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Quality Evaluation of *Cardamine macrophylla* Willd as an Edible and Medicinal Herb of Tibetan and Qiang Ethnic Groups

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Abstract [**Objectives**] To evaluate the quality of *Cardamine macrophylla* Willd as Tibetan and Qiang medicinal materials, so as to improve its quality standard and evaluate the quality of *C. macrophylla* Willd in western Sichuan Province. [**Methods**] *C. macrophylla* Willd produced from western Sichuan Province was used as the sample, and the contents of moisture, total ash, acid-insoluble ash, extract, total flavonoids and quercetin in the ground part of *C. macrophylla* Willd were determined in accordance with the methods of *Chinese Pharmacopoeia* (2020 edition). With the above seven indicators as evaluation indicators, the quality of medicinal materials was comprehensively evaluated by cluster analysis and principal component analysis (PCA). [**Results**] According to the results of each indicator, the moisture content of *C. macrophylla* Willd sample should not exceed 11.00%, the total ash content should not exceed 18%, the acid-insoluble ash content should not exceed 6%, the extract content should not be less than 19%, the total flavone content (calculated by quercetin) should not be less than 2%, and the quercetin content should not be less than 0.15%. [**Conclusions**] The sample S7 has the best quality and S6 has the worst quality. In this study, the quantitative analysis method of total flavonoids (quercetin) and quercetin in *C. macrophylla* Willd was established, and the limits of each indicator were preliminarily formulated.

Key words *Cardamine macrophylla* Willd, Quality standard, Total flavonoids, Multivariate statistical analysis

1 Introduction

Cardamine macrophylla is the whole plant of *Cardamine macrophylla* Willd of Cruciferae[1-3], also known as Shigecai. The Tibetan, Qiang, Han and other ethnic groups in Sichuan Province have a long history of eating, often eating whole plant, and now there are dried products, pickled cabbage and other products on the market. Scholars at home and abroad have studied *C. macrophylla* Willd mainly in its nutritional value, which not only has medical and health care functions and high nutritional value, but also has certain ornamental value, and can be used as ornamental flowers[4-8].

At present, *C. macrophylla* Willd is considered to have the effects of promoting digestion, defecating, regulating blood pressure, blood lipid, blood sugar and so on. Besides, it has been found that *C. macrophylla* Willd has significant weight loss effect and has great development prospects. However, there is no systematic study on *C. macrophylla* Willd, and the quantitative detection of its index components has not been carried out, so the quality standards of *C. macrophylla* Willd need to be improved urgently, and it is necessary to study from the aspects of quality standards.

The purpose of this study is to establish the quantitative analysis method of total flavonoids and quercetin, and to improve the quality standard of *C. macrophylla* Willd. Multivariate statistical analysis was used to evaluate the quality of the ground and underground parts of *C. macrophylla* Willd from different places according to its moisture, total ash, acid-insoluble ash, extract, total flavonoids and quercetin, so as to ensure the safety, effectiveness, stability and controllability of its clinical application, to provide a reference for the further development and rational utilization of *C. macrophylla* Willd.

2 Materials

2.1 Instruments METTLER AE 240 Electronic Analytical Balance (Shanghai Mettler - Toledo Instrument Co., Ltd.); QE-100 high-speed crusher (Zhejiang Yili Industry and Trade Co., Ltd.); HW-12 electric thermostatic water bath (Shanghai Yiheng Scientific Instrument Co., Ltd.); DHG-9240D intelligent electric heating constant temperature blast drying oven (Shanghai Langya Laboratory Equipment Co., Ltd.); RE-2000A rotary evaporator (Shanghai Yarong Biochemical Instrument Factory); Agilent 1100 (Agilent, USA); SX2 box-type resistance furnace (Changsha yuandong Electric Furnace Factory); Persee TU-1810 UV-Vis Spectrophotometer (Beijing Persee General Instrument Co., Ltd.).

2.2 Materials and reagents Ten batches of *C. macrophylla* Willd. (S1-S10) were collected from Aba Tibetan and Qiang Autonomous Prefecture and Ganzi Prefecture of Sichuan Province, and identified by Professor Liu Yuan of Southwest Minzu University as *Cardamine macrophylla* Willd of Cruciferae (Table 1).

