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Original Article

Pharmacokinetic study of rutin and quercetin in rats after oral administration of total flavones of mulberry leaf extract

Zhen Ou-yang^{a,*}, Xu Cao^a, Yuan Wei^a, Wei-Wan-Qi Zhang^a, Ming Zhao^a, Jin-ao Duan^b

^aSchool of Pharmacy, Jiangsu University, Zhenjiang, PR, China

^bJiangsu Key Laboratory for Traditional Chinese Medicine Formulae Research, Nanjing University of Chinese Medicine, Nanjing, PR, China

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Mulberry leaves, a traditional Chinese medicine, are effective in the treatment of diabetes mellitus. Rutin and quercetin are the main components of total flavones of mulberry leaf extract. To study the pharmacokinetics of rutin and quercetin in rat plasma and their metabolites in rat urine and feces after oral administration of total flavones of mulberry leaf extract. At different timepoints after oral administration of total flavones of mulberry leaf extract in rats, plasma concentrations of rutin and quercetin were determined by RP-HPLC. The main pharmacokinetic parameters were estimated using 3P97 software. The metabolites in rat urine and feces were determined by using UPLC-ESI-QTOF/MS and estimated MetaboLynx™ software. The plasma concentration-time curves of rutin and quercetin both were best fitted with a two-compartment model. Rutin and quercetin were absorbed rapidly and then slowly decreased. Two prototype compounds and seven metabolites were identified. The pharmacokinetic and metabolic results may be useful for further studies of the bioactive mechanism of mulberry leaf flavones and potential development of a new TCM.

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Introduction

Mulberry leaves (*Morus alba* L., Moraceae) have been cultivated in many Asian countries including China, Korea, Japan and Thailand. The infusion of its leaves is consumed as antihyperglycemic nutraceutical foods for patients with diabetes mellitus. Scientific and clinical studies have confirmed that mulberry leaves have a good hypoglycemic effect (Yang et al., 2007; Sun et al., 2002). Flavones are the main constituents

of hypoglycemic mulberry leaves. Those flavones mainly contain rutin (**1**), quercetin (**2**), kaempferol-3-O-rutinoside and kaempferol. Rutin and quercetin in mulberry leaves inhibit could enhance antioxidative activity and hyperglycemia (Ana et al., 2010; Mahmood et al., 2003; Yang et al., 2008; Jo S-H et al., 2010). The pharmacokinetics of pure rutin and quercetin has been studied, but not specifically in the mulberry leaf extract.

In this study we used a reliable reversed phase high performance liquid chromatography (RP-HPLC) method for the simultaneous determination of two active components,

*Corresponding author.

E-mail: zhenouyang@ujs.edu.cn (Z.Ouyang)

rutin and quercetin, in rat plasma after oral administration of total flavones of mulberry leaf extract. The pharmacokinetics of rutin and quercetin in plasma were investigated.

Ultra performance liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOF/MS) is a reliable method for detailed chemical analysis. High selectivity and sensitivity characteristics have allowed the wide application of UPLC-ESI-QTOF/MS for quantitative and qualitative analyses, and metabolite analysis and bioassays of identification from complex samples such as traditional Chinese medicines (TCM). We developed a method to analyze and UPLC-ESI-QTOF/MS identify potential metabolites in rat urine and feces following oral administration of total flavones of mulberry leaf extract (Zhang et al., 2003; Bateman et al., 2007; Zhu et al., 2006; Tiller et al., 2008).

Materials and methods

Materials and reagents

Rutin (**1**) and quercetin (**2**) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade methanol was used for analysis and purchased from Jiangsu Hanbon Science & Technology CO., Ltd (Jiangsu, China); HPLC-grade acetonitrile (ACN) was used for analysis and purchased from Tedia (Fairfield, OH, USA). All other reagents were of analytical grade. Water for UPLC analysis was purified using the Millipore water purification system (Millipore, Milford, MA, USA) and filtered with 0.22 µm membranes. Distilled water was used to extract and prepare samples. The mulberry leaves (*Morus alba* L.) used in this study were cultivated in Dongtai (Dongtai, Jiangsu, China). All samples were identified to be authentic leaves of *Morus alba* L., Moraceae, by teacher Zhen Ouyang (School of Pharmacy, Jiangsu University, Zhenjiang, China). Samples were dried below 40 °C and then crushed into fine powder.

