Metabolic profile of Fructus Gardeniae in human plasma and urine using ultra high-performance liquid chromatography coupled with high-resolution LTQ-orbitrap mass spectrometry

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Abstract
1. In China, Fructus Gardeniae was used as a traditional Chinese medicine (TCM) with a wide array of biological activities. The bioactive components identified in Fructus Gardeniae mainly included iridoids, flavonoids, pigments, and so on. Among them, iridoids were regarded as important compounds in Fructus Gardeniae. Though analyses of the constituents in biological samples after oral administration of Fructus Gardeniae effective fraction or its active compounds have been reported, few efforts have been made to investigate the metabolic profile of Fructus Gardeniae in humans. In this study, the constituents and metabolites of Fructus Gardeniae in human blood and urine after oral administration of Fructus Gardeniae were investigated using ultra high-performance liquid chromatography (UHPLC) coupled with high-resolution LTQ-Orbitrap mass spectrometry.

2. Totally, 14 constituents (two parent compounds and 12 metabolites) of Fructus Gardeniae were identified in human plasma and urine either by comparing the retention time and mass spectrometry data with that of reference compounds or by the accurate high-resolution MS/MS data of the chemicals. The compounds identified were mainly iridoid glycosides such as geniposide and the derivatives of genipin-O-glucuronide. Among them, 11 metabolites were detected in human plasma and urine while the other three metabolites including geniposidic acid (M1), demethylation derivative of genipin-O-glucuronide (M2), and dehydration product of mono-hydroxylated genipin-O-glucuronide (M9) were only discovered in human urine. Further, the possible metabolic pathways of Fructus Gardeniae in vivo were proposed and the peak area–time curve of the most abundant metabolite genipin-O-glucuronide (M13) in human plasma after oral administration of Fructus Gardeniae was depicted. The results suggested that a metabolic difference existed between rats and humans.

3. The results obtained in the present research would provide basic information to understand the metabolic profile of Fructus Gardeniae in humans and explore the chemicals responsible for the hepatotoxicity of Fructus Gardeniae in vivo. Moreover, it would be beneficial for us to further study the pharmacokinetic behavior of Fructus Gardeniae in humans systematically.

Keywords
Fructus Gardeniae, human plasma and urine, metabolic profile, UHPLC-HR-MS

Introduction
Fructus Gardeniae (Zhizi) is derived from the ripe fruits of Gardenia jasminoides Ellis and widely used in Asian countries such as China, Korea, and Japan. In China, it has been used as a traditional Chinese medicine (TCM) to treat diseases for a long history. Lines of pharmacology researches demonstrated that Fructus Gardeniae extracts possess a wide array of biological activities on diabetes (Yao et al., 2014), hyperlipidemia (Lee et al., 2006), bacterial infections (Chang et al., 2013; Chu et al., 2013), influenza A virus (Cui et al., 2013; Guo et al., 2014), liver injury (Chen et al., 2012), ligament injury (Chen et al., 2010), acute pancreatitis (Jung et al., 2008), gastritis (Lee et al., 2009), anxiety (Toriizuka et al., 2005), and thrombotic diseases (Wang et al., 2013b; Zhang et al., 2013). In addition, Fructus Gardeniae extracts could inhibit matrix metalloproteinases activity (Yang et al., 2008), down-regulate nitric oxide production (Peng et al., 2013), ameliorate memory impairment (Nam & Lee, 2013), and improve learning (Li et al., 2013) in rats. These effects were closely bound up with the active constituents in Fructus Gardeniae.
The bioactive components identified in Fructus Gardeniae mainly included iridoids (scandoside methyl ester, gardenoside, genipin, geniposide, and acetylgeniposide) (Wang et al., 2012; Zhou et al., 2005, 2007), flavonoids (Cai et al., 2011), pigments (corin, crocetin, and neocrocin A), organic acids (chlorogenic acids, vanillic acid, quinic acid, and their derivatives) (Clifford et al., 2010; Isacchi et al., 2009; Kim et al., 2006), and other lipophilic compounds (Cai et al., 2015). Previous studies mainly focused on the identification or simultaneous determinations of bioactive compounds in Fructus Gardeniae as quality control markers (Bergonzì et al., 2012; Coran et al., 2014; Du et al., 2008; Han et al., 2015; He et al., 2006; Lee et al., 2014; Ouyang et al., 2011; Yang et al., 2011). Among them, eight bioactive constituents including geniposidic acid, chlorogenic acid, genipin-1-β-gentiobioside, geniposide, genipin, rutin, crocin-1, and crocin-2 were suggested as quality control and producing areas differentiation markers of Fructus Gardeniae (Wu et al., 2014; Yin et al., 2015). Nowadays, medicinal herbs are increasingly drawing attention as alternative treatment approaches and the most important consideration involving medicinal plants is to identify the active compounds responsible for the pharmacological activities. Numerous researches have demonstrated that the major active constituents in Fructus Gardeniae were responsible for the majority of medical effects of this fruit. Plasma pharmacochemistry demonstrated that only the constituents absorbed into the blood have the chance to exhibit the effects (Wang et al., 2008). Recently, analyses of the constituents in rat plasma after oral administration of Fructus Gardeniae effective fraction or active compounds (genipin and geniposide) have been reported (Hou et al., 2008; Wang et al., 2013c; Yang et al., 2012; Zhou et al., 2010). Besides, the metabolic profiles of geniposide in rat urine (Han et al., 2011) and genipin in biological samples (rat urine, plasma, feces, and bile) have been clarified (Ding et al., 2013a). However, to our knowledge, few efforts have been made to investigate the metabolic profile of Fructus Gardeniae in human plasma and urine in the literature up to now.

With the development of advanced instrument, high-resolution mass spectrometry (HR-MS) is being used extensively in metabolic analyses owing to its accurate measurements of the mass-to-charge ratio (m/z) of molecular fragments, and retention time (Ding et al., 2013a; Dunn et al., 2013; Han et al., 2011; Ren et al., 2014; Zuo et al., 2015). Considering the importance of drug metabolism in the body, the aim of our study was to investigate the constituents of Fructus Gardeniae in human blood and urine after oral administration of Fructus Gardeniae using ultra high-performance liquid chromatography (UHPLC) coupled with high-resolution LTQ-Orbitrap mass spectrometry.

Materials and methods

Plant materials

Fructus Gardeniae were purchased from Hebei jingcao Pharmaceutical Co., Ltd. (Hebei, China). The batch number was 20150614. The collection of Fructus Gardeniae was permitted by Affiliated Hospital of Inner Mongolia University for the Nationalities (Tongliao, China). The Fructus Gardeniae were authenticated as Gardenia jasminoides Ellis by professor Buhebateer (College of Mongolian Medicine, Inner Mongolia University for the Nationalities).

