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Research Article

Quality Evaluation of Market *Acacia catechu* by Fingerprint-Chemical Pattern Recognition

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Acacia catechu (L.f.) Willd, a leguminous plant, is included in the 2020 edition of the Chinese Pharmacopoeia and is mainly used to treat eczema, mouth ulcers, diarrhea, bruising, and traumatic hemorrhage. However, there are imported and domestic *Acacia catechu* samples available in China, and their quality and price are very different, which seriously affects the safety and stability of their clinical application. Importantly, there is no simple and effective method for identifying or classifying grades of *Acacia catechu*. In this study, 47 batches of commercial *Acacia catechu* were used for identifying or classifying grades of *Acacia catechu* using high performance liquid chromatography (HPLC) combined with chemometric analysis. Firstly, gradient elution was adopted with 0.05% phosphoric acid water (A)-methanol (B) as the mobile phase to establish chromatographic conditions. The HPLC chromatograms of 47 batches of *Acacia catechu* samples were analyzed by the "Similarity Evaluation System for Chromatographic Fingerprint of TCM" software (version 2012A). The common peaks of *Acacia catechu* were identified to evaluate the similarity. Based on the determination results of fingerprint chromatographic peak area, the quality of the collected *Acacia catechu* was evaluated by chemometric methods such as CA, PCA, and OPLS-DA. The results showed that the collected *Acacia catechu* samples were significantly divided into three categories. The first-class samples were all imported *Acacia catechu* except S9 sample, which was domestic *Acacia catechu*; the second-class samples were partly domestic *Acacia catechu* and partly imported *Acacia catechu*; and the third-class samples were all domestic *Acacia catechu*. Moreover, OPLS-DA of 47 batches of samples showed that the contents of catechin and the total contents of catechin and epicatechin could be used as key indicators for assessing the quality of *Acacia catechu*. The developed HPLC fingerprint and quantitative analysis method of multi-indicator components can be used for classification and quality evaluation of market *Acacia catechu*, which has a significant reference value for developing *Acacia catechu* grade quality standards.

1. Introduction

Traditional Chinese medicine *Acacia catechu* is the dried decoction of peeled branches and trunks of *Acacia catechu* (L.f.) Willd. [1], which has antidiabetic [2], antihypertensive [3], hepatoprotective [4], antioxidant [5], antibacterial [6], anticancer [7, 8], anti-inflammatory [9], and immunomodulatory effects [10] and is mainly used to treat eczema, mouth ulcers, diarrhea, bruising, and traumatic hemorrhage [11, 12]. In China, the medicinal use of *Acacia catechu* started from "Compendium of Herbology" in the Ming Dynasty (AD 1552–1578), known as "*Wudieni*" and "*Haiercha*." In addition, it is worth paying attention to that the origin of *Acacia catechu* also includes the dried extract of the decoction of the leafy shoots of *Uncaria gambir* (Hunter) Roxb, which was recorded in the Dictionary of Chinese Pharmacy, National Compilation of Chinese Herbal Medicine (Third Edition), Chinese Basic Medicinal Herbs, etc. Therefore, there is a need for a standardized assessment and monitoring of the quality of market *Acacia catechu*.

Market research by our team found that there are only domestic *Acacia catechu* and imported *Acacia catechu* on the market. Moreover, the price of imported *Acacia catechu* is two times higher than that of domestic products. However, the intrinsic quality of market *Acacia catechu* is also mixed and varies greatly. In terms of color appearance, the surface color of both imported and domestic *Acacia catechu* is black or reddish-brown. After crushing, the color of *Acacia catechu* powder varies from earthy yellow to reddish-brown. Importantly, domestic *Acacia catechu* is mostly reddish-brown, while imported *Acacia catechu* is mostly earthy yellow (Figure 1). Therefore, it is important to explore whether the appearance correlates with the herbs' intrinsic quality. Simultaneously, the research questions need to be urgently studied and explained, including whether the division between imported *Acacia catechu* and domestic *Acacia catechu* in the market can reflect the intrinsic quality of the herbs and whether the price difference has any actual scientific connotation.

The total content of catechin and epicatechin is the key indicator for evaluating *Acacia catechu* quality in the Chinese Pharmacopoeia (2020 version) [1]. However, the complexity and diversity of the chemical components of traditional Chinese medicine determine that the detection results of only a few components as evaluation indicators cannot reflect the quality of traditional Chinese medicine comprehensively and accurately [13]. The chromatographic fingerprint in particular is significant for the evaluation of traditional Chinese medicine, which can better reflect the overall chemical composition of traditional Chinese medicine and is widely used [14–17]. Li and Chen [18] have established the chromatographic fingerprint of *Acacia catechu* by HPLC. Based on this, this study enriched the scientificity, representativeness, and universality of test samples and optimized the chromatographic conditions. Furthermore, HPLC fingerprints of 47 batches of *Acacia catechu* were established, and the contents of catechin and epicatechin in *Acacia catechu* were determined. The similarity evaluation combined with cluster analysis (CA), principal component analysis (PCA), and orthogonal partial least squares discriminant analysis (OPLS-DA) was used to analyze the quality grades of market *Acacia catechu*, in order to provide a scientific basis for developing classification standards and quality evaluation of *Acacia catechu*.

