A Combined Quality Evaluation Method That Integrated Chemical Constituents, Appearance Traits and Origins of Raw Rehmanniae Radix Pieces

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Research

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Abstract

Background The quality control of traditional Chinese medicine (TCM) is a challenge for internationalization of TCM. Modern quality evaluation methods ignore origins and appearance traits, and traditional quality evaluation methods lack quantitative analysis. Therefore, an integrated quality evaluation method is urgent in need. Raw Rehmanniae Radix (RRR) was a widely used TCM. Its quality has caught much attention, and the existing quality evaluation methods have certain limitations. The aim of this study was to establish a comprehensive and practical method for the quality evaluation and control of RRR pieces based on its chemical components, appearance traits and origins.

Methods 33 batches of RRR pieces were collected from 6 provinces, high performance liquid chromatography (HPLC) was applied to determine 5 constituents including catalpol, rehmannioside A, rehmannioside D, leonuride and verbascoside in RRR pieces. The appearance traits were qualitatively observed. Furthermore, correlation analysis, principal components analysis (PCA), cluster analysis and *t*-test were performed to discriminate and evaluate different quality of RRR pieces.

Results 33 batches of RRR pieces could be divided into three categories, the samples of Henan province were in a group, the samples from Shandong province and Shanxi province were classified in a group, and the samples from other provinces were divided in a group. Furthermore, the constituents and appearance traits of RRR pieces were significantly different in diverse origins.

Conclusions The combined method of chemical components, appearance traits and origins could classify and distinguish different quality of RRR pieces, which could provide a basic reference to quality control of TCM.

1. Introduction

Chinese herbal medicine is an essential component of traditional Chinese medicine (TCM). In recent years, its clinical value has been continuously confirmed and recognized[1], and the demand of Chinese herbal medicine has increased greatly[2]. The quality evaluation and control of it have become the focus of scholars. Modern quality evaluation methods quantitatively and accurately determine the contents of active ingredients in Chinese herbal medicine with the aid of advanced analytical techniques[3], but ignore the correlation between origins, medicinal parts and active ingredients. Traditional quality evaluation methods mainly use the origin and appearance traits as indicators, which are subjective and lack of quantification. Therefore, the index of quality control in these methods is single, which cannot comprehensively evaluate the quality of traditional Chinese medicine, and an integrated quality evaluation method is urgently proposed to ensure the quality of Chinese herbal medicine.

Raw Rehmanniae Radix, the dried root of Rehmannia glutinosa Libosch, has been used as a traditional Chinese medicine. It has been applied to treat constipation, vomiting blood, diabetes[4, 5]. Modern pharmacological research has revealed that RRR has the effects of promoting blood clotting[6], anti-anxiety and depression[7], delaying aging[8], and reducing blood sugar[9]. It is because of its significant

and extensive clinical efficacy that RRR has been used widely[10], and the quality of it has also been paid much attention. Studies have demonstrated that the quality of RRR is different, and it is easily affected by regions[11], germplasm[12] and processing methods[13]. Therefore, one of the toughest challenges facing the clinical practice of RRR is its uncontrollable quality.

The quality control of RRR has been paid much attention since ancient time. Traditionally, the quality of RRR is evaluated based on its appearance characters and origins. Doctors obtained the appearance information of RRR through eyes, nose, mouth, and touch, and further summarized their experience and knowledge about the quality of RRR by clinical practice. Due to backward technology and lack of quantitative thinking, both qualitative descriptions and differences in personal experience made it difficult to accurately judge the quality of RRR. At present, the content of bioactive ingredients is the main indicator to evaluate the quality of RRR. Studies have reported that RRR contains major components such as iridoid glycosides and phenylethanol glycosides, among which the iridoid glycosides include catalpol, verbascoside and rehmannioside A and rehmannioside D. Studies suggested that catalpol has wide pharmacological activities, such as protecting nerves[14], improving oxidative stress[15] and anti-diabetes[16]. Verbascoside has biological activities of anti-inflammatory[17] and neuroprotection[18]. Therefore, catalpol and verbascoside are regarded as index components in the quality control of RRR under the Pharmacopoeia of the People's Republic of China (ChP; 2015 edition)[19]. In addition, the method of measuring multi-components and establishing the fingerprint of RRR has been a hot spot for scholars to evaluate and control the quality of RRR. Various analytical methods have been studied, such as high performance liquid chromatography (HPLC), ultra high performance liquid chromatography (UHPLC), chromatography hyphenated mass spectrometer (LC-MS), near infrared reflectance spectroscopy (NIRS)[20-23]. However, some analytical methods require precise and excellent instruments, and their complex operation is not conducive in quality evaluation of RRR. More importantly, the origin and appearance of RRR have been neglected in most studies. Hence, it is crucial to comprehensively evaluate the quality of RRR with a relatively reliable and convenient analytical method, based on its origins, appearance traits and bio-active constituents.This will provide a scientific basis for establishing a convenient and effective way to control the quality of RRR.

Herein, to establish a comprehensive and practical method for the quality evaluation and control of RRR pieces, 33 batches of Raw Rehmanniae Radix pieces from Henan, Shandong, Shanxi, Anhui, Zhejiang, Sichuan provinces were collected and evaluated by the contents of catalpol, verbascoside, leonuride, rehmannioside A, and rehmannioside D, appearance characters and their origins in this study. In addition, a cluster analysis was performed on classification of 33 batches of RRR pieces on the basis of correlation analysis and principal component analysis.

2. Materials And Methods

2.1 Plant materials

Thirty-three batches of raw Rehmanniae Radix (RRR) pieces were collected from different locations (Table 1). The voucher specimens were authenticated by Prof. Huaqiang Zhai (Beijing University of Traditional Chinese Medicine, Beijing, China), and were deposited in the laboratory of Chinese medicine identification, Beijing University of Traditional Chinese Medicine. According to the standards of RRR in ChP (2015 edition), they were all qualified after examination.