Chemicals and reagents

Seven standards including geniposidic acid, chlorogenic acid, shanzhiside methyl ester, genipin-1-β-gentiobiosid, geniposide, rutin, and ursolic acid were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); MS-grade formic acid was purchased from Thermo Fisher Biochemical Products Co., Ltd. HPLC-grade methanol, acetonitrile (Fisher, Fair Lawn, NJ) and purified mili-Q water were used for UHPLC-MS analysis.

Quality control of Fructus Gardeniae

Before oral administration, the quality of Fructus Gardeniae was evaluated by UHPLC-HR-MS. Certain amounts of geniposidic acid, chlorogenic acid, shanzhiside methyl ester, genipin-1-β-gentiobiosid, geniposide, rutin, and ursolic acid were dissolved with methanol individually (100 μg/mL for each compound) and stored at 4 °C. According to the literature (Wu et al., 2014), an accurately weighted 1.0 g pulverized Fructus Gardeniae was extracted in an ultrasonic bath with 50 mL 50% methanol for 60 min to get the Fructus Gardeniae sample. The supernatants of the extract were centrifuged at 12 000 rpm for 10 min at 4 °C prior to UHPLC-MS analysis.

Collection and preparation of biosamples

The pulverized Fructus Gardeniae was orally given to human volunteers (n = 6) at a clinical dose (5 g/60 kg). Blood specimens (2 mL) were obtained before dosing and subsequently at 30 min, 1, 2, 3, 4, 4.5, 5, 6, 8, 10, and 12 h after oral administration of Fructus Gardeniae. Samples were collected in heparinized eppendorf tubes and centrifuged at 3000 rpm for 15 min. The plasma was stored at −80 °C until assay. Urine was also collected before dosing and subsequently during 0–12 h. This study was ethically approved by the Medical Ethics Committee of Affiliated Hospital of Inner Mongolia University for the Nationalities (Tongliao, China). The ethical approval number was NM-LL-2009-07-01. All the people voluntarily joined this study with informed consents. The baseline characteristics of the volunteers are shown in Table 1.

The human plasma and human urine samples were prepared according to the procedure described by Zuo et al. with slight modifications (Zuo et al., 2015; Zuo et al., 2014). At each time point, 100 μL aliquot of the plasma of each volunteer (n = 6) was mixed. Further, 30 μL of 1/100 ascorbic acid (1 g of ascorbic acid dissolved in 100 mL of saline) and 1800 μL of methanol were added to the mixed plasma, followed by vortexing for 5 min and centrifuging at 10 000 rpm for 15 min to remove the precipitation. The supernatant was transferred to a clean tube and evaporated to dryness under nitrogen blow at 35 °C. The residue was redissolved in 100 μL of 60% methanol, vortexed for 3 min, and centrifuged at 10 000 rpm for 10 min. The supernatant was analyzed by UHPLC-HR-MS. The preparation of urine samples was conducted the same as the above procedure with
plasma samples, except that 1 mL of urine (n = 6) was mixed with 50 μL of ascorbic acid (1/100) and 3 mL of methanol and finally the residue of urine samples was redissolved in 200 μL of 60% methanol. The supernatant was analyzed by UHPLC-HR-MS. Plasma and urine samples before administration were regarded as controls.

**Instrumentation and chromatographic conditions**

The assay was performed by a Dionex UltiMate 3000 hyperbaric liquid chromatography system coupled to an LTQ Orbitrap mass spectrometer via an ESI interface from Thermo Fisher Scientific (Bremen, Germany). The liquid chromatography system consisted of a diode array detector, an auto-sampler, a column compartment, and two pumps. Xcalibur 3.0, Metwork 1.3, and Mass Frontier 7.0 software packages (Thermo Fisher Scientific Inc., Fair Lawn, NJ) were used for data collection and data analysis.

Liquid chromatographic separations of Fructus Gardeniae and its metabolites were carried out using a Thermo Hypersil BDS C18 column (Thermo Fisher Scientific Inc., Fair Lawn, NJ) (150 mm × 2.1 mm, 2.4 μm). The LC separation was optimized according to the reported methods with slight modification (Fu et al., 2014a,b; Wu et al., 2014). The mobile phase consisted of 0.1% formic acid in water (solvent A) and methanol (solvent B). The optimized gradient elution was as follows: 5–8% B at 0–5 min; 8% B at 5–15 min; 8–30% B at 15–20 min; 30–95% B at 20–25 min; 95% B at 25–32 min; 5% B for equilibration of the column at 32–38 min. The injection volume was 3.0 μL and the flow rate was 0.3 mL/min. The temperature controlled column oven was set at 30 °C and the sampler was set at 4 °C.

The ESI source parameters were set as follows: the heater temperature and the capillary temperature were 350 °C, the spray voltage was set at 3.5 kV in the positive mode and −3.5 kV in the negative ion mode, the normalized collision energy was 35 eV, sheath gas (N2) flow was 35 psi, and the aux gas flow was 10 psi. The ESI source was operated in the negative mode for Fructus Gardeniae extract. To guarantee accurate identifications of metabolites, both positive and negative ion modes were used for the analysis of human plasma and urine. In the Fourier Transform (FT) cell, full MS scans were acquired in the range of m/z 50–1500 with a mass resolution of 30000. In the MS/MS analysis, data requirements were set as data-dependent scans.

**Results**

**Qualitative analysis of Fructus Gardeniae**

The quality of Fructus Gardeniae was evaluated by UHPLC-MS before oral administration. Totally, seven main bioactive constituents, most of which were reported as quality control and producing areas differentiation markers of Fructus Gardeniae, were confirmed in the Fructus Gardeniae extract with standard references (Figure S1). The results showed that genipin-1-β-gentiobiosid and geniposide were the most abundant bioactive components, indicating that the Fructus Gardeniae used in our study achieved an excellent quality (Wu et al., 2014).

**The proposed fragmentation pathways of four iridoid glycosides**

In the present study, the possible ESI-HR-MS/MS fragmentation pathways of four iridoid glycosides (geniposide, geniposidic acid, shanzhiside methyl ester, and genipin-1-β-gentiobioside) were deduced to assist the identification of metabolites in human plasma and urine. In the negative ion mode, geniposide was detected with [M + HCOOH–H]− ion at m/z 433.1344 (C18H25O12, error 0.89 ppm) and [M–H]− ion at m/z 387.1288 (C17H23O10− error 0.53 ppm). In its MS/MS spectrum, the common ions at m/z 355.1027, 225.0767, and 207.0663 assigned as [M–H–CH3OH]−, [M–H–C6H10O5]−, and [M–H–C6H10O5–H2O]− were observed. Similarly, the [M–H]− ion of geniposidic acid at m/z 373.1116 (C16H21O10− error −3.63 ppm) produced the [M–H–C6H10O5]−, [M–H–C6H10O5–CO2]−, and [M–H–C6H10O5–CH2O3]− fragments at m/z 211.0609, 167.0712, and 149.0607, respectively. Shanzhiside methyl ester exhibited [M–H]− ion at m/z 405.1379 (C17H25O11− error −1.26 ppm) and genipin-1-β-gentiobioside showed [M–H]− ion at m/z 549.1823 (C22H33O15− error 1.55 ppm) in the full mass spectra. According to relevant references (Fu et al., 2014a,b; Wu et al., 2010; Zuo et al., 2015), their fragmentation pathways were proposed (shown in Figure S2). The neutral losses of CH3OH, H2O, and C6H10O5 were the main fragmentation patterns for iridoid glycosides in the negative ionization mode. In addition, the characteristic ions at m/z 225.0767 (C11H21O3) and 123.0452 (C7H7O2) were found in the MS/MS spectra of the four iridoid glycosides, which played an important role in the metabolite identification (Table 2).

