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Component analysis and quality evaluation of *Rheum tanguticum* from Sichuan Plateau area of China

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Abstract

The aim was to measure the total anthraquinone, free anthraquinone, tannin, total ash, moisture and extractum content of *Rheum tanguticum* from Sichuan plateau area of China. The important index specified in the Chinese pharmacopoeia is the content of anthraquinones in *R. tanguticum*, which is determined by HPLC. The secondary indexes moisture, extractum and total ash. The non-Chinese Pharmacopoeia index is tannin content, which is determined by UV method. The result was that the moisture content of 10 samples did not exceed 15.0%, the total ash score did not exceed 10.0%, and the content of the extractum reached over 25.0%, which all meet the pharmacopeia index. In addition, for the determination of anthraquinone content, the result of the total anthraquinone percentage of 10 samples was higher than 1.50%, meeting the pharmacopeia requirements. But in some samples, the percentage content of free anthraquinone did not reach 0.20% and did not meet the Pharmacopoeia standard. The tannin content of 10 samples was up to 52.46 mg/g, but not at about 10%~30%. The conclusion is that the quality difference of different Ruoergai County production can provide a reference value for the germplasm resource identification and production technology improvement of the *R. tanguticum* plantation area.

Keywords: *Rheum tanguticum*, anthraquinone, tannin, analysis

Introduction

Rhubarb is a perennial herb of the genus *Rheum* in *Polygonaceae*. In Chinese Pharmacopoeia includes the *Rheum palmatum L*, *Rheum tanguticum Maxim. Ex Balf* and *Rheum officinale Baill* [1]. It is one of the oldest and most commonly used herbal medicines in China, Korea, Japan, and other Asian countries. At present, rhubarb is mainly distributed in Asia, Europe and North America [2]. In China, rhubarb mainly grows in Gansu, Qinghai, Sichuan provinces and Tibet. As a traditional Chinese medicine, rhubarb contains anthraquinones, polysaccharides, tannins and other active components. Under their combined action, rhubarb has the pharmacological effects of anti-diarrhea, bacteriostasis, anti-tumor, and improving renal function [3]. As a high content in rhubarb, tannins also have a variety of pharmacological effects, such as inhibiting excess free radicals in organisms and reducing serum urea nitrogen. In addition, tannins also have a convergence effect, oral treatment can be used in the treatment of gastrointestinal bleeding, ulcer, and other diseases, external use for trauma, and burns [4-6].

At present, more than 200 chemical components have been found in rhubarb, which are mainly divided into anthraquinones, polysaccharides, tannins, etc. Stilbene, phenyl butanone, naphthalene glycosides, organic acids, and trace elements have been found in rhubarb [7, 8]. More than 30 anthraquinones have been isolated and identified from rhubarb, with a content of about 2%~5%, which can be divided into free anthraquinones and bound anthraquinones. The content of anthraquinones in different parts of rhubarb is different [7]. Zhou *et al*. analyzed the content of anthraquinones in two overground parts (leaves and seeds) and three underground parts (root, rhizome, and fibrous root) of cultivated *R. palmatum* L.by HPLC, and found that the free anthraquinones in fibrous root was the highest [9]. Ge *et al* studied the content of rhubarb acid in three genuine rhubarb and found that for the content, *Rheum palmatum L.*and *Rheum Officinale Baillon* was far less than *R. tanguticum*, which also showed that different varieties are important factors affecting the content of anthraquinone components in rhubarb [10]. Tannins represent a wide variety of compounds that can be found in fruits, vegetables, dry extractum of red wine and grape seeds, tea, and dry not edible plants [11]. The tannin content in rhubarb is about 10%~30%, is a water-soluble polyphenol compound with a molecular weight of 500~3000, widely exists in the plant boundary, mainly including hydrolysis and condensation type [12-14]. The main monomers of these 2 tannins are oxalic acid (gallic acid) and catechin [15]. The common determination methods of tanning are leather powder, potassium permanganate, casein, legality, etc. According to Pharmacopoeia, the indicators of rhubarb quality evaluation are water content, total ash content, leaching objects, total anthraquinone, and free anthraquinone. The quality of rhubarb is affected by climatic conditions, germplasm resources, planting technology, and collection time. Therefore, the composition analysis and quality evaluation of rhubarb can be of great significance to the ability of germplasm resources identification and production technology improvement in the *R. tanguticum* planting area.

