Research Article

UPLC-QTOF/MS Analysis of Alkaloids in Traditional Processed Coptis chinensis Franch.

Xue Jiang, Lin-Fang Huang, La-Bin Wu, Zeng-Hui Wang, and Shi-Lin Chen

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, China

Correspondence should be addressed to Lin-Fang Huang, lfhuang@implad.ac.cn and Shi-Lin Chen, slchen@implad.ac.cn

Received 16 August 2012; Revised 29 November 2012; Accepted 29 November 2012

Academic Editor: Muhammad Nabeel Ghayur

Copyright © 2012 Xue Jiang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The processing technology employed in traditional Chinese medicine (TCM) is significant and distinct. Meanwhile, the processed Coptis chinensis Franch. are significant in clinic based on clinical practice and literature. The current study used ultraperformance liquid chromatography method (UPLC) coupled with quadrupole time of flight mass spectrometry (qTOF/MS) and Marklynx software to analyze the chemical profiles of crude and processed C. chinensis Franch. 13 compounds in these samples are identified, including 3 compounds that are detected in C. chinensis Franch. for the first time. Moreover, the results of the experiment show significant chemical differences between crude and processed C. chinensis Franch. with principal component analysis (PCA). The obvious separation in PCA confirms the traditional processing theory in TCM.

1. Introduction

Traditional processing technology (Paozhi technology) is a significant part of traditional Chinese medicine (TCM). Employing the correct processing technology is necessary in manufacturing clinical decoction pieces. According to the restrictions in the science of TCM, any decoction piece must be processed by certain methods before it can be used in clinical practice. Processing technology was developed almost 5000 years ago, along with a number of processing technology theories and methods. Briefly, processing technology plays three main roles in TCM. First, it decreases the toxicity of some TCMs. Some crude TCMs have high toxicity and various side effects; thus, numerous processing technologies have been developed to remove the toxicity and decrease the side effects. Second, processing technology increases the pharmacological effects. Third, processing technology creates new drugs that have new pharmacological effects as a pharmaceutical method. The reason for developing this is when faced with complex human diseases, crude TCMs are sometimes unable to satisfy the need in clinical practice due to the limitations posed by herbal medicine. Thus, the ancient Chinese created processing technology to produce abundant new drugs. In this paper, we investigate the five kinds of processed Coptis chinensis Franch. in chemical profiles.

Processing technology was developed via clinical practice experience and processing technology theories. The processing technology theories were based on the Traditional Five Elements Theory and the Yin-Yang Theory from ancient China. The five kinds of processed C. chinensis Franch. are originally based on the important theories in processing technology: Processing Synergy Theory (PST) or Cong Zhi and Processing Antagonism Theory (PAT) or Fan Zhi. Processed C. chinensis Franch. with rice wine (Jiu HL), with Zingiber officinale Rosc. (Jiang HL) and with Euodia rutaecarpa (Juss.) Benth (Yu HL) belong to PAT, whereas those processed with vinegar (Cu HL) and with salt (Yan HL) belong to PST. Such classification bases on the PST/PAT theory, the contribution of C. chinensis Franch. and their auxiliary materials. These processed products have different pharmacological effect in clinic according to the traditional literatures. The rice wine processed C. chinensis is especially used for the treatment of inflammation in head. The ginger processed C. chinensis is focusing on the treatment in stomach. The Euodia rutaecarpa (Juss.) Benth processed C.


2.1. Chemicals, Reagents, and Materials

Acetonitrile and formic acid were purchased from Fisher Scientific Co. (MA, USA). Ammonium acetate was purchased from Xilong Company (Shanxi, China). All aqueous solutions were prepared with ultrapure water produced by Milli-Q system (18.2 MΩ, Millipore, Ma, USA). Berberine, palmatine, coptisine, epiberberine, and jatrohzzine standards were purchased from Must Company (Sichuan, China). C. chinensis Franch., Z. officinale Rosc., and E. rutacearca (Juss.) Benth were purchased from Sichuan Chinese Herbs Corporation (Sichuan, China). The botanical materials were identified by Professor Chen Shilin, and the voucher specimens were deposited in the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing, China.

