# Fingerprint Study of Polygonati Rhizoma with Steaming and Exposing to the Sun Alternatively for Different Times

**Min WANG, Chenying YE, Qian WANG, Ting HE, Shenggao YIN, Gailian ZHOU***

Guangxi University of Chinese Medicine, Nanning 530200, China

**Abstract** [**Objectives**] To explore the influence of different times of steaming and exposing to the sun on the fingerprint of Polygonati Rhizoma by studying the HPLC fingerprint of Polygonati Rhizoma processed products with different times of steaming and exposing to the sun, and to provide a basis for the determination of the best processing technology of Polygonati Rhizoma. [**Methods**] SETSAIL II AQ-$C_{18}$ (5 μm × 250 mm × 4.6 mm) was used as the column, the column temperature was 30 ℃, pure water (A) and acetonitrile (B) were eluted gradually, 0 - 10 min, B (5% - 10%), 10 - 30 min, B (10% - 35%), 30 - 40 min, B (35% - 60%), 40 - 45 min, B (60% - 100%), flow rate 1 mL/min, absorption wavelength 200 nm. [**Results**] The relative retained peak area RSDs of the common peaks in the precision, reproducibility and stability tests were all less than 5%. There were 17 common peaks in the fingerprint of nine batches of samples, and the retention time of Peak 2 was basically the same as that of the reference peak of 5-HMF. Peak 4 mainly existed in the chromatogram of Sample 3 to Sample 5, peaks 5 and 11 mainly existed after Sample 3, peaks 9, 14 and 16 mainly existed after Sample 6, and peaks 12 and 17 mainly existed after Sample 4. [**Conclusions**] A total of 17 common peaks were obtained, and the Peak 2 was the designated peak, and the chemical components of each processed product were different.

**Key words** Polygonati Rhizoma, Processing, HPLC, Fingerprint

## 1 Introduction

Polygonati Rhizoma is the dried rhizome of *Polygonatum sibiricum* Red., *Polygonatum kingianum* Coll. et Hemsl., or *Polygonatum cyrtonema* Hua[1]. As a traditional tonic medicine, it often needs to be steamed repeatedly in the traditional processing method[2-3] until the color is black and smooth, and there is no numbness in the mouth to reduce toxicity and enhance efficacy. According to records in *Compendium of Materia Medica*[4], taking Polygonati Rhizoma only needs steaming and exposing drugs to the sun alternatively for nine times, to tonify the deficiency and enhance the essence. The processing times represented by "nine" in the "steaming and exposing drugs to the sun alternatively for nine times" in the traditional steaming processing are still controversial[5], and some scholars believe that the processing technology can be optimized based on modern research on the number of resteaming times. Zheng Xiaoqian *et al.* studied the changes in carbohydrate components in Polygonati Rhizoma with different processing degrees during steaming and exposing drugs to the sun alternatively for nine times, they found that when steaming and exposing drugs to the sun alternatively for five times, the fructose content reached the peak then decreased, but the peak of sucrose content appeared at "steaming and exposing drugs to the sun alternatively for two times", and glucose level reached peak at "steaming and exposing drugs to the sun alternatively for seven times"; finally, they comprehensively evaluated the changes in appearance, nature and taste, and carbohydrate components, and selected Polygonati Rhizoma "steaming and exposing drugs to the sun alternatively for five times" as optimal processing method[6]. Yang Shengxian *et al.* comprehensively evaluated that "steaming and exposing drugs to the sun alternatively for four times" was the best processing technology based on the content changes in polysaccharides and saponins[7].

The change rule and mechanism of Polygonati Rhizoma in the process of resteaming are not clear. Fingerprint is a rapid and effective method to study the quality control and evaluation of traditional Chinese medicine. Some scholars used HPLC fingerprint to study the changes in components of Zingiberis Rhizoma Recens[8] and Pinelliae Rhizoma[9] before and after processing. In this study, we studied the fingerprints of each steaming and exposing drugs to the sun, and observed the fingerprints of Polygonati Rhizoma with different processing degrees. According to the experimental results, we optimized the best processing technology of Polygonati Rhizoma, so as to provide a reference for establishing a more systematic, standardized and perfect quality evaluation system of Polygonati Rhizoma decoction pieces.

