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ORIGINAL ARTICLE

Pharmacokinetic Comparisons of Six Major Bioactive Components in Rats after Oral Administration of Crude and Saltwater Processed *Phellodendri amurensis* Cortex by Ultra-performance Liquid Chromatography–Mass Spectrometry/Mass Spectrometry

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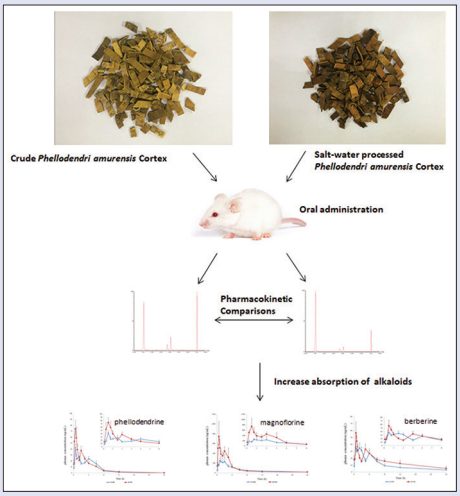
ABSTRACT

Background: The *Phellodendri amurensis* cortex is a traditional Chinese medicine with multiple pharmacodymic uses (antibacterial, anti-inflammation, antitumor, etc). It is often processed by saltwater to strengthen its effects in terms of nourishing yin to reduce pathogenic fire and reducing asthenic fever. To clarify the principle of saltwater processing, an experiment of pharmacokinetic comparison after oral administration from crude *P. amurensis* cortex and its saltwater processed product was carried out. **Materials and Methods:** A validated and sensitive ultra-performance liquid chromatography-mass spectrometry/mass spectrometry (UPLC-MS/MS) method was established for simultaneous quantification of five alkaloids and one triterpene in rat. An UPLC C_{18} column was used for chromatograph separation by an elution program with the mobile phase consisting of 0.1% formic acid and acetonitrile. Each analytes and internal standard, nimodipine, was detected using positive ion scan mode via multiple reaction monitoring mode. All of the validation parameters investigated involving selectivity, precision, accuracy, extraction recovery, matrix effects, and stability shown this approach was suitable to the pharmacokinetic study. **Results:** Pharmacokinetic profiles showed these parameters of maximum of drug concentration and AUC_{0-t} of alkaloids analytes elevated remarkably after oral administration of *P. amurensis* cortex processed with the saltwater. **Conclusion:** The results suggested that the absorption effect from the saltwater processed product was better than those from crude product, which could explain that saltwater processing may enhance the activity of clearing heat and removing toxicity from *P. amurensis* cortex.

Key words: Alkaloids, pharmacokinetic, *Phellodendri amurensis* cortex, saltwater processing, ultra-high-performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry

SUMMARY

- In our study, an ultra-performance liquid chromatography-mass spectrometry/mass spectrometry approach was established for simultaneous quantitation of five alkaloids and one triterpene from *Phellodendri amurensis* cortex. The approach was successfully used to investigate pharmacokinetic differences in rats. The result demonstrated that saltwater processing might enhance the absorption of alkaloids from *Phellodendri amurensis* cortex. It is the first time about the study on comparative pharmacokinetic of crude *Phellodendri amurensis* cortex and its saltwater processed product and the result could suggest that saltwater processing would strengthen its bioactivity of clearing heat via enhancing the absorption of the alkaloids from *Phellodendri amurensis* cortex.



Abbreviations used: GHB: *Phellodendri amurensis* cortex; CGHB: Crude *Phellodendri amurensis* cortex; SGHB: Saltwater processed *Phellodendri amurensis* Cortex; HPLC: High-performance liquid chromatography; UPLC-MS/MS: Ultra-performance liquid chromatography-mass spectrometry/mass spectrometry; UPLC-QqQ-MS: Ultra-high-performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry; MRM: Multiple reaction monitoring mode; QC: Quality control; RE: Relative error; RSD: Relative standard deviation; C_{max}: Maximum of drug concentration; T_{max}: Time for maximum of drug concentration; AUC: Area under concentration-time curve; LLOQ: lower limit of quantification; $T_{½}$: Half-life.

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INTRODUCTION

The *Phellodendri amurensis* cortex, named "Guanhuangbo" (GHB) in Chinese, the dried bark of *P. amurensis* Rupr. (Family: *Rutaceae*), has been widely applied for thousands of years in China. In traditional Chinese medicine (TCM), GHB is used to "clear heat, dry dampness, purge fire, relieve steaming, remove toxin, and treat sore."[1] In clinic, it can be used to treat bacterial malaria, pneumonia, acute conjunctivitis, etc.[2] According to requirements in the science of TCM, most herbs

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must be processed by a certain method to be used in clinical practice. The purpose of processing is to enhance efficacy, reduce toxic effects, or eliminate side effects. GHB is now often processed by stir-frying with saltwater (SGHB). Compared with crude GHB (CGHB), SGHB can moderate its bitter flavor and drastic properties and strengthen its effects in terms of nourishing yin to reduce pathogenic fire and reducing asthenic fever.[3,4]