Instrumentation and analytical conditions

The HPLC system (LC-1500, JASCO, Japan) consisted of a pump (PU-1580 HPLC pump, JASCO, Japan), and a ultra-violet (UV) detector (UV-1575 Spectrometer, JASCO, Japan). The UPLC-ESI-QTOF/MS system consisted of an AcQuity™ ultra performance liquid chromatograph and an AcQuity Synapt mass spectrometer equipped with an electrospray ionization (ESI) source (Waters, Milford, MA, USA). An AcQuity Binary Solvent Manager system and an auto-sampler were used for the analysis UPLC-ESI-QTOF/MS. Data was performed with MassLynx V4.1 software (Waters, USA).

In the pharmacokinetic study, the C18 column (250 mm × 4.6 mm, 5 µm, Kromasil, HanBang, China) was used for the analysis. The column temperature was maintained at 30 °C with methanol (A) and water (0.5% acetic acid, B) as mobile phase (30:70, v/v) at a rate of 1 ml·min⁻¹, the UV detection wavelength was at 368 nm and the column temperature was maintained 30 °C. The injection volume was 20 µl. The mobile phase was filtered through to 0.45 µm Millipore filter and degassed before use.

In the metabolism study, an AcQuity UPLC™ BEH C₁₈ column (1.7 µm, 100 mm × 2.1 mm) (Waters, Milford, MA, USA) was used for the analysis. The column temperature was maintained at 30 °C. The standards and extracts were analyzed by UPLC chromatography using a gradient mobile phase consisting of the water solvent A and ACN as solvent B. The gradient of the mobile phase conditions were: 0 min 95% A, 5.0 min 90% A, 8.0 min 88% A, 10.0 min 85% A, 15.0 min 85% A, 18.0 min 45% A, and 20.0 min 10% A. The flow rate was 0.40 ml·min⁻¹. The mobile phase was passed through a 0.22 µm Millipore filter. The injection volume was 2 µl.

In the metabolism study, the negative ion mode was used to analyze metabolites in rat urine and feces samples. For analysis, the electrospray source parameters were fixed as follows: electrospray capillary voltage was 4.0 kV for positive ionization mode and 3.0 kV for negative ionization mode; source temperature was 100 °C and desolvation temperature was 250 °C. The cone voltage was 30 V. set at Nitrogen was used the cone and collision gases. The cone and desolvation gas flows were 50 and 700 l·h⁻¹, respectively. The collision energy trap Collision energy (CE) was set at 6.0 eV and the transfer was CE set at 4.0 eV. The mass was set range from 100 to 1000 m/z.

Preparation of mulberry leaf flavones

Components were extracted from dry mulberry leaf powder (approximately 3 kg) using 10 l of water decocted at 80 °C for 1 h. The filtrates were collected and the residues were then refluxed twice at 80 °C in 10 l of water for 1 h. All filtrates were combined. The solvent was removed below 70 °C to obtain a certain volume at a density ratio of 1.15; 95% ethanol was added to the extract filtrates until the concentration of ethanol was adjusted to 50% and centrifuged at 865 g·min⁻¹ for 10 min to remove insoluble substances. The supernatant was separated by elution with 60% ethanol from macroporous adsorptive resins. The fraction was freeze-dried for 48 h. Mulberry leaf flavones were obtained. The content of rutin (**1**) and quercetin (**2**) in total flavones of mulberry leaf extract was determined simultaneously by HPLC as 8.714 mg·g⁻¹ and 2.782 mg·g⁻¹.

Animals

The animal protocols were approved by the Animal Care and Use Committee of Jiangsu University, and all experiments animals were conducted according to the guidelines of Jiangsu University.

