Comparative Study of Different Processed Products of *Rubia cordifolia* Based on High-Performance Liquid Chromatography

Juandi WANG[1,2], Ruijuan ZHU[3], Mingtong ZHANG[1,2], Xin LI[1,2], Xiaoxia GUO[1,2], Donghua LI[1,2], Xiao MA[1,2]*

1. Gansu Provincial Institute of Drug Control, Lanzhou 730070, China; 2. NMPA Key Laboratory for Quality Control of TCM, Lanzhou 730070, China; 3. Shandong Vocational Animal Science and Veterinary College, Weifang 261061, China

Abstract [**Objectives**] To establish a high-performance liquid chromatography (HPLC) method for comparing the content differences of xanthopurpurin in raw *Rubia cordifolia* and its charred products processed at different temperatures, sourced from various regions. [**Methods**] The chromatographic separation was performed using an HPLC Column C_{18}. The mobile phase consisted of acetonitrile-0.2% phosphoric acid solution (50∶50, *v/v*) at a flow rate of 1.0 mL/min. Detection was carried out at 245 nm, and the column temperature was maintained at 30 ℃. [**Results**] Xanthopurpurin exhibited a good linear relationship within the range of 6.712 - 83.905 μg/mL (r = 1.000 0). The average recovery rate was 101.3% with an *RSD* of 2.1% (n = 6). Content determination results showed that the xanthopurpurin content in raw *R. cordifolia* ranged from 0.040 0 to 0.169 3 mg/g. In charred *R. cordifolia* processed at 110 - 130 ℃, it ranged from 0.309 2 to 0.380 1 mg/g, while in charred *R. cordifolia* processed at 350 - 370 ℃, it ranged from 0.512 8 to 1.168 8 mg/g. A significant increasing trend in xanthopurpurin content was observed with rising charring temperatures. [**Conclusions**] This method is simple, accurate, feasible, and reproducible. It can effectively distinguish between raw *R. cordifolia* and its charred products processed at different temperatures, providing a scientific basis for the quality evaluation of charred *R. cordifolia* and its formulations in traditional Chinese medicine.

Key words High-performance liquid chromatography (HPLC), Xanthopurpurin, *Rubia cordifolia*, Charred *R. cordifolia*, Processing temperature, Processed product, Content determination, Quality evaluation

1 Introduction

Rubia cordifolia L. (family Rubiaceae) is a medicinal plant known for its effects in cooling blood, dispelling stasis, stopping bleeding, and relieving dysmenorrhea. It is commonly used for conditions such as hematemesis, epistaxis, metrorrhagia, and traumatic bleeding[1]. Its processed products primarily include raw *R. cordifolia* and charred *R. cordifolia*. Raw *R. cordifolia* is characterized by its core functions of promoting blood circulation, dispelling stasis, clearing heat, and cooling blood, while also possessing hemostatic properties. After charring by stir-frying, its cold nature is significantly reduced, and its medicinal properties shift to astringency, with hemostasis becoming its primary function[2]. Modern pharmacological studies have found that after charring, the anti-inflammatory, analgesic, and blood-activating and stasis-resolving effects of *R. cordifolia* are diminished, while its hemostatic effect is enhanced, providing a solid pharmacological basis for its rational clinical application[3-10]. Further research has identified xanthopurpurin, present in charred *R. cordifolia*, as possessing significant hemostatic activity, suggesting its role as an active component responsible for the hemostatic effect of charred *R. cordifolia*[11]. Previously, our research group studied charred *R. cordifolia* using content determination methods specified under the standards for crude drugs and decoction pieces in the *Pharmacopoeia of the People's Republic of China*. The results indicated a significant decreasing trend in the content of rubimaillin and purpurin from the raw material to the charred product, consistent with existing literature reports[12-13]. Consequently, using these two components proves inadequate for the accurate quality evaluation of charred *R. cordifolia*. Literature review[14-17] and subsequent in-depth research revealed significant differences in the xanthopurpurin content between raw *R. cordifolia* and its charred product. Based on this, this study establishes an HPLC method to comparatively analyze different processed products of *R. cordifolia* from various origins. The aim is to provide reliable technical support and a theoretical foundation for the quality control and scientific evaluation of charred *R. cordifolia*.