2.2. Instrumentation and Chromatographic Conditions

UPLC-qTOF/MS analysis was performed using a qTOF Synapt G2 HDMS system (Waters, Pittsburgh, PA, USA) equipped with an ESI source operated in the positive ion mode. N₂ was used as the desolvation gas. The desolvation temperature was set at 450°C with a flow rate of 800 L/h and a source temperature of 120°C. The capillary and cone voltages were set to 2500 and 40 V. Data were collected between 50 Da and 1200 Da, with a scan time of 0.1 s and interscan delay of 0.01 s over an analysis time of 16 min.

2.3. Preparation of the Sample Solution

C. chinensis Franch. samples were cut into 1.5 mm slices and then processed according to the methods described in China pharmacopoeia [1]. Then, the samples were processed at a temperature of 160°C and dried in an oven after mixed with auxiliary material consisting of rice wine (20% w/w), salt (10% w/w), vinegar (20% w/w), E. rutacearca (Juss.) Benth (10% w/w), and Z. officinale Rosc. (10% w/w) [13]. All samples were milled into powder and dried at 30°C in oven until they attained constant weight. A total of 0.150 g powder sample was dissolved in a methanol-sulfuric acid (100:3) solution. Next, the samples were extracted by ultrasonic cleaner for 30 min before a 15 min water bath (60°C). The sample solutions were subsequently filtered through a 0.22 µm membrane and then injected into the UPLC-qTOF system for analysis.

2.4. Preparation of Standard Solution

The standard stock solutions of berberine (0.12 mg/mL), palmatine (0.11 mg/mL), berberrubine (0.118 mg/mL), coptisine (0.115 mg/mL), epiberberine (0.107 mg/mL), and jatrohzzine (0.115 mg/mL) were prepared in methanol and stored at -4°C. The solutions were brought to room temperature and filtered through a 0.22 µm membrane filter before injection.

2.5. Data Analysis

The UPLC-qTOF/MS data of crude C. chinensis Franch. and processed C. chinensis Franch. samples were analyzed to identify discriminant variables. The peak finding, peak alignment, and peak filtering of ES+ raw data were carried out with Marklynx applications manager version 4.1. The parameters used were: within the retention time of 0–10 min, and mass range 50–1200 Da, mass tolerance 0.02 Da. Noise elimination level was set at 6.00, and minimum intensity was set to 15% of base peak intensity.

3. Results and Discussion

3.1. UPLC Method Development

To produce a better chromatogram in UPLC-qTOF, the UPLC method was developed with consideration for such factors as mobile phases, modifiers, and flow rates. Methanol and acetonitrile were tested with different ratios, linear gradients, and flow rates of the mobile phase (0.1, 0.2, and 0.25 mL/min). The modifiers, such as formic acid, ammonium acetate, sodium dodecyl sulfate (SDS), phosphoric acid, and diethylamine, were all detected in the present experiment. As a result, water containing 1% formic acid and 1% ammonium acetate (A)—acetonitrile (B) with a flow rate of 0.25 mL/min was chosen as the optimum chromatographic condition with a linear gradient (0–10 min, 80%–70% A) at room temperature.
3.2. UPLC-qTOF/MS Chemical Analysis. Figure 1(a) presents the representative chromatogram of *C. chinensis* Franch. by UPLC-qTOF/MS. Figure 1(b) shows the five standard compounds chromatograms. Table 1 shows 13 compounds in the chromatograms that have been identified based on [M+H]^+ m/z and retention time analysis by the database and references. In the experiments, the chromatograms of the samples were analyzed using the Marklynx software. More than 3000 markers (differences) were detected to be present among the crude and processed *C. chinensis* Franch.

Figure 1: (a) The representative chromatogram of *C. chinensis* Franch. by UPLC-qTOF/MS (the numbers of peaks are same with the identification in Table 1). (b) The chromatograms of standard compounds (EPI: epiberberine; COP: coptisine; JAR: jatrorrhizine; PAL: palmatine; BER: berberine).