Various bioactive compounds, especially protoberberine-type alkaloids,[5] have been identified in GHB, and most of them have shown anti-inflammatory,[6] antitumor,[7] antiarrhythmic,[8] and antidiabetic activities.[9] Besides alkaloids, there are limonoid-type triterpenes, such as obacunone and limonin in GHB,[10-12] and they exhibit significant antitumor,[13] antibacterial,[14] and antioxidation[15] activities. Nowadays, there are few reports on the pharmacokinetics investigation of alkaloids and limonoides of CGHB and SGHB, either few reports on the effect of the different absorptions of these active ingredients from crude and processed GHB. In this course of the research, a reliable, sensitive, and specific ultra-high-performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry (UPLC-QqQ-MS) approach was established and verified for simultaneous quantitative analysis of six bioactive components in rat plasma. This method was successfully used to a pharmacokinetics study, in which it was found that oral administration of the saltwater processed product could affect the absorption of these bioactive compounds. The *in vivo* pharmacokinetic study of the bioactive components of GHB could be necessary and helpful for further clinical applications and explanations of the processing mechanism.

MATERIALS AND METHODS

Herbal material and chemical reagents

GHB was purchased from Kangmei Pharmaceutical Co. Ltd. It was identified by Professor Bing Wang from Liaoning University of TCM. SGHB was produced in accordance with the Chinese Pharmacopeia (2015 ed). Simply, the CGHB were sealed in a container with the saltwater (100:2, GHB–salt, W/W), until the saltwater showed 100% infiltration (no residual solution in the container) into CGHB, then stir-fried in a wok at the temperature of 160°C for 5 min.

Standard substances (purity >98% by HPLC-UV) phellodendrine, magnoflorine, jatrorrhizine, palmatine, berberine, obacunone, and nimodipine (Internal Standard [IS]) were purchased from Dalian Meilun Biotechnology company (Dalian, China). The acetonitrile, methanol (mass-grade), and formic acid (chromatographic-grade) were obtained from Merck (Darmstadt, Germany). Ultrapure water was prepared by a Milli-Q system (18.2 MΩ, Millipore, Billerica, USA). The other reagents and chemicals were of the highest grade analytical.

Preparation of aqueous extracts

An appropriate amount of CGHB was soaked with a ten-fold volume of distilled water for 30 min, after which it was decocted for 60 min and percolated. The residue was redissolved with an eight-fold volume of distilled water to decocted for 60 min and percolated again, then two filtrates were combined. The final concentration of CGHB aqueous extracts was 1 g/mL.[16] Respectively, the processed CGHB with saltwater aqueous extracts were prepared in the same method. All of the samples were maintained at 4°C before use.

Content determination of six major bioactive components in CGHB extract and SGHB extract

To determine the oral administration dose of GHB, the quantitation of phellodendrine, magnoflorine, jatrorrhizine, palmatine, berberine, and obacunone in CGHB and SGHB extracts were carried out. The aqueous extracts of CGHB and SGHB were diluted ten times with methanol, and the diluted solution was centrifuged at 5000 rpm for 15 min. The supernatants were filtered through a 0.45-μm Millipore filter before HPLC analysis. A 10 μL sample was injected into the HPLC system with a Waters C_{18} column (4.6 mm × 150 mm 5 μm). To determine the content of alkaloids, the mobile phase was 0.025 mol/L KH_2PO_4-acetonitrile (60:40) with the flow rate of 1.0 mL/min at 30°C, via setting the detective wavelength at 284 nm. For obacunone, the mobile phase was 0.01 mol/L H_3PO_4-acetonitrile (55:45) with the flow rate of 1.0 mL/min at 30°C, via setting detective wavelength at 210 nm. The concentration of phellodendrine, magnoflorine, jatrorrhizine, palmatine, berberine, and obacunone in CGHB extract were 0.18, 0.12, 4.31, 5.21, 9.26, and 0.16 mg/g, respectively, and 0.19, 0.13, 4.26, 5.22, 9.77, and 0.16 mg/g, respectively, in SGHB extract.

Ultra-high-performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry conditions

The analysis of pharmacokinetic was carried out by a Waters ACQUITY UPLC system and a Xevo TQ-S mass spectrometer. Moreover, the chromatographic separation was performed on a Waters UPLC BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm) at 35°C. The composition of mobile phase was 0.1% formic acid water (A) and 0.1% formic acid acetonitrile (B). The gradient elution program was shown in Table 1, and run at a flow rate of 0.3 mL/min. Each sample was placed in an autosampler at 4°C.

Most of the bioactive compounds of GHB are alkaloids, and alkaloids have strong signal response in positive scan mode. Therefore, the positive ion mode was applied to detect the samples. To receive a richer mass spectral abundance of precursor and product ions, the MS condition was conducted as follows: source temperature 150°C; capillary 3000 V; cone voltages 50 V; desolvation gas (N2) flow 900 L/h; desolvation temperature 450°C; and cone gas flow 50 L/h. The majorization of collision energy was according to the chemical standards, and using helium for collision gas of collision-induced dissociation. Quantitative analysis was executed by the multiple reactions monitoring (MRM). The precursor → product ion transitions of m/z 342.17 → 192.15, m/z 342.18 → 265.16, m/z 338.15 → 323.03, m/z 352.15 → 336.20, m/z 336.07 → 320.29, m/z 455.23 → 161.14 and m/z 419.21 → 343.17 were employed for quantification of phellodendrine, magnoflorine, jatrorrhizine, palmatine, berberine, obacunone, and nimodipine. The results are shown in Table 2 and Figure 1.

