistent with the product ions of compound 302 in QFPDD. As a result, compound p87 was identified as the prototype of compound 302 (irisflorentin). Similarly, compound p57 shared analogical elution time and identical ions in MS² spectra with compound 189 in the extract of QFPDD, such as \[ m/z = 301.0721 \ [M - H - C_{12}H_{20}O_3]\], 286.0478 \ [M - H - C_{12}H_{20}O_3 - CH_3], 257.0801 \ [M - H - C_{13}H_{22}O_4 - CO_2], 242.0597 \ [M - H - C_{14}H_{20}O_5 - CH_3 - CO_2] \]. For this reason, compound p57 was then deemed to be hesperidin, i.e., the prototype of compound 189.

Triterpene saponins were another major group of bioactive components in QFPDD, and it was found that the majority of them could be absorbed into serum. For example, compound p103 gave molecular formula C_{30}H_{40}O_{14} and deprotonated molecule at m/z 535.3648 \ [M + FA - H] \], along with
Fig. 5 Structures of the absorbed prototype compounds (organic acids, triterpene saponins, and others) in mice serum after oral administration of Qing-Fei-Pai-Du decoction

H$_2$O], which was in keeping with compound 396 in the extract of QFPDD. Then, compound p103 was regarded as prototype of compound 396.

Similarly, compound p50 (an organic acid) occurring in rat serum showed identical retention times and quasi-molecular ion (m/z 515.1202 [M − H]) to compound 158 in QFPDD. Furthermore, they also produced near identical fragment ions in MS$^2$ spectra, e.g., m/z m/z 353.0884 [M − H − C$_3$H$_6$O$_3$], 191.0554 [M − H − C$_9$H$_6$O$_3$ − C$_3$H$_6$O$_3$], 179.0337 [M − H − C$_9$H$_4$O$_3$ − C$_3$H$_6$O$_3$], 161.0234 [M − H − C$_9$H$_6$O$_3$ − C$_3$H$_1$O$_3$]. Thus, compound p50 was identified as the prototype of compound 158, namely isochlorogenic acid A.

Identification of the metabolites in mice serum

Next, the metabolites in serum from QFPDD-treated mice that are derived from the constituents in QFPDD were tentatively characterized. To discriminate the endogenous metabolites from QFPDD-related metabolites, the TIC chromatograms of QFPDD-treated mice serum were compared with that of the blank serum. After that, the endogenous substances could be excluded, while the exogenous compounds could be identified by comprehensive analysis. By contrasting these compounds with already known compounds in vitro, searching for the same compounds and validating them via comparison of the retention time and the product ions. TraceFinder software incorporated in UHPLC-Q-Exactive-Orbitrap HRMS could process the data acquired, provide key information about group changes from prototypes to metabolites by means of exact molecular weight, and provide elementary compositions thereof. These analyses were beneficial to determine the prototypes and the metabolites in biological samples, and to conjecture potential metabolic pathways. Furthermore, in this study, parts of in vitro metabolites of the major constituents in QFPDD were identified via comparison with the retention times and MS/MS spectra of the in vitro metabolites generated in MLMs.

Taking metabolites M27, M30 and M65 as the examples, these three compounds gave similar quasi-molecular ion [M − H]$^-$ at m/z 431.0983, as well as characteristic fragment ions at m/z 255.0665 [−176 Da, M − H − C$_3$H$_6$O$_3$], m/z 135.0090 [M − H − C$_9$H$_6$O$_3$ − C$_3$H$_6$O$_3$], m/z 119.0501 [M − H − C$_9$H$_4$O$_3$ − C$_3$H$_1$O$_3$] in the MS$^2$ spectrum. The fragment ions of these three metabolites were identical with that of compound 205 (liquiritigenin), a major constituent identified from the extract of QFPDD. In vitro metabolism of liquiritigenin in MLMs incubated with both NADPH and UDPGA, three mono-O-glucuronides could be easily detected. The retention times, product ions were consistent with the metabolites M27, M30 and M65. In these cases, these three compounds were identified as liquiritigenin-O-glucuronides. Similarly, the in vitro metabolites M34 and M70 are identified as typical sulfates, both compounds displayed similar quasi-molecular ion [M − H]$^-$ at m/z 335.0231, as well as characteristic fragment ions at m/z 255.0665 [−80 Da, M − H − SO$_3$]$^-$, m/z 135.0090 [M − H − SO$_3$ − C$_3$H$_6$O$_3$], m/z 119.0501 [M − H − SO$_3$ − C$_3$H$_4$O$_3$]$^-$ in the MS$^2$ spectrum, the fragment ions were identical with that of the authentic standard liquiritigenin. In vitro metabolism demonstrated that following incubation of liquiritigenin in MLMs in the presence of both NADPH and PAPS, two mono-O-sulfates could be easily detected. The retention times, product ions were well-matched with the metabolites M34 and M70. Thus, these two peaks were identified as liquiritigenin-O-sulfate.