**Metabolite identification in human plasma and urine**

First, a wide range of mass values from 150 to 1000 one by one in order were extracted without mass defect filter (MDF) by comparing the UPLC-MS chromatograms of the administrated versus control samples to discover the potential metabolites. Based on the obtained m/z of the potential metabolites, extractions of their HR-MS data with MDF were performed by comparing the UPLC-MS chromatograms of the administrated versus control samples to further identify the metabolites. The key parameters were carefully modulated as follows: the maximum tolerance of the MDF was set at 5 ppm; elemental compositions for expected and unexpected metabolite peaks were generated based on the extensively possible formula of the compounds. The structural elucidation of the constituents was further clarified by comparing their retention time and MS/MS fragmentations with those of standards or data reported in the literatures. False positive results were not reported.
M1 was detected in human urine at retention time ($t_R = 4.23$ min with $[\text{M–H}^-]$ and $[\text{M+Na}^+]$ ions at $m/z$ 373.1136 ($\text{C}_{16}\text{H}_{23}\text{O}_{10}$, Cal. 373.1129, error 1.13 ppm) and 397.1099 ($\text{C}_{16}\text{H}_{25}\text{O}_{10}\text{Na}$, Cal. 397.1105, error $–1.66$ ppm), respectively. By comparison with geniposide, the product ions of M1 in negative ion mode at $m/z$ 211.0612, 167.0714, 149.0609, and 123.0453 were all 14 Da ($\text{CH}_2$) less than those of M13 in negative ion mode at $m/z$ 225.0767, 211.0609, 175.0246, and 139.0400, respectively. By comparison with geniposide, the product ions of M13 were 14 Da ($\text{CH}_2$) less than those of the corresponding product of geniposide in negative ion mode at $m/z$ 399.0920, 385.0775, 341.0871, 241.0715, 165.0712, and 139.0400, respectively, in the chromatogram were detected in human plasma and urine. They were observed as metabolites M7 and M8 with the same $[\text{M+H}]^+$ ion at $m/z$ 361.1490 ($\text{C}_{16}\text{H}_{25}\text{O}_{9}$, Cal. 361.1493, error $–0.88$ ppm) and $[\text{M–H}^-]$ ion at $m/z$ 359.1343 ($\text{C}_{16}\text{H}_{25}\text{O}_{9}$, Cal. 359.1337, error 1.90 ppm). In the negative ion mode, the product ion at $m/z$ 175.0245, indicating that a glucuronic acid was attached to the aglycone moiety. The product ion at $m/z$ 175.0246 resulted from the neutral loss of $\text{C}_6\text{H}_{10}\text{O}_7$ and $\text{C}_7\text{H}_8\text{O}_6$, respectively, were the common fragments. All these fragments were 16 Da more than those of M13 (Figure 4). Other two product ions at $m/z$ 193.0355 and 175.0246 resulted from the neutral loss of $\text{C}_6\text{H}_{10}\text{O}_7$, and $\text{C}_6\text{H}_{10}\text{O}_6$ groups were the same as those of M13, suggesting that they were mono-glucuronidated conjugates. For M4 and M5, $[\text{M–H–C}_6\text{H}_2\text{O}_4\text{O}_3]^- \text{ ion at } m/z = 227.0577 (\text{C}_7\text{H}_{12}\text{O}_6)$ was the unique fragment detected. And for M3, M5, and M6, the characteristic $[\text{M–H–C}_6\text{H}_2\text{O}_4\text{O}_3]^- \text{ ion at } m/z = 311.0768$ was observed. However, the concrete hydroxylation site could not be confirmed. Consequently, M3, M4, M5, and M6 were deduced as mono-hydroxylated products of genipin-O-glucuronide.

Two isomers diluted at $t_R = 8.87$ min and 15.30 min, respectively, in the chromatogram were detected in human plasma and urine. They were observed as metabolites M7 and M8 with the same $[\text{M+H}]^+$ ion at $m/z$ 361.1490 ($\text{C}_{16}\text{H}_{25}\text{O}_{9}$, Cal. 361.1493, error $–0.88$ ppm) and $[\text{M–H}^-]$ ion at $m/z$ 359.1343 ($\text{C}_{16}\text{H}_{25}\text{O}_{9}$, Cal. 359.1337, error 1.90 ppm). In the negative ion mode, the product ion at $m/z$ 183.0272 was observed in their MS/MS spectra, with a loss of 176 Da from M13, suggesting that they were mono-hydroxylated products of M13. In their MS/MS spectra, $[\text{M–H–H}_2\text{O}]^–$ ions of M13 were confirmed as mono-hydroxylated products of genipin-O-glucuronide, which was previously reported as a metabolite of genipin in rat bile (Ding et al., 2013a) (Table 3).