Preparation of standard solutions and quality control samples

The stock solutions of phellodendrine, magnoflorine, jatrorrhizine, palmatine, berberine, obacunone, and nimodipine (IS) at concentration of 74.80, 63.40, 63.20, 60.10, 61.20, 62.80, and 5.00 μg/mL were,

Table 1: Gradient elution program of mobile phase

Time (min)	A % (0.1% formic acid water)	B % (0.1% formic acid acetonitrile)
0	75	25
2.5	75	25
4.5	40	60
7.5	0	100
10	0	100
10.01	75	25
12	75	25

respectively, prepared by dissolving the accurately weighed seven corresponding reference substances in methanol. A series of mixed standard stock solutions were obtained by diluting with methanol. The range of the concentrations of each standards were phellodendrine 0.37–119.76 ng/mL, magnoflorine 0.79–2028.80 ng/mL, jatrorrhizine 0.79–252.80 ng/mL, palmatine 0.75–240.4 ng/mL, berberine 1.53–489.60 ng/mL, and obacunone 0.79–121.60 ng/mL. The seven calibration solutions samples were prepared by the appropriate amount of the mixing standard stock solutions (100 μL), IS (20 μL) and blank plasma (100 μL). At last, the seven calibration solutions were obtained at the concentrations of 0.37, 3.74, 7.48, 14.96, 29.94, 59.88, and 119.76 ng/mL for phellodendrine; 0.79, 1.58, 15.85, 63.40, 253.60, 1014.40, and 2028.80 ng/mL for magnoflorine; 0.79, 1.58, 15.80, 31.60, 63.20, 126.40, and 252.8 ng/mL for jatrorrhizine; 0.75, 1.50, 15.00, 30.00, 60.00 120.00, and 240.00 ng/mL for palmatine; 1.53, 15.30, 30.60, 61.20, 122.40, 244.80, nd 489.60 ng/mL for berberine; and 0.79, 1.57, 3.14, 6.28, 31.4, 62.80, and 121.60 ng/mL for obacunone. Quality control (QC) samples were prepared at the concentrations of 0.8, 9, and 90 ng/mL for phellodendrine; 1.5, 40, and 1530 ng/mL for magnoflorine; 1.5, 20, and 200 ng/mL for jatrorrhizine; 4, 20, and 200 ng/mL for palmatine; 4, 40, and 360 ng/mL for berberine; and 2, 15 and 90 ng/mL for obacunone. All the samples were maintained at 4°C before use.

Table 2: Optimized multiple reaction monitoring parameters for analytes and internal standard

Analytes	MRM	Cone voltage (V)	Collision energy (V)
Phellodendrine	342.17→192.15	62	22
Magnoflorine	342.18→265.16	26	28
Jatrorrhizine	338.15→323.03	50	22
Palmatine	352.15→336.20	50	30
Berberine	336.07→320.29	50	28
Obacunone	455.23→161.14	58	44
Nimodipine	419.21→343.17	18	10

→ is used to separate precursor ions and product ions m/z. MRM: Multiple reaction monitoring

Preparation of plasma samples

About 20 μL of IS solution (5 μg/mL nimodipine) was added to 100 μL plasma samples by vortex-mixing for 30 s, then 400 μL of acetonitrile solution was spiked and vortexed for 180 s. After centrifugation (13,000 rpm, 10 min), the supernatant was diverted to another cuvette and dried with nitrogen gas at 37°C. A 100 μL of initial mobile phase was applied to redissolve the residue, then vortexed for 180 s, and centrifuged (13,000 rpm, 10 min). A 2 μL supernatant solution was used for pharmacokinetic analysis.

Animals

The Sprague–Dawley rats (weight: 200 ± 20 g, male) were purchased from Liaoning Changsheng Bio-Technology Co., Ltd. (Certification No.:SCXK [LN] 2010-0001), housed in the plastic cages at the temperature of 22°C–24°C, could drink and eat *ad libitum*. All the animals were kept for 7 days to adapt to the environment before the start of the experiment,