Materials and Methods

Plant material

The rhizomes of *Rheum tanguticum* were collected from ten

sites in Ruoergai County of Sichuan province located in the Northeast edge of Qinghai Tibet Plateau with an average altitude of about 3100 m. The rhizomes of *R. tanguticum* named A1, A2, A3, A4, and A5 were gathered on October 11, 2020, and other five rhizome materials (named B1, B2, B3, B4, and B5) were obtained on April 12, 2021.

The dirt of the fresh rhizomes of *R. tanguticum* was removed, washed under tap water, cut into pieces, and dried in an Electro-thermostatic blast oven at 60℃ for 24 hours. A portion of the dried rhizome pieces of each material was pulverized into powder and stored in a glass desiccator at room temperature.

Analysis of total and free anthraquinone contents

The analysis of total and free anthraquinone contents was determined according to the method of the Chinese Pharmacopoeia in the 2020 edition. For the determination of total anthraquinone, about 0.15 g of rhubarb powder (80 mesh) in a plug conical flask was added to 25 ml methanol and weighed. Heat reflux for 1 hour, cool, and weigh again. Make up the missing weight with methanol and shake well. Take 5 ml of filtrate in a flask to remove the solvent. Add 10 ml 8% hydrochloric acid solution for ultrasonic treatment for 2 minutes and then add 10ml trichloromethane. After heating reflux for 1 hour and cooling, the container is placed into the separating funnel, and a small amount of trichloromethane is used to wash the container and merge it into the separating funnel. Separate the trichloromethane layer of acid and then extractum it with trichloromethane three times 10ml each. Decompressive recovery of solvent to dry by merging trichloromethane solution. After the residue was dissolved in methanol, it was transferred to a 10ml measuring flask and methanol was added to the scale and shaken well. And the test sample was then obtained by filtration [1].

About 0.5 g rhubarb powder (80 mesh) in a plug conical flask was added to 25 ml methanol and weighed. Heat and reflux for 1 hour and weigh after cooling. Replenish the reduced weight with methanol and shake well. Finally, filter the liquid in front and take the filtrate [1].

Aloe-emodin, rhein, emodin, chrysophanol and physcion (the purity was above 98.0%, Chengdu Pusi Biotechnology Co., Ltd., China) were used as the refereneced substances. The control solutions of aloe-emodin, rhein, emodin and chrysophanol were prepared to 16 μg/ml concentration with methanol and physcion to 8 μg/ml concentration.

Analysis was perfomed using a UltiMate 3000DGLC system (Thermo Fisher in the USA) at the Analysis and Testing Center of the Southwest University of Science and Technology, China. This HPLC method was used with Zorbax SB-C18 column (250 mm×4.6 mm, 5.0 m). The mobile phase was a mixture of methanol-0.1% phosphate acid (85:15). The column was thermostated at 30 ℃. Flow rate was 0.8 ml/min. Injection volume was 10 ul. Detection was at 254 nm [1].

Measurement of total phenol, non-adsorbed phenol, and tannin content

About 0.5 g rhubarb powder was transferred into a 250 ml brown volumetric flask and 150 ml water added. After overnight, the solution was treated with ultrasound for 10 minutes and cooled. Dilute with water to the scale and shake well. After standing, filter and discard 50ml of primary filtrate. Measure 20 ml of secondary filtrate, place it in a 100 ml Brown volumetric flask, dilute it with water to the scale and shake it well to obtain the test solution [16].

Measure 2 ml of test solution, 1 ml of phosphomolybdic tungstic acid test solution (Fuzhou Feijing Biotechnology Co., Ltd.), and 10 ml of water into a 25 ml Brown volumetric flask in sequence. Dilute with 29% sodium carbonate solution to the scale and shake well. Leave it for 30 minutes to obtain the total phenol determination solution [16].