M3, M4, M5, and M6 were four isomers eluted at $t_R = 5.17$ min. 7.32 min, 8.44 min, and 16.44 min, respectively in the chromatography (Figure 3). They were detected in both human plasma and urine. In the positive mode, they showed identical $[\text{M+Na}^+]$ ion at $m/z$ 441.0992 ($\text{C}_{17}\text{H}_{22}\text{O}_{12}\text{Na}$, Cal. 441.1004, error $–2.71$ ppm). In the negative ion mode, they exhibited the same $[\text{M–H}^-]$ ion at $m/z$ 417.1036 ($\text{C}_{17}\text{H}_{22}\text{O}_{12}$, Cal. 417.1028, error 2.06 ppm) with an increased 16 Da (O) from M13, suggesting that they were mono-hydroxylated products of M13. In their MS/MS spectra, $[\text{M–H–H}_2\text{O}]^–$, $[\text{M–H–CH}_3\text{OH}]^–$, $[\text{M–H–CH}_3\text{OH–CO}_2]^-$, $[\text{M–H–C}_6\text{H}_2\text{O}_4\text{O}_3]^-$, $[\text{M–H–C}_6\text{H}_2\text{O}_4\text{O}_3]$ and $[\text{M–H–C}_{10}\text{H}_2\text{O}_4\text{O}_3]^- \text{ fragments at } m/z = 399.0920, 385.0775, 341.0871, 241.0715, 165.0556, and 139.0400, respectively, were the common fragments. All these fragments were 16 Da more than those of M13 (Figure 4). Other two product ions at $m/z$ 193.0355 and 175.0246 resulted from the neutral loss of $\text{C}_6\text{H}_{10}\text{O}_7$, and $\text{C}_6\text{H}_{10}\text{O}_6$ groups were the same as those of M13, suggesting that they were mono-glucuronidated conjugates. For M4 and M5, $[\text{M–H–C}_6\text{H}_2\text{O}_4\text{O}_3]^- \text{ ion at } m/z = 227.0577 (\text{C}_7\text{H}_{12}\text{O}_6)$ was the unique fragment detected. And for M3, M5, and M6, the characteristic $[\text{M–H–C}_6\text{H}_2\text{O}_4\text{O}_3]^- \text{ ion at } m/z = 311.0768$ was observed. However, the concrete hydroxylation site could not be confirmed. Consequently, M3, M4, M5, and M6 were deduced as mono-hydroxylated products of genipin-O-glucuronide.

Two isomers diluted at $t_R = 8.87$ min and 15.30 min, respectively, in the chromatogram were detected in human plasma and urine. They were observed as metabolites M7 and M8 with the same $[\text{M+H}]^+$ ion at $m/z$ 361.1490 ($\text{C}_{16}\text{H}_{25}\text{O}_{9}$, Cal. 361.1493, error $–0.88$ ppm) and $[\text{M–H}^-]$ ion at $m/z$ 359.1343 ($\text{C}_{16}\text{H}_{25}\text{O}_{9}$, Cal. 359.1337, error 1.90 ppm). In the negative ion mode, the product ion at $m/z$ 175.0245, indicating that a glucuronic acid was attached to the aglycone moiety. The product ion at $m/z$ 175.0246 was observed in their MS/MS spectra, with a loss of 176 Da from M13, suggesting that they were mono-hydroxylated products of M13. In their MS/MS spectra, $[\text{M–H–H}_2\text{O}]^–$ ions of M13 were confirmed as mono-hydroxylated products of genipin-O-glucuronide.

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Figure 1. Extracted ion chromatograms of M1 and M12 in human plasma and urine.

Figure 2. The MS/MS spectra of geniposidic acid and M2.
Table 3. UHPLC-HR-MS data of the parent compounds and metabolites identified in human plasma and urine.

| Compound no. | \( t_R \) (min) | Ion form          | Molecular formula | Calculated mass | Measured mass | Error (ppm) | Identification                                      | Plasma | Urine |
|--------------|------------------|-------------------|-------------------|-----------------|---------------|-------------|-----------------------------------------------------|--------|-------|
| M1           | 4.23             | \([\text{M}−\text{H}]^-\) | \( \text{C}_{16}\text{H}_{21}\text{O}_{10} \) | 373.1129        | 373.1136      | 1.13        | Geniposidic acid                                    | −      | +     |
|              |                   | \([\text{M}+\text{Na}]^+\) | \( \text{C}_{16}\text{H}_{22}\text{O}_{10}\text{Na} \) | 397.1105        | 397.1098      | −1.66       |                                                     |        |       |
| M2           | 4.35             | \([\text{M}−\text{H}]^-\) | \( \text{C}_{16}\text{H}_{22}\text{O}_{11} \) | 387.0922        | 387.0930      | 2.00        | Demethylation derivative of genipin-O-glucuronide   | −      | +     |
|              |                   | \([\text{M}+\text{Na}]^+\) | \( \text{C}_{16}\text{H}_{22}\text{O}_{10}\text{Na} \) | 441.1004        | 441.0992      | −2.71       |                                                     |        |       |
| M3           | 5.17             | \([\text{M}−\text{H}]^-\) | \( \text{C}_{17}\text{H}_{21}\text{O}_{12} \) | 417.1028        | 417.1036      | 2.06        | Mono-hydroxylated product of genipin-O-glucuronide  | +      | +     |
|              |                   | \([\text{M}+\text{Na}]^+\) | \( \text{C}_{17}\text{H}_{22}\text{O}_{10}\text{Na} \) | 441.1004        | 441.0994      | −2.08       |                                                     |        |       |
| M4           | 7.32             | \([\text{M}−\text{H}]^-\) | \( \text{C}_{17}\text{H}_{21}\text{O}_{12} \) | 417.1028        | 417.1037      | 2.27        | Mono-hydroxylated product of genipin-O-glucuronide  | +      | +     |
|              |                   | \([\text{M}+\text{Na}]^+\) | \( \text{C}_{17}\text{H}_{22}\text{O}_{10}\text{Na} \) | 441.1004        | 441.0994      | −2.08       |                                                     |        |       |
| M5           | 8.44             | \([\text{M}−\text{H}]^-\) | \( \text{C}_{17}\text{H}_{21}\text{O}_{12} \) | 417.1028        | 417.1040      | 2.57        | Mono-hydroxylated product of genipin-O-glucuronide  | +      | +     |
|              |                   | \([\text{M}+\text{Na}]^+\) | \( \text{C}_{17}\text{H}_{22}\text{O}_{10}\text{Na} \) | 441.1004        | 441.0998      | −1.33       |                                                     |        |       |
| M6           | 16.44            | \([\text{M}−\text{H}]^-\) | \( \text{C}_{17}\text{H}_{21}\text{O}_{12} \) | 417.1028        | 417.1024      | −0.94       | Mono-hydroxylated product of genipin-O-glucuronide  | +      | +     |
|              |                   | \([\text{M}+\text{Na}]^+\) | \( \text{C}_{17}\text{H}_{22}\text{O}_{10}\text{Na} \) | 441.1004        | 441.0998      | −1.33       |                                                     |        |       |
| M7           | 8.87             | \([\text{M}−\text{H}]^-\) | \( \text{C}_{16}\text{H}_{23}\text{O}_{9} \) | 359.1337        | 359.1343      | 1.90        | Mono-glucuronidated product of ring opened and methyl formate removal of genipin | +      | +     |
|              |                   | \([\text{M}+\text{H}]^+\) | \( \text{C}_{16}\text{H}_{25}\text{O}_{9} \) | 361.1493        | 361.1490      | −0.88       |                                                     |        |       |
| M8           | 15.30            | \([\text{M}−\text{H}]^-\) | \( \text{C}_{16}\text{H}_{23}\text{O}_{9} \) | 359.1337        | 359.1344      | 2.15        | Mono-glucuronidated product of ring opened and methyl formate removal of genipin | +      | +     |
|              |                   | \([\text{M}+\text{H}]^+\) | \( \text{C}_{16}\text{H}_{25}\text{O}_{9} \) | 361.1493        | 361.1489      | −1.24       |                                                     |        |       |
| M9           | 9.09             | \([\text{M}−\text{H}]^-\) | \( \text{C}_{17}\text{H}_{21}\text{O}_{11} \) | 399.0922        | 399.0932      | 2.46        | Dehydration product of mono-hydroxylated genipin-O-glucuronide | −      | +     |
|              |                   | \([\text{M}+\text{H}]^+\) | \( \text{C}_{17}\text{H}_{23}\text{O}_{11} \) | 423.0898        | 423.0889      | −2.09       |                                                     |        |       |
| M10          | 11.04            | \([\text{M}−\text{H}]^-\) | \( \text{C}_{17}\text{H}_{21}\text{O}_{11} \) | 403.1235        | 403.1242      | 1.64        | Ring-opened derivative of genipin-O-glucuronide      | +      | +     |
|              |                   | \([\text{M}+\text{Na}]^+\) | \( \text{C}_{17}\text{H}_{22}\text{O}_{11}\text{Na} \) | 427.1211        | 427.1207      | −1.02       |                                                     |        |       |
| M11          | 19.01            | \([\text{M}−\text{H}]^-\) | \( \text{C}_{17}\text{H}_{21}\text{O}_{11} \) | 403.1235        | 403.1241      | 1.40        | Ring-opened derivative of genipin-O-glucuronide      | +      | +     |
|              |                   | \([\text{M}+\text{Na}]^+\) | \( \text{C}_{17}\text{H}_{22}\text{O}_{11}\text{Na} \) | 427.1211        | 427.1205      | −1.35       |                                                     |        |       |
| M12          | 15.70            | \([\text{M}−\text{HCOOH-H}]^-\) | \( \text{C}_{18}\text{H}_{25}\text{O}_{12} \) | 433.1341        | 433.1343      | 0.67        | Geniposide                                          | +      | +     |
| M13          | 16.61            | \([\text{M}−\text{H}]^-\) | \( \text{C}_{17}\text{H}_{21}\text{O}_{11} \) | 401.1078        | 401.1090      | 2.92        | Genipin-O-glucuronide                                | +      | +     |
| M14          | 19.75            | \([\text{M}−\text{H}]^-\) | \( \text{C}_{16}\text{H}_{25}\text{O}_{8} \) | 345.1544        | 345.1539      | −1.35       | Reduced derivative of genipin-O-glucuronide         | +      | +     |