Table 1. Resource information for the 33 batches of RRR pieces

No.	Origin	No.	Origin
A1	Wen County, Henan Province	A18	Jinhua, Zhejiang Province
A2	Wen County, Henan Province	A19	Jinhua, Zhejiang Province
A3	Wen County, Henan Province	A20	Jinhua, Zhejiang Province
A4	Wen County, Henan Province	A21	Guangyuan, Sichuan Province
A5	Wen County, Henan Province	A22	Guangyuan, Sichuan Province
A6	Wen County, Henan Province	A23	Guangyuan, Sichuan Province
A7	Wen County, Henan Province	A24	Guangyuan, Sichuan Province
A8	Wen County, Henan Province	A25	Guangyuan, Sichuan Province
A9	Wen County, Henan Province	A26	Pinglu, Shanxi Province
A10	Wen County, Henan Province	A27	Pinglu, Shanxi Province
A11	Bozhou in Anhui Province	A28	Pinglu, Shanxi Province
A12	Bozhou, Anhui Province	A29	Pinglu, Shanxi Province
A13	Bozhou, Anhui Province	A30	Pinglu, Shanxi Province
A14	Bozhou, Anhui Province	A31	Chengwu, Shandong Province
A15	Bozhou, Anhui Province	A32	Chengwu, Shandong Province
A16	Jinhua, Zhejiang Province	A33	Chengwu, Shandong Province
A17	Jinhua, Zhejiang Province		

2.2 Chemicals, reagents and equipment

Catalpol was purchased from the National Institutes for the Control of Food and Drug (Beijing, China, Lot no.110808-201210) and verbascoside was provided by the National Institutes for the Control of Food and Drug (Beijing, China, Lot no.111530-201512). Rehmannioside A, rehmannioside D and leonuride were purchased from Shanghai Yuanye Biotechnology Co., Ltd (Shanghai, China), their Lot numbers are

CAS81720-08-3, YY91599-5mg, B21571-20 mg respectively. Acetonitrile and methanol were chromatographically pure, water was ultra pure water.

The HPLC equipment was a Waters 2695 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a Waters 2996 diode array detector (DAD), a Waters 2420 evaporative light scattering detector and Empower Workstation.

2.3 Quality evaluation method

2.3.1 Chemical compositions measurement

2.3.1.1 Preparation of Sample Solutions and Standard Solutions

All the tested samples were crushed into powder. The powder (400 mg) was accurately weighed and extracted with 25 mL 25% methanol by ultrasonication (40KHz,500W) for 30 min. After cooling down at normal temperature, 25% methanol was added for the lost weight. Then the extraction was filtered through a 0.45 μm membrane filter. The filtrate was used as the test solution.

The individual standard stock solutions of the 5 reference substances were prepared by dissolving reference substances in 25% methanol. Their concentrations were as following: 0.968 mg/mL (catalpol), 0.342 mg/mL (rehmannioside A), 0.302 mg/mL (rehmannioside D), 0.050 mg/mL (leonuride), 0.352 mg/mL (verbascoside), then diluted with 25% methanol to different concentrations to generate the calibration curves. All the standard solutions were filtered through 0.45 μm membrane filters, then stored at 4℃ before analysis.

2.3.1.2 HPLC conditions

The chromatographic separation was carried out on a Waters XBridge C18 column（4.6 mm×250 mm, 5μm) with a gradient elution program using a mixture of acetonitrile (A) and 0.02% aqueous phosphate (v/v) (B) as mobile phase. The elution program was optimized and conducted as follows: 0–10 min, 2–4% A; 10–28 min, 4–15% A; 28–55 min, 15–30% A at 1 mL/min flow rate. All injection sample volumes were 10 μl. The column temperature was maintained at 30 ℃ and the detection wave length at 205 nm.

The reference solution and test solution were precisely absorbed 10 μl and injected into the HPLC under the above chromatographic conditions respectively. The contents of catalpol, rehmannioside A, rehmannioside D, leonuride, verbascoside of each batch of RRR pieces were calculated by means of the external standard method.

2.3.1.3. Validation of the method

The precision, repeatability and stability experiments were conducted in method validation of HPLC. The calibration curves of 5 compounds were evaluated by plotting the peak area against corresponding solution concentration, respectively. The limits of detection (LODs) and limits of quantification (LOQs) were measured at S/N (signal-to-noise ratio) of about 3 and 10, respectively. The precision was assessed

by determining A1 solution six times continuously, whose results were expressed as relative standard deviation (RSD). To evaluate the repeatability, six independently prepared samples of raw Rehmanniae Radix (A1) were analyzed in parallel. The stability was assessed by analyzing prepared A1 solution at 0, 4, 8, 12 and 24 h within 1 day.

2.3.2 Appearance traits measurement

The appearance characteristics of 33 batches of RRR slices were observed, including the color of cut surface, stickiness, size, etc. A vernier caliper was used to measure the width of 100 pieces of 1kg pieces at random, the value was recorded and used to calculate average width. Large pieces whose width were greater than 1.5cm were picked from 100 pieces and weighed by analytical balance, and the value was recorded and calculated as weight ratio of large slices.

2.4 Statistical analysis

Data were expressed as means ± standard deviation. Correlation analysis was applied to explore the relation of appearance traits with the contents of water soluble extract and chemical compositions. Besides, PCA was conducted to find important indexes that could represent the quality information of RRR. Then the selected indicators after standardized were analyzed by Ward's cluster analysis. In addition, for comparison between different groups, data were statistically analyzed by non-parametric-test. Values of $P < 0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed by the SAS 9.2 software (SAS Inc.; Cary, NC, USA).