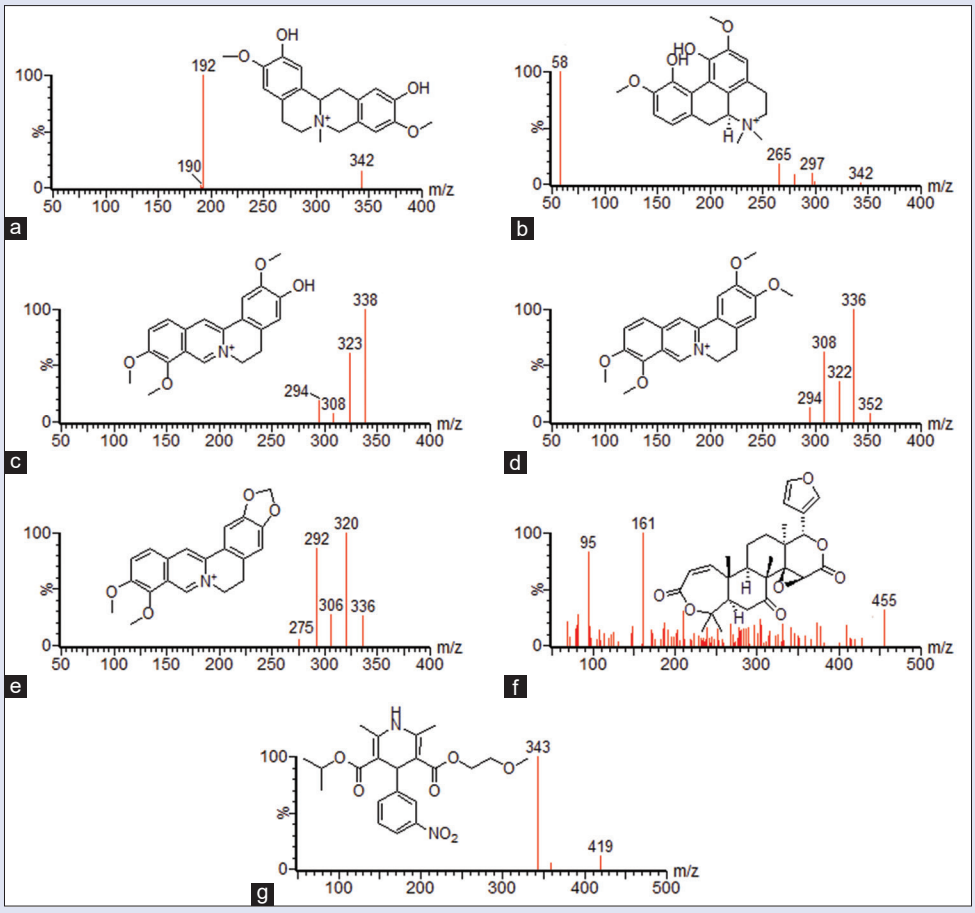

Figure 1: The products spectra and fragmentation reaction of the seven compounds in positive electrospray ionization mode: (a) phellodendrine, (b) magnoflorine, (c) jatrorrhizine, (d) palmatine, (e) berberine, (f) obacunone, and (g) nimodipine

and fasted for 12 h before dosing. The study protocol was authorized by the Animal Ethics Committee of Liaoning University of TCM.

Method validation

Selectivity

The selectivity of the assay was evaluated by comparing the chromatogram peaks of blank plasma and blank plasma added with phellodendrine, magnoflorine, jatrorrhizine, palmatine, berberine, obacunone, and IS, along with plasma samples achieved after administration of GHB aqueous extract.

Linearity of calibration curves and lower limit of quantification

The calibration curve involved seven concentration levels and was established based on the peak area ratios (Y) of phellodendrine, magnoflorine, jatrorrhizine, palmatine, berberine, and obacunone to the nimodipine (IS) versus the concentration standards (X) using the weighted least square linear regression ($1/X^2$). The LLOQ (Lower Limit of Quantification) of the method was evaluated as the lowest concentrations of the calibration curve which would be quantitative analysis by the value of an S/N ≥ 10 with precision and accuracy well applied.

Precision and accuracy

Six replicates of each concentration of QC sample were tested for the precision and accuracy validation. The intraday precision and accuracy were analyzed by the QC samples determined on the same day, and the interday precision and accuracy were measured by the QC samples on the 3 consecutive days. The precision was defined as relative standard deviation (RSD)%, and accuracy was expressed as relative error (RE)%, all of these values should be within ± 15%.

Extraction recovery and matrix effect

The extraction recoveries of the assay were calculated using each QC concentrations by comparing the peak areas from plasma samples with those achieved by the extracted blank plasma added with the corresponding analytes and IS. The matrix effect was determined by comparing the peak areas of the blank plasma which the postextracted matrix was supplemented with analytes and IS with those of the samples in the solution of water/acetonitrile (50:50).

Stability

The stability study was based on the quantitation of the low-, middle-, and high-QC concentration under different conditions: For 6 h at room temperature, at −80°C for 30 days, after three cycles of repeated freezing–thawing (from −80°C to −25°C) for 3 consecutive days and maintaining the extracted samples at 4°C for 24 h in the autosampler.

Pharmacokinetic study

The experimental rats were randomly divided into two groups: CGHB group and SGHB group. Then, the animals were orally treated with the CGHB and SGHB extracts at a single dose of 10.0 g/kg. The whole blood samples 0.25 mL in volume were achieved from orbital vein, after 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h of the oral administration. All the blood samples were processed to centrifuge (3800 rpm, 10 min) to get plasma and then stored the samples at −80°C before analysis.

The verified approach was successfully used to a pharmacokinetic investigation for simultaneous quantitation of the five alkaloids and one triterpene in rat after administration of CGHB and SGHB. Data of sample information were obtained by MassLynx (Version 4.1) and processed by Drug and Statistics software (Version 3.0, Shanghai, China). The statistics of the results were calculated by the SPSS 19.0 software (IBM company, New York, USA). The mean concentration–time curves of each analyte from CGHB and SGHB were showed in Figure 2. The pharmacokinetic profile was analyzed by using a noncompartmental model.

RESULTS AND DISCUSSION

Validation of the analytical method

Selectivity

Representative positive ion chromatograms of blank plasma, blank plasma added with the analytes and IS, along with plasma samples collected after oral administration of GHB for 1 h are illustrated in Figure 3. The figure demonstrated the good resolution chromatogram and without interference. Each retention time of phellodendrine, magnoflorine, jatrorrhizine, palmatine, berberine, obacunone, and IS were approximately 1.03, 1.10, 2.42, 3.69, 4.15, 6.61, and 7.05 min, respectively.