Male Sprague-Dawley (SD) rats (240-260 g) were purchased from the Laboratory Animal Service Center of the Jiangsu University (China). The rats were maintained in an air-conditioned animals quarter at a temperature of 22 ± 2 °C and a relative humidity of 50 ± 10 %. Food and water were allowed *ad libitum*. The animals were acclimatized to the facilities for five days, and then fasted with free access to water for 12 h prior to the experiment.

Preparation of standard solutions, calibration standards and quality samples:

The stock solutions of rutin and quercetin were prepared in methanol. A series of standard solutions were obtained by diluting the mixture of the stock standard solutions with the mobile phase solution.

Calibration standards of the flavones mixture were prepared by spiking the appropriate amount of the standard solutions into 100 μL drug-free rat plasma to give nominal concentration range of 0.1664 - 8.320 $\mu\text{g}\cdot\text{mL}^{-1}$ for rutin (**1**), 0.1528 - 7.640 $\mu\text{g}\cdot\text{mL}^{-1}$ for quercetin (**2**).

Quality control (QC) samples were prepared at low, medium and high concentrations (0.2080 $\mu\text{g}\cdot\text{mL}^{-1}$, 1.664 $\mu\text{g}\cdot\text{mL}^{-1}$, 4.160 $\mu\text{g}\cdot\text{mL}^{-1}$ for rutin (**1**) and 0.1910 $\mu\text{g}\cdot\text{mL}^{-1}$, 1.528 $\mu\text{g}\cdot\text{mL}^{-1}$, 3.820 $\mu\text{g}\cdot\text{mL}^{-1}$ for quercetin (**2**) in the same manner to the calibration standards.

Application to pharmacokinetic study in rat plasma and metabolites study in rat urine, feces

The rats were administered total flavones of mulberry leaf extract oral route at the dose of 4.0 $\text{g}\cdot\text{kg}^{-1}$ (approximately 34.856 $\text{mg}\cdot\text{kg}^{-1}$ rutin, 11.128 $\text{mg}\cdot\text{kg}^{-1}$ quercetin). For the pharmacokinetic study, blood samples (500 μL) were collected from five rats from the fossa vein orbitalis at 0.167, 0.333, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after dosing. Blood samples were immediately transferred to heparinized tubes and centrifuged at 1538 g for 10 min. Plasma was collected and then frozen at $-20\text{ }^{\circ}\text{C}$. For the metabolite study, urine and feces samples were collected at the same time between 0 to 24 h using the metabolic cage. Samples were stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

Pretreatment of plasma, urine and feces samples

Plasma samples of 100 μL were added to 200 μL ACN/acetic acid 100:5 (v/v). Each tube was mixed thoroughly using a vortex mixer for 3 min and centrifuged at 13839 g for 10 min. The supernatant liquid was collected for the HPLC analysis.

Urine samples of 500 μL were added to 1 ml of ACN. Each tube was mixed thoroughly using a vortex mixer for 3 min and centrifuged at 13839 g for 10 min. The supernatant liquid was collected for the analysis UPLC-ESI-QTOF/MS.

Dried feces samples of 1 g were added to 5 ml of 70% ACN and dissolved using ultrasound for 10 min. The mixture was centrifuged at 13839 g for 10 min at $4\text{ }^{\circ}\text{C}$ and the supernatant was collected for the analysis UPLC-ESI-QTOF/MS.

Data analysis

The plasma concentration of the analytes at different time points were expressed mean \pm SD and the mean concentration-time curves were plotted. Data were processed using 3P97 software. The area under the plasma concentration-time curve (AUC), the maximum concentration in plasma (C_{max}) and the corresponding time (t_{max}), the half-life of absorption ($t_{1/2\text{ka}}$), the half-life of distribution ($t_{1/2\alpha}$)

and the half-life of elimination ($t_{1/2\beta}$) were determined. All results were expressed as arithmetic mean \pm SD. Samples were analyzed by QTOF/MS. The post-acquisition data were processed using MetaboLynxTM.

Results and discussion

In vivo pharmacokinetic study of rutin and quercetin in rat plasma

Selectivity

The selectivity of the method was tested by comparing the chromatograms of blank plasma, spiked plasma and actual plasma samples after orally administered total flavones of mulberry leaf extract at a dose of 4.0 $\text{g}\cdot\text{kg}^{-1}$. Analytes were well separated and no interferences were detected from endogenous substances.

