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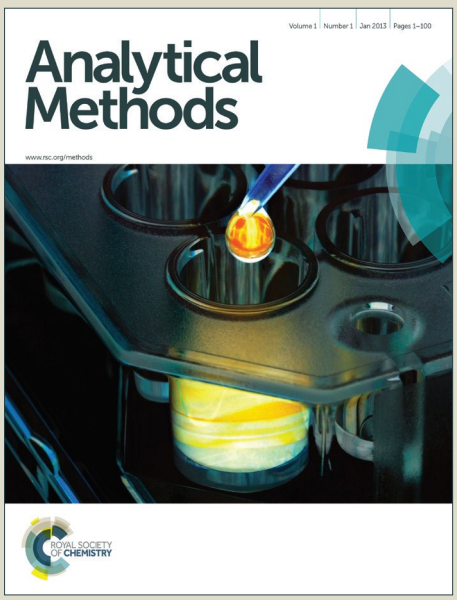

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UHPLC-MS/MS determination and pharmacokinetic study of three active compounds in male rats after oral administration of *Saxifraga stolonifera* (L.) Meerb extract

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Abstract *Saxifraga stolonifera* (*S. stolonifera*) has been used to cure various diseases effectively while little is known about the pharmacokinetic properties of the bioactive components of *S. stolonifera*. The aim of this study is to develop an UHPLC-ESI-MS/MS method for simultaneous determination of gallic acid (GA), bergenin (BG) and quercetin-3-O-*β*-L-rhamnopyranoside (QR), three bioactive compounds of *S. stolonifera*, and to apply the method for pharmacokinetic study to learn how dosage variations of *S. stolonifera* alters the pharmacokinetics of GA, BG and QR in treated rats. The decoctions at low dose, middle dose, and high dose of *S. stolonifera* extract were administered orally to rats. The results showed that variations of *S. stolonifera* extract doses altered the contents of GA, BG and QR in rat blood. GA, BG and QR could be rapidly absorbed into the circulation. T_{max} of GA was 40-100 min. T_{max} of BG was 80-100 min. T_{max} of QR was 20 min. The AUC_{0-t} of three compounds increased with the dose of *S. stolonifera* extract. These results provide a meaningful basis for evaluation of the interactions between the components in a complex prescription on their pharmacokinetics.

Keywords:

Benign Prostatic Hyperplasia,

Saxifraga stolonifera,

Pharmacokinetics,

Gallic acid,

Bergenin,

Quercetin-3-O-*β*-L-rhamnopyranoside

1. Introduction

Saxifraga stolonifera (L.) Meerb. (*S. stolonifera*), a traditional Miao herbal medicine in China, has been used to treat otitis media, erysipelas, and hemoptysis with low toxicity or non-toxicity for centuries [1, 2]. Clinical studies of Ju [3] indicated that *S. stolonifera* could be used to cure benign prostatic hyperplasia (BPH). In addition, modern pharmacological investigations indicated that *S. stolonifera* remained the abilities on anti-inflammation, anti-prostate cancer and anti-BPH [4-7]. Studies of Zhang [7] indicated that *S. stolonifera* extract could be used to treat BPH via inhibitions to fibroblasts. Evidences from animal models suggested a potential role for anti-BPH after oral administration of aqueous extract of *S. stolonifera* according to our previous work[8]. It's known that pharmacokinetic studies of bioactive compounds are essential programs in preclinical and clinical processes and are indispensable for learning the efficacies of the plant [9, 10]. Given its low toxicity or non-toxicity and important functions, pharmacokinetics study of bioactive compounds in this extract is very essential for further understanding of *S. stolonifera*. However, no papers about the pharmacokinetic study of *S. stolonifera* were reported.

Natural products have been used in traditional cures and herbal remedies throughout the world[11, 12]. Extracts of herbal medicine were usually administrated because the pharmacokinetic properties of the bioactive components in their pure forms are significantly different from that in herbal medicines [13, 14]. Considering the complexity of the compounds, several compounds are generally selected to demonstrate the pharmacokinetic properties of the herbal extracts [15, 16].