Rutin standard substance (batch No.: 19012405, Chengdu Kangbang Biotechnology Co., Ltd.); anhydrous glucose (batch No.: 20200520, Fuchen (Tianjin) Chemical Reagent Co., Ltd.); cellulase (Chengdu Chron Chemical Reagent Factory); quercetin (batch No.: 100081-201610; National Institute for the Control of Pharmaceutical and Biological Products); acetonitrile was chrom-

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atographically pure; sodium hydroxide, sodium nitrite, aluminum nitrate, phenol, sulfuric acid, methanol, ethanol, and anhydrous aluminum chloride were all commercially available analytical reagents; water was double distilled water.

Table 1 Source of *Cardamine macrophylla* Willd samples

Sample No.	Sample source	Altitude//m	Longitude(E)/Latitude(N)
S1	Zhonggu Village, Daofu County, Ganzi Prefecture	3 718.0	101°32′59.28″ E/30°32′27.15″ N
S2	Mengbi Mountain, Xiaojin County	3 713.6	102°19′24.48″ E/30°40′39.33″ N
S3	Wolong Dengshenggou, Wenchuan County	2 867.0	102°57′00.99″ E/30°51′00.88″ N
S4	Gengda Huangcaoping, Wenchuan County	2 315.4	103°20′31.21″ E/31°08′08.00″ N
S5	Gengda Tizigou Dahekou, Wenchuan County	2 400.0	103°03′24.01″ E/30°55′34.02″ N
S6	Houcun Village, Yadu Township, Mao County	2 439.5	103°10′33.46″ E/31°52′40.98″ N
S7	Bailongchi River, Jiuding Mountain, Mao County	3 251.0	103°96′21.31″ E/31°30′33.72″ N
S8	Downstream of Bailongchi River, Jiuding Mountain, Mao County	3 240.0	103°46′33.52″ E/31°30′33.31″ N
S9	Jiuding Mountain Ski Resort, Mao County	3 185.0	103°45′23.33″ E/31°30′55.39″ N
S10	Entrance of Bailongchi River, Jiuding Mountain, Mao County	3 264.0	103°46′19.59″ E/31°30′34.65″ N

3 Methods and results

3.1 Determination of moisture, total ash, acid-insoluble ash and extract content First, 2 g of each of 10 batches of *C. macrophylla* Willd sample powder (screened with No. 4 sieve) was taken for moisture detection according to the second method under "General Rule 0832 Moisture Determination Method"; the total ash and acid-insoluble ash were determined according to the total ash determination method and acid-insoluble ash determination method under "General Rule 2302 Ash Determination Method"; the content of extract was determined by the cold extraction method under "General Rule 2201 Method for Determination of Water-Soluble Extract" in *Chinese Pharmacopoeia* (Volume IV, Edition 2020). Each sample was measured in parallel for 3 times, and the average value was taken.

The results showed that the moisture content of the ground part of *C. macrophylla* Willd was 6.15% – 10.38%, the total ash content was 12.85% – 17.79%, the acid-insoluble ash content was 0.87% – 5.69%, and the extract content was 19.54% – 36.29% (Table 2). Taking into account the differences in the sources of medicinal materials, this study set the limit slightly higher than the highest measured value, and preliminarily proposed that the moisture content of *C. macrophylla* Willd should not exceed 11%, the total ash content should not exceed 18%, the acid-insoluble ash content should not exceed 6%, and the extract content should not be less than 19%.

Table 2 Contents of moisture, total ash, acid-insoluble ash and extract in ground part of *Cardamine macrophylla* Willd %

No.	Moisture	Total ash	Acid-insoluble ash	Extract
S1	9.25	14.17	2.11	30.46
S2	6.99	15.34	2.82	23.87
S3	8.67	14.20	2.93	27.84
S4	9.25	16.77	1.52	26.46
S5	9.63	15.98	2.15	25.76
S6	6.91	12.85	1.03	24.00
S7	10.38	12.90	0.87	36.29
S8	6.15	15.63	3.36	19.54
S9	7.87	17.79	5.69	25.79
S10	8.73	16.92	5.02	23.81

3.2 Determination of total flavonoids

3.2.1 Preparation of reference solution. Precisely weighed a certain amount of rutin reference substance, added 70% ethanol to a constant volume of 50 mL, and shook up to obtain rutin reference solution with concentration of 0.29 mg/mL.