Measure about 25 ml of test solution into a 100 ml conical flask containing 0.6 g casein (Chengdu Cologne Chemical Reagent Co., Ltd.) and seal it. Place the conical flask in a 30 ℃ water bath for 1 hour and shake from time to time. Remove to cool and shake well. After filtration, 2 ml of continuous filtrate is taken to prepare the non-adsorbed phenol determination solution according to the total phenol determination method. During the determination, the casein adsorption blank test shall be carried out at the same time and the blank value shall be calculated and deducted [16].

Standard curves were drawn according to pharmacopeia methods using 0.05 mg/ml gallic acid solution as a control [16].

The absorbance of all samples at the wavelength of 760 nm was measured on the UV-Vis spectrophotometer (Shanghai Yuan Analytical Instrument Co., Ltd., UV-8000S), and the content was obtained according to the standard curve. The calculation formula of tannin content is as follows: tannin content = total phenol-non-absorbed polyphenols [16].

Measurement of moisture, extractum, and total ash content

About 0.1 g of ten samples (40 mesh) were weighed in a weighing bottle and dried for about 5 h in an intelligent temperature controller at 105℃. It was covered and cooled in a desiccator and then weighed. The sample was further dried for 1h and reweighed until a constant weight was obtained [1].

About 2 g of ten samples (over 40 mesh) was weighed accurately into a porcelain crucible and heated first over a low flame till all the materials were completely charred and then heated in a muffle furnace to a constant weight to ensure complete conversion to ash [1].

The content of the extractum was determined by the hot immersion method. Take about 0.5 g of 10 sample powder (above 40 mesh) into a conical flask. It was extractumed with water as a solvent for 1h, heated and refluxed in a water bath for 1 h, and then filtered. Evaporate 6.25 ml filtrate on a rotary evaporator (Shanghai Yalong biochemical instrument factory, RE-52AA) and dry at 105℃ for 3 h, cool, and weigh again [1].

Results

Total and free anthraquinone contents of *Rheum tanguticum* rhizome

Anthraquinone chromatogram

The results showed that the anthraquinones in ten samples of rhubarb were separated well under the chromatographic conditions. When the retention time was about 5.14 min, aloe-emodin was isolated. When the retention time was about 5.99 min, rhein was also separated. When the retention time was about 8.46 min, emodin was isolated. Chrysophanol was isolated when the retention time was about 11.03 min. When the retention time was about 14.02 min, the physcion was separated. HPLC images of free

anthraquinone and total anthraquinone in the first batch of *R. tanguticum* were shown in Fig. 1 and Fig. 2 respectively. HPLC images of free anthraquinone and total anthraquinone in the second batch of *R. tanguticum* were shown in Fig. 3 and Fig. 4.

The number of theoretical plates of emodin peaks in the chromatograms of free anthraquinone and Total Anthraquinone can reach about 9000, indicating that the chromatographic system is applicable and effective.