Linearity, limits of detection and quantification

The linear regression of the investigated flavones in rat plasma was constructed by plotting peak area with concentration of standard solutions. The calibration curves showed good linearity over the concentration range 0.1664 - 8.320 $\mu\text{g}\cdot\text{mL}^{-1}$ for rutin with a correlation coefficient (R^2) larger than 0.9981, and 0.1528 - 7.640 $\mu\text{g}\cdot\text{mL}^{-1}$ for quercetin with R^2 larger than 0.9981. The mean standard curves were typically described by the equations: $Y = 7327.9 X + 900.75$ for rutin and $Y = 7982.5 X + 145.32$ for quercetin. The limits of detection (LOD, $S/N = 3$) and the lower limit of quantification (LLOQ, $S/N = 9$) were 0.0555 $\mu\text{g}\cdot\text{mL}^{-1}$ and 0.1664 $\mu\text{g}\cdot\text{mL}^{-1}$ for rutin and 0.0509 $\mu\text{g}\cdot\text{mL}^{-1}$ and 0.1528 $\mu\text{g}\cdot\text{mL}^{-1}$ for quercetin.

Accuracy and precision:

The method's precision was assessed by determination of QC samples ($n = 6$) on three different validation days. Intra-day precision was determined by assaying standard solutions of the analyte at different times during the same day. Inter-day precision was determined by assaying standard solutions of the analyte over three consecutive days. The concentration of each sample was determined using a calibration curve prepared each day Table 1.

Extraction recovery

The mean extraction recoveries of rutin from rat plasma at concentrations of 0.2080, 1.664 and 4.160 $\mu\text{g}\cdot\text{mL}^{-1}$ were 80.6, 88.4 and 85.5%, respectively. The mean extraction recoveries of quercetin from rat plasma at concentrations of 0.1910, 1.528 and 3.820 $\mu\text{g}\cdot\text{mL}^{-1}$ were 79.1, 80.8 and 81.2 %, respectively.

Stability

The stabilities of 0.2080, 1.664 and 4.160 $\mu\text{g}\cdot\text{mL}^{-1}$ rutin, or 0.1910, 1.528 and 3.820 $\mu\text{g}\cdot\text{mL}^{-1}$ quercetin in rat plasma were tested in room temperature for 12 h, $4\text{ }^{\circ}\text{C}$ for 24 h and $-20\text{ }^{\circ}\text{C}$ for one week. No significant degradation of rutin (**1**) or quercetin (**2**) was detected under any of these conditions (Table 2).

Table 1
Precision and accuracy of rutin and quercetin in rat plasma (n = 6).

| Nominal conc. ($\mu\text{g}\cdot\text{l}^{-1}$) | Intra-day | | | Inter-day | | |
|--|---|-----------------|---------------------|---|-----------------|---------------------|
| | Measured conc. ($\mu\text{g}\cdot\text{l}^{-1}$) | Accuracy (%) | Precision RSD(%) | Measured conc. ($\mu\text{g}\cdot\text{l}^{-1}$) | Accuracy (%) | Precision RSD(%) |
| rutin | | | | | | |
| 0.208 | 0.201 \pm 0.007 | 96.6 | 3.48 | 0.197 \pm 0.008 | 94.7 | 4.06 |
| 1.664 | 1.651 \pm 0.037 | 99.2 | 2.25 | 1.644 \pm 0.040 | 98.8 | 2.43 |
| 4.160 | 3.970 \pm 0.086 | 95.4 | 2.16 | 3.920 \pm 0.091 | 94.2 | 2.32 |
| quercetin | | | | | | |
| 0.191 | 0.184 \pm 0.004 | 96.3 | 1.98 | 0.181 \pm 0.006 | 94.8 | 3.31 |
| 1.528 | 1.489 \pm 0.026 | 97.4 | 1.77 | 1.481 \pm 0.029 | 96.9 | 1.96 |
| 3.820 | 3.700 \pm 0.056 | 96.9 | 1.52 | 3.650 \pm 0.067 | 95.5 | 1.84 |

