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Original Article

Determination of orcinol glucoside by LC-MS in *Curculigo orchioides* and its application to a pharmacokinetic study

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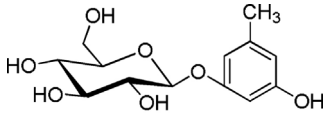
ABSTRACT

This study was designed to explore the pharmacokinetic regularity of the plasma concentration, tissue distribution and excretion of orcinol glucoside from aqueous extracts of raw and processed *Curculigo orchioides* Gaertn., Hypoxidaceae. The experiment first used an ultrahigh-performance liquid chromatography-tandem mass spectrometry approach with multiple reaction monitoring and a positive mode to separate orcinol glucoside from naringin to obtain the plasma concentration curves, bar graph of tissue distribution and excretion curves. These results might be beneficial for reasonable clinical application of *C. orchioides* and for further development of its wine and salt-processing mechanism.

Introduction

The dried rhizome of *Curculigo orchioides* Gaertn., Hypoxidaceae, is a traditional Chinese herbal medicine that has been used for centuries in the treatment of declining strength (Wang et al., 2017), jaundice, asthma etc. (Wang et al., 2019). *Curculigo orchioides* has various processed products, which have a long history of medical use in China. Wine-processing and salt-processing are specialized technology involving sautéing Chinese rice wine and saline solution in an applied processing technology. Wine-processed *C. orchioides* (WXM) is part of Chinese Pharmacopoeia. Salt-processed *C. orchioides* (SXM) is an innovative technology designed by our research group, guided by the theories of traditional Chinese medicine of "Ru Yan Zou Shen" and "Xiang Fan Wei Zhi". These theories have thousands of history in clinical uses in China (Tao et al., 2017; Chen et al., 2018; Hejazi et al., 2018). Furthermore, wine-processing and salt-processing can reduce or remove the side-effects of *C. orchioides*, while also changing its properties and strengthening its efficacy.

On the basis of prophase pharmacodynamics studies by our group, the increasing absorption of *C. orchioides in vivo* was most likely due to processing. The major active ingredients of *C. orchioides* are phenolic glycosides and mainly include curculigoside, orcinol glucoside (**1**), curculigine A, anacardoside etc.; the most common ingredient is orcinol glucoside, an active constituent that is a common traditional Chinese medicine with diverse beneficial biological and pharmacological activities. Constituent orcinol glucoside (**1**) has high safety margins and novel pharmacotherapeutic applications in cancer treatment that include diverse beneficial biological and pharmacological functions (Nahak et al., 2018), as well as antioxidant, antidepressant, adaptogenic, and neuroprotective activities, among others (Ge et al., 2014); in addition, **1** may be a pharmacodynamic substance that enhances the immune activity of RAW264.7 cells, according to our research results.



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Ultimately, orcinol glucoside (**1**) is chosen as the analyte for this experiment, in which we study its absorption, distribution and excretion in rats. The method involves the support of time pharmacology and an effective, rapid and sensitive UPLC-MS/MS approach combined with multiple reactions monitoring by optimizing separation and finding the best daughter ion for determination of orcinol glucoside (**1**). This study reports, for the first time, the pharmacokinetic parameters of the oral bioavailability, tissue distribution and excretion of orcinol glucoside and compares the influence of W

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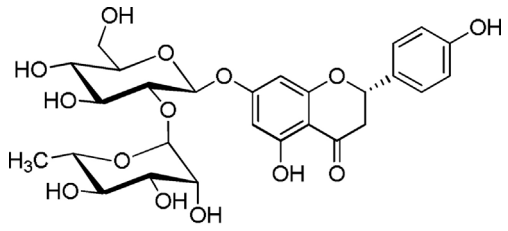
and S with raw *C. orchioides* (RXM), which can contribute to further clinical applications of *C. orchioides.*

Material and method

Chemicals and reagents

Orcinol glucoside (**1**) and naringin (**2**) were purchased from the Must Company (Sichuan, China). Chinese rice wine and salt were purchased from Zhejiang Brand Tower Shaoxing Wine Co., Ltd. (Zhejiang, China) and Dalian Salt Industry Co., Ltd. (Dalian, China), respectively. Water for UPLC analysis was purified with a Milli-Q academic water purification system (Millipore, USA). UPLC-MS grade methanol (Merck KGaA, Darmstadt, Germany), acetonitrile (Merck KGaA, Darmstadt, Germany), spectroscopy grade formic acid (Merck KGaA, Darmstadt, Germany) and analysis grade methanol (Kermol Chemical Reagent, Tianjin, China). *C* samples were collected from Kangmei Pharmaceutical Co., Ltd. in China and authenticated by Professor Yanjun Zhai from the Department of Identification of Chinese Medicine, Liaoning University of Traditional Chinese Medicine (Liaoning, China) (180200431). RXM, WXM and SXM were prepared in accordance with the Pharmacopoeia, *i.e.*, the method of stir-frying with wine and stir-frying with salt-water (Chinese Pharmacopoeia, 2015). The above procedure was completed under the guidance of the Processing of Chinese Materia Medica.