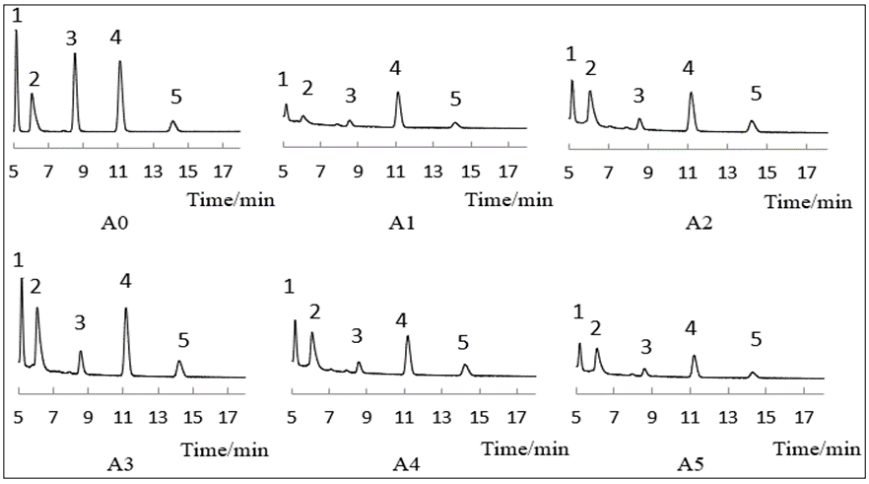

Fig 1: HPLC chromatography of free anthraquinone in standard substance (A0) and five samples of *R. tanguticum* rhizome (A1~A5). Peak 1~5 represents aloe-emodin, rhein, emodin, chrysophanol, and physcion respectively.

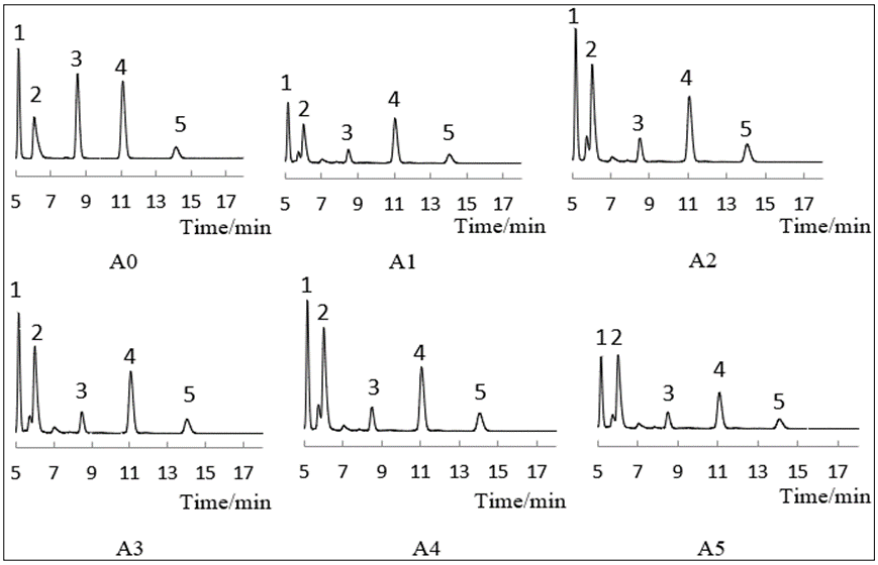

Fig 2: HPLC chromatography of total anthraquinone in standard substance (A0) and five samples of *R. tanguticum* rhizome (A1~A5). Peak 1~5 represents aloe-emodin, rhein, emodin, chrysophanol, and physcion respectively.

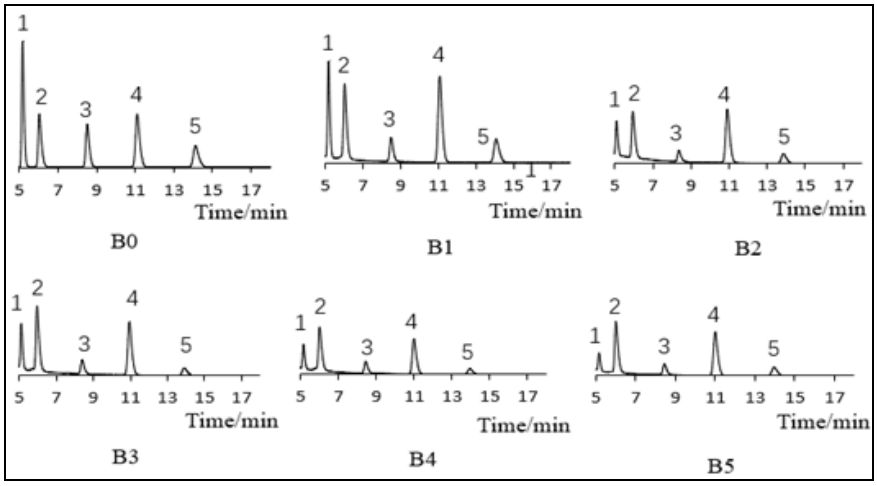

Fig 3: HPLC chromatography of free anthraquinone in standard substance (B0) and five samples of *R. tanguticum* rhizome (B1~B5). Peak 1~5 represents aloe-emodin, rhein, emodin, chrysophanol, and physcion respectively.