Table 2
Stability of rutin and quercetin in rat plasma (n = 5, mean \pm SD).

| Nominal conc. ($\mu\text{g}\cdot\text{l}^{-1}$) | RT for 24 h | | 4 °C for 72 h | | -20 °C for 2 week | |
|--|---|---------|---|---------|---|---------|
| | Conc. ($\mu\text{g}\cdot\text{l}^{-1}$) | RSD (%) | Conc. ($\mu\text{g}\cdot\text{l}^{-1}$) | RSD (%) | Conc. ($\mu\text{g}\cdot\text{l}^{-1}$) | RSD (%) |
| rutin | | | | | | |
| 0.832 | 0.833 \pm 0.039 | 4.7 | 0.825 \pm 0.044 | 5.3 | 0.823 \pm 0.048 | 5.8 |
| 1.664 | 1.651 \pm 0.064 | 3.9 | 1.642 \pm 0.084 | 5.1 | 1.636 \pm 0.075 | 4.6 |
| 4.160 | 4.013 \pm 0.128 | 3.2 | 3.971 \pm 0.131 | 3.3 | 3.923 \pm 0.149 | 3.8 |
| 0.764 | 0.757 \pm 0.035 | 4.6 | 0.748 \pm 0.028 | 3.7 | 0.744 \pm 0.040 | 5.4 |
| quercetin | | | | | | |
| 1.528 | 1.495 \pm 0.063 | 4.2 | 1.474 \pm 0.075 | 5.1 | 1.481 \pm 0.076 | 5.1 |
| 3.820 | 3.759 \pm 0.105 | 2.8 | 3.687 \pm 0.159 | 4.3 | 3.713 \pm 0.167 | 4.5 |

Pharmacokinetics study

The mean plasma concentration-time profiles of the investigated components were shown in Figure 1, demonstrating that the concentration-time profile was best described by the two-compartment model for rutin (**1**) and quercetin (**2**). The pharmacokinetics and model parameters were calculated by the practical pharmacokinetic program 3P97 software. The pharmacokinetic parameters showed that rutin was fastly absorbed after oral administration of total flavones of mulberry leaves, the C_{max} of rutin in rat plasma was $1.546 \pm 0.188 \text{ mg}\cdot\text{l}^{-1}$, and the T_{max} was 1 h. Quercetin was absorbed fastly also in rat, but it has the second C_{max} . The C_{max} of quercetin in rat plasma was $1.127 \pm 0.329 \text{ mg}\cdot\text{l}^{-1}$ and the T_{max} was 0.333 h. It had a secondary absorption peak at 6 h. There might be two reasons: the quercetin has a second absorption in the intestines; rutin was hydrolyzed in the intestinal tract. The main pharmacokinetics parameters were summarized in Table 3.

Table 3
Pharmacokinetic parameters for rutin and quercetin after oral administration of total flavones of mulberry leaf extract in SD rats (at a dose containing $34.856 \text{ mg}\cdot\text{kg}^{-1}$ rutin and $11.128 \text{ mg}\cdot\text{kg}^{-1}$ quercetin).

| Parameter | Rutin | Quercetin |
|--|--------------------|--------------------|
| $t_{1/2\alpha}$ /h | 0.619 \pm 0.127 | 0.092 \pm 0.043 |
| $t_{1/2\beta}$ /h | 10.355 \pm 7.339 | 36.454 \pm 14.08 |
| $t_{1/2ka}$ /h | 0.358 \pm 0.089 | 0.040 \pm 0.030 |
| AUC / $\text{mg}\cdot\text{h}\cdot\text{l}^{-1}$ | 9.947 \pm 2.705 | 5.623 \pm 3.183 |
| T_{max} /h | 1 | 0.333 |
| C_{max} / $\text{mg}\cdot\text{l}^{-1}$ | 1.546 \pm 0.188 | 1.127 \pm 0.329 |