The metabolite M21 and M25 were produced from an organic acid. They gave similar quasi-molecular ion [M − H]$^-$ at m/z 367.1034, as well as characteristic fragment ions at m/z 353.0883 [−15 Da, M − H − CH$_3$], m/z 191.0549 [M − H − CH$_3$ − C$_3$H$_6$O$_3$], m/z 179.0337 [M − H − CH$_3$ − C$_3$H$_6$O$_3$], m/z 173.0442 [M − H − C$_9$H$_6$O$_3$ − H$_2$O], m/z 161.0228 [M − H − CH$_3$ − C$_3$H$_9$O$_2$ − H$_2$O] in the MS$^2$ spectrum, the fragment ions were identical with the compound 59 (neochlorogenic acid), 80 (chlorogenic acid), 87 (cryptochlorogenic acid) in the extract of QFPDD. In vitro metabolism demonstrated that following incubation of chlorogenic acid in MLMs in the presence of NADPH and SAM, two O-methylated metabolites were detected. The retention times, product ions were well-matched with that of the metabolites M21 and M25. Thus, these two metabolites were identified as O-methylated chlorogenic acid. Previous study have reported that two O-methylated metabolites of chlorogenic acid were found in urine samples after intramuscular administration of chlorogenic acid injection to healthy adults, and the pure O-methylated metabolites were synthesized by chlorogenic acid and followed by isolation, purification and identification [27]. According to the chromatographic behavior of them, M21 was tentatively identified as 3′-methyl-chlorogenic acid, and M25 was tentatively identified as 4′-methyl-chlorogenic acid.

Following carefully metabolite identification as mentioned above, a total of 91 metabolites were tentatively identified (Supplementary Table S3). A variety of metabolic reactions, such as hydroxylation, O-methylation, O-glucuronidation, O-sulfation, demethylation, were involved in the metabolism of the major constituents in QFPDD. Among them, 20 metabolites are fully confirmed by the in vitro metabolites generated in MLM.

Characterization of the absorbed prototypes and metabolites in mice tissues

With the help of the information about the absorbed prototypes and metabolites in mice serum, the absorbed prototypes and metabolites in various tissues from QFPDD-treated mice were explored. Subsequently, total of 165, 177, 112, 120, 44 and 53 prototypes and metabolites were identified in the lung, liver, heart, kidney, brain, and spleen of QFPDD-treated mice, respectively. The peak areas of absorbed prototypes and metabolites were summarized in Supplementary Table S2 and Supplementary Table S3, while Fig. 6 showed the heat map of the peak areas of both the absorbed components and the metabolites occurring in serum or six different
On the basis of the peak area, the top ten components in the mice serum were ranked as follows, stachydrine (p3), salicylic acid (p32), ephedrine (p7), caffeic acid-sulfate (M4), baicalin (p66), baicalein 6-O-β-D-glucuronide (p82), wogonoside (p80), methylephedrine (p10), baicalein-glucuronide methylation (M78), quinic acid (p2).

The top ten components in the lung tissue were ranked as follows: ephedrine (p7), naringin (p53), neohesperidin (p59), baicalin (p66), wogonoside (p80), stachydrine (p3), quinic acid (p2), methylephedrine (p10), oroxylin A 7-O-glucuronide (p75), pseudoe Ephedrine (p9).

The top ten components in the liver tissue were ranked as follows: ephedrine (p7), naringin (p53), neohesperidin (p59), baicalein-glucuronide methylation (M78), irisin florentin (p87), stachydrine (p3), wogonoside (p80), wogonin (p88), baicalin (p66), methylephedrine (p10).

The top ten components in the heart tissue were ranked as follows: ephedrine (p7), naringin (p53), stachydrine (p3), methylephedrine (p10), neohesperidin (p59), O (p102), wogonoside (p80), oroxylin A 7-O-glucuronide (p75), pseudoe Ephedrine (p9).

The top ten components in the kidney tissue were ranked as follows: ephedrine (p7), naringin (p53), neohesperidin (p59), baicalin (p66), wogonoside (p80), stachydrine (p3), methylephedrine (p10), quinic acid (p2), oroxylin A 7-O-glucuronide (p75), pseudoe Ephedrine (p9).

The top ten components in the brain tissue were ranked as follows: ephedrine (p7), pseudoe Ephedrine (p9), methylephedrine (p10), stachydrine (p3), mandelonitrile (M1), phenylpropanolamine (p4), salicylic acid (p32), alisol O (p102), D-demethyl pseudoe Ephedrine (p5), oroxylin A (p93).