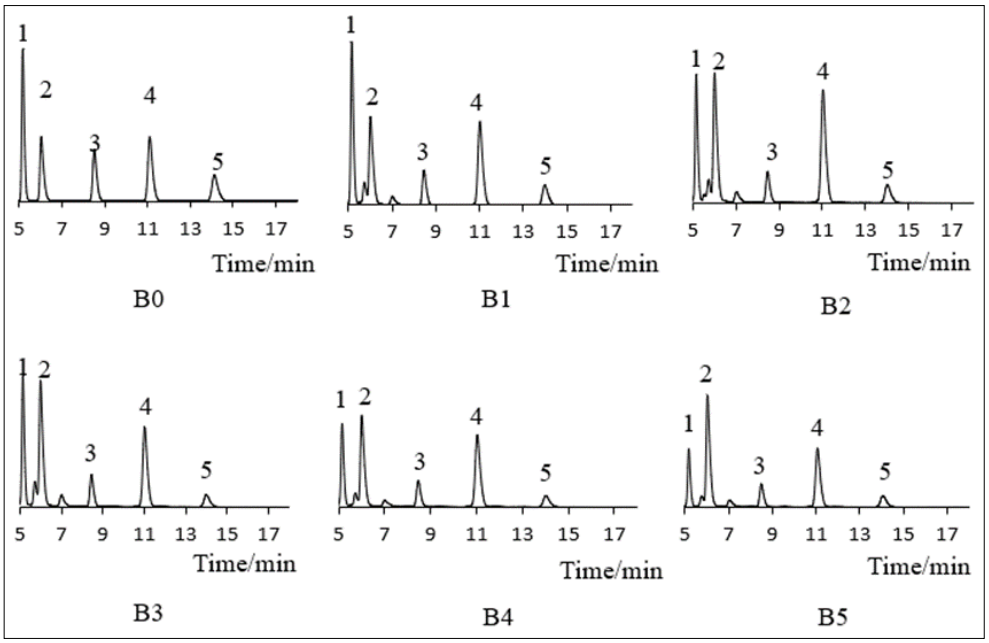

Fig 4: HPLC chromatography of total anthraquinone in standard substance (B0) and five samples of *R. tanguticum* rhizome (B1~B5). Peak 1~5 represents aloe-emodin, rhein, emodin, chrysophanol, and physcion respectively.

Content of total anthraquinones

The analyzed result is shown in Table 1. The total anthraquinone content of 10 samples of *R. tanguticum* reached more than 1.50%, which was in line with the standard of pharmacopeia. Among them, the A3 sample is the best, the percentage can reach 3.10%. The percentages of B2 and A2 samples were also over 3%, reaching 3.07% and 3.05%, respectively. The contents of A4, B1, and B3 samples exceeded 2%, which were 2.99%, 2.76%, and 2.46%, respectively. The samples with percentage content less than 2% were B4, B5, A5, and A1, among which the lowest sample was A1, whose total anthraquinone percentage content was only 1.59%, which was only slightly higher than that stipulated by the pharmacopeia, and the other three samples were 1.92%, 1.81%, and 1.72%, respectively.

In addition, the results showed that among the five total anthraquinone components in the root of 10 kinds of *R. tanguticum*, the highest content was rhein with 10.51 mg/g. The second was aloe-emodin with 8.06 mg/g. Emodin content was the lowest and the highest was only 2.20 mg/g, which was far less than the other four anthraquinones.