Received: January 13, 2026 Accepted: February 14, 2026
Supported by the Key Laboratory Project for Quality Control of Chinese Materia Medica and Decoction Pieces (2023GSMPA-KL06, 2024GSMPA-KL16).
* Corresponding author. Xiao MA, bachelor's degree, chief TCM pharmacist.

2 Instruments and reagents

2.1 Instruments and solutions High-Performance Liquid Chromatograph (Agilent Technologies Co., Ltd., model: 1260 Infinity II); Electronic Balance (Mettler Toledo Instrument Shanghai Co., Ltd., model: XS 204); Electronic Balance (Mettler Toledo Instrument Shanghai Co., Ltd., model: ME 204).

High-Frequency Digital Ultrasonic Cleaner (Kunshan Shumei Ultrasonic Instrument Co., Ltd., model: KQ-700TDE). Methanol (Merck KGaA, chromatographic grade); Acetonitrile (Merck KGaA, chromatographic grade); all other reagents were of analytical grade, and water was purified water; Xanthopurpurin (Chengdu Efa Biotechnology Co., Ltd., purity: 100%).

2.2 Analytical samples Samples were collected from different manufacturers; specific sample information is provided in Table 1.

3 Methods and results

3.1 Sample preparation Dried *R. cordifolia* decoction pieces were taken. The temperature of the stir-frying machine was set to 110 - 130 ℃ and 350 - 370 ℃, respectively. Once the target

temperature was reached, the material was added and stir-fried for 7 min. Stir-frying continued until dense smoke emerged, the surface turned charred black, and the interior became dark brown. A fine mist of water was then sprayed to extinguish any sparks completely. The material was removed, yielding charred *R. cordifolia* (T = 110 – 130 ℃) and charred *R. cordifolia* (T = 350 – 370 ℃).

Table 1 Information on *Rubia cordifolia* and charred *Rubia cordifolia* samples

No.	Product name	Specification	Origin
1	A	Decoction piece	Shaanxi
2	B	Processing 1	Shaanxi
3	A	Decoction piece	Hebei
4	B	Processing 1	Hebei
5	A	Decoction piece	Hebei
6	B	Processing 1	Hebei
7	A	Decoction piece	Hebei
8	B	Processing 1	Hebei
9	A	Decoction piece	Shaanxi 1
10	B	Processing 2	Shaanxi 1
11	B	Processing 1	Shaanxi 1
12	A	Decoction piece	Shaanxi 2
13	B	Processing 2	Shaanxi 2
14	B	Processing 1	Shaanxi 2
15	A	Decoction piece	Shaanxi 3
16	B	Processing 2	Shaanxi 3
17	B	Processing 1	Shaanxi 3
18	A	Decoction piece	Henan 1
19	B	Processing 2	Henan 1
20	B	Processing 1	Henan 1
21	A	Decoction piece	Henan 2
22	B	Processing 2	Henan 2
23	B	Processing 1	Henan 2
24	A	Decoction piece	Henan 3
25	B	Processing 2	Henan 3
26	B	Processing 1	Henan 3
27	A	Decoction piece	Shanxi 1
28	B	Processing 2	Shanxi 1
29	B	Processing 1	Shanxi 1
30	A	Decoction piece	Shanxi 2
31	B	Processing 2	Shanxi 2
32	B	Processing 1	Shanxi 2
33	A	Decoction piece	Shanxi 3
34	B	Processing 2	Shanxi 3
35	B	Processing 1	Shanxi 3

NOTE A. *Rubia cordifolia*; B. Charred *R. cordifolia*. Processing 1: Processing temperature 350 – 370 ℃; Processing 2: Processing temperature 110 – 130 ℃.