From the above results, it was obvious that ephedrine (p7) and methylephedrine (p10) from Herba Ephedrae, and stachydrine (p3) from Fructus Aurantii Immaturus displayed relatively high exposure levels in mice serum and all tested tissues. Additionally, baicalin (p66), oroxylin A 7-O-glucuronide (p75), wogonoside (p80), wogonin (p88) and oroxylin A (p93) from Radix Scutellariae, alisol O (p102) and alisol A (p103) from Rhizoma Alismatis also showed relatively high exposure levels in most of mice tissues.

**Discussion**

To fight against COVID-19, some Chinese medicines were recommended by National Health Commission of the People’s Republic of China or the local government in China, for alleviating the major symptoms of COVID-19 or prevent-
ing disease deterioration \[^{10}\]. As the first Chinese medicine compound formula recommended for combating COVID-19, QFPDD is made by 20 herbs and a mineral drug, which is composed by hundreds of ingredients. In most cases, for treating COVID-19, the absorbed constituents (including the prototypes and the metabolites) into blood circulation can be delivered to the target organ (such as the lung) and then exert various pharmacological effects, such as anti-inflammatory and anticoagulant effects \[^{28, 29}\]. Therefore, it is necessary to investigate the absorbed constituents of QFPDD following oral administration of this super Chinese medicine compound formula, which will be very helpful for better understanding the key material basis of QFPDD, as well as for guiding the quality control and clinical applications of this formula.

In this study, an UHPLC-Q-Orbitrap HRMS based method was established for profiling the constituents in QFPDD and the absorbed constituents in QFPDD-treated mice. The research strategy and the key findings for clarifying the chemical composition and absorption components of QFPDD have been summarized in Fig. 7. The chemical constituents in QFPDD were tentatively identified via comparison with exact molecular weight, the retention times and MS/MS spectra of the standards or refereed by TCM databases and literature. The absorbed components (including the prototypes and the metabolites) occurring in mice serum and tissues were identified via profiling the serum and tissue samples from QFPDD-treated mice. The absorbed prototype components were tentatively identified via comparison with the retention times and MS/MS spectra of components in QFPDD. The in vivo metabolites were tentatively identified via comparison with fragment ions of the parent compounds or refereed by literature, while parts of in vivo metabolites of the major constituents were identified via comparison with the retention times and MS/MS spectra of the in vitro metabolites of each constituent generated in MLMs. Finally, a total of 405 chemicals (including alkaloids, flavonoids, organic acids, triterpene saponins and other compounds) in the extract of QFPDD were identified, while 195, 165, 177, 112, 120, 44, 53 constituents were identified in the serum, lung, liver, heart, kidney, brain, and spleen of QFPDD-treated mice, respectively. All these findings might be very helpful for deep understanding the fates of the constituents in QFPDD, as well as offered key information of the tissue distribution of the absorbed components of this anti-COVID-19 Chinese medicine.

It is well known that it is always a challenging task for identification of the prototypes and the metabolites of TCM prescription. For example, oroxylin A 7-O-glucuronide (p75) is a trace amount prototype constituent occurring in QFPDD, but this agent is a highly exposed component in the serum and various tissues of QFPDD-treated mice. The reasons may include i) produced by the O-glucuronidation of oroxylin A (p103) in vivo; ii) produced by baicalein (p66) through 6-O-
methyltion and O-glucuronidation. It is also suggested that it is very difficult to differentiate some components detected in QFPDD-treated mice as the prototypes or the metabolites, owing to that some in vivo components are derived from both the prototypes and the metabolites of the homologous compounds occurring in this TCVM prescription. Meanwhile, the exposure of some prototype constituents in blood are extremely low and thus are difficult to be detected, since these natural constituents could be readily transformed into the corresponding metabolites (glucuronides or sulfates) in vivo. For instances, trihydroxy-methoxyflavone-O-glucuronides (M35 and M60) and daidzein-sulfates (M33 and M41) can be easily detected in mice serum, but the related prototypes are hardly detected in mice serum. In addition, many nature products have isomers that cannot be distinguished by MS spectral analysis alone, thus it is hard to correlate the parent compounds with the metabolites, such as vanillic acid and its isomer.