Table 1: Total anthraquinone con tent in *Rheum tanguticum* rhizome

No.	Aloe-emodin (mg/g)	Rhein (mg/g)	Emodin (mg/g)	Chrysophanol (mg/g)	Physcion (mg/g)	Total anthraquinone (%)
A1	3.69	4.38	1.20	3.81	2.85	1.59
A2	7.56	10.05	1.90	5.34	5.60	3.05
A3	8.06	10.51	1.98	5.77	5.05	3.14
A4	7.52	9.64	1.89	5.24	5.56	2.99
A5	3.80	6.70	1.23	2.79	2.72	1.72
B1	7.55	7.04	2.20	5.51	5.29	2.76
B2	6.11	10.18	2.02	7.55	4.80	3.07
B3	5.75	9.06	1.89	4.93	3.02	2.46
B4	3.79	6.48	1.58	4.58	2.81	1.92
B5	2.64	7.44	1.44	3.73	2.85	1.81

Content of free anthraquinones

It was found that the content of free anthraquinone was different from the content of total anthraquinone in the 10 samples, and the content of free anthraquinone in the sample B1 was the best, and the percentage of anthraquinone reached 0.39% (Table 2). The percentage of B3 and B5 reached 0.22% and 0.21%, respectively. The contents of A3 and B2 samples reached the pharmacopeia standard of 0.20%. The percentage of free anthraquinone in other samples did not reach 0.20%, which did not reach the standard of pharmacopeia. Among the samples that did not meet the standard of pharmacopeia, B4 was the best, with a percentage of 0.17%. A2 and A4 followed with 0.11% and 0.10%, respectively. The content of free anthraquinone in A5 and A1 was lower, which was 0.07% and 0.05%, respectively.

In addition, for the contents of five free anthraquinones, the trend of the results was roughly the same as that of the total anthraquinones except for the B1 sample. Rhein was the highest, and the highest content of rhein in the *R. tanguticum* sample was 0.76 mg/g. Chrysophanol and physcion were the next, with the highest values of 0.60 mg/g and 0.45 mg/g, respectively. Emodin was the lowest and the highest was only 0.16 mg/g, which was far lower than the other four chemical components. However, the highest content of physcion in the B1 sample was 1.03 mg/g. Chrysophanol and rhein were the next, which were 0.97 mg/g and 0.93 mg/g, respectively. The lowest was emodin, which was only 0.27 mg/g.

Table 2: Free anthraquinone content in *Rheum tanguticum* rhizome

No.	Aloe-emodin (mg/g)	Rhein (mg/g)	Emodin (mg/g)	Chrysophanol (mg/g)	Physcion (mg/g)	Free anthraquinone (%)
A1	0.08	0.06	0.05	0.22	0.13	0.05
A2	0.19	0.31	0.08	0.25	0.27	0.11
A3	0.39	0.59	0.16	0.45	0.41	0.20
A4	0.18	0.30	0.08	0.23	0.25	0.10
A5	0.13	0.23	0.06	0.16	0.14	0.07
B1	0.75	0.93	0.27	0.97	1.03	0.39
B2	0.27	0.55	0.13	0.60	0.44	0.20
B3	0.34	0.76	0.16	0.56	0.33	0.22
B4	0.22	0.61	0.15	0.43	0.22	0.17
B5	0.18	0.73	0.15	0.60	0.45	0.21

Total phenol, non-adsorbed phenol and tannin content of *Rheum tanguticum* rhizome

The standard graph for gallic acid with UV spectrophotometry was plotted by taking concentration of gallic acid on x-axis and absorbance on y-axis and was shown in Fig 5. The results show that the regression equation is Y=90.6491x+0.1011 (R^2=0.9996). Therefore, the linear range of gallic acid solution was 0.001~0.10mg/ml.