Linearity of calibration curves and LLOQ

Table 3 shows the linear regression analysis of phellodendrine, magnoflorine, jatrorrhizine, palmatine, berberine, and obacunone exhibited good linearity, with all of the correlation coefficients higher than 0.9906 over ranges of 0.37–119.76 ng/mL, 0.79–2028.80 ng/mL, 0.79–252.80 ng/mL, 0.75–240.00 ng/mL, 1.53–489.60 ng/mL, and 0.79–121.60 ng/mL, respectively. The values of the LLOQs were measured at a (S/N) ratio of ≥10, which were suitable for pharmacokinetic investigation.

Precision and accuracy

The precision was demonstrated as RSD%, and the accuracy was demonstrated as RE% of the QC samples. The intra- and inter-day precision and accuracy were calculated by six replicates of the QC samples at low, medium, high concentration on the same day for 3 consecutive days. Table 4 showed the precision and accuracy of the method were acceptable and satisfactory, indicating this method could quantify the analytes in rat reliably.

Extraction recovery and matrix effects

Table 5 summarized that the extraction recoveries were ranged from 85.20%–96.27% for each analyte and 90.05% for IS. The results indicated this means was within the acceptance criteria. Meanwhile, the matrix effects of all the analytes at each QC level ranged from 87.72% to 107.25%, could be expressed no notable matrix effects.

Stability

The stability test results were demonstrated in Table 6. It showed all the analytes were stable for 6 h at room temperature, for 30 days at −80°C, after repeated freeze-thaw cycles for 3 days and maintaining the extracted samples at 4°C for 24 h in the autosampler. The results indicated that the developed method is acceptable for pharmacokinetic investigation.

Table 3: The regression equations, linear ranges, and lower limit of quantifications for the determination of the analytes in rat plasma

Analytes	Regression equation	*R*	Linear range (ng/mL)	LLOQ (ng/mL)
Phellodendrine	y=5.1667x+12.6354	0.9972	0.37-119.76	0.37
Magnoflorine	y=1.6274x+0.6420	0.9981	0.79-2028.80	0.79
Jatrorrhizine	y=3.2443x−24.4222	0.9953	0.79-252.80	0.79
Palmatine	y=13.9116x−90.8682	0.9906	0.75-240.00	0.75
Berberine	y=11.2165x−16.3284	0.9944	1.53-489.60	1.53
Obacunone	y=0.09613x+0.876629	0.9951	0.79-121.60	0.79

LLOQ: Lower limit of quantification

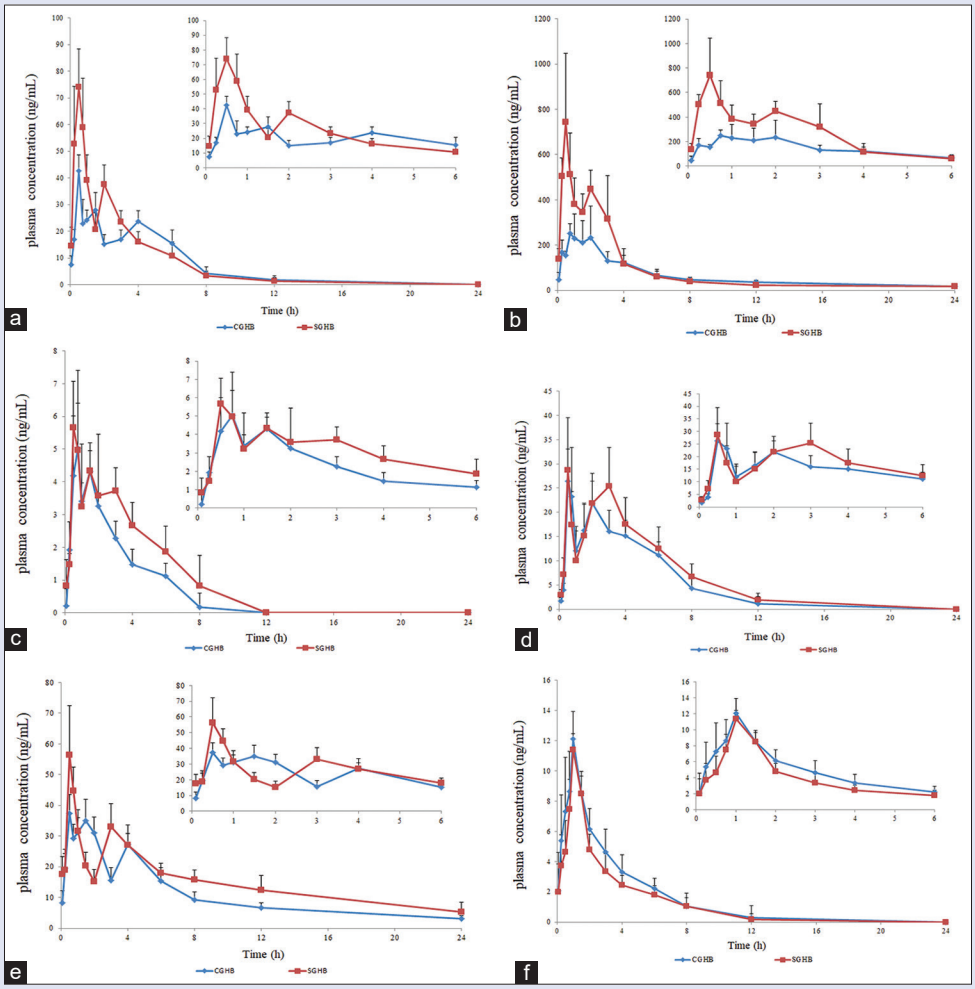

Figure 2: Mean plasma concentration–time curve for (a) phellodendrine, (b) magnoflorine, (c) jatrorrhizine, (d) palmatine, (e) berberine, and (f) obacunone in rat plasma after oral administration of CGHB and SGHB