3.2 Chromatographic conditions Column: HPLC Column C_{18} (250 mm × 4.6 mm, 5 μm); mobile phase: Acetonitrile-0.2% phosphoric acid solution (50 ∶ 50, *v*/*v*); detection wavelength: 245 nm; column temperature: 30 ℃; flow rate: 1.000 mL/min.

3.3 Preparation of reference solution An appropriate amount of xanthopurpurin reference standard was accurately weighed and dissolved in methanol to prepare a solution containing 1 mg of xanthopurpurin per 1 mL, which served as the reference stock solution.

3.4 Preparation of test solution Approximately 0.5 g of the sample powder (passed through a No. 2 sieve) was accurately weighed and placed into a stoppered conical flask. Exactly 25 mL of methanol was added, the flask was stoppered, and the total weight was recorded. The mixture was subjected to ultrasonic extraction (power: 250 W, frequency: 40 kHz) for 30 min, then allowed to cool to room temperature. The weight was recorded again, and the weight loss was replenished with methanol. The mixture was shaken well, filtered, and the successive filtrate was collected for use.

3.5 Specificity test The test solution (Shaanxi 2 Charred *R. cordifolia*) and the reference solution were injected and analyzed according to the chromatographic conditions described in Section **3.2**. The results indicated that xanthopurpurin in the test solution was not interfered with by other components, and the resolution was greater than 1.5, meeting the requirement (Fig. 1).

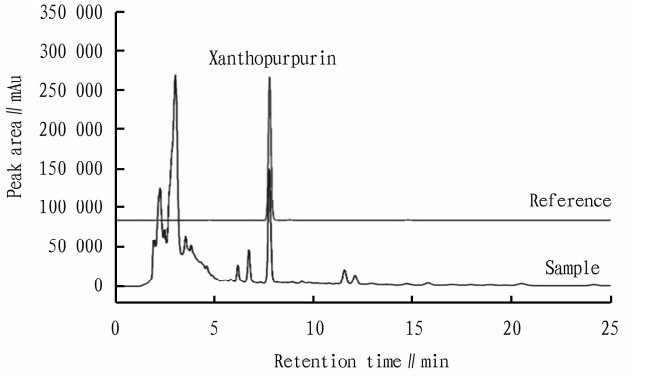

Fig. 1 HPLC chromatograms of test solution (Shaanxi 2 Charred *Rubia cordifolia*) and reference solution

3.6 Linearity investigation Precise volumes of the reference stock solution were diluted to prepare reference solutions at different concentrations: 0.005 27, 0.010 54, 0.021 09, 0.042 18, 0.052 7, and 0.105 4 mg/mL. A linear curve was plotted with the peak area as the ordinate (*y*-axis) and the concentration as the abscissa (*x*-axis). The resulting linear regression equation was $y = 59\,042.607\,5x - 30.461\,3$, with an R^2 value of 0.999 3. This indicates good linearity for xanthopurpurin within the concentration range of 0.005 27 to 0.105 54 mg/mL. The linearity curve is shown in Fig. 2.