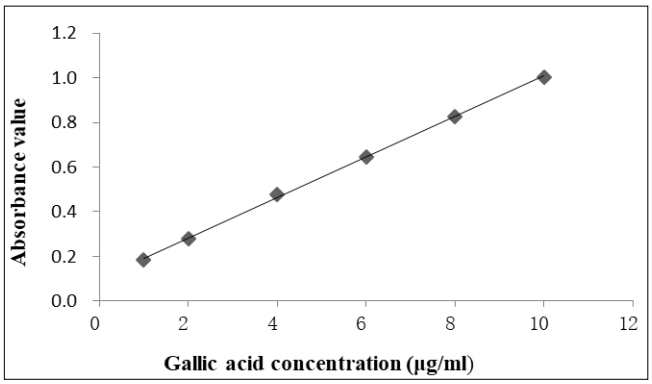

Fig 5: Standard curve of gallic acid with UV spectrophotometry

The tannin content is obtained by subtracting the non-adsorbed phenol from the total phenol. As can be seen from the results, the highest content of B5 was 52.46 mg/g. The content of A3 was also more than 50 mg/g, reaching 52.12 mg/g. The contents of A4, B2, A2, and B1 were 47.71 mg/g, 47.75 mg/g, 46.88 mg/g, and 45.91 mg/g, respectively. B4, B3, A5, and A1 were lower than 40mg/g, and the tannin content of A1 was significantly lower than that of other samples (26.61 mg/g). Others were 37.50 mg/g, 37.23 mg/g and 35.98 mg/g, respectively. The results are shown in Table 3.

Table 3: Total phenol, non-adsorbed phenol, and tannin content in *Rheum tanguticum* rhizome

No.	Total phenol (mg/g)	Non-adsorbed phenol (mg/g)	Tannin (mg/g)
A1	34.18	7.16	26.61
A2	51.97	4.68	46.88
A3	60.66	8.12	52.12
A4	63.14	15.02	47.71
A5	42.28	10.88	35.98
B1	61.91	15.58	45.91
B2	61.98	14.06	47.50
B3	58.05	20.40	37.23
B4	50.26	12.34	37.50
B5	73.01	20.13	52.46

Moisture, extractum and total ash content of *Rheum tanguticum* rhizome

The analyzed result is shown in Table 4.The contents of extractum and total ash in sample A4 were the highest, which were 42.81±0.12 and 8.84±0.04, respectively. The lowest extractum content of B1 was 32.66±0.11, which was not significantly different from B2 and B4. The total ash content of B4 was only 3.47±0.03, which was significantly lower than other samples.

The highest moisture content of A5 was 7.28±0.03, but there was no significant difference between A5 and A2, B2, B4, and B5. The moisture content of A1 was only 5.42±0.09, which was significantly lower than that of other samples. However, the total ash content of the 10 samples was not more than 10.0%, the moisture content was not more than 15.0%, the extractum was much higher than 25.0%, and the average was about 38%, all of which met the requirements of pharmacopeia.

Table 4: Moisture, extractum, and total ash content of *Rheum tanguticum* rhizome

No.	Moisture/%	Extractum/%	Total ash/%
A1	5.42±0.09 ^{d}	39.25±0.41 ^{ab}	8.04±0.01 ^{b}
A2	6.68±0.01 ^{abc}	42.68±0.21 ^{a}	7.91±0.01 ^{b}
A3	6.52±0.02 ^{bc}	42.56±0.19 ^{a}	7.45±0.02 ^{b}
A4	6.39±0.06 ^{bc}	42.81±0.12 ^{a}	8.84±0.04 ^{a}
A5	7.28±0.03 ^{a}	42.75±0.17 ^{a}	6.18±0.09 ^{c}
B1	6.29±0.03 ^{c}	32.66±0.11 ^{c}	4.73±0.06 ^{e}
B2	6.77±0.03 ^{abc}	34.46±0.11 ^{c}	5.67±0.04 ^{cd}
B3	6.47±0.03 ^{bc}	36.26±0.02 ^{bc}	4.26±0.02 ^{e}
B4	6.66±0.02 ^{abc}	33.23±0.09 ^{c}	3.47±0.03 ^{f}
B5	7.03±0.06 ^{ab}	39.54±0.11 ^{ab}	5.45±0.03 ^{d}

The data are the average of 3 repeated determinations ±SE; Superscript letters indicate the significance of the difference (LSD, $p<0.05$). The same superscript letters in the same column indicate that there is no significant difference among the samples.