Table 4: Precision and accuracy for the analytes in rat plasma (*n*=6)

Analytes	QC concentration (ng/μL)	Intraday (*n*=6)			Interday (*n*=18)		
		Measured concentration (ng/μL)	RSD (%)	RE (%)	Measured concentration (ng/μL)	RSD (%)	RE (%)
Phellodendrine	0.8	0.78±0.09	10.86	−2.58	0.81±0.07	8.88	1.11
	9	9.06±0.86	9.46	0.66	9.00±1.16	12.95	−0.05
	90	89.26±5.51	6.18	−0.82	91.73±7.54	8.22	1.92
Magnoflorine	1.5	1.61±0.21	12.88	7.78	1.52±0.17	10.97	1.63
	40	39.48±4.95	12.54	−1.31	36.71±3.98	10.83	−8.21
	1530	1575.01±132.28	8.40	2.94	1512.35±166.15	10.99	−1.15
Jatrorrhizine	1.5	1.62±0.16	9.92	8.11	1.55±0.18	11.32	0.18
	20	19.31±2.43	12.59	−3.45	20.91±2.57	12.30	4.53
	200	201.61±10.18	5.05	0.81	195.64±16.40	8.38	−2.18
Palmatine	4	3.98±0.36	9.04	−0.44	4.05±0.40	9.89	1.15
	20	19.75±0.85	4.31	−1.24	20.86±0.78	3.73	4.30
	200	203.08±13.18	6.49	1.54	193.49±14.13	7.30	−3.25
Berberine	4	4.03±0.45	11.11	0.75	4.02±0.44	11.00	0.40
	40	39.26±1.10	2.80	−1.84	39.19±3.20	8.15	−2.03
	360	367.93±12.76	3.47	2.20	364.00±21.94	6.03	1.11
Obacunone	2	2.12±0.19	8.92	6.25	2.08±0.24	11.58	4.01
	15	15.26±1.89	12.35	1.76	14.74±1.84	12.48	−1.71
	90	89.70±4.40	4.90	−0.33	83.70±4.10	4.91	−7.00