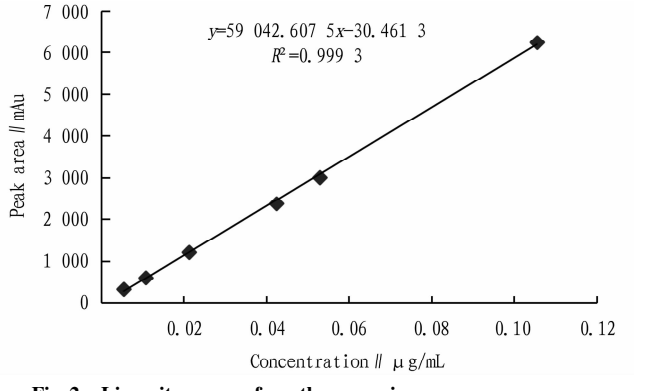

Fig. 2 Linearity curve of xanthopurpurin

3.7 Investigation of precision, stability, and repeatability The reference solution prepared in Section **3.3** was injected six times consecutively under the chromatographic conditions described in Section **3.2**, and the peak areas were recorded. The results showed that the *RSD* of the peak areas was 0.6%, indicating good precision of the analytical instrument. The same test solution (Shaanxi 2 Charred *R. cordifolia*) was analyzed at 0, 4, 8, 12, 16, and 24 h under the chromatographic conditions described in Section **3.2**. The peak areas were recorded, and the xanthopurpurin content was calculated. The results showed an *RSD* of 1.8% for the xanthopurpurin content, indicating good stability of the test solution within 24 h. Powder from the same batch of sample (Shaanxi 2 Charred *R. cordifolia*) was accurately weighed into six portions of 0.5 g each. Test solutions were prepared from each portion according to the method described in Section **3.4**. Each test solution was then analyzed under the chromatographic conditions described in Section **3.2**. The peak area was recorded, and the xanthopurpurin content was calculated. The results showed an *RSD* of 2.1% for the xanthopurpurin content, demonstrating that the analytical method possesses good stability and repeatability.

3.8 Recovery test Powder of a sample with known xanthopurpurin content (Shaanxi 2 Charred *R. cordifolia*) was accurately weighed into six portions of approximately 0.25 g each. Each portion was placed into a separate stoppered conical flask. Exactly 1.0 mL of the xanthopurpurin reference solution was added precisely to each conical flask. The mixtures were then processed according to the method for preparing the test solution described in Section **3.4**. They were subsequently injected and analyzed under the chromatographic conditions described in Section **3.2**. The peak area corresponding to xanthopurpurin was recorded. Calculation showed that the recovery rates of xanthopurpurin ranged from 99.7% to 105.2%, with an average recovery rate of 101.3%. The results of the recovery test are presented in Table 2. These results indicate that the sample extraction method and content determination method employed in this experiment exhibit good accuracy and meet the requirements for experimental analysis.

Table 2 Recovery test results for xanthopurpurin ($n=6$)

Sample weight // g	Content in sample // mg	Amount added // mg	Amount measured // mg	Recovery // %	Average recovery // %	*RSD* // %
0.250 6	0.294 4	0.292 1	0.585 9	99.78	101.12	1.73%
0.251 1	0.292 6	0.292 1	0.583 8	99.70		
0.250 1	0.293 7	0.292 1	0.588 8	101.01		
0.250 9	0.294 0	0.292 1	0.585 8	99.90		
0.250 2	0.294 6	0.292 1	0.593 2	102.21		
0.250 2	0.294 5	0.292 1	0.598 6	104.10		

3.9 Sample determination results Powder from 35 batches of samples was taken. Test solutions were prepared according to the method described in Section **3.4** and injected under the chromatographic conditions specified in Section **3.2**. The peak area of xanthopurpurin was recorded, and its content was calculated. The results showed that the xanthopurpurin content in raw *R. cordifolia* (decoction pieces), charred *R. cordifolia* (T = 110 – 130 ℃), and charred *R. cordifolia* (T = 350 – 370 ℃) from different origins ranged from 0.040 0 to 0.169 3 mg/g, 0.309 2 to 0.380 1 mg/g, and 0.512 8 to 1.168 8 mg/g, respectively. Based on the content results and bar charts, it is evident that after processing to varying degrees, the xanthopurpurin content in *R. cordifolia* from different origins demonstrated an increasing trend from raw *R. cordifolia* to charred *R. cordifolia* (T = 110 – 130 ℃), and further to charred *R. cordifolia* (T = 350 – 370 ℃). The effect of temperature on the content was consistent across samples (Table 3).