2. Materials and Methods

2.1. Materials and Reagents. Control samples of *Acacia catechu* (Batch No. 121397-201502, National Institutes for Food and Drug Control), catechin (Batch No. HC019123198, 98%), and epicatechin (Batch No. HE019124198, 98%) were purchased from Chenguang Biotechnology Co., Ltd. Epicatechin gallate (Batch No. C07J8Y39406, 98%) and quercetin (Batch No. C28J11Y116820, 98%) were purchased from Lvyuan Biotechnology Co., Ltd. Methanol (chromatographic pure, Shanghai Macklin Biochemical Technology Co., Ltd.), phosphoric acid (analytical pure, Sinopharm Chemical Reagent Co., Ltd.), and water (Hangzhou Wahaha Co., Ltd.) were used as received. The sample information of 45 batches of *Acacia catechu* is shown in Table1, collected from the Anguo medicinal materials market in Hebei Province, Bozhou medicinal materials market in Anhui Province, Hehuachi medicinal material market in Chengdu, and Yulin medicinal material market in Guangxi Province. Domestic samples were numbered 1–21, and imported samples were numbered 22–45. The control samples of *Acacia catechu* were numbered 46–47.

2.2. Apparatus and Conditions. HPLC analysis was performed on a Shimadzu LC-2030 HPLC system, which consisted of a PDA detector, LabSolutions software, a quaternary pump, and an autosampler. All separations were performed on an Agilent C18 column (250 mm × 4.6 mm, 5 μM). The mobile phase was composed of 0.05% aqueous phosphoric acid (v/v) (A) and methanol (B) with the following gradient elution: 0–12 min: 8–25% B; 12.01–40 min: 25–40% B; 40.01–55 min: 40–70% B; 55.01–60 min: 8% B. The column temperature and flow rate were set at 25°C and 1 mL/min. The injection volume was 20 μL, and the detection wavelength was 265 nm.

2.3. Preparation of Sample Solutions. Acacia catechu was crushed and filtered through a 65-mesh sieve. An amount of 100 mg *Acacia catechu* powder was accurately weighed and placed in a 25 mL volumetric flask with a stopper, and 50% methanol was added to the scale. Ultrasonication was performed for 20 min, and then 50% methanol was used to compensate for the weight loss during the extraction. The extract was filtered through a 0.22 μm membrane and stored at 4°C for further experiments.

2.4. Similarity Analysis. The raw HPLC chromatographic data of 47 batch samples were exported as AIA format files. The software "Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine" (Version 2012, Chinese Pharmacopoeia Committee) was used to analyze similarity. The reference fingerprint was obtained automatically by the median method with a time window width of 0.5 min, and the similarity values of all the samples were calculated.

2.5. Chemical Pattern Recognition Analysis. In order to further analyze the quality grade of *Acacia catechu*, the possible grades of market *Acacia catechu* were clarified, and the differences of different grades were evaluated. The 218 peak areas with different retention times from the chromatographic peak matching data of *Acacia catechu* in the "Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine" were used as variables, and 47 batches of *Acacia catechu* were analyzed using CA, PCA, and OLPS-DA.

2.6. Software. The software "Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese

Figure 1: The powder color of market *Acacia catechu*. The samples are S1 sample of domestic *Acacia catechu* (a), S19 sample of domestic *Acacia catechu* (b), and S38 sample of imported *Acacia catechu* (c).

Table 1: Contents of 47 batches of *Acacia catechu* ($n = 2$).

No.	Origin	Content of catechin (%) (A)	Content of epicatechin (%) (B)	The sum of catechin and epicatechin contents (%) (C)	The ratio of catechin and epicatechin contents (%) (D)	Class
S9	Bozhou medicinal material market (Domestic)	34.69	4.21	38.89	8.25	1
S22	Bozhou medicinal material market (Imported)	33.12	3.82	36.94	8.66	1
S23	Bozhou medicinal material market (Imported)	35.19	2.69	37.88	13.09	1
S24	Bozhou medicinal material market (Imported)	28.33	2.26	30.59	12.52	1
S25	Bozhou medicinal material market (Imported)	34.47	3.97	38.44	8.67	1
S27	Bozhou medicinal material market (Imported)	27.02	2.94	29.97	9.18	1
S28	Anguo medicinal material market (Imported)	36.99	3.59	40.58	10.31	1
S29	Anguo medicinal material market (Imported)	34.63	1.89	36.52	18.27	1
S30	Anguo medicinal material market (Imported)	36.7	3.56	40.26	10.3	1
S32	Anguo medicinal material market (Imported)	32.71	4.3	37.01	7.61	1
S33	Anguo medicinal material market (Imported)	33.22	3.76	36.98	8.84	1
S34	Hehuachi medicinal material market (Imported)	33.34	3.75	37.1	8.88	1

Table 1: Continued.