QC: Quality control; RSD: Relative standard deviation; RE: Relative error

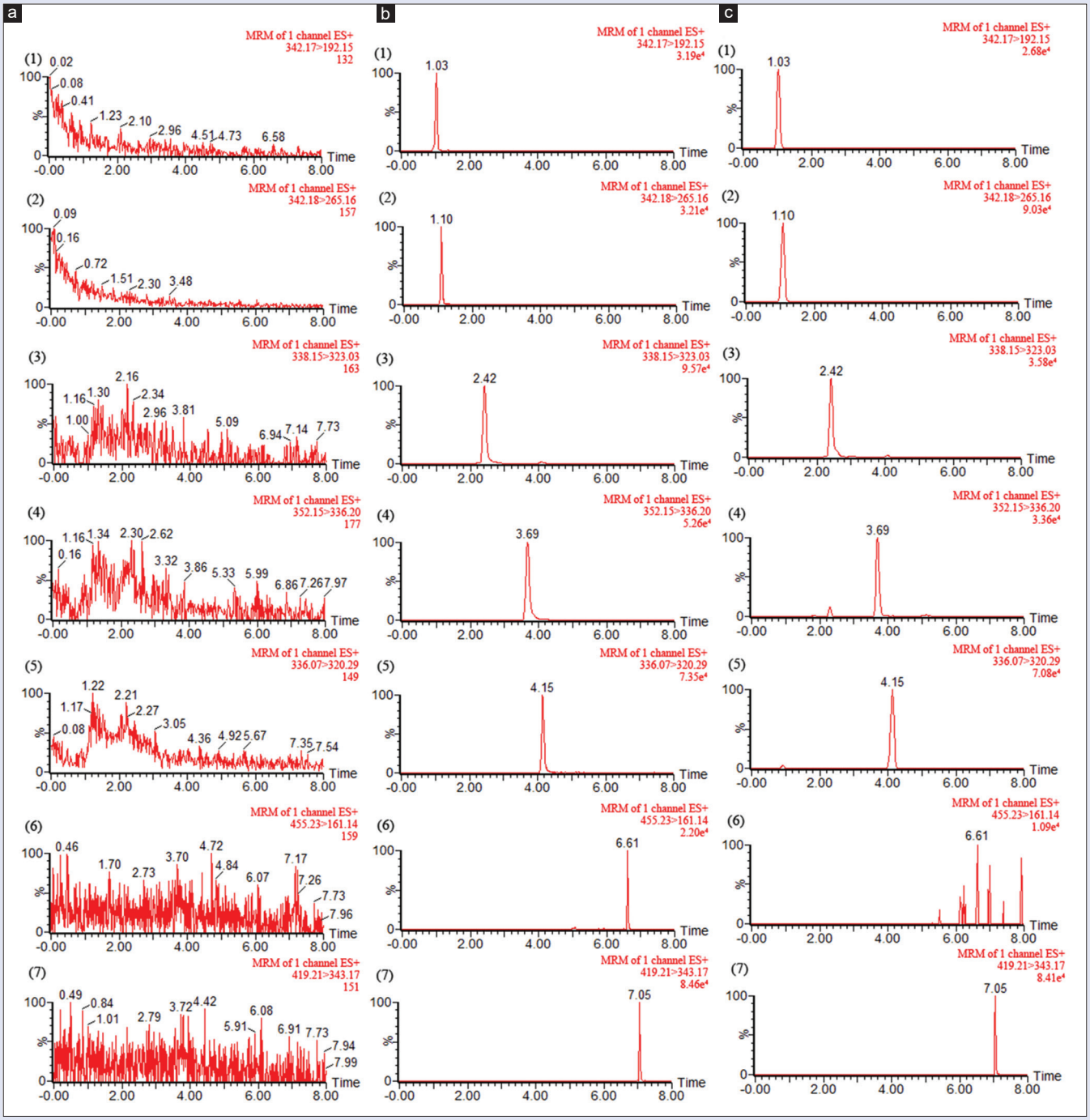

Figure 3: Typical multiple reaction monitoring chromatogram of (1) phellodendrine, (2) magnoflorine, (3) jatrorrhizine, (4) palmatine, (5) berberine, (6) obacunone, and (7) IS. Blank plasma from six rats (a); spiked plasma samples with the analytes and IS (b); plasma samples from rats 1 h after oral administration of GHB stir-fried in saltwater (SGHB) extract (c)

Application to pharmacokinetic study

We established a validated and successful method for simultaneous quantitation of five alkaloids and one triterpene in rat after oral administration of CGHB and SGHB. The mean concentration–time curves and pharmacokinetic profiles of each analyte from CGHB and SGHB were shown in Figure 2 and Table 7.

In GHB, phellodendrine, magnoflorine, jatrorrhizine, palmatine, and berberine are alkaloids compounds. As shown in Figure 2, most alkaloids compounds from GHB to reach the maximum concentration within 1 h of oral administration. At the same time, there was another small peak observed at almost 2 h of the alkaloids. The double-peak phenomenon of alkaloids was consistent with the literature and very likely owing to distribution, reabsorption, and enterohepatic circulation.[16-18] compared with alkaloid compounds, obacunone is a limonin-type triterpene ingredient from GHB, and there was only one peak in the concentration–time curve.

Table 7 demonstrated that no remarkable difference between CGHB and SGHB in parameters of half-life ($T_{½}$) and time for maximum of

Table 5: Matrix effects and extraction recovery for the analytes in rat plasma (*n*=6)

Analytes	QC concentration (ng/μL)	Extraction recovery (%)	RSD (%)	Matrix effects (%)	RSD (%)
Phellodendrine	0.8	89.62	2.03	103.25	13.14
	9	91.15	7.07	107.25	10.87
	90	90.54	9.89	96.67	7.86
Magnoflorine	1.5	88.57	13.5	103.25	0.28
	40	93.05	5.47	100.58	7.79
	1530	86.32	12.50	97.58	0.76
Jatrorrhizine	1.5	95.25	14.06	92.25	2.74
	20	91.33	3.94	108.25	8.68
	200	89.67	3.45	91.56	3.58
Palmatine	4	85.57	4.11	93.57	1.27
	20	91.30	12.16	105.60	4.47
	200	87.59	1.97	93.57	1.32
Berberine	4	96.27	4.77	87.72	8.01
	40	91.75	3.66	96.35	7.91
	360	90.37	10.58	101.77	4.87
Obacunone	2	87.25	11.87	106.51	3.47
	15	85.20	9.45	104.85	2.49
	90	95.21	9.74	93.71	12.23
IS	1000	90.05	6.21	95.52	4.25

QC: Quality control; RSD: Relative standard deviation; IS: Internal standard

Table 6: Stability of each analyte in rat plasma under different storage conditions (*n*=6)

Analytes	QC concentration (ng/μL)	Stability (percentage RE)			
		Room temperature for 6 h	Storage at -80°C for 30 days	Three Freeze-Thaw cycles	Posttreatment for 24 h at 4°C
Phellodendrine	0.8	0.19	3.14	−1.83	5.23
	9	2.72	−2.74	3.00	3.12
	90	−1.77	−1.50	1.42	−5.36
Magnoflorine	1.5	2.75	−1.18	6.02	1.23
	40	−5.79	−1.73	−1.48	2.98
	1530	2.78	1.71	2.86	−3.15
Jatrorrhizine	1.5	10.55	12.46	13.30	2.08
	20	−6.84	−2.70	−0.91	1.74
	200	−1.10	1.12	0.66	3.98
Palmatine	4	4.70	−3.08	−1.35	−4.25
	20	−0.68	−0.41	−1.02	1.30
	200	0.57	−2.05	4.84	1.08
Berberine	4	4.41	0.19	1.86	5.12
	40	−2.30	−0.02	−3.42	4.09
	360	2.58	7.63	0.42	2.78
Obacunone	2	6.42	3.79	11.57	−1.56
	15	4.77	1.82	−0.61	5.27
	90	1.92	0.18	1.18	1.87