Table 3 Xanthopurpurin content in samples ($n=3$)

Origin	No.	Specification: Charred *Rubia cordifolia* (T = 350 – 370 ℃)	Specification: Charred *R. cordifolia* (T = 110 – 130 ℃)	Specification: Raw *R. cordifolia*
Shaanxi 3	1	1.021 2	0.343 4	0.169 5
Shaanxi 2	2	1.163 3	0.303 1	0.161 1
Shaanxi 1	3	0.982 3	0.383 1	0.148 6
Shanxi 3	4	0.916 1	0.351 2	0.125 4
Shanxi 2	5	0.772 1	0.374 5	0.114 2
Shanxi 1	6	0.850 2	0.352 1	0.113 4
Henan 3	7	0.742 1	0.374 3	0.103 3
Henan 2	8	0.814 1	0.361 2	0.110 1
Henan 1	9	0.755 4	0.312 3	0.086 5
Guangjitang	10	0.511 1	–	0.096 4
Antaitang	11	0.560 5	–	0.102 3
Renxin Pharmaceutical	12	0.540 1	–	0.051 1
Juyaotang	13	1.030 1	–	0.041 2

NOTE " – " indicates the absence of the sample; "T" represents processing temperature.

4 Discussion

Traditional Chinese medicine (TCM) processing methods mainly comprise three categories: purification, cutting, and processing. Among these, over 90% of TCM herbs subjected to processing undergo a transformation in properties between their raw and processed forms. The distinction between "raw" and "processed" forms of TCM herbs manifests not only in significant changes in medicinal properties and appearance but also in alterations to their pharmacological actions and chemical compositions[18]. Historical processing methods for *R. cordifolia* include filing, roasting, stir-frying, processing with wine, processing with vinegar, and charring, among others[19]. Modern research has confirmed that the

hemostatic effect of *R. cordifolia* is enhanced after charring by stir-frying. This enhancement is attributed to the astringent and adsorptive properties of the carbon generated during charring, as well as changes in trace elements and chemical composition resulting from the process[20-21].

However, the current processing technology for charred *R. cordifolia* is only vaguely described as "stir-fried using the charring method until the surface turns charred black and the interior becomes dark brown". This description lacks quantifiable parameters such as specific temperature and duration. Furthermore, the quality standard for charred *R. cordifolia* included in the *Chinese Pharmacopoeia* only encompasses items such as description, identification, testing, and extractives. It does not incorporate content determination indicators for key components, making it difficult to achieve precise and objective quality evaluation of charred *R. cordifolia*. Based on this, our research group specifically collected samples of charred *R. cordifolia* prepared under different conditions. The aim was to screen for core indicator components that can characterize the quality of charred *R. cordifolia* and to establish a corresponding content determination method.

As evident from the experimental results, the xanthopurpurin content in *R. cordifolia* from different origins increases significantly from the raw form to the charred product. This characteristic can serve as a key criterion for clearly distinguishing between non-charred *R. cordifolia* and charred *R. cordifolia*. Moreover, the HPLC method established in this study is simple, practical, and demonstrates good resolution, high sensitivity, excellent specificity, and robustness. It not only provides a scientific basis for the quality control of charred *R. cordifolia* and its formulations in TCM but also offers a reference for the precise control of the charring degree during the processing of *R. cordifolia*.

References

[1] Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China: 2020 Edition Volume I[S]. Beijing: China Medical Science Press, 2020: 245. (in Chinese).

[2] YE DJ, ZHANG SC. Science of Chinese materia medica processing[M]. Beijing: People's Medical Publishing House, 1999: 341. (in Chinese).

[3] ZHANG ZL, HUANG XF, ZHANG CS, *et al.* Pharmacologic action comparison of India madder root before and after charcoal[J]. China Journal of Traditional Chinese Medicine and Pharmacy, 2008, 23(10): 879-881. (in Chinese).

[4] GENG QB, HUANG HL, WENG ZF, *et al.* Effects of *Rubia cordifolia* and charred *R. cordifolia* on coagulation-fibrinolytic system of normal rats [J]. Chinese Journal of Experimental Traditional Medical Formulae, 2013, 19(13): 279-282. (in Chinese).

[5] WANG HL, CHEN Y, XIAO LY, *et al.* Screening of effective parts in Rubiae radix et rhizome carbonisatum with resisting coagulation tendency in the acute blood stasis model rat[J]. Natural Product Research and Development, 2016, 28(11): 1797. (in Chinese).

[6] JIA XH, CHOU L, ZHOU XL. Influence of madder and charcoal mixed extract on eutopic endometrium proliferation and apoptosis and intimal tissue VEGF and NF-κB in model rats with endometriosis[J]. Practical Pharmacy and Clinical Remedies, 2018, 21(6): 615-619. (in Chinese).