3. Results

3.1. Quantitative determination of five components in raw Rehmanniae Radix pieces

The contents of five components were calculated from the regression equations obtained from calibration curves. The results were presented in Fig. 1. All raw Rehmanniae Radix pieces samples were rich in rehmannioside D and catalpol, the content of rehmannioside D varied from 0.21 to 0.50 mg/g, and the content of catalpol ranged from 0.71 to 1.71 mg/g. In addition, the chemical component with the largest content variation was verbascoside, its content range was 0.020–0.268 mg/g. As for leonuride and rehmannioside A, the ranges of them were 0.040–0.135 mg/g, 0.043–0.069 mg/g, respectively. It was worth mentioning that the contents of catalpol and verbascoside in all samples were higher than 0.20 mg/g, 0.020 mg/g, respectively, which suggested that the quality of 33 batches of RRR pieces conforms to the standard of ChP (2015 edition). Besides, Fig. 1 presented that verbascoside content in RRR pieces from Henan province was obvious higher than that in other origins. However, the contents of rehmannioside D and catalpol were less than that in other origins. The results clearly suggested that there were substantial differences in the contents of the five components in 33 batches of RRR pieces from different origins.

3.2. Appearance traits of raw Rehmanniae Radix pieces

The measurement results of appearance traits of raw Rehmanniae Radix pieces were shown in Table 2. The average width ranged from 1.57 to 1.82 cm, and the weight ratio of large pieces varied from 23 to 41%. As for viscosity, samples form Henan and Shandong province were stickier than that from other origins. In addition, except for the samples from Anhui, Zhejiang, and Sichuan province, whose cut surface color was sepia, the samples from other origins were dark brown. The results demonstrated that the appearance traits were obvious different in 33 batches of RRR pieces.

Table 2
Appearance traits of raw Rehmanniae Radix pieces

No.	Average width (cm)	Weight ratio of large slicesa (%)	Viscosity	Section color
A1	1.79 ± 0.2	35	sticky	dark brown
A2	1.82 ± 0.2	40	sticky	dark brown
A3	1.78 ± 0.2	41	sticky	dark brown
A4	1.82 ± 0.2	36	sticky	dark brown
A5	1.75 ± 0.2	34	sticky	dark brown
A6	1.81 ± 0.2	37	sticky	dark brown
A7	1.82 ± 0.2	38	sticky	dark brown
A8	1.81 ± 0.2	40	sticky	dark brown
A9	1.80 ± 0.2	37	sticky	dark brown
A10	1.81 ± 0.2	38	sticky	dark brown
A11	1.57 ± 0.2	23	slightly sticky	sepia
A12	1.57 ± 0.2	33	slightly sticky	sepia
A13	1.58 ± 0.2	29	slightly sticky	sepia
A14	1.61 ± 0.2	27	slightly sticky	sepia
A15	1.62 ± 0.2	25	slightly sticky	sepia
A16	1.61 ± 0.2	31	slightly sticky	sepia
A17	1.59 ± 0.2	25	slightly sticky	sepia
A18	1.60 ± 0.2	30	slightly sticky	sepia
A19	1.59 ± 0.2	28	slightly sticky	sepia
A20	1.58 ± 0.2	29	slightly sticky	sepia
A21	1.61 ± 0.2	26	slightly sticky	sepia
A22	1.60 ± 0.2	29	slightly sticky	sepia
A23	1.61 ± 0.2	28	slightly sticky	sepia
A24	1.62 ± 0.2	29	slightly sticky	sepia
A25	1.61 ± 0.2	28	slightly sticky	sepia

[a]The weight ratio of large slices with the width greater than 1.5 cm

No.	Average width (cm)	Weight ratio of large slicesa (%)	Viscosity	Section color
A26	1.70 ± 0.2	31	slightly sticky	dark brown
A27	1.69 ± 0.2	31	slightly sticky	dark brown
A28	1.70 ± 0.2	32	slightly sticky	dark brown
A29	1.71 ± 0.2	33	slightly sticky	dark brown
A30	1.70 ± 0.2	32	slightly sticky	dark brown
A31	1.71 ± 0.2	31	sticky	dark brown
A32	1.70 ± 0.2	34	sticky	dark brown
A33	1.72 ± 0.2	32	sticky	dark brown
[a]The weight ratio of large slices with the width greater than 1.5 cm				

3.3. Correlation analysis of the appearance traits and chemical compositions in raw Rehmanniae Radix pieces.

Correlation analysis was applied to explore the correlation between appearance traits and chemical components of RRR pieces, and the results were presented in Fig. 2. Both the average width and the weight ratio of large slices of RRR pieces were positively correlated with the contents of verbascoside ($P < 0.0001$), while negatively correlated with the contents of catalpol, rehmannioside D and leonuride ($P < 0.0001$), and had nothing to do with the content of rehmannioside A. In fact, rehmannioside A had not only nothing to do with the appearance traits, as well as 4 chemical components. In addition, catalpol, rehmannioside D and leonuride had positive correlation with each other($P < 0.0001$), but all had negative correlation with verbascoside($P < 0.0001$).

3.4. Principal component analysis of samples

The principal component analysis was performed on six indicators: the average width, the weight ratio of large slices, the contents of verbascoside, catalpol, leonuride, and rehmannioside D. The results presented that the first principal factor, the variance contribution rate reached 75.601%; for the first two principal factors, the cumulative variance contribution rate accounted for 88.240% (> 80.0%) of the total contribution rate. These data indicated that the first two principal factors could reflect most of the information of RRR pieces, and can be applied to quantitatively classify 33 batches of RRR pieces. The PCA score plot showed that PC1 and PC2 scores of samples from Henan province were mainly positive; samples from Shandong and Shanxi province were located together; and the PC1 scores of samples from Anhui, Zhejiang, Sichuan province were negative (Fig. 3). The result demonstrated that there are significant differences in appearance traits and constituents of different origins of RRR pieces.