Alkaloids comprise the main compounds in *C. chinensis* Franch., including berberine, palmatine, coptisine, epiberberine, jatrorrhizine, columbamine, and magnoflorine. Berberine, palmatine, coptisine, epiberberine, and jatrorrhizine are the five main alkaloids in *C. chinensis* Franch., with their contents range from 5% to 10% in total [1]. Among them, berberine, coptisine, epiberberine, jatrorrhizine are the chemical indicators in evaluating the quality of *C. chinensis* Franch. in China pharmacopoeia [1]. Although the compounds, like magnoflorine and columbamine, with very lower contents, are difficult to identify in chromatographs [20], they help explain the pharmacological effects of *C. chinensis* Franch. Therefore, identifying these compounds is significant. In our study, the UPLC-qTOF/MS provides the information of 13 compounds in *C. chinensis* Franch.

Except lincangenine/stephabine, lycoranine B, and dihydrochelerythrine, all these identified compounds have been reported in *C. chinensis* Franch. in [13–15, 21] with significant bioactivities. And all the identified compounds are protoberberine alkaloids with the similar structures chemically. Lincangenine/stephabine and lycoranine B have been isolated from *Stephania siberosa* and *Lycoris radiata*, respectively [16, 18]. And dihydrochelerythrine could be transformed for berberine chemically [19]. These references show a higher possibility for the existence of lincangenine/stephabine, lycoranine B, and dihydrochelerythrine in *C. chinensis* Franch. And they are the first time to be reported in *C. chinensis* Franch. Lincangenine and stephabine are isomeric compounds, and they both have the possibility to exist in *C. chinensis* Franch. Jatrorrhizine/columbamine and thalifendine/berberrubine are the isomeric compounds, respectively. And they could all exist in *C. chinensis* Franch. because they could transform into each other with biosynthesized way [22].

3.3. Dihydrochelerythrine Contained in Crude and Processed *C. chinensis* Franch. Dihydrochelerythrine was detected in this study. Figure 2 presents the relative content (based on the peak area) in crude and processed *C. chinensis* Franch. It shows that the relative content of dihydrochelerythrine in crude *C. chinensis* Franch. is very lower than other samples. And the relative content of dihydrochelerythrine in Cu HL
Table 1: Identified compounds in *C. chinensis* Franch. by UPLC-QTOF/MS.

| Peak no. | \( t_R \) (min) | Identified compounds | Molecular formula | Mean measured mass (Da) | \([M+H]^+ \) m/z | Theoretical exact mass (Da) | Mass error (ppm) | Reference |
|----------|-----------------|----------------------|-------------------|-------------------------|---------------|---------------------------|-----------------|-----------|
| 1        | 1.79            | Magnoflorine         | C\(_{20}\)H\(_{23}\)NO\(_4\) | 342.1703                | 342.1705      | −0.6                      | [13–15]         |
| 2        | 3.32            | Groenlandicine       | C\(_{19}\)H\(_{18}\)NO\(_4\) | 322.1064                | 322.1079      | −4.7                      | [14]            |
| 3        | 3.81            | Berberastine         | C\(_{20}\)H\(_{21}\)NO\(_3\) | 352.1184                | 352.1185      | −0.3                      | [14]            |
| 4        | 3.71            | Lincangeneine/stephanine | C\(_{21}\)H\(_{26}\)NO\(_5\) | 368.1506                | 368.1498      | 2.2                       | [16]            |
| 5        | 4.02            | Demethyleneberberine | C\(_{19}\)H\(_{17}\)NO\(_4\) | 324.1220                | 324.1236      | −4.9                      | [17]            |
| 6        | 4.32            | Lycoranine B         | C\(_{18}\)H\(_{13}\)NO\(_4\) | 308.0903                | 308.0923      | −6.5                      | [18]            |
| 7        | 4.50            | Jatrorrizine/columbamine | C\(_{20}\)H\(_{15}\)NO\(_4\) | 338.1386                | 338.1392      | −1.8                      | [13, 14]        |
| 8        | 4.66            | Epiberberine         | C\(_{20}\)H\(_{17}\)NO\(_4\) | 336.1228                | 336.1236      | −2.4                      | [13, 14]        |
| 9        | 4.95            | Coptisine            | C\(_{19}\)H\(_{15}\)NO\(_4\) | 320.0915                | 320.0923      | −2.5                      | [13, 14]        |
| 10       | 5.81            | Thalifendine/berberrubine | C\(_{18}\)H\(_{16}\)NO\(_4\) | 322.1070                | 322.1079      | −2.8                      | [14]            |
| 11       | 6.68            | Palmitane            | C\(_{21}\)H\(_{22}\)NO\(_4\) | 352.1556                | 352.1549      | 2.0                       | [13, 14]        |
| 12       | 7.01            | Berberine            | C\(_{20}\)H\(_{17}\)NO\(_4\) | 336.1236                | 336.1236      | 0                         | [13, 14]        |
| 13       | 8.90            | Dihydrochelerythrine | C\(_{21}\)H\(_{19}\)NO\(_4\) | 350.1394                | 350.1392      | 0.6                       | [19]            |