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Figure 3. Extracted ion chromatograms of M3, M4, M5, and M6 in human plasma and urine.

Figure 4. The MS/MS spectra of M3, M4, M5, M6, and M13.
literature (Ding et al., 2013a; Han et al., 2011). The unique products of M8 at \(m/z\) 299.1133 and \(m/z\) 283.1184 were detected. By comparison with the published papers (Ding et al., 2013a; Han et al., 2011), they were deduced as mono-glucuronidated products of ring opened and methyl formate removal of genipin. But the accurate site of glucuronated action could not be confirmed because it could be occurred on any of the three hydroxyl groups.

M9 was eluted at 9.09 min and displayed \([M + Na]^+\) ion at \(m/z\) 423.0889 (C_{17}H_{20}O_{11}Na, Cal. 423.0898, error 2.09 ppm) and \([M–H]^-\) ion at \(m/z\) 399.0932 (C_{17}H_{19}O_{11}, Cal. 399.0922, error 2.46 ppm). Accurate mass measurement showed that the chemical formula of M9 was C_{17}H_{20}O_{11}, which was 18 Da (H_{2}O) less than that of M3. In addition, the main fragment of M9 at \(m/z\) 223.0613 was 18 Da (H_{2}O) less than that of M3 at \(m/z\) 241.0715, suggesting the loss of one H_{2}O from metabolite M3. By comparison with the \([M–H–C_6H_8O_6–CH_3OH]^-\) fragment of M3 at \(m/z\) 209.0453, the character \([M–H–C_6H_8O_6–CH_3]^-\) product ion of M9 at \(m/z\) 208.0376 was detected, revealing that one H_{2}O might lose from the CH_{3}OH group. The \([M–H]^-\) ion of M9 could generate the product ion at \(m/z\) 223.0613 by losing a C_{6}H_{8}O_{6} unit, indicating that there was a glucuronic acid group in M9. Thus, M9 was identified as a dehydration product of mono-hydroxylated genipin-O-glucuronide based on its MS/MS fragments.

M10 (\(t_R = 11.04\) min) and M11 (\(t_R = 19.01\) min) detected in the plasma and urine were two isomers with identical \([M + Na]^+\) ion at \(m/z\) 427.1207 (C_{17}H_{24}O_{11}Na, Cal. 427.1211, error 1.02 ppm) in the positive ion mode and the same \([M–H]^-\) ion at \(m/z\) 403.1242 (C_{17}H_{23}O_{11}, Cal. 403.1235, error 1.64 ppm) in the negative mode. Accurate mass measurement showed that their chemical formula was 2 Da (2H) more than that of M13, suggesting two dihydrogen derivatives of M13. Their product ions at \(m/z\) 385.1134, 371.0974, 327.1069, and 227.0925 were 2 Da more than those of M13 as well. Their fragment at \(m/z\) 175.0248 indicated that a glucuronic acid was attached to the aglycone moiety. According to the literature, the fragments of M10 and M11 were the same as those of the metabolites reported (Ding et al., 2013a; Zuo et al., 2015) and they were deduced as ring-opened derivatives of genipin-O-glucuronide.

M12 was a prototype in the plasma and urine. It diluted at \(t_R = 15.70\) min with \([M + HCOOH–H]^-\) ion at \(m/z\) 433.1343 (C_{18}H_{25}O_{12}, Cal. 433.1341, error 0.66 ppm) in the full mass spectrum. In its MS/MS spectrum, the common neutral losses of \([M–H–HCOOH]^-\), \([M–H–HCOOH–C_6H_{10}O_5]^-\) and \([M–H–HCOOH–C_6H_{10}O_5–C_4H_6O_3]^-\) fragmentations at \(m/z\) 387.1297, 255.0769, and 123.0452 were observed. The \(t_R\) and \([M + HCOOH–H]^-\) ion of M12 in the full MS were the same as those of geniposide (Figure 3). In addition, all the fragments of M12 were identical with those of geniposide in the MS/MS spectra. Thus, M12 was confirmed as geniposide.