3.5. Cluster analysis of samples

The average group linkage was chosen as the clustering method, and the Squared Euclidean Distance was the distance measurement method. After the data were standardized, a systematic cluster analysis was carried out. The results were presented in Fig. 4.
33 batches of raw Rehmanniae Radix pieces were clustered based on the score of the first two principal components of samples. Figure 4 showed that 33 batches of RRR pieces could be divided into three categories. Samples A1-A4, A6-A10 were in Group A, which was from Wen County, Henan Province. Group B included samples A26-A33, A5, A22. Except A5 and A22, the other was from Shandong and Shanxi Province. Samples A11-A21, A23-A25 were in Group C. The results of cluster analysis demonstrated that there were similarity and differences between various samples from different origins, which may provide more references for further quality studies of RRR pieces.

3.6. t-Test of samples

The average width, weight ratio of large slices and contents of detected constituents were analyzed by t-test to evaluate the variation of appearance traits and 4 constituents in different groups of RRR pieces, the values of $p < 0.05$ were considered statistically significant differences. As shown in Fig. 5, the average width, weight ratio of large slices, the contents of verbascoside and catapol were remarkably different in Group A, B, C. The average width and weight ratio of large slices in Group A were greater than those in Group B and C ($p < 0.001$), and verbascoside content in Group A was higher than that in Group B and C ($p < 0.001$). However, the contents of catalpol and leonuride in Group A were less than those in Group C. There were no significant differences of rehmannioside D and leonuride between Group A and Group B.

4. Discussion

With the widespread application of RRR pieces, its quality control has attracted more and more attention. Li H.Y. et al.[12] established HPLC-ultraviolet detection-2, 2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) free radical scavenging (HPLC-UV-ABTS) method to determine the content of iridoid glycosides and its antioxidant activity to evaluate the quality of RRR; Wang X. et al.[21] took iridoid glycosides and oligosaccharides as detection indicators to study the quality of RRR; and some scholars analyzed the quality of RRR by establishing its fingerprint[24, 25]. However, previous studies ignored the investigation of the appearance traits and origins of RRR. Studies have found that the content of active ingredients of RRR from different origins is varied[26, 27], which suggested that the quality of RRR is affected by many factors, and the evaluation methods of RRR have been limited. Based on previous research and analysis, we combined the chemical constituents, appearance traits and origins of RRR to comprehensively evaluate the quality of RRR pieces.

Traditionally, the quality control of traditional Chinese medicine mainly takes origins and appearance traits as indicators. The origin is of great importance, it includes many affected herbs growth factors, such as soil conditions, light, moisture, and climate[28]. Rehmannia glutinosa is widely planted in China. It mainly grows in Henan, Hebei, Shandong, Shanxi, Hubei, Sichuan and other provinces. Among them, Henan Province has been considered as the genuine producing area of RRR, which means the RRR from Henan province is of excellent quality. The PCA and cluster analysis results showed that the samples from Henan could be obviously discriminated from other origins’, and t-test revealed that the chemical

components, appearance traits in RRR pieces from Henan and other origins were significant different. Those results suggested that origin is a vital factor of quality control in RRR pieces.

The contents of chemical constituents of Chinese medicinal materials vary from place to place. Tan M.X. et al.[29] applied ultra-fast performance liquid chromatography coupled with triple quadrupole-linear ion trap mass spectrometry (UFLC-QTRAP-MS/MS) to measure 36 components of Ophiopogonis Radix and found the bioactive constituents in Ophiopogonis Radix from different regions were significantly different; Shu Z. et al.[30] employed reversed-phase high-performance liquid chromatography coupled with hierarchical clustering analysis to evaluate the quality of Vitex negundo seeds, and observed significant differences in the content of lignans of Vitex negundo seeds from different origins; Cao Y. et al.[31] in order to evaluate the quality consistency of Chansu, established HPLC fingerprints of Chansu and discovered that the fingerprint profile of Chansu from different geographical origins were different. These studies have fully revealed that the contents of chemical components of traditional Chinese medicine are affected by origin. In the 2015 edition of the ChP, the quality control components of RRR are verbascoside and catalpol[19], so we determined the contents of the two in RRR pieces by HPLC, and found that the content of verbascoside of RRR pieces from Henan province was higher than that in other origins. While catalpol content in RRR pieces from Henan province was less than that in other origins, which is same as Xue Shujuan's[32], and opposite of Li Min's[33]. The reason why the content of catalpol in RRR varies greatly is due to its unstable chemical properties. It is easily affected by temperature, light, processing methods and other factors[34, 35]. Moreover, as rehmannioside D, and leonuride are the contents into the blood with in prototype forms[36], and rehmannioside D could significantly reduce blood glucose, and rehmannioside A has a pharmacological effect of increasing immunity and protecting nerves[37, 38], we determined them, and discovered that RRR pieces was low in rehmannioside A and leonuride, and there were no significant differences of rehmannioside D and leonuride in RRR pieces from different origins. Therefore, considering the stability of chemical properties and the results of *t*-test, verbascoside may be regarded as the chemical marker to control the quality of RRR pieces.