3.2.2 Preparation of the test solution. Precisely weighed *C. macrophylla* Willd sample powder, separately weighed 3 portions in parallel, added appropriate amount of 70% ethanol, added 0.07 mg enzyme, ultrasonically extracted at 37 ℃ for 43 min, then filtered, fixed the volume of the filtrate to a 50 mL volumetric flask, shook well, and the total flavonoids of *C. macrophylla* Willd test solution was obtained for later use.

3.2.3 Linear relationship investigation. Accurately pipetted 2.0, 4.0, 6.0, 8.0 and 10.0 mL of rutin reference solution, put into a 25 mL volumetric flask, added water to 6 mL, added 1 mL of 5% sodium nitrite solution, shook well, and placed them for 6 min. Added 1 mL of 10% aluminum nitrate solution, shook up, and placed for 6 min. Added 10 mL of sodium hydroxide solution, and then added water to the scale line, shook up, and placed for 15 min, and replaced the rutin reference solution with 70% ethanol. Added the reagent according to the above method to obtain a blank solution, immediately measured the absorbance at the wavelength of 510 nm by UV-spectrophotometry (General Rule 0401), and performed linear regression with the value of absorbance (A) as the ordinate and the mass concentration of rutin (C) as the abscissa, to obtain the regression equation $Y = 11.034X - 0.0206$, and the correlation coefficient $R = 0.9997$. The standard curve is shown in Fig. 1, indicating that the rutin reference solution has a good linear relationship with the absorbance in the range of 0.023 – 0.115 mg/mL.

3.2.4 Precision test. Precisely measured 2 mL of rutin reference solution, developed the color in accordance with the method in Section **3.2.3**, performed parallel determination for 6 times, recorded the absorbance A, and calculated the *RSD* value to be 0.35%, showing that the precision of the instrument was good.

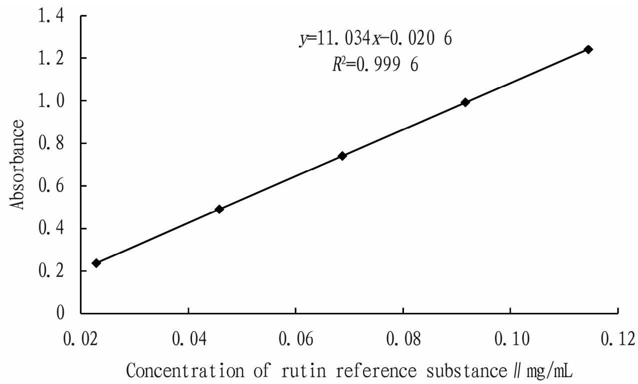

Fig. 1 Standard curve of rutin reference substance

3.2.5 Repeatability test. Precisely weighed 6 portions of 0.5 g sample powder, prepared the solution in accordance with the method in Section **3.2.2**, developed the color in accordance with the method in Section **3.2.3**, measured and recorded the absorbance A, and calculated the *RSD* value to be 1.68%, indicating that the method had good repeatability.

3.2.6 Stability test. Took *C. macrophylla* Willd test solution and developed color in accordance with the method in Section **3.2.3**, and measured the absorbance at the wavelength of 510 nm at 0, 15, 30, 45, 60, 75 and 90 min, calculated the *RSD* value to be 0.32%, indicating that the stability was good within 90 min.

3.2.7 Sample recovery test. Precisely prepared 6 portions of test solutions, separately added a certain amount of rutin reference substance, developed the color in accordance with the method in Section **3.2.3**, the absorbance A was measured at 510 nm, and calculated the average recovery rate and the average value was 98.09%, and the *RSD* value was 1.13%, indicating that the method was accurate and reliable.