No.	Origin	Content of catechin (%) (A)	Content of epicatechin (%) (B)	The sum of catechin and epicatechin contents (%) (C)	The ratio of catechin and epicatechin contents (%) (D)	Class
S35	Hehuachi medicinal material market (Imported)	35.14	4.47	39.61	7.86	1
S36	Hehuachi medicinal material market (Imported)	26.56	2.85	29.41	9.31	1
S37	Hehuachi medicinal material market (Imported)	33.3	3.01	36.3	11.08	1
S38	Hehuachi medicinal material market (Imported)	29.97	3.05	33.03	9.82	1
S41	Yulin medicinal material market (Imported)	39.57	2.66	42.23	14.89	1
S42	Yulin medicinal material market (Imported)	41.89	3.2	45.08	13.11	1
S43	Yulin medicinal material market (Imported)	34.2	2.77	36.97	12.34	1
S44	Yulin medicinal material market (Imported)	36.68	2.77	39.46	13.23	1
S45	Yulin medicinal material market (Imported)	32.97	3.61	36.59	9.12	1
S2	Bozhou medicinal material market (Domestic)	15.43	7.06	22.5	2.19	2
S4	Bozhou medicinal material market (Domestic)	16	6.57	22.58	2.44	2
S5	Bozhou medicinal material market (Domestic)	15.94	8.34	24.28	1.91	2
S11	Bozhou medicinal material market (Domestic)	13.01	5.53	18.54	2.35	2
S19	Hehuachi medicinal material market (Domestic)	18.1	7.01	25.1	2.58	2
S26	Bozhou medicinal material market (Imported)	13.87	5.27	19.14	2.63	2
S31	Anguo medicinal material market (Imported)	22.18	8.44	30.62	2.63	2
S39	Hehuachi medicinal material market (Imported)	20.71	8.77	29.48	2.36	2
S40	Yulin medicinal material market (Imported)	21.65	2.75	24.4	7.86	2
S46	National institutes for food and drug control	18.71	9.81	28.52	1.91	2
S47	National institutes for food and drug control	18.13	10.24	28.37	1.77	2
S1	Bozhou medicinal material market (Domestic)	0.4	0.02	0.42	17.32	3

Table 1: Continued.

No.	Origin	Content of catechin (%) (A)	Content of epicatechin (%) (B)	The sum of catechin and epicatechin contents (%) (C)	The ratio of catechin and epicatechin contents (%) (D)	Class
S3	Bozhou medicinal material market (Domestic)	2.37	0.8	3.17	2.95	3
S6	Bozhou medicinal material market (Domestic)	1.3	0.1	1.4	13.58	3
S7	Bozhou medicinal material market (Domestic)	0.23	0.03	0.26	7.65	3
S8	Bozhou medicinal material market (Domestic)	0.23	0.06	0.29	4.16	3
S10	Bozhou medicinal material market (Domestic)	0.52	0.18	0.69	2.92	3
S12	Anguo medicinal material market (Domestic)	0.05	0	0.05	0	3
S13	Anguo medicinal material market (Domestic)	2.81	0.82	3.63	3.44	3
S14	Hehuachi medicinal material market (Domestic)	0.2	0.01	0.21	13.14	3
S15	Hehuachi medicinal material market (Domestic)	0.41	0.05	0.46	8.2	3
S16	Hehuachi medicinal material market (Domestic)	2.42	1.09	3.51	2.22	3
S17	Hehuachi medicinal material market (Domestic)	7.17	2.2	9.37	3.26	3
S18	Hehuachi medicinal material market (Domestic)	1.98	0.78	2.76	2.56	3
S20	Yulin medicinal material market (Domestic)	0.4	0.16	0.56	2.52	3
S21	Yulin medicinal material market (Domestic)	0.27	0.04	0.31	6.35	3

Medicine" (Version 2012, Chinese Pharmacopoeia Committee) was used to assess the fingerprint similarity of *Acacia catechu*. SPSS 23.0 (IBM Corporation, USA) was used to build a CA unsupervised pattern recognition model. Unscrambler X 10.4 (CAMO Corporation, Norway) was used to establish a PCA unsupervised pattern recognition model. SIMCA-P 11.5 (Sartorius Stedim Data Analytics AB, Germany) was used to establish an OPLS-DA supervised pattern recognition model.

3. Results and Discussion

3.1. Optimization of Sample Preparation. Previous literature review revealed that the extraction of *Acacia catechu* was mostly done by ultrasonic extraction with 50% methanol, but the concentration of the test preparation varied widely from 0.4 to 12 mg/mL [19]. In this study, sample concentrations including 0.4 mg/mL, 8 mg/mL, and 12 mg/mL were prepared for the relevant experiments. The results showed that the fingerprints of most domestic samples were almost a straight line without useful peaks at the concentration of 0.4 mg/mL (Figure 2). At the concentration of 12 mg/mL, most samples had poor separation of catechins, while the test samples were able to obtain relatively reasonable peaks at the concentration of 8 mg/mL. Thus, a sample concentration of 8 mg/mL was used in the study (Figure 3).

3.2. Optimization of Chromatographic Conditions. The mobile phase compositions, including water-methanol, water-acetonitrile, 0.01% aqueous phosphoric acid-methanol, and 0.05% aqueous phosphoric acid-methanol, were screened for establishing chromatographic fingerprint. The results showed that acetonitrile and methanol had similar elution effects and 0.05% aqueous phosphoric acid could greatly improve the peak shape and separation effect of the

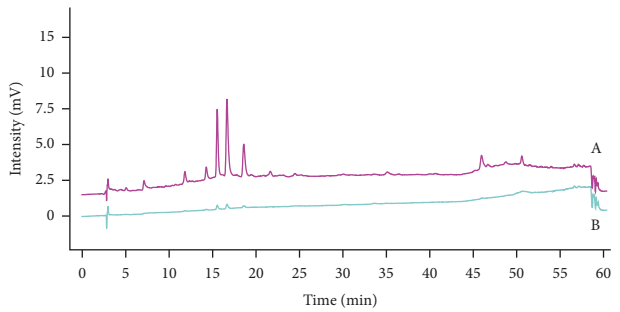

Figure 2: Effect of different concentrations of S7 sample on chromatogram (A for 8 mg/mL, B for 0.4 mg/mL).