Appearance characters of Chinese medicinal materials are diverse in different origins. Xu Z.Z. et al.[39] employed electronic nose technology to obtain the odor information of Aucklandiae Radix, and found that the odor fingerprints of Aucklandiae Radix from different origins were different; Ren W.H. et al.[40] compared the color of Angelicae sinensis radix with the color chip, and discovered that the color of Angelicae sinensis from different regions was various, and the darker the Angelicae sinensis is, the higher contents of active ingredients it contained; Zhou Y.F. et al.[41] reported that there were great differences in the color, 100-sees weight, and pulp content of Schisandrae Chinensis Fructus(SCF) from different origins. The above studies have demonstrated that there is a definite relationship between the origin and the appearance of Chinese medicinal materials. In our study, it was found that the appearance of RRR pieces from diverse origins had great differences. The average width and weight ratio of large slices of RRR pieces from Henan province were greater than those in other origins, which accords with the traditional experience in evaluating the quality of RRR. Traditional experience holds the view that RRR from Henan province has black color, large size and heavy weight, and is of good quality[42].

The contents of chemical components in Chinese herbal medicines are diverse with different appearance traits. For instance, one study revealed that Asian and American ginsengs with thicker main roots contained higher contents of ginsenosides[43]; for another example, Liu, W et al. reported that the color of Salvia miltiorrhiza had significant positive correlation with Tanshinone Ⅱand the total content of lipsoluble components[44]. The above research has indicated that the appearance traits of traditional Chinese medicine are relevant to chemical composition. In our study, it was found that both the average width and the weight ratio of large slices of RRR pieces were positively correlated with the contents of verbascoside, which suggested that the heavier and bigger RRR pieces are, the higher contents of verbascoside they contained.

Based on the above analysis, the chemical components, appearance traits and origins of traditional Chinese medicine are not isolated, but are interrelated with each other, and each has its own emphasis. The chemical compositions is an indirect efficacy index. Measuring its content can characterize the efficacy to a certain extent; the appearance traits are intuitive physical indicators, they are easier to detect and observe than chemical components; the origin is a comprehensive geographical indicator, which can represent environmental factors that affect the quality of traditional Chinese medicine. Therefore, the method of evaluating the quality of traditional Chinese medicine that integrates the chemical compositions, appearance traits and origins could learn from each other' s strengths and offset weaknesses, which is beneficial to control the quality of traditional Chinese medicine more comprehensively and effectively.

5. Conclusions

In our study, we found that the contents of chemical components and the appearance traits of RRR pieces were different in diverse origins, which indicated that origin is an important factor that could affect the quality of RRR pieces. Furthermore, the results of this study demonstrated that the combined method of chemical compositions, appearance characters and origins could classify and distinguish different quality of RRR pieces, which suggested that the combined method not only avoids the neglect of the attributes of some features in Chinese herbs by the quality evaluation methods based on chemical constituents; but also overcomes the ambiguity and subjectivity of quality assessment of Chinese medicine by origins and appearance traits. Therefore, it is commendable and favorable to carry out further research about quality evaluation and control of Chinese medicine by the combined method. Although we had classified different qualities of RRR pieces into three categories with the combined method, we still think that the results of classification based on the combined method should be verified by pharmacology or clinical experiments. Therefore, the scientific, practical and comprehensive quality evaluation and control of TCM still require more and more efforts.