Polyphenols, famous secondary metabolites with wide pharmacological activities [17,18], such as gallic acid (GA), bergenin (BG) and quercetin-3-O-β-L-rhamnopyranoside (QR) were thought to be the bioactive compounds [19,20] of *S. stolonifera*. These compounds have been studied for properties against

various diseases, such as cardiovascular diseases [21], inflammation [22] and cancer [23]. Beyond that, the activities on anti-cancer and antioxidant of GA [24,25], anti-inflammatory, anti-HIV agent and antitumor of BG [26,27] and the good resistance to PC-3 of QR [5] were reported. GA, BG and QR were also selected as markers [2] to evaluate the *S. stolonifera* plant.

The current study described an UHPLC-ESI-MS/MS method with a simple protein precipitation, satisfying recovery and minimal matrix effect for simultaneous determination of GA, BG and QR in male rats. Pharmacokinetic interactions among three compounds after oral administration of *S. stolonifera* extract in three doses were characterized.

2. Materials and methods

2.1 Chemicals and reagents

Gallic acid (GA) and Puerarin (internal standard) were purchased from the National Institute for the Control of Biological and Pharmaceutical Products of China (Beijing, China). Bergenin, (BG) was purchased from Guizhou Dida Technology Co. Ltd. Quertecin-3-O-*β*-L-rhamnoside (QR, purity > 98%) was extracted from S. *stolonifera*. Their structures were showed in (Fig.1). HPLC-grade acetonitrile and methanol were purchased from Tedia Co. Inc. (Fairfield, OH, USA). Formic acid was MS grade (Roe Scientific Inc, USA). Super purified water was used for preparations. All other solvents in the presents study were of analytical grades and commercially available.

2.2 Method and validation

2.2.1 UHPLC-MS/MS system

UHPLC-MS/MS system contained an Accela 1250 UHPLC system coupled with a TSQ quantum

ultra-triple-quadrupole mass spectrometer (Thermo fisher Scientific Inc, Waltham, MA, USA).

Chromatographic separation was achieved using a Weltch ultimate UHPLC XB-C18 column (2.1 × 150 mm, 1.7 μm). The mobile phase consisted of acetonitrile containing 0.2% formic acid (A) and water containing 0.2% formic acid (B). The gradient program was as follows: 0-3.0 min, 3% A; 3.0-6.0 min, 25% A; 6.0-12.0 min, 25% A; 12.0-13.0 min 3% A; 13.0-20.0 min 3% A. The column temperature was maintained at 25 °C. The flow rate was 200 μL/min and the injection volume was 5 μL.

Mass spectrometric analysis was performed on a TSQ quantum ultra-triple-quadrupole mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with an electro-spray ionization (ESI) interface in negative mode. All analytes, including the IS, were monitored under negative ionization conditions and quantified in multiple reactions monitoring (MRM) mode with transitions of *m/z* 169.012→125.063 for GA, *m/z* 326.942→191.997 for BG, *m/z* 415.051→266.999 for IS, and *m/z* 447.014→300.028 for QR. Other parameters of the mass spectrometer were as follows: sheath gas flow rate at 40 (arbitrary units); auxiliary gas flow rate at 10 (arbitrary units); spray voltage at 2500 V; vaporizer temperature at 350˚C; capillary temperature at 350˚C. Helium was used as the collision gas for collision-induced dissociation (CID).

2.2.2 Plasma sample preparation

A 100 μL aliquot plasma sample was transferred into a 1.5 mL Eppendorf tube (EP tube), 10 μL IS solution (44.24 ng/mL) and 400 μL acetonitrile (0.1% formic acid) were individually added. The mixture was vortexed for 1 min and the centrifuged at 13000 rpm for 10 min at 4 ˚C. Subsequently, the supernatant was transferred into a clean 1.5 mL EP tube and evaporated to dryness under a nitrogen stream at 40 ˚C. The residue was dissolved in 100 μL of 0.2% formic acid aqueous solution and

centrifuged at 13000 rpm for 10 min at 4 ˚C. A 5 μL aliquot was injected into UHPLC-MS/MS for analysis.

2.2.3 Standard and quality control samples preparation

A mixed stock solution containing 6.65 μg/mL QR, 6.23 μg/mL GA and 8.69 μg/mL BG was dissolved in methanol, and further successive diluted into 0.66-132.96 ng/mL of QR, 4.34-434.40 ng/mL of GA, and 3.31-331.40 ng/mL of BG as calibration carves and the IS was prepared to 44.24 ng/mL in methanol separately. All the solutions were stored at 4 ˚C.