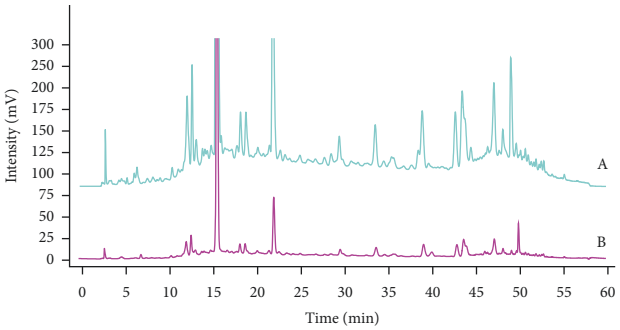

Figure 3: Effect of different concentrations of S22 sample on chromatogram (A for 12 mg/mL, B for 8 mg/mL).

chromatographic fingerprint. Finally, 0.05% aqueous phosphoric acid (A)-methanol (B) was utilized as the optimum mobile phase (Figure 4). Detection wavelengths of 245 nm, 265 nm, 280 nm, and 300 nm were investigated to obtain useful chemical information. It was found that using the wavelength of 265 nm could get a smoother baseline and more detectable peaks. Therefore, the wavelength of 265 nm was selected (Figure 5).

3.3. HPLC-DAD Fingerprint

3.3.1. Method Validation of Fingerprint. The analytical method was validated through precision, repeatability, and stability. One sample was prepared, and the chromatographic fingerprints were recorded in six consecutive injections. The similarity between the fingerprints was calculated and was not less than 0.999, indicating that the instrument has good precision. Six independent samples were prepared in parallel, and the chromatographic fingerprints were recorded. The similarity between the fingerprints was calculated and was not less than 0.997, indicating that the method has good repeatability. One sample was prepared and stored at room temperature for 0, 2, 4, 8, 12, and 24 h for the evaluation of stability. The results showed that the similarity between the fingerprints was calculated and was not less than 1.000, indicating that the samples were stable for 24 h. All in all, the method was suitable for fingerprint analysis.

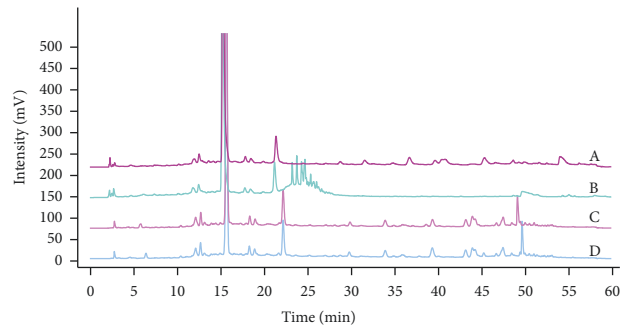

Figure 4: Effect of different chromatographic conditions on chromatogram (S38 sample: A for water-methanol, B for water-acetonitrile, C for 0.01% phosphoric acid water-methanol, D for 0.05% phosphoric acid water-methanol).

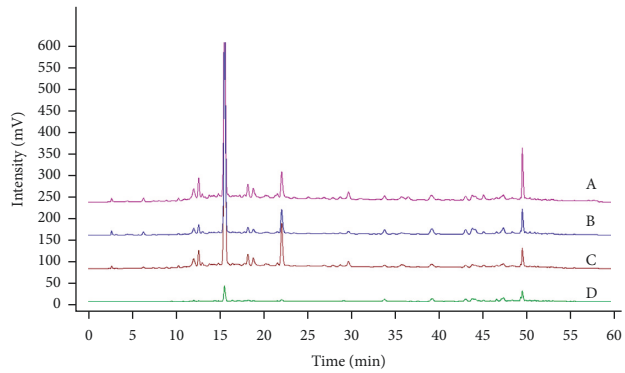

Figure 5: Effect of different detection wavelengths on chromatogram (S38 sample: A for 245 nm, B for 265 nm, C for 280 nm, D for 300 nm).

3.3.2. HPLC-DAD Fingerprint Analysis. The results of fingerprints analysis are shown in Figures 6 and 7. In the fingerprints, 14 peaks were defined as common peaks, and peaks 5, 7, 8, and 14 were identified as catechin, epicatechin gallate, epicatechin, and quercetin, respectively. The similarities were calculated according to the sample fingerprints and reference fingerprints. The results of the similarity analysis are shown in Table 2. The similarities were in the range of 0.965–0.996 for imported *Acacia catechu* and 0.111–0.996 for domestic *Acacia catechu*. Among the domestic *Acacia catechu* samples, the similarities of nine samples (S16, S15, S20, S21, S10, S14, S7, S8, S12) were lower than 0.900. More unusually, the S9 sample had a similarity of 0.996, demonstrating that the chemical compositions of the batches of imported *Acacia catechu* were very similar. In contrast, the chemical compositions of domestic *Acacia catechu* varied significantly from batch to batch.