M13 (\(t_R = 16.61\) min) was the most abundant metabolite in human plasma and urine on the basis of the chromatographic peak area. It displayed \([M + Na]^+\) and \([M + H]^+\) ions at \(m/z\) 425.1052 (C_{17}H_{22}NaO_{11}, calculated 425.1054; error –0.64 ppm) and \(m/z\) 403.1231 (C_{17}H_{21}O_{11}, calculated 403.1230; error 0.86 ppm) in the positive ion mode. In the negative ion mode, M13 showed an \([M–H]^-\) ion at \(m/z\) 401.1090 (C_{17}H_{21}O_{11}, calculated 401.1078; error 2.92 ppm). In its negative MS/MS spectrum, the characteristic product ion at \(m/z\) 225.0764 with neutral loss of a glucuronide unit (176 Da)
was detected. Fragments at m/z 207.0661, 147.0449, and 123.0451 were the same as those of geniposide. Additionally, [M−H−H2O]−, [M−H−CH3OH]−, [M−H−CH3OH−CO2]−, [M−H−H2O−C6H8O6]− ions at m/z 383.0981, 369.0827, 325.0930, 307.0819, and 207.0661, respectively, (Figure 4) assured its structure as genipin-O-glucuronide (Ding et al., 2013a; Zuo et al., 2015). For steric reasons, the glucuronation site of M13 at C-1 was more likely.

The chromatographic peak eluted at tR = 19.75 min (M14) exhibited [M + H]+ and [M−H]− ions at m/z 345.1539 (C16H25O8, calculated 345.1544; error −1.34 ppm) and m/z 343.1399 (C16H23O8, calculated 343.1387; error 3.43 ppm). The characteristic neutral loss of 176 Da were detected at m/z 175.0248 in the MS/MS spectrum of M14, indicating the presence of glucuronide residue on the structure. Considering that the elemental composition of M14 was one O (16 Da) less than that of M7 and M8, it was deduced that M14 underwent deoxidation from M7 or M8. In the MS/MS analysis, the fragment of M14 at m/z 325.1288 was 16 Da less than that of M7 and M8 at m/z 341.1238 (Figure 5). Other fragments at m/z 193.0355, 175.0248, and 113.0244 were the same as those of M7 and M8. According to the above evidence, M14 was identified as a reduced product of M7 or M8, namely, the ring opened and reduced derivative of genipin-O-glucuronide (Han et al., 2011).

In general, 14 constituents (two parent compounds and twelve metabolites) of Fructus Gardeniae were identified in human plasma and urine by UHPLC-MS/MS data (Table S1). Among them, 11 metabolites were detected in human plasma and urine while the other three metabolites (M1, M2, and M9) were only discovered in human urine. Based on the metabolites detected in human plasma and urine, the possible metabolic pathways of Fructus Gardeniae in vivo were proposed (Figure 6).

**Discussion**

Fructus Gardeniae, a commonly used TCM called Zhizi in Chinese, was first recorded in the book named “Shen Nong’s Herbal Classic” in China. In Chinese medical theory, with a kind of cool and bitter character, it could ease the mind, reduce pathogenic fire, eliminate damp-heat, and remove heat-toxicity from blood. Nowadays, the exploitations of gardenia plants had been involved in food additives, dyestuffs, cultivation of ornamental plant, antiseptic, and new medicines (Liu et al., 2013; Park et al., 2013). Zhang et al. (2014) reviewed that there are nearly 18 healthcare functions of the health food containing Fructus Gardeniae, and 23 formulas containing Fructus Gardeniae have assisted function to protect chemical liver injury and modulate the immune system. Liu et al. (2013) reviewed that crocins and iridoid glycosides exhibited considerable biological activities. At present, consumers are becoming more and more interested in naturally occurring colorants with bioactives, which could offer health benefits. Natural colorants derived from Fructus Gardeniae and its processed products are particularly of significance in the food industry (Wrolstad & Culver, 2012). In Japan, gardenia blue iridoid pigment extracted from Gardenia jasminoides is approved for food use. In Asian countries, geniposide is used as a functional food and traditional medicine, and is also applied as a food coloring (Cai et al., 2015). However, Fructus Gardeniae extracts were reported to exhibit hepatotoxicity (Ding et al., 2013b; Liu et al., 2013;
Wang et al., 2013a; Yang et al., 2006). Thus, being a functional food and TCM with multi-pharmacological activities and hepatotoxicity, an in-depth study of its constituents absorbed into the blood and its metabolites in vivo was necessary and significant.

In previous studies, Yang et al. (2012) detected 13 compounds in rat blood after oral administration of Fructus Gardeniae extract. Zhou et al. (2010) reported that 7 major iridoid glycosides (parent compounds) were characterized in rat plasma after intravenous administration of Gardenia jasminoides Ellis. In their studies, no conjugated metabolite was found in rat serum. Further, Wang et al. (2013c) identified four iridoid glucosides (parent compounds) and one of their metabolite in rat serum after administration of Fructus Gardeniae target fraction. By comparison, in our study, 11 constituents (one parent compound and 10 metabolites) in human plasma and 14 compounds (two parent compounds and 12 metabolites) in human urine were tentatively characterized after oral administration of Fructus Gardeniae (shown in Figure 6). Although glucuronidated derivatives of iridoids and iridoid glycosides were the main compounds detected in humans and mainly parent compounds were discovered in rats after oral administration of Fructus Gardeniae, the metabolic pathway of Fructus Gardeniae in humans agreed with those of geniposide and genipin in rats (Han et al., 2011; Ding et al., 2013a,b), except that hydroxylation was another metabolic pathway of Fructus Gardeniae in humans. The results suggested that a metabolic difference existed between rats and humans. It was reported that the noxious property of Gardeniae extracts was related to the hepatotoxicity and genotoxic of genipin (Liu et al., 2013) and geniposide (Ding et al., 2013b; Wang et al., 2013a; Yang et al., 2006). Our results provided a collaborative evidence that iridoid glycosides, especially M13 (the most abundant metabolite in human plasma and urine), a genipin-O-glucuronide metabolite, might be the chemicals responsible for the hepatotoxicity of Fructus Gardeniae in vivo. Further, the peak area–time curve of M13 in human plasma after oral administration of Fructus Gardeniae was depicted based on the chromatographic peak area (Figure 7). As shown in Figure 7, the time of M13 to reach the maximum plasma concentration ($T_{\text{max}}$) was at 4.5 h. Zheng et al. (2012) reported that the $T_{\text{max}}$ of geniposidic acid in rat was 1.0 h, demonstrating an obvious pharmacokinetic difference of iridoid glycosides between humans and animals. In addition, by comparison with one blood collection time (20 min) in rats (Yang et al., 2012), our study collected human plasma at different time points (30 min, 1, 2, 3, 4, 4.5, 5, 6, 8, 10, and 12 h) to get a more scientific, reasonable, and comprehensive metabolite identification result. However, the structures of the compounds in TCM were so complex and many metabolites contain similar moieties that it would be hard to unambiguously confirm all the constituents solely by mass spectrometry (Zuo et al., 2015). Thus, the metabolite identification in the present study is preliminary and speculative, but it might be helpful to understand the metabolic profile of Fructus Gardeniae in vivo and discover the active constituents of Fructus Gardeniae responsible for its hepatotoxicity and pharmacological activities.