Calibration standards were prepared by spiking working standard solutions and the IS (10 μL, 44.24 ng/mL) into 100 μL of blank plasma to the yield concentrations of 0.66, 3.32, 6.65, 13.29, 33.24, 66.48, 132.96 ng/mL of QR, 3.31, 6.23, 12.46, 31.14, 62.28, 124.56, 311.40 ng/mL of BG and 4.34, 8.69, 17.38, 43.44, 86.88, 173.76, 434.40 ng/mL of GA.

Quality control samples (QCs) at three levels of 3.32, 33.24, 132.96 ng/mL for QR, 3.31, 31.14, 124.56 for BG and 4.34, 43.44, 173.76 ng/mL for GA samples.

2.2.4 Method validation

The method was validated according to the accepted FDA Guidance for Industry, Bioanalytical Method Validation (US-FDA, 2001) [28] in this matter.

Matrix effects were assessed by analyzing the potential interference of endogenous compounds to the analytes and the IS. Blank plasma samples from six rats were measured using the preparation procedures and instrument conditions mentioned previously. The matrix effects of GA, BG and QR at three QC levels and the IS were tested comparing peak areas of the analytes spiked in post-extraction

blank plasma samples with those of the reference compounds diluted in methanol.

The plasma recoveries of GA, BG, QR, as well as IS were conducted as follows: A1 blank matrix was extracted and then spiked with standards. A2 standards were spiked in and extracted from blank plasma. Plasma recovery was calculated as the equation:

$$\mathrm{Re}\% = (\mathrm{A2}/\mathrm{A1}) \times 100 \quad \text{Eq. (1)}$$

Method linearity of GA, BG and QR were calculated by spiking standards into the blank plasma at concentrations ranging from 0.66-132.96 ng/mL for QR, 3.31-331.40 ng/mL for BG, and 4.43-434.40 ng/mL for GA with weighed ($1/x^2$) least square linear regression method through measurement of the peak area ratio of analyte to IS. The lower limit of quantification (LLOQ) was established based on signal-to-noise (S/N) ratio approach. LLOQ was expressed as S/N=10 from the chromatograms of the samples spiked at the lowest level validated and defined as the lowest concentration on the calibration curve.

Precision was expressed as the relative standard deviation (RSD) and accuracy was calculated as the relative error (RE). Acceptance criteria for precision and accuracy were defined as ≤15 %. In this paper, the QC samples of three levels were run in six replicates at the same day to determine the intra-day precision, and three consecutive days to analyze the inter-day precision.

The accuracy was calculated from the nominal concentration (C_{nom}) and the mean value of the measured concentration (C_{mes}) as follows:

$$\text{accuracy (Bias,\%)} = [(\mathrm{Cnom} - \mathrm{Cmes})/\mathrm{Cnom}] \times 100 \quad (2)$$

The precision was calculated from the standard deviation and measured concentration as follows:

$$\text{precidion(RSD,\%)} = [\text{standard-deviation(SD)}/\mathrm{Cmes}] \times 100 \quad (3)$$

Plasma stability was assessed in samples under different conditions. The short-term stability was

assessed by placing the analytes at room temperature for 6 hours and keeping at 4˚C in the autosampler for 12 hours. The freeze-thaw stability was evaluated over three freeze-thaw cycles (-20˚C to room temperature as one cycle). The long-term stability was assessed after the untreated QC samples had been stored at -20 ˚C for 19 days.

2.3 Pharmacokinetics study and statistical analysis

2.3.1 Preparation of aqueous extract from *S. stolonifera*

S. stolonifera, collected at Anshun (Guizhou, China), was identified by professor Deyuan Chen. *S. stolonifera* extract was prepared as follows: 200 g of the dried powder was accurately weighed into a 3-L glass pocket flask and extracted with 2 L of water for 2 h at 80 °C, followed by two more extractions. The extracts were combined, then the supernatant was evaporated to dryness. *S. stolonifera* extract was determined according to the method reported with some minor modification [2]: Briefly, approximately 0.1 g of *S. stolonifera* extract was accurately weighed into a 100 mL conical flask with 50 mL of 50% methanol (v/v) added, which was then dissolved via ultra-sonication for 20 min (100W, 40 kHz). The supernatant was filtered through a 0.45 μm membrane for the HPLC analysis. Chromatographic conditions were modified on the Dionex Ultimate 3000 (California, USA) system with a Diamonsil C18 column (250 mm×4.6 mm, 5 μm) to obtain a good response and a resolution. 400 mg/mL (equivalent of GA 0.88 mg/mL, BG 2.82 mg/mL, and QR 0.58 mg/mL) of *S. stolonifera* extract was suspended in water for oral administration.