3.4. Cluster Analysis. Cluster analysis is an unsupervised pattern recognition method, which is widely used to classify samples with similar properties into one class, and samples with large differences in properties into different classes [20]. In this study, centroid based clustering was used to perform a systematic cluster analysis with squared Euclidean distance as the measure, and the results are

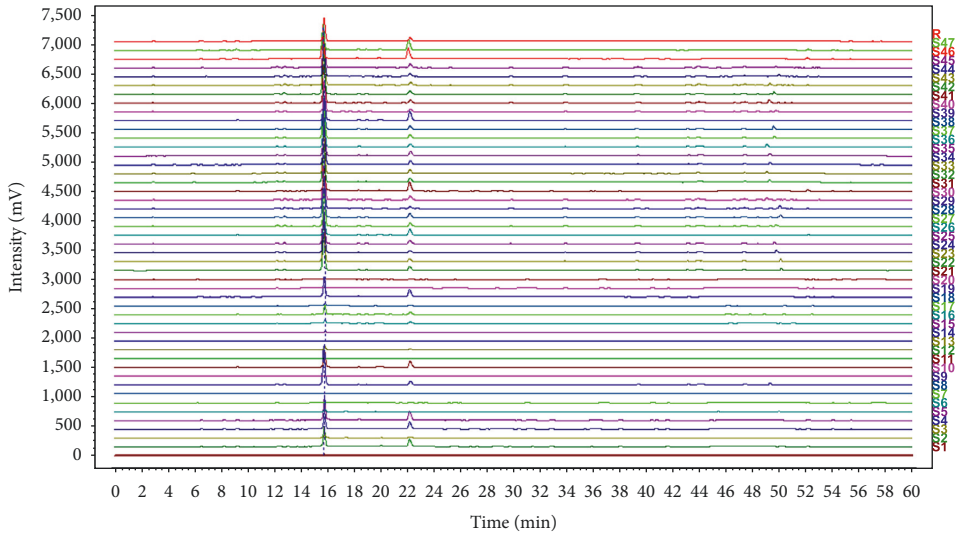

Figure 6: HPLC fingerprints of 47 batches of *Acacia catechu*.

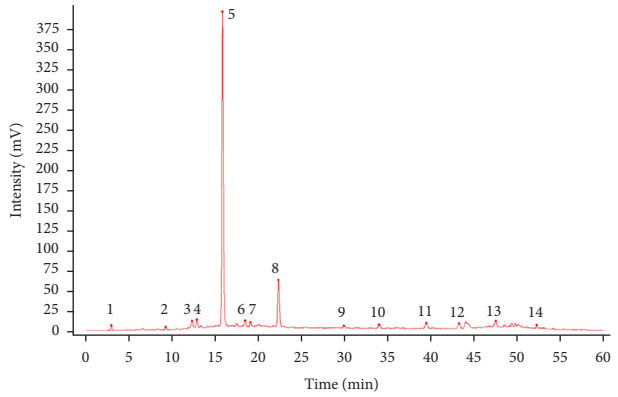

Figure 7: Common peaks of HPLC fingerprints of *Acacia catechu*.

Table 2: Similarity evaluation of 47 batches of *Acacia catechu*.

No.	Similarity
S1	0.980
S2	0.944
S3	0.964
S4	0.968
S5	0.938
S6	0.916
S7	0.578
S8	0.633
S9	0.996
S10	0.641
S11	0.954
S12	0.111
S13	0.981
S14	0.569
S15	0.792
S16	0.829
S17	0.969
S18	0.932
S19	0.973
S20	0.730
S21	0.658
S22	0.996
S23	0.994
S24	0.993
S25	0.996
S26	0.972
S27	0.994
S28	0.995
S29	0.989
S30	0.995
S31	0.973
S32	0.995
S33	0.996
S34	0.995
S35	0.996
S36	0.993
S37	0.995
S38	0.994
S39	0.965
S40	0.987
S41	0.992
S42	0.994
S43	0.993
S44	0.993
S45	0.995
S46	0.935
S47	0.921
Ref.	1

shown in Figure 8. The cluster analysis classified the samples into two groups: group A (class 3: S1, S3, S6–8, S10, S12–18, S20, S21) and group B, which could be further divided into group B1 (class 1: S9, S22–25, S27–30, S32–38, S41–45) and group B2 (class 2: S2, S4, S5, S11, S19, S26, S31, S39, S40, S46, S47). Additionally, 5 samples (S3, S13, S16, S17, S18) in the three groups had high dispersion. The CA model could classify market *Acacia catechu* into three groups to some extent, indicating that market *Acacia catechu* had significant differences in chemical composition.

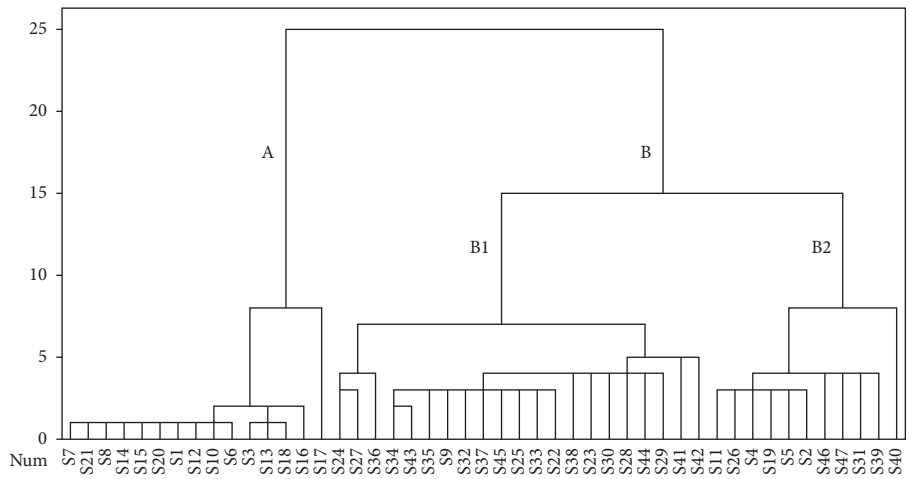

Figure 8: Hierarchical CA of 47 batches of *Acacia catechu*.