Figure 3: PCA of crude and processed *C. chinensis* Franch.

is the highest. It is possible that the auxiliary material or heating process could enhance the transformation of dihydrochelerythrine from berberine. In our previous study, the transformation of berberine to berberrubine, palmitine to palmatine, could be increased with an acidic condition. And the result in this study confirm the general rule of transformation with protoberberine alkaloids in processed *C. chinensis* Franch. that acidic condition in processing could enhance the protoberberine transformation. Dihydrochelerythrine has been reported with many pharmacological activities, such as antiparasitic and antitumor effects [23, 24]. And the reason for increasing the content of dihydrochelerythrine in processed *C. chinensis* Franch. would be explored further.

3.4. Confirmation of TCM Processing Theories with Crude and Processed *C. chinensis* Franch. There are 5 kinds of processed *C. chinensis* Franch.: Jiu HL, Jiang HL, Yu HL, Cu HL, and Yan HL. Crude *C. chinensis* Franch. and 5 different processed products were analyzed by PCA. Figure 3 shows the differences of crude and processed *C. chinensis* Franch. In the PCA, crude and 5 processed *C. chinensis* Franch. have been separated clearly. Jiu HL, Jiang HL, and Yu HL (PAT) are clustered into one side, and Cu HL and Yan HL (PST) are clustered into the other side. Among them, Jiu HL and Jiang HL are positioned in positive region; crude, Cu HL and Yan HL are positioned in the negative region, while Jiu HL group is in the middle of these two regions.

According to the TCM theories, all the TCMs could be grouped into 4 classifications, which are cold, heat, warm, and cool. And *C. chinensis* Franch. is in the classification of “cold,” and it belongs to the extreme cold level. This contribution of *C. chinensis* Franch. could lead to some side-effects if patients take it for a long period of time or some patients with special physiques take it. Therefore, the contribution of *C. chinensis* Franch. should be modified to fit the specific requirement in clinic. On one side, *C. chinensis* Franch. should be adjusted into a little “warm” to eliminate the side-effect of the extreme cold character. On the other side, *C. chinensis* Franch. could be modified into more “cold” to meet the extremely heat syndrome in clinic. The former one should be treated as the method of PAT, while the latter one should be treated as the method of PST. Among the PAT, Jiang HL and Yu HL could transform more of *C. chinensis* Franch. into the “warm” side, while Jiu HL could transform less compared with Jiang HL and Yu HL. And this could be confirmed in the PCA that Jiang HL and Yu HL are in the positive region while Jiu HL is in the middle of the two regions. And crude, Cu HL and Yan HL belong to the contribution of “cold” with the positions of negative region in PCA. Thus, the result of PCA conforms with the traditional processing theories and illustrates the methods of PAT and PST.

Our experiment shows the differences in the 5 processed *C. chinensis* Franch. which can be identified by UPLC-qTOF
and analyzed by Markerlynx software. And this method could demonstrate the TCM theories markedly. This is the first time to elucidate TCM processing theories by modern technology.

4. Conclusion

Forms of processed *C. chinensis* Franch. have been recorded in the long history of TCM. Their curative effects have also been verified in clinical setting. The chemical analysis of processed *C. chinensis* Franch. via UPLC-qTOF/MS demonstrates significant differences. Such information demonstrates the significance of processed *C. chinensis* Franch. as well as of PAT and PST drugs. The processing technology helps create new pharmacological drugs that possess distinctive clinical effects. This result, therefore, is a new finding in traditional alternative medicine.