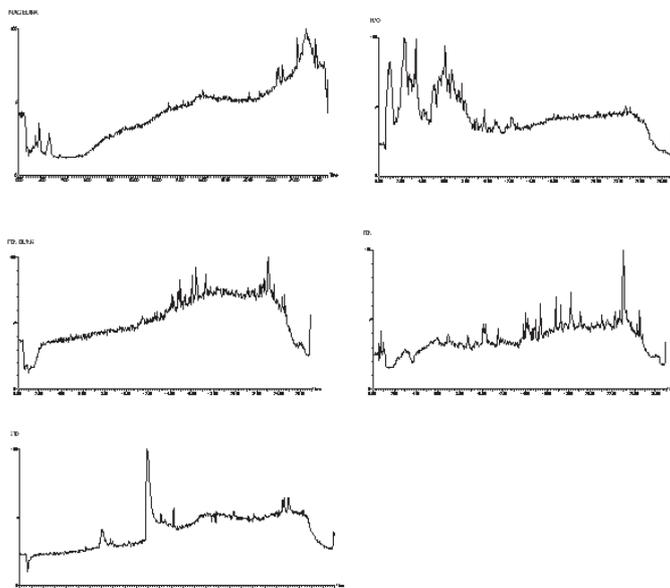


Fig. 1 - UPLC-ESI-QTOF/MS total ion current chromatograms in negative ion mode of urine and feces samples after oral administration of mulberry leaf flavones (a, blank urine; b, urine sample; c, blank feces; d, feces sample; e, rutin and quercetin).

UPLC-QTOF-MS analysis and identification of metabolite components in rat urine and feces

Data analysis

Gradient reversed phase UPLC with absorbance detection and dependent full scan MS data were used to analyze and identify the absorption and metabolic components in rat urine and feces after oral administration of total flavones of mulberry leaf extract.

Urine and feces collected 0-24 h after ingestion of mulberry leaf flavones, and urine and feces samples were analyzed by UPLC-ESI-QTOF/MS. The total ion current chromatograms detected in the negative ion mode were shown in Figure 2.

By comparing the peak retention time (t_R) values and the MS ion fragments characteristics with reference compounds and published data, different classes of metabolites were summarized in Table 4.

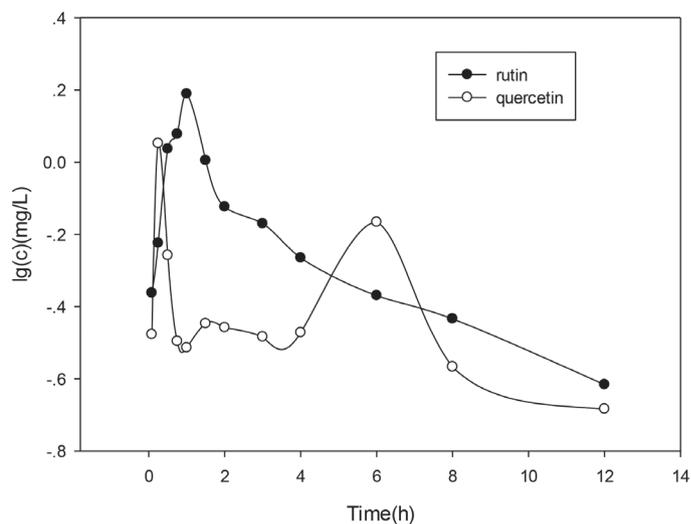
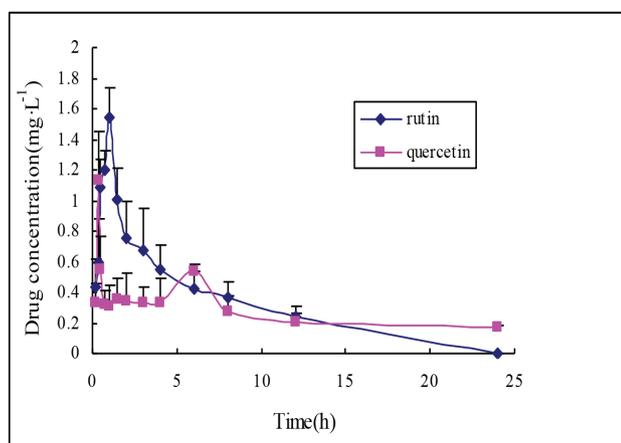


Fig. 2 - A. Mean concentration-time profiles of rutin and quercetin in rats after oral administration of mulberry leaf flavones (each point represents the mean \pm S.D., $n = 5$; at a dose containing $34.856 \text{ mg}\cdot\text{kg}^{-1}$ rutin and $11.128 \text{ mg}\cdot\text{kg}^{-1}$ quercetin); B. semi-log plot of mean plasma concentration of rutin and quercetin in rats after oral administration of mulberry leaf flavones.