3.5. Principal Component Analysis. PCA is one of the most widely used dimensionality reduction algorithms, which is able to extract the main characteristics of things through data processing, greatly reducing the difficulty of processing problems [21]. The peak areas of each batch of *Acacia catechu* at different retention times were used as variables, and the data were imported into Unscrambler X 10.4 software. After preprocessing by SNV, PCA was utilized to observe the natural aggregation of the samples, and the score and loading plots of each peak area were obtained. The results showed that the contribution rate of the first 4 principal components to the original data is 53.579%, 17.479%, 14.764%, and 3.737%, respectively, with a cumulative contribution of 89.558%, and the model had good prediction ability. The PCA score plot of 47 batches of *Acacia catechu* is shown in Figure 9, which indicates that the discrete degrees of samples S12 and S7 were greater. Combining this with the results of the similarity analysis, we could judge S12 and S7 to be abnormal samples.

Furthermore, the first-class and second-class samples in CA could be well aggregated, while the five samples in class 3 (S3, S13, S16, S17, S18) were closer to the second-class samples, and the rest of the samples in class 3 were more discrete and could not be well aggregated into one category. Combining the determination results of principal component groups and index components in the fingerprint of the samples, we could infer that the principal component groups of these 5 samples were consistent with the second-class sample, but the content of chemical components was very low, which could be treated as defective *Acacia catechu* samples. However, the principal component groups of the other 10 samples in the third-class of samples were significantly different from those of the reference herbs, and the content determination results of each component were all low, suggesting that the 10 samples were fakes.

The loading plot is based on the distance of the variable from the origin to judge the influence of the variable on the weight of the principal component. The farther away the variables are from the center, the more they contribute to the weight of the principal component [22]. The PCA loading plot in this study is depicted in Figure 10, indicating that the six variables with greater influence on the weight of principal components 1 and 2 were peaks 1 (17.402), 2 (15.775, catechin), 3 (22.247, epicatechin), 4 (58.354), 5 (19.572, epicatechin gallate), and 6 (57.622). All the findings suggested that the PCA model had a good ability to identify class 1 *Acacia catechu*, while the two chemical components, including catechin and epicatechin, are important indicators for responding to the intrinsic composition of *Acacia catechu*.

3.6. Orthogonal Partial Least Squares Discriminant Analysis. OPLS-DA is a supervised pattern recognition method based on partial least squares, which is suitable for the case of few numbers of sample observations, many explanatory variables, and presence of multicollinearity and has some advantages in quality control in combination with fingerprinting [23]. In this study, the peak areas of each batch of *Acacia catechu* at different retention times were introduced into SIMCA 13.0 software, where the OPLS-DA model was developed better to analyze the rank classification of market *Acacia catechu*. The explanatory rate parameter R2X (cum), the model differentiation parameter R2Y (cum), and the model prediction parameter Q2 (cum) of the data matrix were 0.490, 0.971, and 0.897, respectively. With three new principal component variables, the explanatory capacities of the model to variable X and variable Y were 49% and 97.1%. Furthermore, the fraction of the variation of variable Y predicted by the model based on cross validation was 89.7%, indicating that the established model was stable and had good prediction ability.

The OPLS-DA score is presented in Figure 11, revealing that the sample S38 in class 1 had a large degree of dispersion and the other two classes were better clustered. Variable importance in projection (VIP) values was used as indicator to screen the main contributing chemical components. The higher the VIP value, the greater the contribution of the variable. With VIP >1 as the threshold, the common peaks with great

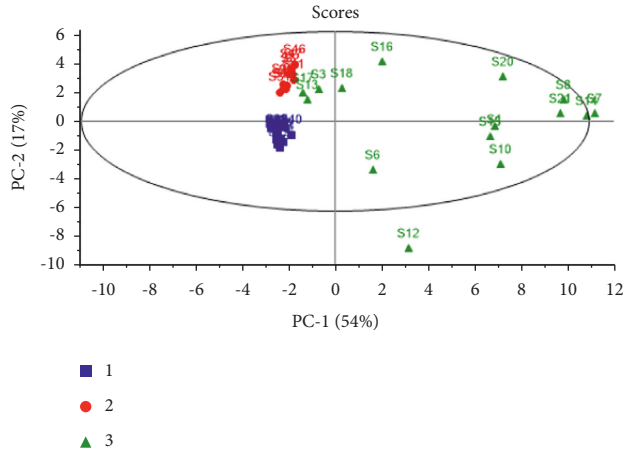

Figure 9: PCA score plot of 47 batches of *Acacia catechu*.

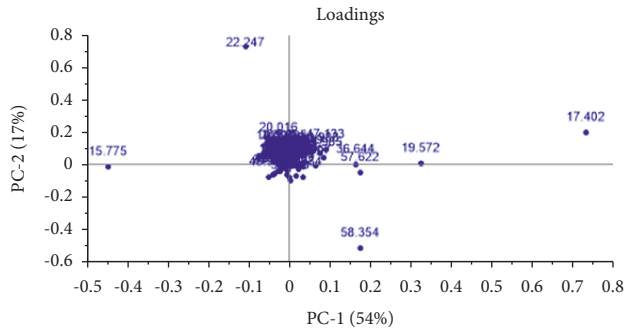

Figure 10: PCA Loading plot of 47 batches of *Acacia catechu*.