2.3.2 Animals and statistical analysis

Pathogen-free adult male Wister rats, weighted 200-260 g, were purchased from Changsha Tianqin

Bio-technology. (Changsha, China, Certificate No. SCXK 2015-0011). All rats were acclimated for at least a week in environmentally controlled quarters (24 ± 1 ˚C and 12/12 h light/dark cycle) with free access to standard chow and water. The rats were fasted overnight but supplied with water ad libitum before the experiments. All experimental protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animal (National Institutes of Health Publication 85-23, revised edition 1985). This study was approved by the Animal Ethics Committee of Guizhou Normal University.

18 male rats were divided into 3 groups randomly and were orally administrated 0.74 g/kg 1.48 g/kg, and 2.96 g/kg of *S. stolonifera* extract, respectively, in each group. 250 μL of blood samples were collected into 1.5 mL heparinized tubes from the suborbital vein at pre-dose (0 min) and 10, 20, 30, 40, 60, 80, 100 min and 2, 3, 5, and 8 hour post dose. Plasma was separated immediately by centrifuging at 6000 rpm for 15 min, stored at -20 °C before analysis.

The pharmacokinetics parameters were calculated by non-compartmental analysis using PK Solver software.[24] A non-compartmental model was applied to the data fitting and parameter estimation. Following parameters were achieved to evaluate the analytes: (1) half-life ($T_{1/2}$) is the time required for the concentration of the drug to reach half of its beginning value; (2) C_{max} is the maximum plasma concentration after oral administration; (3) time to reach the maximum concentrations (T_{max})； (4) the area under the plasma level time curve (AUC), which is related to the extent of drug absorption in the systemic circulation; (5) the clearance (CL), an indicator of drug elimination from the body; and (6) apparent volume of distribution (Vd). Statistical analysis was performed using Microsoft Excel, Origin 8.0, and SPSS 20.0 software (SPSS, Inc., Chicago, USA). Data were expressed as mean ± SD and a P value < 0.05 was considered to be statistically significant.

3. Results

3.1 Optimization for mass and chromatographic conditions

To obtain optimal separation conditions, chromatographic conditions and mass analytical parameters and were optimized. Chromatographic conditions were optimized by screening a few columns and finally the Weltch ultimate UHPLC XB-C18 column (2.1 × 150 mm, 1.7 μm) was selected. In the gradient optimization, gradient time, shape, and the mobile phase were taken into consideration. As a result, acetonitrile (0.2% formic acid)-water (0.2% formic acid) system consists the mobile phase. Under these optimal conditions, satisfactory resolution values, sharp and symmetrical peaks were obtained. Better ionization effects of the analytes were obtained in negative ion mode. In the precursor ion full-scan spectra, the most abundant ions were deprotonated molecules $[M-H]^-$ *m/z* 169.012, 326.942, 415.05, and 447.014 for GA, BG, IS, and QR, respectively. The optimized values of helium collision gas pressure, tube lens offset, and collision energy for each parent ion-product ion transition were displayed in Table 1.

The precursor to product transition was assigned in the multi-reaction- monitoring (MRM) mode as follows: *m/z* 169.012→125.063 for GA, *m/z* 326.942→191.997 for BG, *m/z* 415.051→266.999 for IS, and *m/z* 447.014→300.028 for the QR. Under the optimized parameters, efficient ionizations, high abundances and sensitive detections of the analytes and the IS were achieved.

3.2 Plasma sample preparation

To exhaustively extract analytes and fully reduce the endogenous-related substances in plasma, extraction approaches were conducted. Precipitation of protein was conducted by a single-step protein

precipitation with acetonitrile containing 0.01% formic acid.

3.3 Matrix effects and plasma recovery

Under the optimized LC-MS/MS conditions, there were no interfering peaks at the elution times for markers. The typical MRM chromatograms of blank plasma (A), spiked plasma containing GA, BG, QR and IS (B), and plasma collected at 20 min after oral administration of *S. stolonifera* extract (C) are shown in (Fig.2). Plasma recoveries are in Table 2. Nominal concentrations of the analytes are 43.44 ng/mL of GA, 31.14 ng/mL of BG, and 33.24 ng/mL of QR.