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Standard solution preparation

Standard stock solutions of **1** and **2** were prepared in methanol at a concentration of 630 and 3.128 μg/ml. The stock solutions were diluted with methanol to prepare working standard solutions. Plasma calibration standards were prepared by spiking blank plasma with an appropriate amount of standard solutions and **2** working solution to ensure final series concentrations. Quality control (QC) samples at three levels were prepared using the same procedure at low, middle and high concentrations. **2** stock solutions of urine and tissues were prepared in the same way as plasma. All the solutions were stored at 4 °C.

Equipment and operating conditions

Quantitative analysis of **1** was achieved on the Waters Acquity UPLC H-Class Xevo TQD triple quadrupole mass spectrometer system (Waters Corporation, Milford, MA, USA) equipped with electrospray ionization operated in the positive ionization mode by multiple reaction monitoring. Data acquisition and analysis were accomplished with Masslynx software. Chromatographic separation was optimized on a Waters ACQUITY UPLC BEH C18 column (1.8 μm) at 30 °C, along with a mobile phase flow rate at 0.3 ml/min. (A) acetonitrile and (B) 0.1% formic acid in water constituted the mobile phase, and the final elution condition was applied as follows: 0–4 min: 3% A; 4–5 min: 13% A; 5–7 min: 21% A; 7–9 min: 25% A; 9–12 min: 28% A. The injection volume was 1 μl. The autosampler was maintained at 8 °C.

The analysis was performed on a Waters H-class UPLC- xevo TQD. The ESI source of the mass spectrometer was operated in the positive mode. Detection was performed by multiple reaction monitoring at m/z 287.13→ 125.02 (CE: 8 V, CV: 22 V) for **1**, m/z 581.32→ 273.12 (CE: 20 V, CV: 32 V) for **2** as the internal standard. Source temperature and desolvation temperature were 150 °C and 350 °C. The desolvation gas flow was 700 l/h. The capillary voltage and cone voltage were set to 3.0 kv and 25 v, respectively. MS/MS spectra of **1** and **2** are shown in Fig. 1 in the supporting information.

Biological sample treatment

Rat plasma (100 μl) was spiked with 40 μl of **2** in a 1.5-ml Eppendorf tube. The mixture was vortexed for 15 s. A conventional protein precipitation method was applied by 360 μl methanol. Then, the mixture was vortexed for 3 min and centrifuged at 12,800 × *g* force for 5 min, following transfer of supernatant to an EP tube and evaporation to dryness under a gentle nitrogen stream. Finally, the residue was reconstituted in 150 μl acetonitrile water solution (50:50), mixed for 30 s on a vortex-mixer and then centrifuged at 12,800*g* force for 5 min. Then, 1 μl of supernatant was injected for analysis. Rat urine (100 μl) sample was pretreated following a similar procedure to that of plasma. Tissues (0.5 *g*) were homogenized in normal saline at a proportion of 1:8 (w/v), and tissue homogenate was centrifuged at 12,800*g* force for 10 min at 4 °C. Then, tissue supernatant (100 μl) was processed using a similar approach as plasma samples. Defining the intestine as the blank tissue for the follow-up study, blank tissue supernatant was processed using the same procedure as the tissues samples.

Bioanalytical method validation (FDA, 2013)

Selectivity

The selectivity of this method was tested and ensured by analyses of blank samples of the plasma, urine and tissue supernatant obtained from six rats, shown in Fig. 2 and Fig. 3. There was no interference, and the selectivity of the method was ensured at the lower limit of quantification.

Accuracy, precision, recovery and matrix effect

The accuracy of the analytical method was measured using samples containing known amounts of the analyte, and the mean value was within 15%. The precision of the analytical method was measured using six determinations per concentration, and it did not exceed 15% of the coefficient of variation at each concentration level, as shown in Table 1 (Supporting information). Recovery pertains to the extraction efficiency of the analytical method within the limits of variability. Recovery of **1** did not need to be 100%, but the extent of recovery of **1** and of the internal standard was consistent, precise, and reproducible. In this study, the recovery and matrix effect experiments were performed by comparing the analytical results for extracted samples at three concentrations (low, medium, and high) with unextracted standards that represent 100% recovery in Table 2 (Supporting information).