Table 4
Reviewed metabolite summary for quercetin and rutin by Metabolynx software.

| N° | t _R /min | Molecular weight | Metabolite | Formula | Mass difference | Source |
|----|---------------------|------------------|---|---|-----------------|--------------|
| M1 | 10.90 | 302.0427 | A: Parent | C ₁₅ H ₁₀ O ₇ | -0.0125 | Urine, feces |
| M2 | 6.98 | 610.1534 | B: Parent | C ₂₇ H ₃₀ O ₁₆ | 0.0080 | Urine, feces |
| M3 | 12.28 | 316.0583 | A+CH ₂ | C ₁₆ H ₁₂ O ₇ | 14.0067 | Urine |
| M4 | 9.01 | 624.1690 | B+CH ₂ | C ₂₈ H ₃₂ O ₁₆ | 14.0216 | Urine, feces |
| M5 | 6.83 | 381.9995 | A+SO ₃ | C ₁₅ H ₁₀ O ₁₀ S | 79.9699 | Feces |
| M6 | 5.93 | 690.1102 | B+SO ₃ | C ₂₇ H ₃₀ O ₁₉ S | 79.9651 | Urine, feces |
| M7 | 7.51 | 478.0747 | A+C ₆ H ₈ O ₆ | C ₂₁ H ₁₈ O ₁₃ | 176.0172 | Urine |
| M8 | 6.22 | 786.1855 | B+C ₆ H ₈ O ₆ | C ₃₃ H ₃₈ O ₂₂ | 176.0369 | Urine, feces |
| M9 | 4.13 | 654.1068 | A+2C ₆ H ₈ O ₆ | C ₂₇ H ₂₆ O ₁₉ | 352.0678 | Urine |

A, quercetin; B, rutin.

Quercetin (2) (M1): The urine and feces of three rats contained traces of aglycone, quercetin which had a [M-H]⁻ at *m/z* 301 and MS fragment ions at *m/z* 179 and 151 in the negative ion mode.

Rutin (1) (M2): The urine and feces of three rats contained traces of the rutin which had a [M-H]⁻ at *m/z* 609 and MS fragment ions at *m/z* 463 and 301 in the negative ion mode.

Methylated quercetin (M3): In the negative ion mode at 12.28 min, there was a [M-H]⁻ at *m/z* 315 with a major ion at *m/z* 301 ([M-H]⁻ - 14) in rat urine. According to mass spectrometry (MS) data, the compound at 12.28 min was identified as methylated quercetin; however, methylated quercetin was not identified in rat feces.

Methylated rutin (M4): In the negative ion mode at 9.01 min, there was a [M-H]⁻ at *m/z* 623 with a major ion at *m/z* 609 ([M-H]⁻ - 14) in rat urine and feces. According to MS data, the compound at 9.01 min was identified as methylated rutin.

Quercetin sulfates (M5): In the negative ion mode at 6.83 min, there was a [M-H]⁻ at *m/z* 381 with a major ion at *m/z* 301 ([M-H]⁻ - 80) in rat feces. According to MS/MS data, the compound at 6.83 min was identified as quercetin sulfate; however, quercetin sulfate was not identified in rat urine.

Rutin sulfates (M6): In the negative ion mode at 5.93 min, there was a [M-H]⁻ at *m/z* 689 with a major ion at *m/z* 609 ([M-H]⁻ - 80) in rat urine and feces. According to MS data, the compound at 5.93 min was identified as rutin sulfate.