3.4 Linearity, precision, accuracy, and lower limit of quantification (LLOQ)

Methods in this study showed a very good linearity over 4.34-434 ng/mL range for GA, 3.11-311 ng/mL range for BG, and 0.66-132.96 ng/mL for QR. The best linear fit and least-square residual for the calibration curve was achieved with a $1/x^2$ weighting factor. The regression equations were Y=0.0608X - 0.0264 (γ^2=0.992, n=7), Y=0.0382X + 0.0281 (γ^2=0.994, n=7), and Y=0.134X - 0.0819 (γ^2=0.990, n=7) for GA, BG and QR, respectively. Where Y refers to peak area ratios (anlayte/IS) and X is the concentration. The present UHPLC-MS/MS method offered an LLOQ were 0.66 ng/mL, 3.11 ng/mL and 4.23ng/mL for QR, BG and GA, respectively.

According to the guidance mentioned above, the accuracy was required to be within ± 15% (20% for LLOQ), and the intra- and inter-day precisions were not to exceed ± 15% (20% for LLOQ). The results demonstrated that the values are within the acceptable range mentioned above and the method is accurate and precise. The results of the intra-day and inter-day precision and accuracy of the analytes in QC samples are displayed in Table 3.

All the analytes in this study were stable in all the conditions mentioned above and were listed in

Table 4.

3.5 Pharmacokinetic study

The established method was applied to analyses pharmacokinetic of GA, BG and QR in rat plasma after oral administration of the aqueous extract of *S. stolonifera* with three dosages at 0.74, 1.48 and 2.96 g/kg equivalent to 1.62, 3.24, 6.48 mg/kg of GA, 5.21, 10.42, 20.84 mg/kg of BG, and 1.08, 2.16, 4.32 mg/kg of QR. The plasma concentrations of analytes were tested at each time point, the concentration-time curves of GA, BG and QR were displayed in (Fig.3), (Fig.4) and (Fig.5). The pharmacokinetic parameters were calculated on non-compartment model and presented in Table 5, Table 6, and Table 7. The observed T_{max} and C_{max} were 40, 100, 100 min and 16.38, 29.68, 62.91 ng/mL for GA, 80, 100,100 min and 10.24, 18.54, 28.74 ng/mL for BG and 20 min and 1.40, 2.73, 3.62 ng/mL for QR, respectively. AUC_{0-t} were 4572.80, 9560.22, 17844.47 ng/mL for GA, 2877.44, 9560.22, 17844.47 ng/mL for BG and 454.55, 567.58, 619.94 ng/mL for QR.

4. Discussion

A rapid and highly sensitive method for simultaneous determination of GA, BG and QR after administration of *S. stolonifera* extract was developed. The LLOQ of three analytes were 0.66 ng/mL, 3.11 ng/mL and 4.23ng/mL for QR, BG and GA, respectively.

As shown, GA, BG and QR exhibited relatively rapid absorption processes, of which the plasma concentration achieved the peak from 20 to 100 min and showed a relatively sharp peak shape. The T_{max} of three compounds were within 100 min. C_{max} and AUC_{0-t} of three compounds increased with the increase of dose, indicating that the pharmacokinetic parameters of GA, BG and QR extracted from *S.*

stolonifera showed a dose-dependent profile [26]. The Vd values of GA, BG and QR was greater than 40 L/kg which indicated that the three markers might be distributed to some specific tissues selectively [32,33].

The pharmacokinetic profiles of the three bioactive compounds were closely related to their chemical structures and metabolism mechanisms. Through comparing C_{max} and AUC, the quantity detected of QR was lower than other two analytes. The molecular structure of QR contains glucose, which might be easily hydrolyzed. Studies [34, 35] showed that transglucosylase might be inhibited by QR which reduced the absorption of QR. Bimodal phenomenon of QR might be due to multiple-sites absorption or enterohepatic circulation. The $T_{1/2}$ and T_{max} of GA were prolonged, to some extent, by comparing with that of its pure form[31]. A proper reason might be that other compounds in the *S. stolonifera* extract were metabolized to gallic acid in vivo, such as some of tannins might translate into GA by taking off the gluside [31]. The T_{max} of BG was advanced compared with that of its pure form[27] which indicated that BG could be influenced by other compounds in the *S. stolonifera* extract. Nevertheless, additional studies should be carried out in order to confirm the pharmacokinetic mechanism involved.