Abbreviations

TCM: Traditional Chinese medicine

RRR: Raw Rehmanniae Radix

HPLC: High performance liquid chromatography

PCA: Principal components analysis

ChP: Pharmacopoeia of the People's Republic of China

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

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Figures

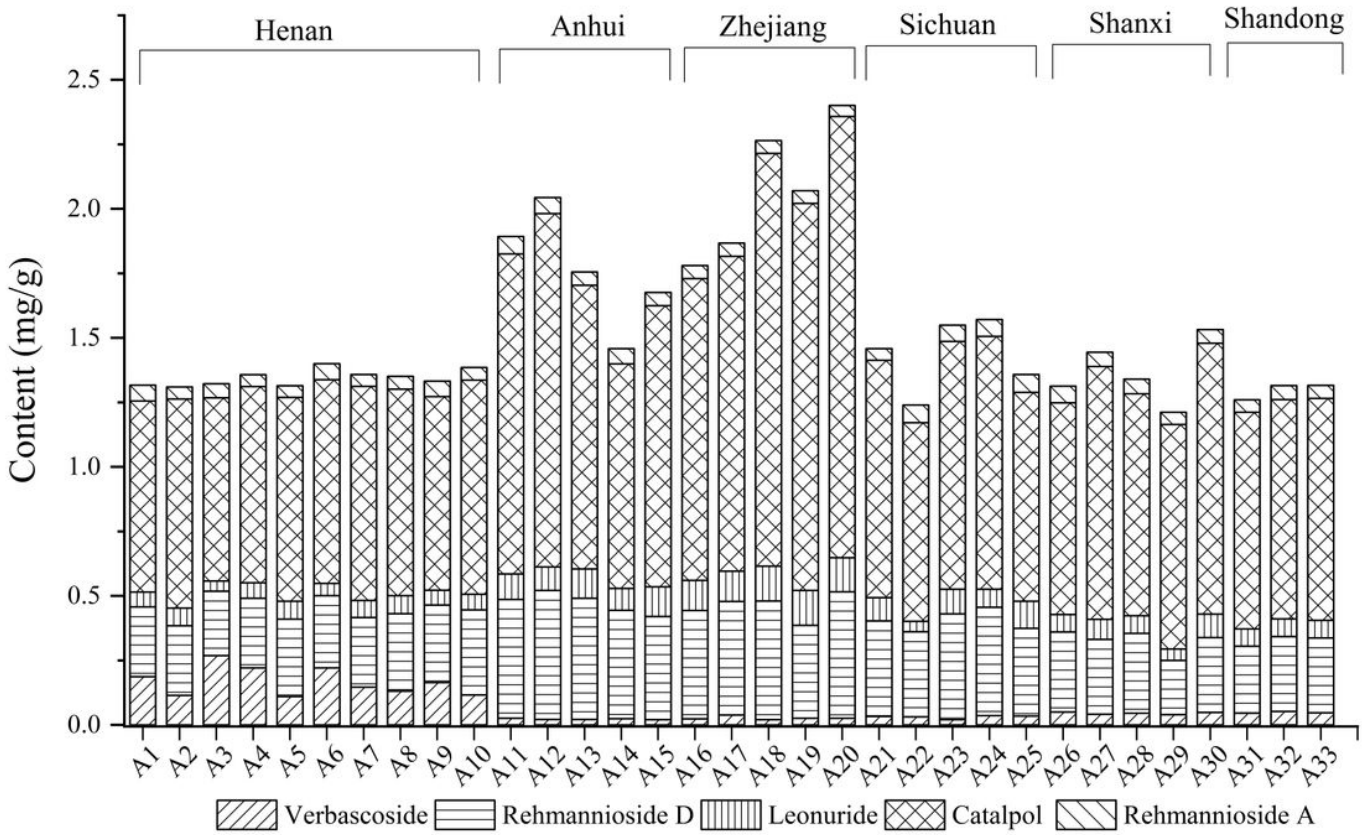

Figure 1

The contents of five chemical components in raw Rehmanniae Radix pieces from different origins.

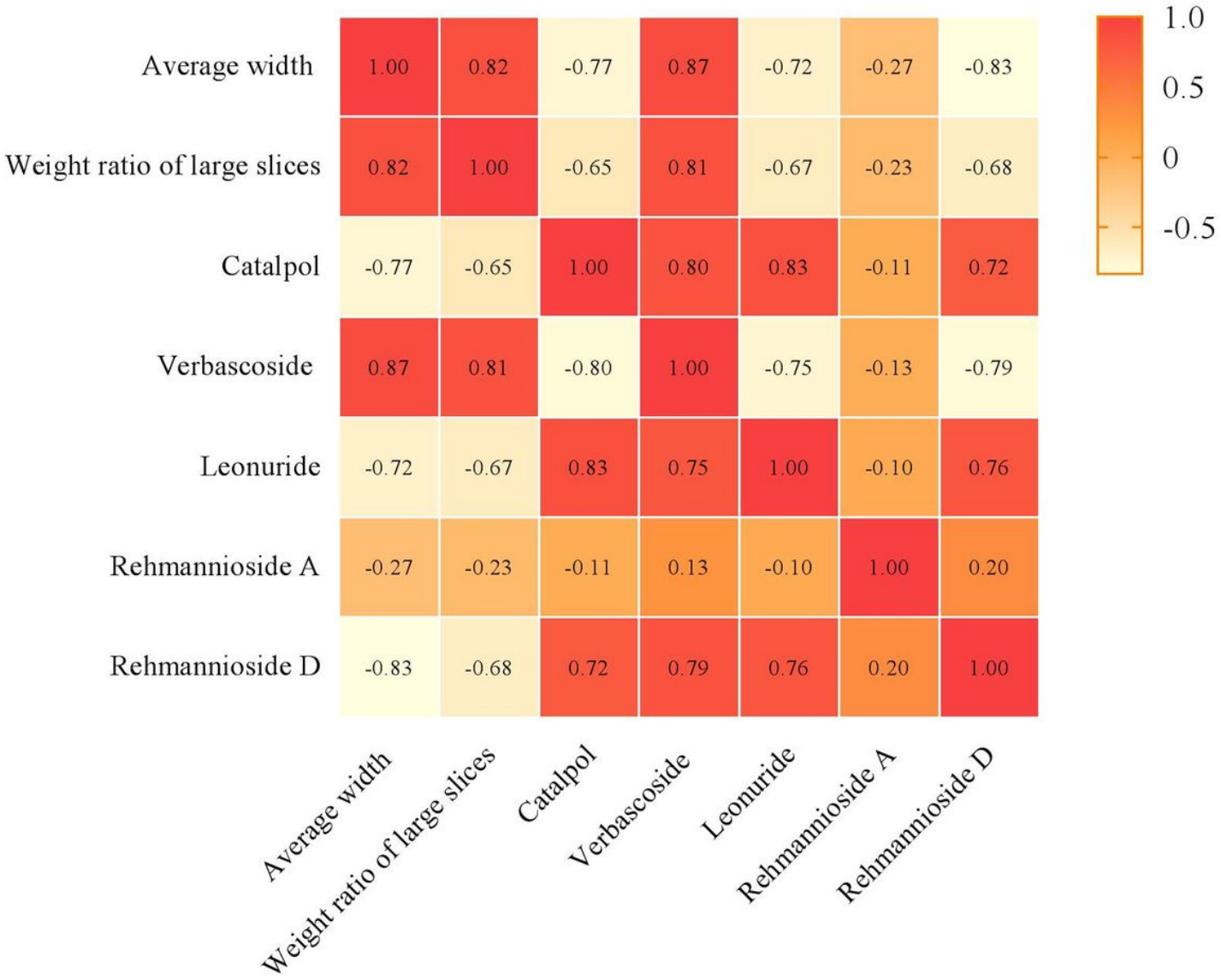

Figure 2

Correlation matrix heat map of appearance traits and chemical constituents of Rehmanniae Radix pieces