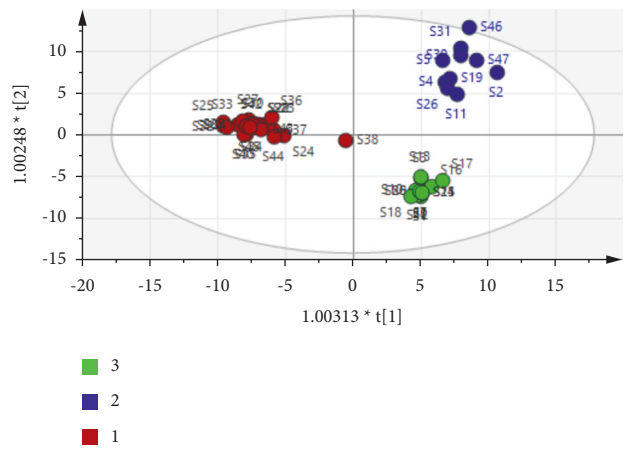

Figure 11: OPLS-DA score plot of 47 batches of *Acacia catechu*.

influence on the classification of three classes of market *Acacia catechu* were screened out. The result of VIP values is given in Figure 12, indicating that there were 98 components with VIP >1 and six compounds, which were the main markers of quality difference between 47 batches of market *Acacia catechu*, were successively represented by peak 1 (22.247, epicatechin), peak 2 (15.775, catechin), peak 3 (12.247), peak 4 (33.923), peak 5 (14.079), and peak 6 (43.159). The results indicated that catechin and epicatechin were the main differential components in the three classes of market *Acacia catechu* and had a significant effect on the quality of each batch.

3.7. Quantitative Analysis

3.7.1. Method Validation of Quantitative Analysis. 50% methanol stock solutions containing catechins and epicatechins were prepared. By analyzing different concentrations of the two analytes, the calibration curves were generated to determine the contents of catechins and epicatechins. The calibration curves for the two components were $y = 6861.3x$ and $y = 7047.2x$, and the measurement ranges were 0–1684.00 μg/mL and 0–1404.00 μg/mL, respectively, with good linearity ($r^2 > 0.999$). The apparatus precision of the two analytes was determined, and their relative standard deviation (RSD) values were less than 2.0% ($n = 6$), indicating the instrument had good precision. The method's repeatability was assessed, proving the method had good reproducibility with RSD less than 2% ($n = 6$) for the two analytes. Stability tests were performed by analyzing the same samples stored for 0, 2, 4, 8, 12, and 24 h. The results showed that the RSD values of both components were less than 2.0% ($n = 6$), indicating that the samples were stable for 24 h. The spiked recovery test was performed to assess the accuracy of the content determination method. The average spiked recoveries of the two analytes were 96.73% and 97.95% with RSD = 1.5% and 1.2%, indicating that the method was accurate. Therefore, the above results demonstrated that the method was considered to be accurate for quantitation analysis of market *Acacia catechu*.

3.7.2. Quantitative Analysis of Market Acacia catechu. The developed HPLC method was used to determine two compounds, catechin and epicatechin, in the market *Acacia catechu* from different batches. The contents of catechin and epicatechin, the sum of catechin and epicatechin contents, and the ratio of catechin and epicatechin contents were calculated as four factors for the quality evaluation of market *Acacia catechu*, and the results of the determination are shown in Table 1. Through the analysis of the origin of the herbs and the content of the two compounds, it was found that the content of the main components of the imported *Acacia catechu* was higher (only the sample S9 in the domestic *Acacia catechu* had high content, and the sample S9 could be considered as an outlier), which indicated that the price of imported *Acacia catechu* in the market was significantly higher than that of domestic *Acacia catechu* with broad scientific interest and significance. In addition, in terms of analysis of sample powder color, class 1 of samples was earthy yellow, while class 3 of samples was more reddish-brown to red-black, presuming that the earthy yellow samples were high-quality products. However, this was a deviation from the records of "reddish-brown or brown-black" in various ancient books. Therefore, the study of sample powder color and intrinsic quality correlation was worth further in-depth exploration.

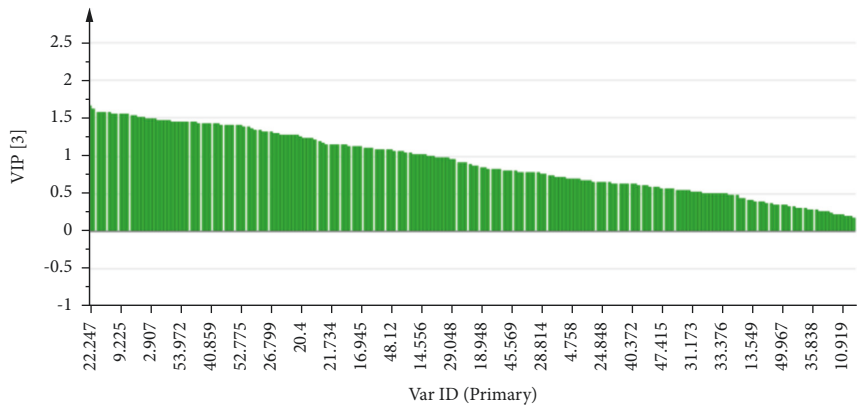

Figure 12: VIP of 218 chromatographic peaks in OPLS-DA model of *Acacia catechu*.