Calibration curve

The calibration curve for **1** showed good linearity over the concentration ranges. The results were fitted to linear regression analysis using $1/x^2$ as the weighting factor. The coefficients of determination (r^2) were greater than 0.99 in all validation batches in Table 3 (Supporting information).

Stability

Stability testing was performed by evaluating the stability of **1** during sample collection and handling, after long-term (frozen at the intended storage temperature) and short-term (bench top, room temperature) storage, and after three freeze and thaw cycles and the analytical process in Table 4 (Supporting information).

Biological sample preparation

Plasma sample preparation: Blood samples were collected from the ocular vein of 18 rats classified randomly into three groups: RXM, WXM and SXM. Blood samples were contained in polypropylene tubes with heparin as an anti- coagulant at 0.0833, 0.25, 0.4167, 0.6667, 1, 1.5, 2,3.5, 5.5, 8.5, 12 and 24 h after intragastric administration of water decoction of R, W and S at a dose of 20 g/kg. The volume of blood at each time point was 0.5 ml. The samples were centrifuged at 3733 *g* × force for 15 min and the supernatant plasma was gathered and stored at −80 °C until being injected into the UPLC-MS system for analysis. Blank plasma samples were acquired at predose.

Urine sample preparation: For the excretion study, **1** was not discovered in all feces. The urine was collected from 18 rats kept in metabolic cages and classified randomly into three groups of R, W and S; the collections were taken at time intervals of 0–3, 3–6, 6–9, 9–12, 12–24, 24–36, 36–48 and 48–72 h after intragastric administration of water decoction of RXM, WXM and SXM at a dose of 20 g/kg and stored at −80 °C until analysis.

Tissue distribution study: The tissues, including kidney, gonad, spleen, lung, stomach, intestine, liver, heart and brain, were collected after execution at 0.1667, 0.6667, 1.5, 0.5455, 5.5 h after intragastric administration of a water decoction of RXM, WXM and SXM at a dose of 20 g/kg from 90 rats classified randomly into three groups and at each time point for 6 rats. The tissues samples were stored at −80 °C until treated.

Results and discussion

The routine dose of C for human consumption, according to the Chinese Pharmacopoeia (2015), was 3–10 g/day. The dose in rats was calculated as 0.9 g/kg. We determined 20 times the dose. The content of **1** in R, W and S was quantitatively analyzed as 456.8, 430.7 and 432.1 μg/ml in aqueous extract of RXM, WXM and SXM using our previous method. Ensuring the difference between groups and reducing the impact of the loss of the extraction process of the drug on the experiment, the dose was appropriately amplified based on the prescribed dose in the Pharmacopoeia. The specific gastric concentration was 20 g/kg, and the volume was 1 ml/100 g.

The most fundamental advantage of noncompartmental modeling is the use of fewer and less restrictive assumptions than in the compartmental methods. The assumptions of the noncompartmental methods are often more readily subject to experimental verification; these methods eliminate the problem resulting from the inability to fit all datasets in a study to the same compartmental model and the resulting difficulty in making comparisons of parameter estimates (William R. Gillespie, 1991). Therefore, a noncompartmental method was chosen in this study, and the mean concentration time curves of **1** fitted a noncompartmental analysis model (Fig. 1). In addition, the pharmacokinetic parameters after oral administration of water decoction of RXM, WXM and SXM were calculated and are shown in Table 1.

Orcinol glucoside (**1**) of RXM was quickly absorbed to achieve maximum plasma concentration 2 h after administration, while **1** of WXM and SXM were absorbed to achieve the maximum plasma concentration more rapidly. The area under the curve

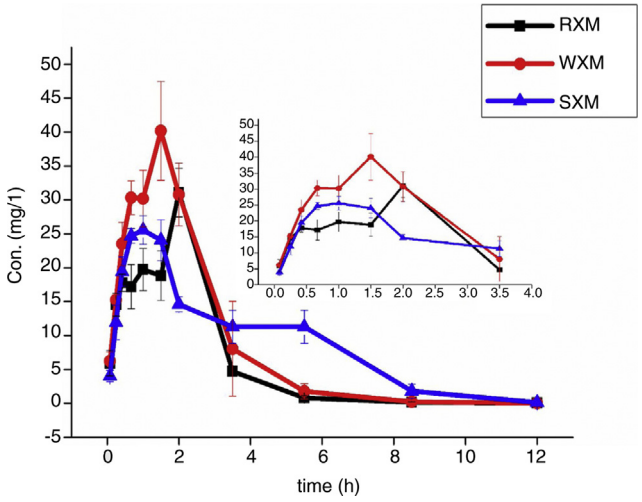

Fig. 1. Plasma concentration curves of orcinol glucoside from different processing products.