5. Conclusions

A rapid, sensitive and specific UHPLC-MS/MS method with a simple protein precipitation, satisfying recovery and minimal matrix effect for simultaneous quantification of GA, BG and QR in male rat plasma was developed and validated according to FDA Guidance. This method was applied to a pharmacokinetic study after oral administration of *S. stolonifera* extract successfully. Three compounds of *S. stolonifera* extract might display their in vivo pharmacological activities at different

levels and different time periods after oral administration. Pharmacokinetic profiles of QR were obtained for the first time.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Figure caption

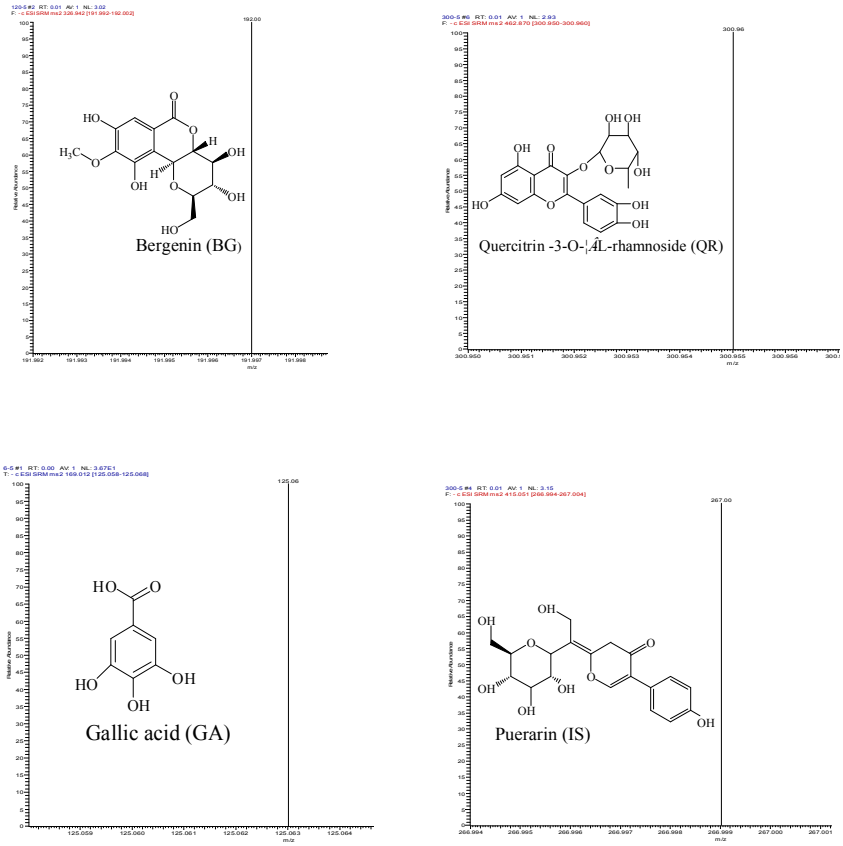
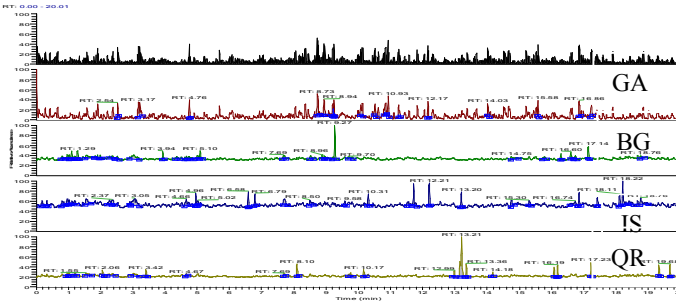


Fig.1 Chemicals and spectras of bergenin (BG), quercitrin-3-O-*β*-L-rhamnoside (QR), gallic acid (GA) and Puerarin (IS)



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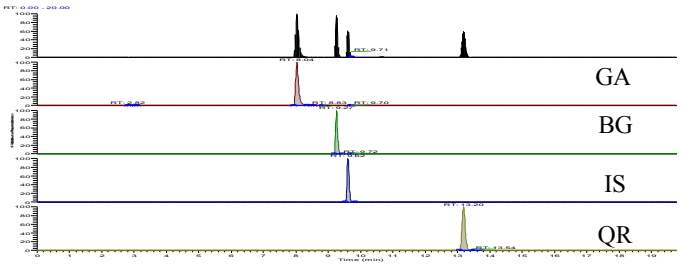