Conclusions

In the present study, a simple and specific UHPLC-HR-MS method for the quality control of Fructus Gardeniae and its metabolite profile in human plasma and urine was developed. Totally, two parent compounds and 12 metabolites of Fructus Gardeniae were identified in human plasma and urine based on the characteristic fragmentation behaviors of four iridoids and the accurate high-resolution MS/MS data of the chemicals. Further, the possible metabolic pathways of Fructus Gardeniae in vivo were proposed and the peak area–time curve of the most abundant metabolite M13 in human plasma after oral administration of Fructus Gardeniae was depicted based on the chromatographic peak area. The results obtained in the present research would provide basic information to understand the metabolic process of Fructus Gardeniae in humans. Moreover, it would be beneficial for us to further study the pharmacokinetic behavior of Fructus Gardeniae in humans systematically.

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Declaration of interest

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References

Bergonzzi MC, Righeschi C, Isacchi B, Bilia AR. (2012). Identification and quantification of constituents of Gardenia jasminoides Ellis (Zhizi) by HPLC-DAD-ESI-MS. Food Chem 134:1199–204. Cai DJ, Shu Q, Xu BQ, et al. (2011). Orthogonal test design for optimization of the extraction of flavonoid from the Fructus Gardeniae. Biomed Environ Sci 24:688–93. Cai X, Zhang R, Guo Y, et al. (2015). Optimization of ultrasound-assisted extraction of gardenia fruit oil with bioactive components and their identification and quantification by HPLC-DAD/ESI-MS2. Food Funct 6:2194–204.
Chang CH, Chen YS, Chiou MT, et al. (2013). Application of Scutellariae Radix, Gardeniae Fructus, and probiotics to prevent salmonella enterica serovar choleraesuis infection in swine. Evid Based Complement Alternat Med 2013:568528.

Chen QC, Zhang WY, Kim H, et al. (2010). Effects of Gardeniae Fructus extract and geniposide on promoting ligament cell proliferation and collagen synthesis. Phytother Res 24:S1–5.

Chen SD, Li J, Zhou HH, et al. (2012). Study on effects of zhi zi (Fructus Gardeniae) on non-alcoholic fatty liver disease in the rat. J Tradit Chin Med 32:82–6.

Chu W, Zhou S, Jiang Y, et al. (2013). Effect of traditional Chinese herbal medicine with antiquorum sensing activity on pseudomonas aeruginosa. Evid Based Complement Alternat Med 2013:e648257.

Clifford MN, Wu WG, Kirkpatrick J, et al. (2010). Profiling and characterisation by liquid chromatography/multi-stage mass spectrometry of the chlorogenic acids in Gardenia Fructus. Rapid Commun Mass Spectrom 24:3109–20.

Coran SA, Mulas S, Vasconi A. (2014). Profiling of components and validated determination of iridoids in Gardenia Jasmoinoides Ellis fruit by high-performance-thin-layer-chromatography/mass spectrometry approach. J Chromatogr A 1325:221–6.

Cui XL, Guo SS, Shi YJ, et al. (2013). The inhibitory effect of iridoid glycoside extracted from Fructus Gardeniae on ion channel activity of influenza A virus M2 protein. Acta Pharmacol Sin 34:22–33.

Ding Y, Hou JW, Zhang Y, et al. (2013a). Metabolism of genipin in rat and identification of metabolites by using ultra-performance liquid chromatography/quadrupole time-of-flight tandem mass spectrometry. Evid Based Complement Alternat Med 2013:957030.

Ding Y, Zhang T, Tao JS, et al. (2013b). Potential hepatotoxicity of geniposide, the major iridoid glycoside in dried ripe fruits of Gardenia jasminoides (Zhi-zi). Nat Prod Rev 27:29–33.

Du W, Cai H, Wang M, et al. (2008). Simultaneous determination of six active components in crude and processed Fructus Corni by high performance liquid chromatography. J Pharm Biomed Anal 48:194–7.

Dunn WB, Erban A, Weber RJ, et al. (2013). Mass appeal: metabolite identification in mass spectrometry-focused untargeted metabolomics. Metabolomics 9:44–66.

Fu ZW, Ling Y, Li ZX, et al. (2014a). HPLC-Q-TOF-MS/MS for analysis of major chemical constituents of Yinchen-Zhizi herb pair extract. Biomed Chromatogr 28:799–807.

Fu ZW, Xue R, Li ZX, et al. (2014b). Fragmentation patterns study of iridoid glycosides in Fructus Gardeniae by HPLC-Q-TOF-MS/MS. Biomed Chromatogr 28:1795–807.

Guo SS, Gao YJ, Jin YH, et al. (2014). The inhibitory effect of iridoid glycoside extracted from Fructus Gardeniae on intracellular acidification and extracellular Ca2+ influx induced by influenza A virus. Exp Biol Med 239:986–97.

Han H, Yang L, Xu Y, et al. (2011). Identification of metabolites of geniposide in rat urine using ultra-performance liquid chromatography combined with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. Rapid Commun Mass Spectrom 25:3339–50.

Han Y, Wen J, Zhou T, Fan G. (2015). Chemical fingerprinting of Gardeniae jasminoides Ellis by HPLC-DAD-ESI-MS combined with chemometrics methods. Food Chem 188:648–57.

He ML, Cheng XW, Chen JK, Zhou TS. (2006). Simultaneous determination of five major biologically active ingredients in different parts of Gardenia jasminoides Fruits by HPLC with diode-array detection. Chromatographia 64:713–17.

Hou YC, Tsai SY, Lai PY, et al. (2008). Metabolism and pharmacokinetics of genipin and geniposide in rats. Food Chem Toxicol 46:2764–9.

Isacchi B, Bergonzoni MC, Righeschi C, et al. (2009). Problems for the validation of analyses of TCM herbal drugs and herbal drug preparations: the case Fructus Gardeniae (Zhizi) and its preparata. Planta Med 75:1004.

Jung WS, Chae YS, Kim DY, et al. (2008). Gardenia jasminoides protects against cerulein-induced acute pancreatitis. World J Gastroenterol 14:6188–94.

Kim HJ, Kim EI, Seo SH, et al. (2006). Vanillic acid glycoside and quinic acid derivatives from Gardeniae Fructus. J Nat Prod 69:600–3.

Lee EJ, Hong JK, Whang WK. (2014). Simultaneous determination of bioactive marker compounds from Gardeniae Fructus by high performance liquid chromatography. Arch Pharm Res 37:992–1000.