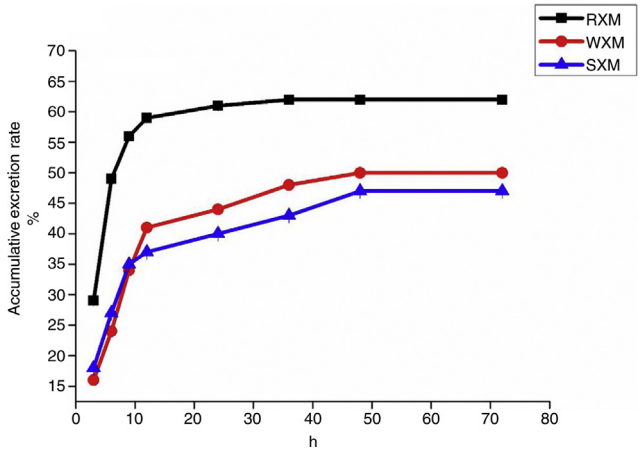

Fig. 2. Excretion stability of orcinol glucoside from different processing products in rat urine.

(AUC0-∞) for **1** was found to be 99.73 ± 14.325 μg/l*h in WXM, 71.458 ± 1.378 μg/l*h in RXM and 75.646 ± 7.216 μg/l*h in SXM, which suggests the same efficacy due to the reabsorption and enterohepatic circulation. (Feng et al., 2018; Zhang et al., 2018; Zhou et al., 2018; Tao et al., 2018). Moreover **1** was not found in rat feces but was found in rat urine, along with a 100% degradation after 48 h (Fig. 2). From the Fig. 2, we could see that the accumulative excretion rates of **1** in WXM and SXM were always lower than RXM, where **1** in RXM had 62.24% excretion rate, while 50.02% in WXM and 47.05% in SXM. The pharmacokinetic parameters of **1** in tissue matrices are summarized in Fig. 3. Considerable levels were detected in all studied tissues. Compared with plasma exposure, higher levels of **1** were detected in tissues including the kidney, stomach and intestine, while lower levels of **1** were detected in liver, gonad and lung tissues (Zhang et al., 2013; Xu et al., 2017; Wang et al., 2018). And, hardly any **1** was detected in spleen, heart and brain tissues, which suggests that **1** cannot cross the blood-brain barrier. As shown in Fig. 3, after intragastric administration, **1** rapidly distributed to various tissues, including the kidney, stomach, intestine, liver, gonad and lung. The WXM and SXM exhibited a similar pattern to the RXM, which probably explains the efficacy and legitimacy of wine-processing and saline- processing. In all of the RXM, WXM and SXM groups, the highest concentration of **1** in the tissues showed this ten-

Table 1
Pharmacokinetic parameters of orcinol glucoside from different processing products in rat plasma.

Pharmacokinetic parameters	RXM	WXM	SXM
AUC(0-t) (mg/l*h)	71.443 ± 1.376	99.591 ± 14.473	75.48 ± 7.112
AUC(0-∞) (mg/l*h)	71.458 ± 1.378	99.730 ± 14.325	75.646 ± 7.216
MRT(0-∞) (h)	1.892 ± 0.056	1.913 ± 0.285	2.130 ± 0.191
Tmax (h)	2.000 ± 0.000	1.417 ± 0.204	0.973 ± 0.304
T1/2 (h)	0.864 ± 0.227	0.819 ± 0.211	0.821 ± 0.117
Vz/F (l/kg)	347.554 ± 87.013	242.17 ± 79.243	313.654 ± 39.196
CLz/F (L/h/kg)	279.972 ± 5.430	204.003 ± 29.054	266.380 ± 25.062
Cmax (mg/l)	31.089 ± 3.596	40.786 ± 6.611	26.381 ± 2.638

Each value represents mean ± SD (n = 6); AUC(0-t), area under the plasma concentration- time from zero to the last quantifiable time-point; AUC(0-∞) area under the plasma concentration-time curve from zero to infinity; MRT(0-∞), mean residence time zero; Cmax, maximum plasma concentration; Tmax, time to maximum concentration; t1/2, elimination half-life;