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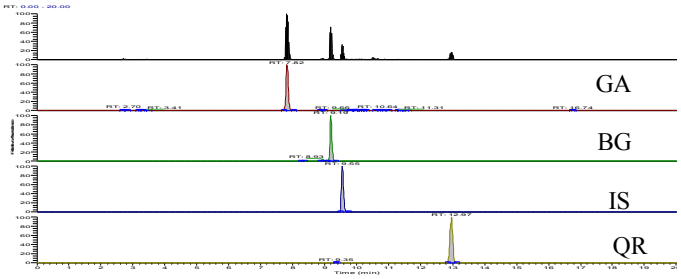

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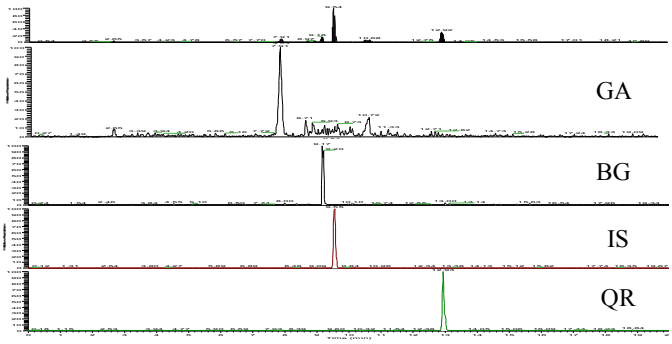

D

Fig.2. Representative MRM chromatograms of GA, BG, QR, and puerarin (internal standard, IS) in (A) blank plasma, (B) blank plasma spiked with GA, BG, QR and IS at concentrations of 43.44, 31.14, 33.24 and 4.42 ng/mL, respectively, (C) plasma at 30 min after oral administration of 1.48 g/kg S. stolonifera extract, and (D) blank plasma spiked with GA, BG, QR and IS at concentrations of 4.34, 3.11, 0.66 and 4.42 ng/mL.

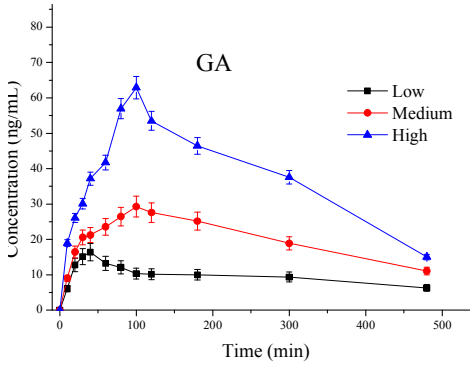

Fig.3 Plasma concentration-time curves of GA in rats (n=6) after oral administration of *S. stolonifera* extract for different dose levels.

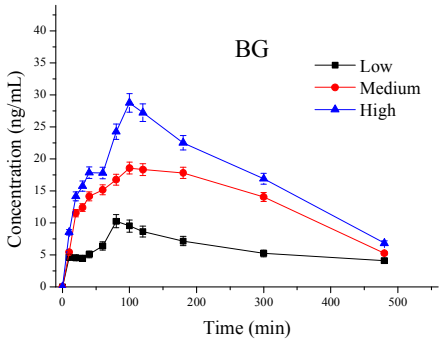

Fig.4 Plasma concentration-time curves of BG in rats (n=6) after oral administration of *S. stolonifera* extract for different dose levels.

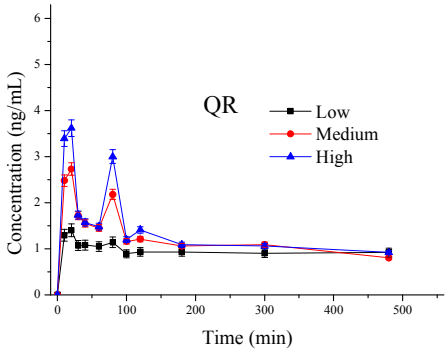

Fig. 5 Plasma concentration-time curves of QR in rats (n=6) after oral administration of *S. stolonifera* extract for different dose levels

Table 1 Values of tube lens offset (V), collision pressure (mTorr) and collision energy (eV) for the parent ions-product ions transitions.