Quercetin glucuronide (M7): In the negative ion mode at 7.51 min, there was a [M-H]⁻ at *m/z* 477 with a major ion at *m/z* 301 ([M-H]⁻ - 176) in rat urine. According to MS data, the compound at 7.51 min was identified as quercetin glucuronide; however, quercetin glucuronide was not identified in rat feces.

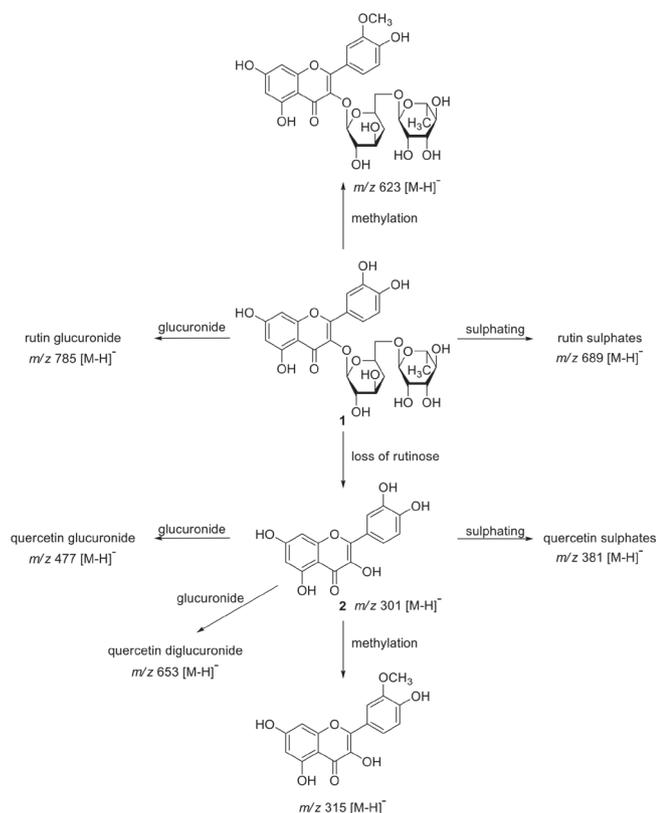
Rutin glucuronide (M8): In the negative ion mode at 6.22 min, there was a [M-H]⁻ at *m/z* 785 with a major ion at *m/z* 609 ([M-H]⁻ - 176) in rat urine and feces. According to MS data, the compound at 6.22 min was identified as rutin glucuronide.

Quercetin diglucuronides (M9): One diglucuronide quercetin was detected at 4.13 min in three rats urine during 0-24 h. The data indicated that two glucuronyl units were attached at different positions on the quercetin skeleton. Disaccharides conjugate fragments with the loss of the intact disaccharide moiety. If these two glucuronyl units had been attached at the same position, it is unlikely that an M-176 fragment would have been produced at *m/z* 477.

Possible metabolic pathway of rutin and quercetin after oral administration of mulberry leaf flavones

In this study, the mulberry leaf flavones quercetin and rutin, and their potential metabolites, were analyzed. Our data provide guidance for studying the absorption and metabolism of complex active components of TCM. The presumed metabolic pathway including phase I and II is shown in Scheme 1.

In this study we developed and validated a RP-HPLC method for simultaneous determination of two flavones, including quercetin and rutin, in rat plasma after oral administration of the mulberry leaf flavones. We also developed the UPLC coupled with the QTOF-MS method for the analysis and identification of potential metabolites in rat urine and feces. In this experiment, two components and seven metabolites were identified. Notably, the new metabolite, di-glucuronide conjugation of compound quercetin (M9) was observed. The pharmacokinetic and metabolic results may be useful for further study of the bioactive mechanism of mulberry leaf flavones and potential development of a new TCM.



Scheme 1 - The potential metabolic biotransformation pathway of rutin (1) and quercetin (2).

Authorship

YW and MZ contributed in collecting plant and sample identification, running the laboratory work, analysis of the date and drafted the paper. JD contributed to in plant identification and critical reading of the manuscript. XC and WZ contributed to sample processing and chromatographic analysis. ZO designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

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