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| UPLC-qTOF/MS | Ultra-performance liquid chromatography-quadrupole time of flight mass spectrometry |
| PCA | Principal component analysis |
| TCM | Traditional Chinese medicine |
| PST | Processing synergy theory |
| PAT | Processing antagonism theory |
| Jiu HL | Processed *C. chinensis* Franch. with rice wine |
| Jiang HL | Processed *C. chinensis* Franch. with *Zingiber officinale* Rosc. |
| Yu HL | Processed *C. chinensis* Franch. with *Evodia rutaecarpa* (Juss.) Benth |
| Cu HL | Processed *C. chinensis* Franch. with vinegar |
| Yan HL | Processed *C. chinensis* Franch. with salt |

**Acknowledgment**

This work was supported by the National Natural Science Foundation of China (81274013, 81130069), the program for Changjiang Scholars and Innovative Research Team in University of Ministry of Education of China (IRT1150), the Chinese National S&T Special Project on Major New Drug Innovation (2011ZX09307-002-01), Selected Program of Personnel Department for Oversea Scholar.

**References**

[1] State Pharmacopoeia Committee. Pharmacopoeia of People's Republic of China (2010 version), pp. 285, 2010.

[2] J. P. Kou, Y. Wu, Q. Z. Wang et al., “Studies on the sedative and hypnotic activities of JiaotaiWan prepared by raw or wine-processed *Coptidis Rhizoma,*” Pharmacology and Clinics of Chinese Materia Medica, vol. 23, no. 5, pp. 15–17, 2007.

[3] J. Jiang, X. B. Jia, X. H. Lu et al., “Empirical study of Fructus Evodiae processed *Rhizoma Coptidis*’ synergistic effect on breadboard gastric ulcer of rats,” *China Journal of Traditional Chinese Medicine and Pharmacy*, vol. 25, no. 12, pp. 2130–2132, 2010.

[4] J. C. Li, X. L. Meng, R. Cui et al., “Comparison in pharmacodynamic effects of different types of processed *Rhizoma Coptis* on mouse diabetes,” *Chinese Traditional Patent Medicine*, vol. 32, no. 11, pp. 1922–1925.

[5] S. H. Zhou, W. J. Pan, X. H. Xiao et al., “Biothermokinetic studies on four properties of traditional Chinese materia medica—Comparison of different preparation properties of *Coptidis Rhizoma* by microcalorimetry,” *Chinese Traditional and Herbal Drugs*, vol. 35, no. 11, pp. 1230–1232, 2004.

[6] C. Yang, X. Qiu, and L. D. Kong, “Effects of different processing products of *Rhizoma Coptidis* on scavenging oxygen free radical and anti lipid peroxidation,” *Journal of Nanjing University*, vol. 37, no. 5, pp. 559–663, 2001.

[7] L. H. Liu and Z. L. Chen, “Analysis of four alkaloids of *Coptis chinensis* in rat plasma by high performance liquid chromatography with electrochemical detection,” *Analytica Chimica Acta*, vol. 737, pp. 99–104, 2012.

[8] F. Yang, T. Zhang, R. Zhang, and Y. Ito, “Application of analytical and preparative high-speed counter-current chromatography for separation of alkaloids from *Coptis chinensis* Franch.” *Journal of Chromatography A*, vol. 829, no. 1-2, pp. 137–191, 1998.

[9] D. Yan, C. Jin, X. H. Xiao, and X. P. Dong, “Antimicrobial properties of berberines alkaloids in *Coptis chinensis* Franch by microcalorimetry,” *Journal of Biochemical and Biophysical Methods*, vol. 70, no. 6, pp. 845–849, 2008.

[10] P. Giri and G. S. Kumar, “Self-structure induction in single stranded poly(A) by small molecules: studies on DNA intercalators, partial intercalators and groove binding molecules,” *Archives of Biochemistry and Biophysics*, vol. 474, no. 1, pp. 183–192, 2008.

[11] M. M. Islam and G. Suresh Kumar, “RNA targeting by small molecule alkaloids: studies on the binding of berberine and palmatine to polyribonucleotides and comparison to ethidium,” *Journal of Molecular Structure*, vol. 875, no. 1–3, pp. 382–391, 2008.