3.7.3. OPLS-DA of Four Factors in Three Classes of Market Acacia catechu. By comparing the mean values of the four factors in the three classes of market *Acacia catechu*, it was found that the contents of catechin (A) and epicatechin (B), the sum of the contents of the two compounds (C), and the ratio of the contents of the two compounds (D) were from high to low: 1 > 2 > 3, 2 > 1 > 3, 1 > 2 > 3, and 1 > 3 > 2, respectively. To further examine the scientificity of grade classification of market *Acacia catechu*, the OPLS-DA classification model was established, using the above four factors as independent variables, and the results are shown in Figure 13. The explanatory rate parameter R2X (cum), the model differentiation parameter R2Y (cum), and the model prediction parameter Q2 (cum) of the data matrix were 1.000, 0.920, and 0.909, respectively. With two new principal component variables, the explanatory capacities of the model to variable *X* and variable *Y* were 100% and 92.3%, indicating that the established model was stable and had good prediction ability. The order of VIP values for the four factors was VIPC = 1.1931 > VIPA = 1.1395 > 1 > VIPB = 0.8011 > VIPD = 0.7977 (Figure 14). Among them, both VIPC and VIPA were greater than 1, indicating that these two influencing factors needed to be given great attention in the quality control of *Acacia catechu*, which was of great significance to the development of *Acacia catechu* quality standards and could be used as the preferred parameters for the development of *Acacia catechu* grade standards.

3.8. Comprehensive Analysis of the Intrinsic Quality of Three Classes of Market Acacia catechu. The PCA of the fingerprint found that the first- and second-class samples could be well aggregated, and the two control samples (S46 and S47) were all aggregated in the second class. Based on the main component groups of the fingerprint, it could be judged that the first-class samples were consistent with the control samples and the content of the index components was high, indicating that the first-class samples could be judged to be superior. However, the third-class samples were more discrete, and five (S3, S13, S16, S17, S18) were closer to the second-class samples. Combining this with CA, we found that the five samples in class 3 were clustered into a separate category. Further, it could be inferred from the results of the

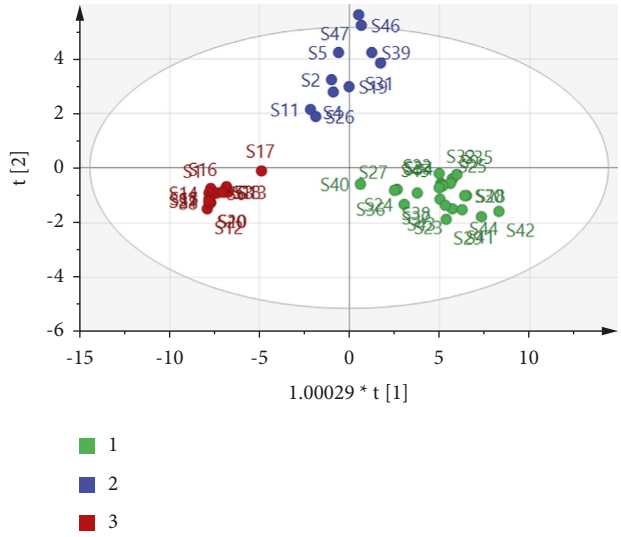

Figure 13: OPLS-DA score plot of 46 batches of *Acacia catechu*.

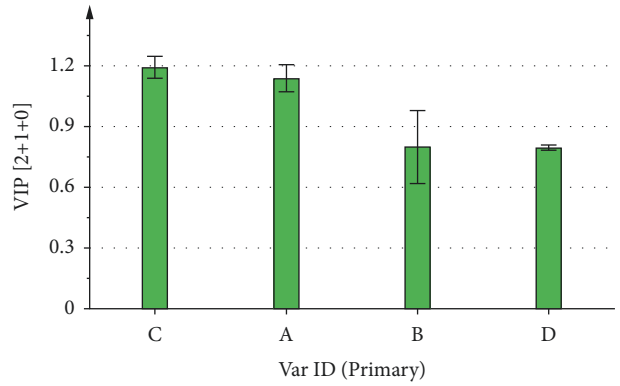

Figure 14: VIP of 4 variables in OPLS-DA model of *Acacia catechu*.

determination of the content of the index components that the main components of the five samples were consistent with the first- and second-class samples, but the contents of the index components were extremely low, which could be presumed to be the residual products of *Acacia catechu*. The fingerprints of the other ten samples in class 3 were different from the control samples, and the content of each

component was determined to be low, so it was presumed that the ten samples were counterfeit. Thus, the third-class samples could be inferred to be substandard samples.

4. Conclusions

In this study, the established fingerprint and multi-indicator component analysis method were stable and feasible and could be used for the classification and quality evaluation of market *Acacia catechu*. The results of fingerprint similarity were highly consistent with the results of unsupervised clustering CA and PCA and supervised OPLS-DA, which could systematically classify market *Acacia catechu* into three classes. The results of the multi-indicator component analysis demonstrated that catechin (A) and the sum of catechin and epicatechin (C) could be used as the preferred indicator parameters for developing quality standards of *Acacia catechu* grade. All in all, this study provides a new method of *Acacia catechu* quality evaluation and identification based on fingerprint-chemical pattern recognition, which is an important reference value for the development of *Acacia catechu* quality standards.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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