## 2 Materials and methods

**2.1 Source and processing of medicinal materials** Polygonati Rhizoma was purchased from the traditional Chinese medicine market in Yulin City, Guangxi, and was identified as the fresh rhizome of *Polygonatum cyrtonema* Hua by Yin Shenggao, associate professor of the Department of Medicinal Plants, Guangxi University of Chinese Medicine.

Taking fresh *Polygonatum cyrtonema* Hua to remove impurities and fibrous roots, cleaning, airing, steaming for 6 h, placing in a sieve, exposing to the sun until half dry, adding the distilled liquid for soaking, steaming for 6 h again, exposing to the sun until half dry, adding the distilled liquid again, uniformly mixing, steaming for 6 h, exposing to the sun until half dry, repeating the operation for 9 times to obtain processed products with different times of steaming and exposing to the sun, namely, Sample 1, Sample 2, Sample 3, Sample 4, Sample 5, Sample 6, Sample 7, Sample 8 and Sample 9. Exposing the steamed Rhizoma polygonati to the sun until the processed Rhizoma polygonati is half dry for later use.

Received: January 5, 2024 Accepted: March 17, 2024

Supported by General Program of Guangxi Natural Science Foundation (2022GXNSFAA035599).

* Corresponding author. E-mail: 40508549@qq.com

**2.2 Reagents** Methanol (Lot 193728, Thermo Fisher Technology Co., Ltd.), 5-Hydroxymethylfurfural (5-HMF, wkq19112208 HPLC≥98%, Sichuan Weikeqi Biotechnology Co., Ltd.), acetonitrile (Lot 19199, Thermo Fisher Technology Co., Ltd.), glacial acetic acid (Lot 2019022701).

**2.3 Instruments** Shimadzu HPLC (LC-2030 Plus, Shimadzu, Japan), chromatographic column (SETSAIL II AQ $C_{18}$, 5 μm × 250 mm × 4.6 mm), ultrasonic cleaner (KQ-250B, Kunshan Ultrasonic Instrument Co., Ltd.), centrifuge (TGL-16G, Shanghai Anting Scientific Instrument Factory), Merck MilliQ Pure Water Instrument (Merck Chemicals Shanghai Co., Ltd.).

**2.4 Chromatographic conditions** Column: SETSAIL II AQ-$C_{18}$ (5 μm × 250 mm × 4.6 mm), mobile phase: A is pure water, B is acetonitrile, gradient elution, 0 – 10 min, B (5% – 10%), 10 – 30 min, B (10% – 35%), 30 – 40 min, B (35% – 60%), 40 – 45 min, B (60% – 100%), column temperature: 30 ℃, flow rate: 1 mL/min, absorption wavelength 200 nm, and injection volume: 10 μL.

**2.5 Preparation of standard solution** Precisely weighed 13.3 mg of 5-hydroxymethylfurfural reference standard and added methanol to prepare the standard solution solution of 1.33 mg/mL.

**2.6 Preparation of the test solution** Precisely weighed 2 g of Polygonati Rhizoma processed product powder, added 40 mL of 70% ethanol, performed ultrasonic treatment for 30 min, cooled down, performed suction filtration, kept the filtrate for use, added 40 mL of 70% ethanol into the filter residue, performed ultrasonic treatment for 30 min, cooled down, performed suction filtration, combined two times of the filtrate, evaporated, added methanol to dissolve the extract, transferred and fixed the volume to 10 mL, then added 100 μL of chitosan glacial acetic acid solution overnight, performed centrifugation at 12 000 rpm for 10 min, filtered with 0.22 μm filter membrane, and took the filtrate as the test solution.