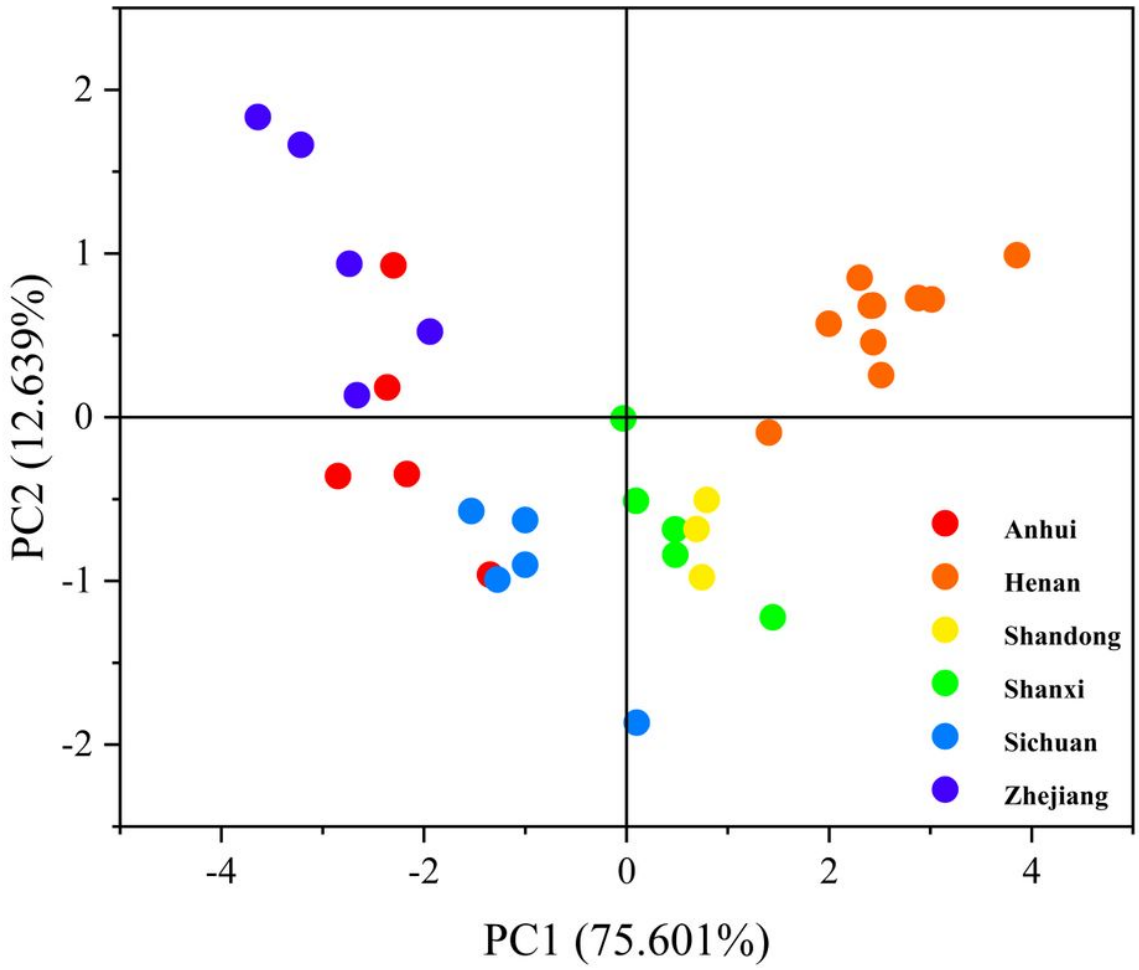

Figure 3

The PCA of 33 batches Rehmanniae Radix pieces from different origins.