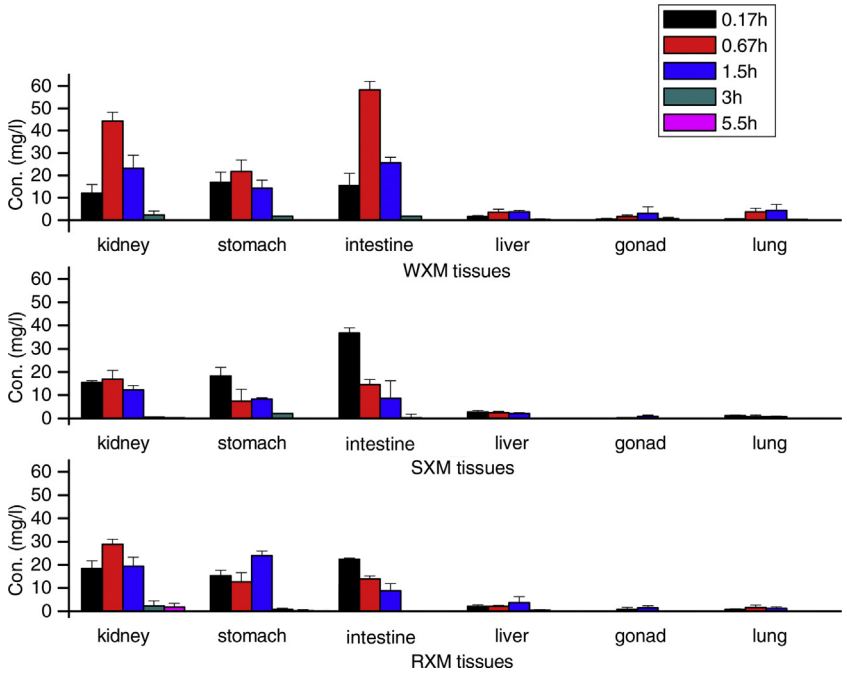

Fig. 3. Pharmacokinetic profile of orcinol glucoside from different processing products in the tissues.

dency: RXM (intestine > kidney > stomach > liver > gonad = lung); WXM (intestine > kidney > stomach > liver > lung > gonad); SXM (intestine > stomach > kidney > liver > gonad = lung).

In addition, the significant increase in AUC0-t and Cmax values suggested that wine-processing could increase the bioavailability. Compared with RXM and WXM, the pharmacokinetic curve of SXM has no bimodal phenomenon, which may explain the effect of salt-processing on C. There were significant differences in the three groups based on the parallel sampling points. **1** from SXM and WXM reached Cmax more rapidly than R. This outcome could be explained by intestinal absorption of **1** in the prior Caco-2 experiment. Wine-processing could enhance intestinal absorption of **1**. At 0.67 h and 1.5 h, the concentration of **1** was highest in the spleen compared with other organs, which suggests the kidney and intestine may serve as the receptor of **1**. Urine excretion is the major excretion route of oral **1** from water decoction. We can further examine its metabolites in future experiments. These results might show that processing products were excreted more rapidly and no resorption and explain the difference in absorption of **1** in rat plasma. Although the content of **1** in WXM was higher than RXM and reversed in SXM, the wine-processing and salt-processing might play a crucial role in enhancing the absorption and bioavailability of the **1**, which might be due to the processing technique of traditional Chinese medicine used for thousands of years.

Conclusion

Furthermore, all data were analyzed using Masslynx4.1, DAS2.1 and Origin 7. In this study, an accurate and precise H-Class XEVO TQD approach based on methanol precipitation and the processing technique was established and validated for the simultaneous determination of **1** in rat plasma, urine and various tissues and for an application to a comparative pharmacokinetic study of aqueous extracts of raw and processed C. The study investigated rat plasma, tissue and urine excretion and explored the regular absorption, tissue distribution and metabolic pathway of **1** in vivo by using aqueous extracts of raw and processed C, which could save resources and permit comparisons between raw and processed C.

Conflict of interest

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Authors' contributions

TL and CJ wrote the manuscript. TL and GS carried out the data analyses. TL and CJ designed the study. The remaining authors con-

tributed to refining the ideas, carrying out additional analyses and finalizing the paper. All the authors have read the final manuscript and approved submission.

Ethical disclosures

Protection of human and animal subjects.

The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data.

The authors declare that no patient data appear in this article.

Right to privacy and informed consent.

The authors declare that no patient data appear in this article.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bjp.2019.08.005.

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