3.2.8 Determination of total flavonoids in samples. Precisely weighed an appropriate amount of total flavonoids extract of *C. macrophylla* Willd in a 20 mL test tube, precisely added 70% ethanol to 10 mL, shook well, used 70% ethanol as blank control, developed the color in accordance with the method in Section **3.2.3**, measured its absorbance at 510 nm, and calculated the total flavonoids content according to the standard curve.

From Table 3, it can be seen that the content of total flavonoids in the ground part of *C. macrophylla* Willd was 2.42% – 6.09%; considering the differences in the sources of medicinal materials, we set the limit slightly higher than the highest measured value, and preliminarily proposed that the content of total flavonoids (calculated by quercetin) in *C. macrophylla* Willd should not be less than 2%.

3.3 Determination of quercetin content

3.3.1 Selection of chromatographic conditions. DIKMA C_{18} column (250 mm × 4.6 mm, 5 μm); the mobile phase was acetonitrile-0.4% phosphoric acid solution (35 : 65); column temperature 30 ℃; the flow rate was 1 mL/min, injection volume 5 μL; detection wavelength 360 nm[9-10]; the chromatograms of the reference solution and samples are shown in Fig. 2.

Table 3 Determination results of total flavonoids in *Cardamine macrophylla* Willd %

No.	Total flavonoids	No.	Total flavonoids
S1	5.41	S1 – 2	0.88
S2	3.22	S2 – 2	0.70
S3	4.79	S3 – 2	0.90
S4	2.88	S4 – 2	0.99
S5	4.23	S5 – 2	0.79
S6	2.53	S6 – 2	1.04
S7	6.09	S7 – 2	0.60
S8	2.87	S8 – 2	0.48
S9	2.42	S9 – 2	0.60
S10	4.04	S10 – 2	0.64

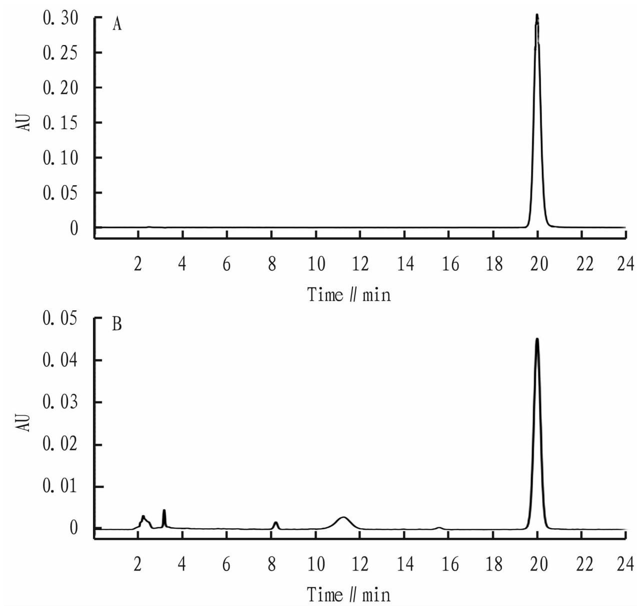

Fig. 2 HPLC chromatograms of quercetin reference solution (A) and sample (B)

3.3.2 Preparation of reference solution. Precisely weighed a proper amount of quercetin reference substance, added methanol to prepare the solution containing 20 μg of quercetin per 1 mL, and filtered through a 0.45 μm microporous membrane for later use.

3.3.3 Preparation of test solution. Precisely weighed 0.5 g of *C. macrophylla* Willd sample powder (screened with No. 4 sieve) and placed in a 250 mL conical flask with a stopper, and precisely added 50 mL of mixed solution of methanol-hydrochloric acid solution (4 : 1), shook up, weighed, heated and refluxed for 1 h, cooled, and weighed again. Made up the weight loss with the mixed solution of methanol-concentrated hydrochloric acid solution (4 : 1), shook well, filtered, took the filtrate, and filtered it with 0.45 μm microporous membrane for later use.