**2.7 Methodological investigation**

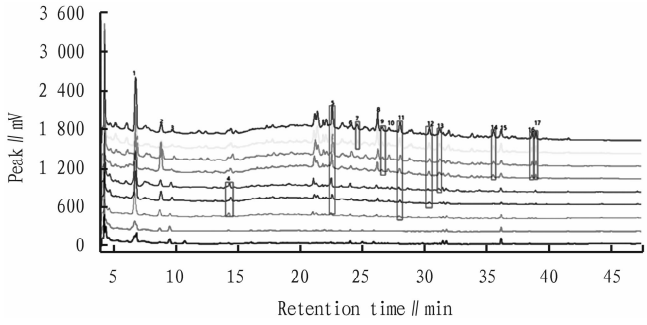
**2.7.1** Precision test. Took Sample 3 and injected for 5 times successively. The *RSD* of relative retention time of common peaks was 0.03% – 1.1%, and the *RSD* of relative retention peak area was 0.57% – 2.82%.

**2.7.2** Reproducibility test. Precisely weighed 5 pieces of Sample 3, extracted and injected. The relative retention time *RSD* of the common peak was 0.04% – 0.38%, and the relative retention area *RSD* was 0.18% – 2.07%.

**2.7.3** Stability test. Took Sample 3, and injected at 0, 3, 6, 12 and 24 h time points in turn, and the relative retention time *RSD* of the common peak was 0.03% – 0.71%, and the relative retention area *RSD* was 0.19% – 2.84%.

## 3 Results and analysis

**3.1 Establishment and analysis of fingerprints and assignment of chromatographic peaks** Took samples of Polygonati Rhizoma processed by "steaming and exposing drugs to the sun alternatively for one time" to "steaming and exposing drugs to the sun alternatively for nine times", prepared the test solution in accordance with methods in Section **2.6** and analyzed it according to the chromatographic conditions in Section **2.4**. The chromatogram was imported into the "Similarity Evaluation of Traditional Chinese Medicine Fingerprint" software, and the results were shown in Fig. 1. Through multi-point correction, 17 common peaks were obtained. Peak 2 of each sample can be identified as 5-HMF by comparison with the reference chromatogram, and the reference chromatogram is shown in Fig. 2.




**NOTE** samples from top to bottom are Sample 1, Sample 2, Sample 3, Sample 4, Sample 5, Sample 6, Sample 7, Sample 8, and Sample 9.

**Fig. 1 Fingerprint of Polygonati Rhizoma samples processed by steaming and exposing drugs to the sun alternatively for different times**

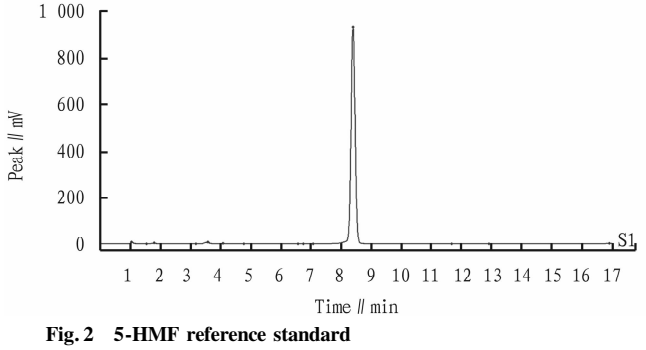



**Fig. 2 5-HMF reference standard**

**3.2 Fingerprint analysis** Among the 17 common peaks, Peak 4 mainly existed in the spectrum of Sample 3 to Sample 5, Peak 5 and Peak 11 mainly existed in the spectrum of Sample 3 to Sample 9, Peak 9, Peak 14 and Peak 16 mainly existed in the spectrum of Sample 6 to Sample 9, and Peak 12 and Peak 17 mainly existed in the spectrum of Sample 4 to Sample 9. Peak 1 had a better peak shape in Sample 3 and had the highest peak height in Sample 1. Peak 2 was 5-HMF, and its peak area and peak height in Sample 6 were higher than those in other samples. As the main effective part of Polygonati Rhizoma is polysaccharide of Polygonati Rhizoma, and 5-HMF is a thermal degradation product of the polysaccharide and has certain pharmacological activity, the content of 5-HMF has a certain relation with the quality of Polygonati Rhizoma. The results indicate that the times of steaming and exposing to the sun may affect the quality of Polygonati Rhizoma.