[12] J. Chen, F. Wang, J. Liu, F. S. C. Lee, X. Wang, and H. Yang, “Analysis of alkaloids in *Coptis chinensis* Franch by accelerated solvent extraction combined with ultra performance liquid chromatographic analysis with photodiode array and tandem mass spectrometry detections,” *Analytica Chimica Acta*, vol. 613, no. 2, pp. 184–195, 2008.

[13] X. Luo, B. Chen, and S. Yao, “Simultaneous analysis of protopine, indolequinine and quinoline alkaloids in coptis-evodia herb couple and the Chinese herbal preparations by high-performance liquid chromatography-electrospray mass spectrometry,” *Talanta*, vol. 66, no. 1, pp. 103–110, 2005.

[14] Q. Liu, S. Qiu, H. Yu, Y. Ke, Y. Jin, and X. Liang, “Selective separation of structure-related alkaloids in *Rhizoma Coptidis* with “click” binaphthyl stationary phase and their structural elucidation with liquid chromatography-mass spectrometry,” *Analyst*, vol. 136, no. 20, pp. 4357–4365, 2011.

[15] T. M. Hung, J. P. Lee, B. S. Min et al., “Magnoflorine from *CoptidisRhizoma* protects high density lipoprotein during oxidant stress,” *Biological and Pharmaceutical Bulletin*, vol. 30, no. 6, pp. 1157–1160, 2007.

[16] A. Patra, C. T. Montgomery, A. J. Freyer et al., “The protopine-berine alkaloids of *Stephania suberosa,*” *Phytochemistry*, vol. 26, no. 2, pp. 547–549, 1987.
[17] Y. Li, G. Ren, Y. X. Wang et al., “Bioactivities of berberine metabolites after transformation through CYP450 isoenzymes,” *Journal of Translational Medicine*, vol. 9, no. 62, pp. 1–10, 2011.

[18] L. Wang, X. Q. Zhang, Z. Q. Yin, Y. Wang, and W. C. Ye, “Two new amaryllidaceae alkaloids from the bulbs of Lycoris radiata,” *Chemical and Pharmaceutical Bulletin*, vol. 57, no. 6, pp. 610–611, 2009.

[19] M. Hanaoka, T. Motonishi, and C. Mukai, “Chemical transformation of protoberberines. Part 9. A biomimetic synthesis of oxychelerythrine, dihydrochelerythrine, and chelerythrine from berberine,” *Journal of the Chemical Society, Perkin Transactions 1*, pp. 2253–2256, 1986.

[20] Y. H. Kuang, J. J. Zhu, Z. M. Wang, and Q. W. Zhang, “Simultaneous quantitative analysis of five alkaloids in rhizoma of *Coptis chinensis* by multi-components assay by single marker,” *Chinese Pharmaceutical Journal*, vol. 44, no. 5, pp. 390–394, 2009.

[21] H. A. Jung, B. S. Min, T. Yokozawa, J. H. Lee, Y. S. Kim, and J. S. Choi, “Anti-Alzheimer and antioxidant activities of *Coptidis Rhizoma* alkaloids,” *Biological and Pharmaceutical Bulletin*, vol. 32, no. 8, pp. 1433–1438, 2009.

[22] M. Rueffer and M. H. Zenk, “Columbamine, the central intermediate in the late stages of protoberberine biosynthesis,” *Tetrahedron Letters*, vol. 27, no. 8, pp. 923–924, 1986.

[23] J. Vrba, P. Doležel, J. Vičar, M. Modrianský, and J. Ulrichová, “Chelerythrine and dihydrochelerythrine induce G1 phase arrest and bimodal cell death in human leukemia HL-60 cells,” *Toxicology in Vitro*, vol. 22, no. 4, pp. 1008–1017, 2008.

[24] J. Y. Yao, Z. M. Zhou, X. L. Li et al., “Antiparasitic efficacy of dihydrosanguinarine and dihydrochelerythrine from Macleaya microcarpa against Ichthyophthirius multifilis in richadsin (Squaliobarbus curriculus),” *Veterinary Parasitology*, vol. 183, no. 1-2, pp. 8–13, 2011.