Quality evaluation of *Rheum tanguticum*

According to the requirements of the Chinese Pharmacopoeia in the 2020 edition [1], the qualified *R. tanguticum* shall meet the following requirements: the water content shall not exceed 15.0%, the total ash content shall not exceed 10.0%, the extractumed content shall not be less than 25.0%, the total anthraquinone content shall not be less than 1.5% and the free anthraquinone content shall not be less than 0.20%. The moisture, extractum, total ash and total anthraquinone contents of all *R. tanguticum* met the requirements prescribed by the Chinese Pharmacopoeia. The main reason for the unqualified quality is that the content of

free anthraquinone did not meet the standard. The qualified samples were A3, B1, B2, B3, and B5 respectively, while A1, A2, A4, A5, and B4 failed to meet the standard. The results are shown in Table 5.

Table 5: Quality evaluation of *Rheum tanguticum* rhizome

No.	Moisture /%	Extractum /%	Total ash /%	Total anthraquinone /%	Free anthraquinone /%	Up to standard or not
Standard	≤15.00	≥25.00	≤10.00	≥1.50	≥0.20	Y
A1	5.42	39.25	8.04	1.59	0.05	N
A2	6.68	42.68	7.91	3.05	0.11	N
A3	6.52	42.56	7.45	3.14	0.20	Y
A4	6.39	42.81	8.84	2.99	0.10	N
A5	7.28	42.75	6.18	1.72	0.07	N
B1	6.29	32.66	4.73	2.76	0.39	Y
B2	6.77	34.46	5.67	3.07	0.20	Y
B3	6.47	36.26	4.26	2.46	0.22	Y
B4	6.66	33.23	3.47	1.92	0.17	N
B5	7.03	39.54	5.45	1.81	0.21	Y

Y stands for compliance with China Pharmacopoeia standards and N stands for not.

Discussion

This study used 10 kinds of *Rheum tanguticum* as experimental materials and collected them from different places in Ruoergai County, Sichuan Province. The content of anthraquinone was determined by the HPLC method, the rhubarb tanning content was determined by UV method, and the content of moisture, total ash, and the extractum was determined according to the 2020 Chinese Pharmacopoeia, thus performing the quality analysis of rhubarb materials.

Early studies showed that the main difference between genuine and pseudorhubarb is whether it contains rhein [17]. All samples of this test contain rhein so that all rhubarb materials are genuine. According to the moisture, total ash, and extractum of 10 kinds of *R. tanguticum* samples, all met the standard of pharmacopeia. Anthraquinones, as the main chemical component in rhubarb, have a variety of physiological activities. Neyrinck A M *et al*., found that a rhubarb extractum rich in anthraquinones could reduce hepatic tissue damage [18]. However, the composition content of rhubarb anthraquinone in different places, mainly due to the altitude, light, and life, and other conditions of its growth [19]. Wang *et al*. found that the total anthraquinone amount of wild rhubarb was twice that of planting rhubarb [20]. The total anthraquinone content in all 10 rhubarb samples measured by HPLC was higher than 1.50% and met the Pharmacopoeia standard. However, some materials may have limited growth conditions, with their free anthraquinone content falling below 0.20%, and they did not meet the pharmacopeia criteria.

The tank components are very high in rhubarb material around 10%~30% [12]. All test samples did not reach 10%~30%, the main reason is that tannins contain gallic acid, catechin, and tannic acid. According to the measurement method of Chinese Pharmacopoeia, gallic acid is mainly used as the reference for standard curve detection, and gallic acid is also used as the reference in European Pharmacopoeia, but skin powder is used as the adsorbent [21]. However, Wang *et al* found that the most rhubarb tannic content is tannic acid, and the content of the tannic composition is affected by the amount of casein [22-23]. Therefore, the rhubarb tannic content measured according to the pharmacopeia is generally low, and the tannic acid should be measured as the control product.

Conclusion

The test results showed that all the *Rheum tanguticum* materials tested in the institute were genuine rhubarb and the active component contents between the rhubarb were distinct and all different. The main reason is affected by the geographical, climatic conditions, great differences in germplasm resources, and planting technology. The detection of the effective components of *R. tanguticum* will be important for the germplasm resources identification and production technology improvement of the *R. tanguticum* planting area.

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