[7] XIAO LY, WANG HL, CHEN Y, *et al.* Protective effect of different polar fractions of Carbonized Rubiae Radix et Rhizoma(cRRR) against oxidative injury[J]. China Journal of Chinese Materia Medica, 2017, 42(23): 4604-4610. (in Chinese).

[8] SHAN MQ, CHEN X, LI J, *et al.* Comparative study on effects of Rubiae Radix et Rhizoma and carbonized Rubiae Radix et Rhizoma on acute blood stasis rat model[J]. China Journal of Chinese Materia Medica, 2014, 39(3): 493-496. (in Chinese).

[9] CUI LL, LIANG ZH, GAO L, *et al.* Analysis of the infrared spectrum of *Rubia cordifolia* before and after processing[J]. Journal of Henan University (Medical Edition), 2020, 39(4): 240-243. (in Chinese).

[10] YUAN Y, DONG SS, FENG WP, *et al.* Research progress on chemical constituents and physiological activities of *Rubia cordifolia* L.[J]. Jiangxi Feed, 2016(4): 14-18. (in Chinese).

[11] WANG HL, SHAN MQ, ZHANG LX. Research progress on carbonized Rubiae Radix et Rhizoma[J]. Chinese Traditional Patent Medicine, 2016, 38(6): 1367-1371. (in Chinese).

[12] YANG YT, CUI CY. Comparative study on different processed products of *Rubia cordifolia* L.[J]. Shandong Chemical Industry, 2020, 49(9): 47-48. (in Chinese).

[13] YANG YT, KANG WY. Determination of rubimaillin contents in *Rubia cordifolia* L and different processed products by HPLC[J]. Chinese Traditional Patent Medicine, 2011, 33(12): 2125-2127. (in Chinese).

[14] SHAN MQ, CHEN X, WANG K, *et al.* Determination of 1,3,6-trihydroxy-2-methylanthraquinone in Rubiae Radix et Rhizoma before and after Prepared by UPLC[J]. Modern Chinese Medicine, 2012, 14(12): 9-11. (in Chinese).

[15] CHEN X, WANG K, SHAN MQ, *et al.* Simultaneous determination of four quinone compounds in carbonized Rubiae Radix et Rhizoma by UPLC[J]. China Journal of Chinese Materia Medica, 2012, 37(19): 2922-2925. (in Chinese).

[16] CHEN Y, SHAN MQ, WANG HL, *et al.* Changes of chemical constituents in Rubiae Radix et Rhizoma before and after carbonized by UPLC-Q-TOF-MS method[J]. China Journal of Chinese Materia Medica, 2017, 42(5): 923-930. (in Chinese).

[17] KANG H, ZHANG ZL, ZHOU Y, *et al.* HPLC determination of 1,3-dihydroxyanthraquinone in carbonized Rubiae Radix et Rhizoma decoction pieces[J]. Chinese Traditional Patent Medicine, 2010, 32(6): 980-982. (in Chinese).

[18] CHEN X. Study on material basis of raw and processed *Rubia cordifolia* L. in compound prescriptions[D]. Nanjing: Nanjing University of Chinese Medicine, 2013. (in Chinese).

[19] WANG XT, YE DJ. Compilation of traditional Chinese medicine processing methods through the ages: Ancient part[M]. Nanchang: Jiangxi Science and Technology Press, 1998: 110. (in Chinese).

[20] GAO mL, LAN JS, SHAN MQ, *et al.* Research strategies thinking and research progress for carbonized traditional Chinese medicine[J]. Journal of Nanjing University of Traditional Chinese Medicine, 2020, 36(5): 696-703. (in Chinese).

[21] YANG YY, TANG JF, LI QW, *et al.* Pharmacological research and clinical applications of carbonized Chinese medicine[J]. Journal of Liaoning University of Traditional Chinese Medicine, 2020, 22(10): 186-190. (in Chinese).