3.3.4 Quercetin content in the ground parts of *C. macrophylla* Willd. It can be seen from Table 4 that the quercetin content in ground parts of *C. macrophylla* Willd samples was 0.15% – 0.68%. Considering the differences in the sources of medicinal materials, we set the limit slightly higher than the highest determination value, and preliminarily proposed that the quercetin content in *C. macrophylla* Willd should not be less than 0.15%.

Table 4 Quercetin content in ground parts of *Cardamine macrophylla* Willd %

No.	Content	No.	Content
S1	0.47	S6	0.15
S2	0.32	S7	0.68
S3	0.63	S8	0.28
S4	0.24	S9	0.22
S5	0.55	S10	0.49

3.4 Comprehensive evaluation of quality Moisture (X_1), total ash (X_2), acid-insoluble ash (X_3), extract (X_4), total flavonoids (X_5) and quercetin (X_6) were used as evaluation indicators, and principal component analysis (PCA) and cluster analysis were carried out to comprehensively evaluate the quality of ground parts of *C. macrophylla* Willd[11-14], as shown in Table 5.

Table 5 Quality indicators of ground parts of *Cardamine macrophylla* Willd

No.	X_1	X_2	X_3	X_4	X_5	X_6
S1	9.25	14.17	2.11	30.46	5.41	0.47
S2	6.99	15.34	2.82	23.87	3.22	0.32
S3	8.67	14.20	2.93	27.84	4.79	0.63
S4	9.25	16.77	1.52	26.46	2.88	0.24
S5	9.63	15.98	2.15	25.76	4.23	0.55
S6	6.91	12.85	1.03	24.00	2.53	0.15
S7	10.38	12.90	0.87	36.29	6.09	0.68
S8	6.15	15.63	3.36	19.54	2.87	0.28
S9	7.87	17.79	5.69	25.79	2.42	0.22
S10	8.73	16.92	5.02	23.81	4.04	0.49
Average value	8.383	15.255	2.750	26.382	3.848	0.403
Standard deviation	1.358	1.692	1.598	4.510	1.274	0.185

NOTE X_1 - X_6 represent moisture, total ash, acid-insoluble ash, extract, total flavonoids, and quercetin content, respectively.

3.4.1 Principal component analysis (PCA). The principal component analysis results of the quality indexes of the ground parts of *C. macrophylla* Willd are shown in Table 6, and the scree plot is shown in Fig. 3. It can be seen from Table 6 that the common factor variance of each indicator was ≥0.508 (the common factor variance of total ash was the largest, which was 0.912; the common factor variance of acid-insoluble ash was the smallest, which was 0.508), there were 3 principal components with characteristic values >0.5, and the cumulative variance contribution rate was 61.899%, which can basically objectively reflect the quality information of ground parts of *C. macrophylla* Willd. The comprehensive scores and ranking results are shown in Table 7.

Table 6 Principal component factor load matrix and common factor variance of quality indicators

Indicator	1	2	Common factor variance
X_1	-0.320	0.706	0.602
X_2	0.927	-0.229	0.912
X_3	0.670	-0.243	0.508
X_4	0.731	0.326	0.640
X_5	0.076	0.775	0.607
X_6	0.720	0.572	0.845
Principal component eigenvalues	2.555	1.778	
Contribution rate of principal component//%	36.501	25.398	
Cumulative contribution rate of principal component//%	36.501	61.899	

$$F_1 = -0.125X_1 + 0.363X_2 + 0.262X_3 + 0.286X_4 + 0.030X_5 + 0.282X_6$$

$$F_2 = 0.397X_1 - 0.129X_2 - 0.137X_3 + 0.183X_4 + 0.436X_5 + 0.322X_6$$

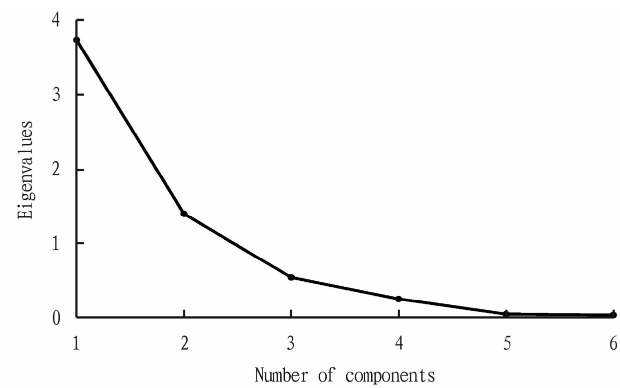

Fig.3 Scree plot

Table 7 Comprehensive score and ranking results of ground parts of *Cardamine macrophylla* Willd