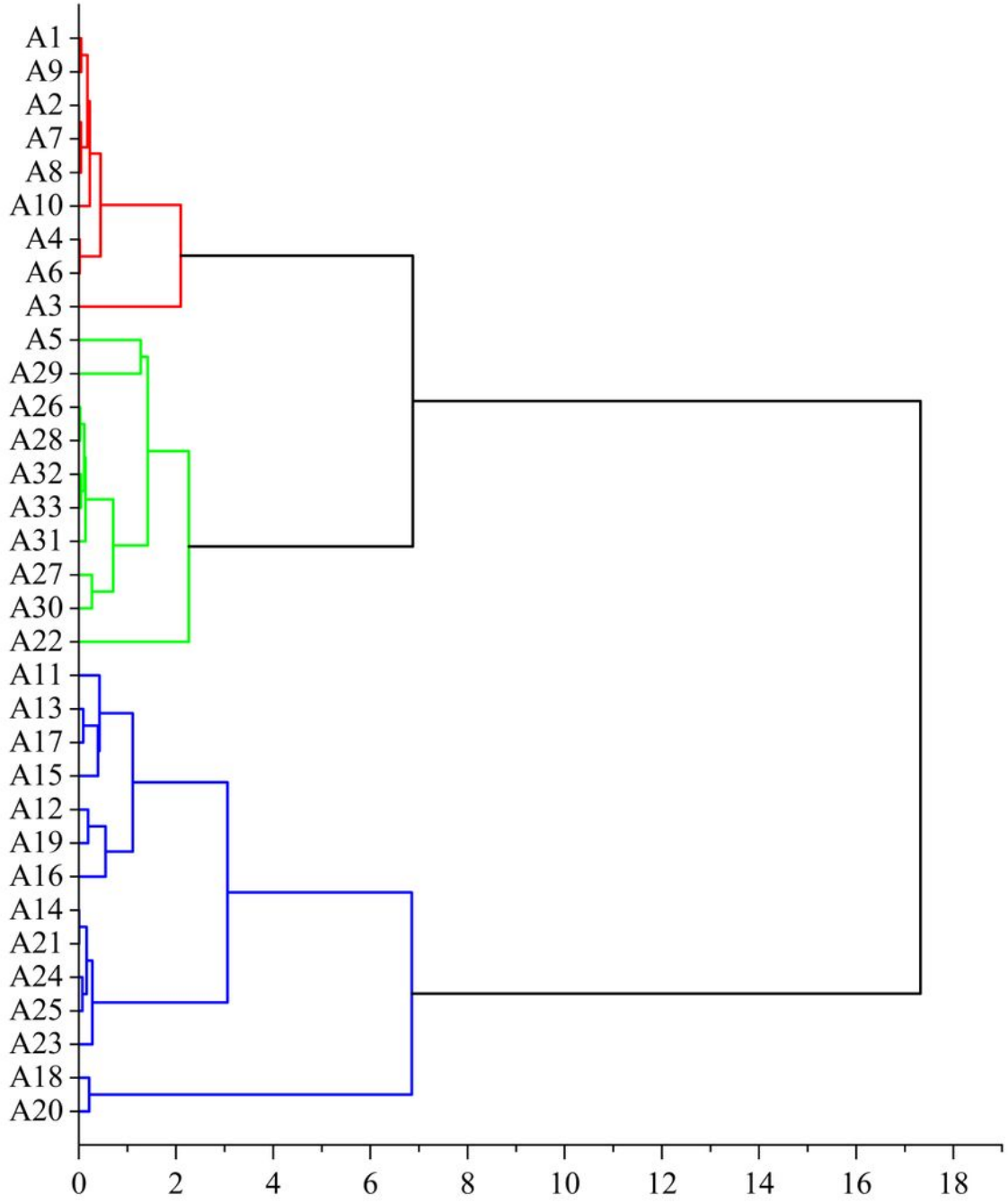

Figure 4

The dendrogram of 33 batches of raw Rehmanniae Radix pieces pieces by cluster analysis

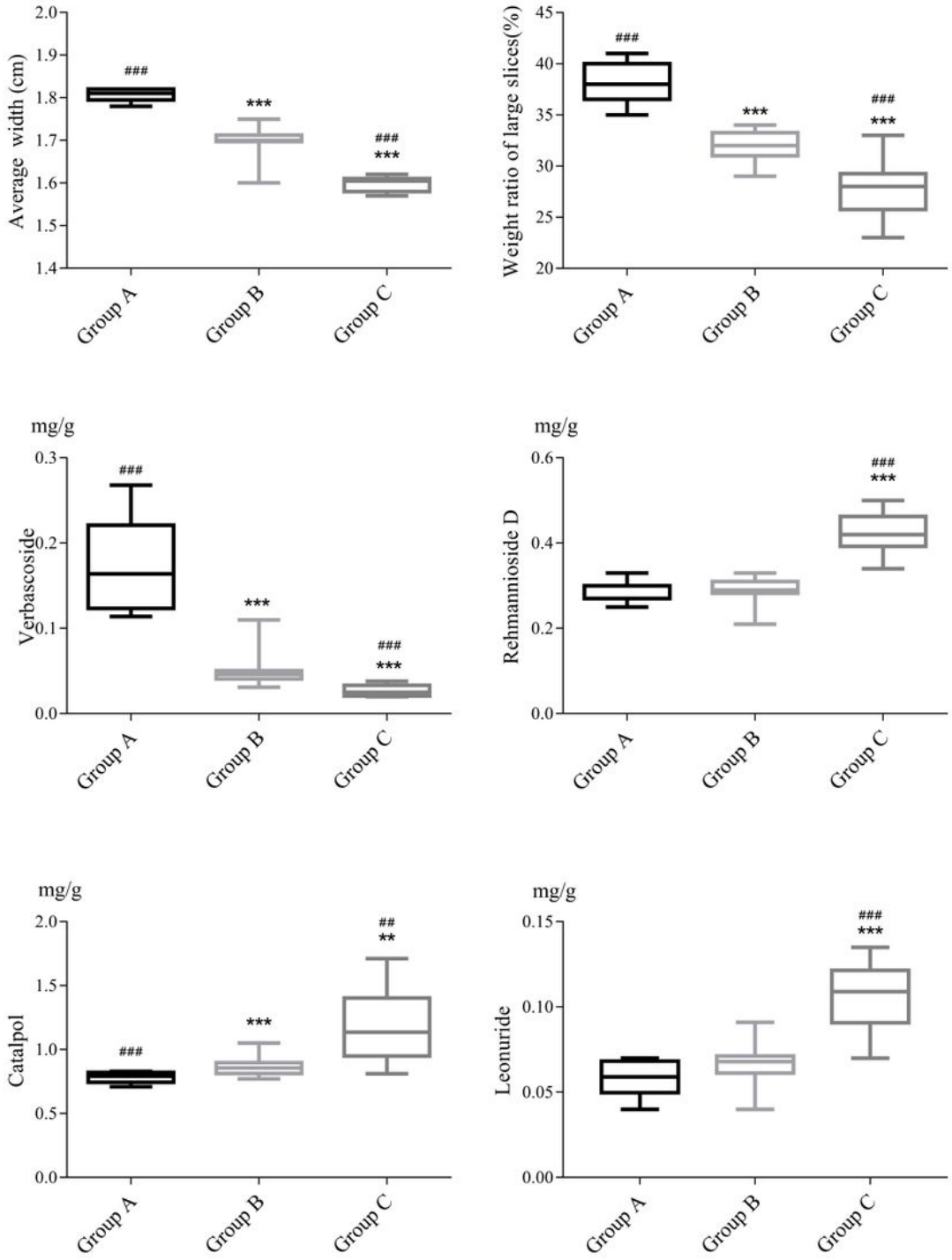

Figure 5

The box plot of five indexes in different groups of raw Rehmanniae Radix pieces. **P<0.01, ***P< 0.001, compared with Group A; ##P<0.01, ###P< 0.001, compared with Group B.