QC: Quality control; RE: Relative error

Table 7: Pharmacokinetic parameters of each analyte in rat plasma after oral administration of crude *Phellodendri amurensis* cortex and saltwater processed *Phellodendri amurensis* cortex (*n*=6)

Analytes	Group	$T_{½}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-t} (h ng/mL)	$AUC_{0-\infty}$ (h ng/mL)
Phellodendrine	CGHB	1.515±0.510	0.500±0.000	42.562±5.908	164.559±11.974	164.598±10.266
	SGHB	1.073±0.415	0.583±0.129	74.838±13.720*	189.374±31.909	189.376±31.909
Magnoflorine	CGHB	11.243±3.977	1.042±0.485	320.013±101.449	1510.984±304.049	1829.265±481.732
	SGHB	12.658±9.798*	0.417±0.129	762.045±276.405*	2114.721±540.042*	2407.518±495.864*
Jatrorrhizine	CGHB	0.859±0.379	0.875±0.345	5.940±1.419	15.865±2.635	15.865±2.635
	SGHB	1.121±0.475	0.833±0.585	5.970±1.093	23.580±5.270*	23.581±5.271*
Palmatine	CGHB	1.754±1.036	0.958±0.641	31.198±5.596	125.091±18.526	125.373±18.692
	SGHB	1.306±0.032	1.5±1.225	34.123±7.877	153.032±24.552*	153.034±24.552*
Berberine	CGHB	10.479±6.113	0.833±0.408	39.848±5.659	260.034±26.131	310.902±61.904
	SGHB	12.51±8.007	0.542±0.102	57.335±14.474*	352.011±50.696	477.494±200.313
Obacunone	CGHB	1.695±0.195	1.000±0.000	12.145±3.142	38.435±10.558	38.437±10.559
	SGHB	1.466±0.386	1.000±0.000	8.447±2.227	31.106±7.301	31.108±7.302

$T_{½}$, peak time (T_{max}), peak plasma concentration (C_{max}), extent of absorption (AUC) of each analyte in rats (mean±SD, *n*=6) after oral administration of CGHB and SGHB. *P<0.05 versus CGHB. GHB: *Phellodendri amurensis* cortex; CGHB: Crude GHB; SGHB: Salt-water processed GHB; $T_{½}$: Half time; C_{max}: Maximum of drug concentration; T_{max}: Time for maximum of drug concentration; AUC: Area under concentration-time curve; SD: Standard deviation

drug concentration except magnoflorine, suggesting that saltwater processing did not have influence the absorption rates of this compounds obviously. However, the mean maximum of drug concentration (C_{max}) values of phellodendrine, magnoflorine, jatrorrhizine, palmatine, and berberine (74.838 ± 13.720, 762.045 ± 276.405, 6.587 ± 1.413, 34.123 ± 7.877 and 57.335 ± 14.474 ng/mL, respectively) from the SGHB group were 1.76-, 2.38-, 1.10-, 1.01-, and 1.83-fold higher than the values from the CGHB group. The AUC_{0-t} values of phellodendrine, magnoflorine, jatrorrhizine, palmatine, and berberine (189.374 ± 31.909, 2114.721 ± 540.042, 23.685 ± 5.281, 153.032 ± 24.552, and 352.011 ± 50.696 h•ng/mL, respectively) were 1.15-, 1.40-, 1.49-, 1.22-, and 1.25-fold higher than the values of the CGHB group. On the contrary, the C_{max} value of obacunone from SGHB is lower than those in the CGHB group, similar to the AUC_{0-t} value. However, there is no remarkable difference between the two groups ($P > 0.05$). It can be inferred that the saltwater processing might mainly affect the absorption of alkaloids compounds and increase them other than the triterpene compounds from GHB.

The significant raises in C_{max} value of alkaloid components from the SGHB group confirmed the saltwater processing could increase these compounds exposure in plasma. Moreover, the remarkable increase of the alkaloid compounds in the plasma from the SGHB group might have caused a substantial increase in clearing heat and removing toxicity activity of GHB. This result appeared to indicate the saltwater processing might have influence on the absorption of the active ingredient. In TCM, a high amount of Chinese Materia medicine should be processed with saltwater, and it is often reported that saltwater processing could enhance the absorption of bioactive compounds, such as those from *Achyranthes bidentata,*[19] *Semen cuscutae,*[20] *Psoralea corylifolia* L.,[21] and *Anemarrhenae rhizoma.*[22] This could indicate that the scientific nature of Chinese Materia medicine processing could increase the absorption of drugs through the corresponding processing methods, thereby enhancing efficacy. However, how Chinese Materia medicine processing specifically enhances absorption, e.g., increasing the permeability of endothelial cells, still requires further research.

CONCLUSION

In summary, a simultaneous quantitative analysis method was established for the determination of five alkaloids and one triterpene from *P. amurensis* cortex in rat plasma. We evaluated the complete pharmacokinetic method and found the different pharmacokinetic profiles of these six components in plasma between the crude and saltwater processed GHB. It was shown that the pharmacokinetic behavior of the alkaloids compounds, including AUC_{0-t} and C_{max}, exhibited significant differences. In addition, the results indicated that the saltwater processing could increase the absorption of the alkaloids components, which might clarify the principle of bioactivity enhancement after processing from GHB.

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Conflicts of interest

There are no conflicts of interest.

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