Analytes	Transition	Tube Lens Offset (V)	Transition (m Torr)	Collision Energy (eV)
GA	m/z 169.012 → 125.06	68	1.5	17
BG	m/z 326.942 → 191.997	94	1.5	27
IS	m/z 415.051 → 266.999	97	1.5	36
QR	m/z 447.014 → 300.028	107	1.5	29

Table 2 Plasma recovery of GA, BG, QR, and IS (n=3).

Aanalyte	A1 Mean±SD	A2 Mean±SD	Plasma recovery Re%
GA	5.65E4	4.41E4	78.06
BG	5.13E4	4.39E4	85.56
QR	1.50E5	1.18E5	78.19
IS	4.58E4	3.81E4	83.21

$$Re\% = (A2/A1) \times 100$$

Table 3 Precision of intra-day and inter-day, accuracy, and recovery of the analytes in QC samples (n=6)

Analyte	Norminal Concentration (ng/mL)	Intra-day			Inter-day		
		Measured concentration (ng/mL)	Precision %R.S.D	Accuracy % Bias	Measured concentration (ng/mL)	Precision %R.S.D	Accuracy % Bias
GA	4.34	3.74	7.01	13.82	4.15	14.16	3.02
	43.44	41.54	8.46	4.38	41.31	4.72	4.90
	173.76	163.48	2.46	5.92	164.77	4.03	5.17
BG	3.11	2.90	6.84	6.75	2.76	10.41	11.36
	31.14	30.59	5.58	1.77	29.49	6.87	5.29
	124.56	122.06	4.23	2.05	118.05	5.70	5.23
QR	3.32	2.88	4.57	13.27	3.04	4.57	8.45
	33.24	30.75	3.79	7.49	118.88	4.49	9.05
	132.96	118.88	5.40	10.59	118.33	4.08	11.00

Table 4 Stability of the analytes under different conditions (n=3)

Condition	Analyte	Norminal concentration (ng/mL)	Measured concentration (mean SD) (ng/mL)	Precision (%)R.S.D	Accuracy %Bias
Room temperature	GA	173.76	161.53±11.69	7.24	7.04
	BG	124.56	122.15±1.46	1.19	1.93
	QR	132.96	122.64±2.62	2.14	7.76
4 ˚C in the autosampler	GA	173.76	172.00±2.30	1.34	1.01
	BG	124.56	122.65±7.78	6.35	1.53
	QR	132.96	114.18±0.06	0.06	14.12
Three freeze-thaw cycles	GA	173.76	164.87±5.09	3.09	5.12
	BG	124.56	120.86±6.75	5.58	2.97
	QR	132.96	120.37±5.84	4.85	9.47
Long-term stability (19d)	GA	173.76	179.59±9.74	5.42	3.35
	BG	124.56	121.19±15.51	12.80	2.71
	QR	132.96	124.69±6.25	5.01	6.22

Table 5 Pharmacokinetic parameters of GA in rats after oral administrations of *S. stolonfera* aqueous extract

Dose	$T_{1/2}$ (min)	Tmax (min)	Cmax (ng/mL)	AUC_{0-t} (ng/mL)	Vz_F_obs (L/kg)	Cl_F_obs (L/kg/min)
Low	513.07	40	16.38±5.76	4572.80	130	0.18
Middle	251.35	100	29.28±11.78	9560.22	85.67	0.24
High	197.32	100	62.91±34.55	17844.47	6921558.08	0.92

Table 6 Pharmacokinetic parameters of BG in rats after oral administrations of *S. stolonfera* aqueous extract

Dose	$T_{1/2}$ (min)	Tmax (min)	Cmax (ng/mL)	AUC_{0-t} (ng/mL)	Vz_F_obs (L/kg)	Cl_F_obs (L/kg/min)
Low	301.77	80	10.24±2.68	2877.44	130	0.18
Middle	251.35	100	18.54±2.59	9560.22	85.67	0.24
High	197.32	100	28.74±14.13	17844.47	6921558.08	0.92

Table 7 Pharmacokinetic parameters of QR in rats after oral administrations of S. stolonfera aqueous extract

Dose	$T_{1/2}$ (min)	Tmax (min)	Cmax (ng/mL)	AUC_{0-t} (ng/mL)	Vz_F_obs (L/kg)	Cl_F_obs (L/kg/min)
Low	1835.95	20	1.40±0.74	454.55	97.72	0.37
Middle	717.49	20	2.73±1.58	567.58	158.76	1.53
High	1187.15	20	3.62±1.75	619.94	334.66	1.95