Modern pharmacological studies have found that some of absorbed components of QFPDD displayed a wide range of benefical effects or biological activities. Some pharmacological activities of them are considered to be beneficial for COVID-19 patients. For example, ephedrine, pseudoephedrine and amyladgin displayed significant anti-cough and anti-asthmatic effects [30], as well as anti-inflammatory [31, 32] and immunomodulatory effects [33, 34], which are considered as the major active components of TCM prescriptions for treating respiratory diseases. Zhang et al. [33] report that amyladgin can induce LPS by inhibiting NF-κB and NLRP3 signaling pathways, while baikalin has antiviral, anti-inflammatory, antioxidant, and immunomodulatory pharmacological activities [35]. A recent study has reported that baikalin can significantly inhibit the catalytic activity of SARS-CoV-2 3CLpro and then exert antiviral activity [36]. The virtual screening of active ingredients of SARS-CoV-2 virus also suggests that hesperidin and baikalin have potential inhibitory effects [37]. The glycyrrhizin and glycyrrhizic acid in licorice have been found with extensive anti-inflammatory [38] and anti-damage effects [39]. Some previous studies have shown that glycyrrhizin can serve as an alternative agent for treating COVID-19 infection and its associated respiratory syndrome [40-43]. The extract of Rhizoma Alismatis processed exerts anti-inflammatorv activity via inhibiting cytochrome P450 enzymes [44], the key enzymes responsible for the metabolism of arachidonic acid, thereby reducing the production of inflammatory factors [45-47].

Furthermore, some in vivo metabolites of QFPDD are more likely to be the active ingredients. Previous studies clearly demonstrated that a panel of O-glucuronides of flavonoids displayed good anticoagulant activities, and their anticoagulant activities are better than that of the prototypes [48]. Recent studies have shown that the blood of COVID-19 patients was in a hypercoagulable state, thus the O-glucuronides of flavonoids were more likely to promote blood circulation through anticoagulation and then relieve the symptoms of patients [49-51]. This study was not only found a considerable number of prototypes and metabolites in serum, but also found a large number of chemical components in lung, liver and other tissues. Among them, the active ingredients derived from Herba Ephedrae (ephedrine, pseudoephedrine, methylephedrine, phenylpropanolamine etc.), Fructus Aurantii Immaturus (stachydrine, naringin, hesperidin, neohesperidin, quinic acid etc.), Radix Scutellariae (baicalein, oxorilin A 7-O-glucuronide, wogonoside, wogonin, oxorilin A etc.) and Rhizoma Alismatis (alisol O, alisol A etc.) displayed relatively high exposure levels in most of tested organs. However, the kinds and exposure levels of each component in various tissues are much different. These findings suggest that these in vivo metabolites of QFPDD may play potential role in the treatment of COVID-19 via targeting multiple targets or pathways. In future, the bioactive ingredients and mechanisms of action can be explored.

Conclusion

In summary, a practical and sensitive UHPLC-Q-Exactive-Orbitrap HRMS approach was developed for chemical profiling of the constituents in QFPDD, as well as the absorbed prototypes and the metabolites in mice serum and tissues following oral administration of QFPDD. With the help of the authentic standards and in vitro metabolites generated by MLM, a total of 405 constituents (including 40 kinds of alkaloids, 162 kinds of flavonoids, 44 kinds of organic acids, 71 kinds of triterpene saponins and 88 kinds of other compounds) were identified from the water extract of QFPDD, while 195 components (including 104 prototypes and 91 metabolites) were identified from mice serum after oral administration of QFPDD to mice. Meanwhile, 165, 177, 112, 120, 44, 53 components were identified from the lung, liver, heart, kidney, brain, and spleen of QFPDD-treated mice, respectively. Additionally, the metabolic pathways of some major absorbed components occurring in the serum of QFPDD-treated mice were also described. All these findings provided key information and guidance for further investigations on the pharmacologically active substances and quality control of QFPDD.

Abbreviations

CMs, Chinese Medicines; COVID-19, Corona Virus Disease 2019; CYPs/P450s, cytochrome P450 enzymes; ESI, electrospray ionization; G-6-P, D-Glucose-6-phosphate; G-6-PDH, glucose-6-phosphate dehydrogenase; MLMs, mice liver microsomes; β-NADP+, nicotinamide adenine dinucleotide phosphate disodium salt; PAPS, 3-phosphoadenosine 5-phosphosulfate; PBS, potassium phosphate buffer; QFPDD, Qing-Fei-Pai-Du decoction; SAM, S-adenosyl-L-methionine; SARS, Severe Acute Respiratory Syndrome; UDPGA, uridine diphosphate glucuronic acid; UHPLC-Q-Orbitrap HRMS, ultra-high-performance liquid chromatography-Q ex-active hybrid quadrupole orbitrap high-resolution accurate
mass spectrometric.

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**Supplementary Materials**

Supplementary materials are available as Supporting Information, and can be requested by sending E-mail to the corresponding author.

**References**


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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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