No.	Comprehensive score	Ranking
S1	0.768	2
S2	-1.187	8
S3	0.335	3
S4	-0.748	6
S5	0.163	4
S6	-1.667	10
S7	1.783	1
S8	-1.596	9
S9	-0.943	7
S10	-0.193	5

3.4.2 Cluster analysis. SPSS 21.0 software was used to perform systematic cluster analysis on the determination results of the contents of moisture, total ash, acid-insoluble ash, extract, total flavonoids and quercetin in the ground parts of *C. macrophylla* Willd, and the cluster results are shown in Fig. 4. The ground parts of *C. macrophylla* Willd can be divided into two clusters. Among them, Cluster I was S7, only the total ash and acid-insoluble ash of *C. macrophylla* Willd were lower than the average, the other indicators were higher than the average, and the comprehensive score of the average was high. The rest were the Cluster II, and the comprehensive scores of the quality indicators of this cluster were less than 1.

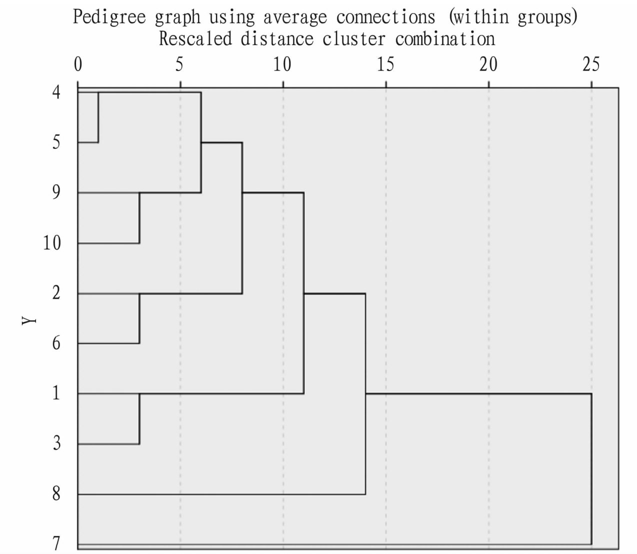

Fig. 4 Cluster analysis of the ground part quality of *Cardamine macrophylla* Willd

4 Discussion

4.1 Significance of this study in formulating standards The 10 batches of medicinal materials used in the study were collected from Sichuan Province, and the quality standards of *C. macrophylla* Willd have not yet been found on the basis of the quality standards of Chinese medicinal materials commonly used in various places. In this study, the quality of *C. macrophylla* Willd was comprehensively evaluated by combining the quantitative detection methods of indicator components in the *Chinese Pharmacopoeia* (2020 edition) and the detection and analysis methods such as principal component analysis (PCA) and cluster analysis. The results showed that the quality of 10 batches of medicinal materials collected from Sichuan Province in this study was better. According to the results of the determination, the quality of *C. macrophylla* Willd can be more effectively controlled by setting a limit slightly higher than the maximum value of the determination results.

4.2 Comparison and analysis of multivariate statistical analysis results At the same time of determining the indincator components of the ground parts of *C. macrophylla* Willd, the corresponding contents of the ground parts were also determined. The results showed that the ground parts of *C. macrophylla* Willd were generally better than the underground parts, and the quality of *C. macrophylla* Willd would be improved if the ground parts and the underground parts of *C. macrophylla* Willd were processed separately after harvesting.

In summary, this study established the quantitative analysis method of total flavonoids and quercetin, and initially worked out the limits of each indincator, and established the quality standard of *C. macrophylla* Willd. Thus, it will provide a theoretical basis for the development and utilization of *C. macrophylla* Willd resources.

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