## 4 Discussion

In this experiment, we investigated the extraction conditions (including solvent ratio, extraction method, extraction time) and chromatographic conditions (including column, column temperature, wavelength, mobile phase), and finally determined the above method.

The extraction conditions were mainly determined by the size

of the peak area of 5-HMF, and finally determined as 70% ethanol, ultrasonic for 30 min. The chromatographic conditions were determined according to the peak shape and resolution. In this experiment, the maximum absorption wavelength was determined between 200 – 210 nm by DAD full-wavelength scanning, and finally 200 nm was determined as the optimal wavelength.

From the experimental results, it can be seen that there are great differences in the chemical composition of the nine batches of samples with different processing degrees. If we need to explore which specific chemical components are different, we need to further analyze them with the help of mass spectrometry. Because the quality of Polygonati Rhizoma from different varieties and different places may be different, the best steaming times of Polygonati Rhizoma with large quality difference may be different. In the future, more batches of Polygonati Rhizoma from different varieties and different places can be selected for further study.

## References


[1] Pharmacopoeia Commission of the People's Republic of China. Pharmacopoeia of the People's Republic of China (2020 edition)[M]. Beijing: China Medical Science Press, 2020: 319 – 320. (in Chinese).

[2] SUN SM. Qianjin Yifang[M]. Beijing: People's Medical Publishing House (PMPH), 1955: 5. (in Chinese).

[3] TANG SW, WANG JK, JIANG M. A review of the Classified Materia Medica (Volume 2)[M]. Beijing: China Medical Science Press, 2021: 9. (in Chinese).

[4] LI SZ, ZHU F, WANG J, *et al.* Compendium of Materia Medica[M]. Nanchang: 21st Century Publishing Group, 2014: 5. (in Chinese).

[5] PENG XX, WANG DQ, PENG HS. Brief discussion on evolution and changes of processing of medicinal materials in "Steamed for Nine Times and Shined for Nine Times" in ancient materia medica[J]. Journal of West Anhui University, 2018, 34(2): 92 – 99. (in Chinese).

[6] ZHENG XQ, JIN CS, ZHANG YZ, *et al.* Dynamic changes of saccharides from *Polygonatum cyrtonema* during Nine Steam Nine Bask processing[J]. Chinese Traditional Patent Medicine, 2020, 42(7): 1837 – 1841. (in Chinese).

[7] YANG SX, YANG ZM, CHEN YJ, *et al.* Study on the polysaccharides and saponins constituents of *Polygonatum sibiricum* Red. in "Nine-steam-nine-bask" processing[J]. Journal of Hunan Normal University (Medical Sciences), 2015, 12(5): 141 – 144. (in Chinese).

[8] WANG WH, LI J, GAO HM, *et al.* Chemical ingredients change of ginger processing with HPLC specific chromatogram[J]. Chinese Journal of Pharmaceutical Analysis, 2009, 29(8): 1248 – 1252. (in Chinese).

[9] YANG BY, LI M, SHI J, *et al.* A system research on HPLC characteristic fingerprint of Pinelliae Rhizoma and Pinelliae Rhizoma Praeparatum cum Zingibere et Alumine[J]. Chinese Traditional and Herbal Drugs, 2014, 45(5): 652 – 658. (in Chinese).


(From page 17)

Therefore, methanol concentration was the factor that had statistical significance on the extraction effect of umbelliferone from *P. tomentellal*. In consequence, the extraction schedule of this experiment was $A_2B_3C_2$, whereas the optimal extraction schedule of this experiment was $A_2B_2C_2$ combined with the experimental cost and energy saving, that is, the extraction effect was the best when the methanol concentration, extraction solvent volume and reflux extraction time were 80%, 30 mL, and 1.5 h, respectively.