Lee IA, Min SW, Kim DH. (2006). Lactic acid bacteria increases hypolipidemic effect of crocin isolated from fructus of Gardenia jasminoides. J Microbiol Biotechnol 16:1084–9.

Lee JH, Lee DU, Jeong CS. (2009). Gardenia jasminoides Ellis ethanol extract and its constituents reduce the risks of gastritis and reverse gastric lesions in rats. Food Chem Toxicol 47:1127–31.

Li B, Fu Z, Hu R, et al. (2013). Semen Ziziphi Spinosae and Fructus Gardeniae extracts synergistically improve learning and memory of a mouse model. Biomed Rep 1:247–50.

Liu H, Chen YF, Li F, Zhang HY. (2013). Fructus Gardenia (Gardenia jasminoides L. Ellis) phytochemistry, pharmacology of cardiovascular, and safety with the perspective of new drugs development. J Asian Nat Prod Res 15:94–110.

Nam Y, Lee D. (2013). Ameliorating effect of zhi zi (Fructus gardeniae) extract and its glycosides on scopolamine-induced memory impairment. J Tradit Chin Med 33:223–7.

Ouyang E, Zhang C, Li X. (2011). Simultaneous determination of geniposide, chlorogenic acid, crocin1, and rutin in crude and processed Fructus Gardeniae extracts by high performance liquid chromatography. Pharmazie Mag 7:267–70.

Park KH, Kim TY, Park JY, et al. (2013). Photochemical properties of dye-sensitized solar cell using mixed natural dyes extracted from Gardenia jasminoides Ellis. J Electro Chem 689:21–5.

Peng K, Yang L, Zhao S, et al. (2013). Chemical constituents from the fruit of Gardenia jasminoides and their inhibitory effects on nitric oxide production. Bioorg Med Chem Lett 23:1127–31.

Ren W, Li Y, Zuo R, et al. (2014). Species-related difference between limonin and obacunone among five liver microsomes and zebrafish using ultra-high-performance liquid chromatography coupled with a LTQ-Orbitrap mass spectrometer. Rapid Commun Mass Spectrom 28:2292–300.

Toriizuka K, Kamiki H, Ohmura NY, et al. (2005). Anxiolytic effect of Gardeniae Fructus-extract containing active ingredient from Kamishoyosan (KSS), a Japanese traditional Kampo medicine. Life Sci 77:3010–20.

Wang K, Jin RM, Chen CX. (2013a). Comparative study on hepatic toxicity of Gardeniae Fructus and Huanglian Jiedu decoction. Chin J Chin Mater Med 38:2365–9.

Wang P, Wang QX, Luo CH, et al. (2013b). Iridoid glycosides extracted from Fructus Gardenia decreased collagen-induced platelet aggregation and reduce carotid artery thrombosis in an in vivo rat model. J Tradit Chin Med 33:531–4.

Wang X, Sun W, Sun H, et al. (2008). Analysis of the constituents in the rat plasma after oral administration of Yin Chen Hao Tang by UPLC-Q-TOF-MS/MS. J Pharm Biomed Anal 46:477–90.

Wang XJ, Li ZX, Wu B, et al. (2013c). Characterization of the constituents absorbed in rats after oral administration of Fructus Gardeniae effective fraction. Asian J Chem 25:7899–903.

Wang XS, Wu YF, Dai SL, et al. (2012). Ultrasound-assisted extraction of geniposide from Gardenia jasminoides. Ultrason Sonochem 19:1155–9.

Wrolstad RE, Culver CA. (2012). Alternatives to those artificial FD&C food colorants. Annu Rev Food Sci Technol 3:59–77.

Wu Q, Yuan Q, Liu EH, et al. (2010). Fragmentation study of iridoid glycosides and phenylpropanoid glycosides in Radix Scrophulariae by rapid resolution liquid chromatography with diode-array detection and electrospray ionization time-of-flight mass spectrometry. Biomed Chromatogr 24:877–85.

Wu YY, Zhou Y, Yin FZ, et al. (2014). Quality control and producing areas differentiation of Gardeniae Fructus for eight bioactive constituents by HPLC-DAD-ESI/MS. Phytomedicine 21:551–9.

Yang BJ, Fu MH, Wu ZL, et al. (2006). Experimental studies on hepatotoxicity of rats induced by Fructus Gardeniae. Chin J Chin Mater Med 31:1091–3.

Yang YG, Shen YH, Hong Y, et al. (2008). Stir-baked Fructus gardeniae (L.) extracts inhibit matrix metalloproteinases and alter cell morphology. J Ethnopharmacol 117:285–9.

Yang QJ, Fan MS, Wu B, et al. (2011). Quality assessment for Fructus gardeniae by multi-component quantification, chromatographic fingerprint and related chemometric analysis. J Med Plants Res 5:4126–37.

Yang QJ, Wu B, Shi YJ, et al. (2012). Bioactivity-guided fractionation and analysis of compounds with anti-influenza virus activity from Gardenia jasminoides Ellis. Arch Pharm Res 35:9–17.
Yao DD, Shu L, Yang L, Jia XB. (2014). Advance in studies on anti-diabetic mechanism of Gardeniae Fructus and its active ingredient geniposide. Chin J Chin Mater Med 39:1368–73.

Yin F, Wu X, Li L, et al. (2015). Quality control of Gardeniae Fructus by HPLC-PDA fingerprint coupled with chemometric methods. J Chromatogr Sci 53:1685–94.

Zhang HY, Liu H, Yang M, Wei SF. (2013). Antithrombotic activities of aqueous extract from Gardenia jasminoides and its main constituent. Pharm Biol 51:221–5.

Zhang YG, Tang SH, Jia Q, Meng FY. (2014). Analysis on formula raw materials application of health food containing Gardeniae fructus. Chin J Chin Mater Med 39:4470–4.

Zheng X, Huang XT, Li N, et al. (2012). Determination of geniposidic acid in rat plasma by LC-MS/MS and its application to in vivo pharmacokinetic studies. J Chromatogr B 8878:138–42.

Zhou T, Fan G, Hong Z, et al. (2005). Large-scale isolation and purification of geniposide from the fruit of Gardenia jasminoides Ellis by high-speed counter-current chromatography. J Chromatogr A 1100:76–80.

Zuo R, Ren W, Bian BL, et al. (2015). Metabolic fate analysis of Huang–Lian–Jie–Du Decoction in rat urine and feces by LC-IT-MS combining with LC-FT-ICR-MS: a feasible strategy for the metabolism study of Chinese medical formula. Xenobiotica 18:1–17.

Zuo R, Wang HJ, Si N, et al. (2014). LC-FT-ICR-MS analysis of the prototypes and metabolites in rat plasma after administration of Huang–Lian–Jie–Du decoction. Acta Pharm Sin 49:237–43.

Supplementary material available online.