**Table 6 Analysis of variance for extraction of umbelliferone from *Pileostegia tomentellal***

| Source of error | *SS* | *f* | *S* | *F* | *P* |
|---|---|---|---|---|---|
| A | 0.016 8 | 2 | 0.008 4 | 19.043 0 | <0.05 |
| B | 0.007 4 | 2 | 0.003 7 | 8.431 6 | >0.05 |
| C | 0.000 3 | 2 | 0.000 2 | 0.375 5 | >0.05 |
| D (error) | 0.000 9 | 2 | 0.000 4 | | |

## 5 Validation test

Three batches of validation tests were conducted according to the optimal technology determined by orthogonal test. The results showed that the content of umbelliferone in *P. tomentellal* was 0.425 0 mg/g, and the *RSD* was 2.36%, indicating that the process had good reproducibility and could be used as the extraction technology of umbelliferone from *P. tomentellal*.

## 6 Discussion

The content of umbelliferone in *P. tomentellal* was determined by HPLC, and the effects of different pH, composition and flow rates of mobile phase were studied. The results showed that the method had good precision, reproducibility and stability, and could be used for the determination of umbelliferone content in *P. tomentellal*, which provides a basis for establishing and improving the quality standard of Yao medicine *P. tomentellal*.

## References


[1] Institute of Botany, Chinese Academy of Sciences. Illustrated catalogue of higher plants of China (Volume II)[M]. Beijing: Science Press, 1972. (in Chinese).

[2] LI YY, PEI YY, HUANG SY, *et al.* Pharmacognosy of Zhongliuteng (*Pileostegia tomentella* Hand. Mazz) of Yao Medicine[J]. Crop Journal, 2018(1): 62 – 65. (in Chinese).

[3] LIU Y, LU GS, WANG L, *et al.* Antitumor effect of alcohol extract of *Pileostegia tomentella* on H22 tumor-bearing mice[J]. Chinese Journal of Pharmacology, 2016, 51(12): 981 – 984. (in Chinese).

[4] Editorial Committee of Chinese Materia Medica, State Administration of Traditional Chinese Medicine. Chinese materia medica (Book IV)[M]. Shanghai: Shanghai Science and Technology Press, 1999: 40 – 41. (in Chinese).

[5] Guangxi Institute of Chinese Medicine & Pharmaceutical Science. List of medicinal plants of Guangxi[M]. Nanning: Guangxi People's Publishing House, 1986: 203. (in Chinese).

[6] Guangxi Institute of Botany. Flora of Guangxi (Volume II)[M]. Nanning: Guangxi Science and Technology Press, 2005: 298. (in Chinese).

[7] XIAO BM, SHE PG, LI YM, *et al.* The study on quality of *Pileostegia tomentella*[J]. Chinese Journal of Experimental Traditional Medical Formulae, 2012, 18(13): 131 – 133. (in Chinese).

[8] WANG YQ, MA GX, LIANG QP, *et al.* Chemical constituents from stems of Yao medicine Zhongliuteng(cane of *Pileostegia tomentella*)[J]. Chinese Traditional and Herbal Drugs, 2016, 47(24): 4326 – 4330. (in Chinese).

[9] KIM WJ, LEE SJ, MOON SK, *et al.* Decursin inhibits growth of human bladder and colon cancer cells via apoptosis, G1-phase cell cycle arrest and extracellular signal-regulated kinase activation[J]. International Journal of Molecular Medicine, 2010, 25(4): 635 – 641.

[10] XIA LT, ZHANG JL, CHEN YF, *et al.* Simultaneous determination of 7-Hydroxycoumarin in Aurantii Fructus by HPLC[J]. Lishizhen Medicine and Materia Medica Research, 2019, 30(8): 1795 – 1797. (in Chinese).

[11] CHEN Y, LI JZ, WEI JC, *et al.* TLC identifi cation and caffeic acid extraction technology and content determination of *Argyreia aceta*[J]. Jilin Journal of Chinese Medicine, 2019, 39(1): 92 – 97. (in Chinese).