Quality Standard of Barberry Branches

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Abstract [**Objectives**] To establish the quality standard of barberry branches. [**Methods**] Microscopic identification and thin layer identification were used to qualitatively identify barberry branches. Berberine content was determined by HPLC method, and the content of water, total ash, acid insoluble ash and extract was detected according to the method of the *Chinese Pharmacopoeia* (2020 edition). [**Results**] The microscopic identification showed that the features were obvious, and stone cells, cork cells, epidermal cells, stomata, fibers and catheter with reticulated pores could be found. Berberine was detected in barberry branches by thin layer chromatography, and the characteristic spots were separated clearly. Moisture, total ash, and acid insoluble ash content shall not exceed 13%, 6%, and 3%, respectively, and extract content shall not be less than 10%. Berberine hydrochloride ($C_{20}H_{18}ClNO_4$) should be calculated in the branches of *Berberis wilsonae* Hemsley and *Berberis aggregata* C. K. Schneid., and berberine ($C_{20}H_{17}NO_4$) content should not be less than 0.05%. The linear relationship was good in the range of 0.002 - 0.240 mg/mL (R^2 = 0.999 5). The average recovery was 89.63%, and *RSD* was 5.28%. [**Conclusions**] The method was simple, accurate and reproducible, and can be used for the quality control of barberry branches.

Key words Barberry branches, Berberine, *Berberis*, *Berberis wilsonae* Hemsley, *Berberis aggregata* C. K. Schneid., TLC, HPLC

1 Introduction

Barberry branches, which are the dry ground part of *Berberis wilsonae* Hemsley and *Berberis aggregata* C. K. Schneid., are one of the commonly used clinical medicines in traditional Qiang medicine, have the effect of clearing heat and dampness, purging fire and detoxifying[1-2]. It is used to cure abdominal pain and diarrhea, sores of mouth and tongue, eye swelling and pain, carbuncle swelling and sore poison, *etc.* The main chemical components of plants in this genus are alkaloids, including proberberine type, bibenzyl isoquinoline, apophen, apophen-isoquinoline and simple isoquinoline alkaloids, *etc.*, and there are many chemical components, such as sterols, lignans and organic acids[3-5]. Modern pharmacological studies have shown that the plants in this genus have antibiosis, blood pressure lowering, blood lipid lowering, anti-tumor, antidiabetics, analgesia, liver protection, antioxidation and other effects[6-10]. Its main component berberine compound has antibiosis, anti-inflammation, antioxidation, liver protection and heart protection activities[11-13]. barberry branches are rich in wild resources, but have not been recorded in the quality standards of medicinal materials, and there are few related reports on them. In order to fully consider the protection and sustainable utilization of the plant resources, the original plant, characters, identification, inspection items, content determination, *etc.* of barberry branches were studied to provide experimental data for the *Standard for Qiang, Yi and Miao Medicinal Materials in Sichuan Province* (2022 edition).

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2 Materials

2.1 Instruments Main instruments included Agilent 1100 Series high performance liquid chromatography (Agilent, USA), ATY124 Electronic Balance (SHIMADZU, Japan), AUW220D electronic balance (SHIMADZU, Japan), ultrasonic cleaning machine (Shenzhen Jie Meng Cleaning Equipment Co., Ltd., China), and CAMAG ATS 4 automatic thin layer point tester (Kamar, Switzerland).

2.2 Reagents and drugs The main reagents were control product berberine hydrochloride (batch number: RP200607, purity > 99%) (Chengdu Medesheng Technology Co., Ltd.), silica gel G sheet (Qingdao Marine Chemical Plant), acetonitrile (chromatographically pure), and potassium dihydrogen phosphate (Chengdu Colon Chemical Co., Ltd.). All other reagents were analytically pure. The water was ultrapure water.

2.3 Medicinal materials 8 batches of barberry branches were collected in 2021, and identified by Professor Liu Yuan of Southwest University for Nationalities as the dry ground part of *B. wilsonae* Hemsley and *B. aggregata* C. K. Schneid. The information is shown in Table 1.

3 Methods and results

3.1 Identification

3.1.1 Identification of the original plants. *B. wilsonae* Hemsley is a semi-evergreen shrub, about 1 m tall. Old branches are brown

Table 1 Information of samples

Sample	No.	Collecting location	Altitude m	Collecting time	Longitude and latitude
Berberis wilsonae	J1	Lvziping, Fengyi Town, Maoxian County, Aba Prefecture	2 435	2021 – 08 – 16	103°50′07.95″ E, 31°41′29.41″ N
Hemsley (J1-J4)	J2	Meigu County, Liangshan Prefecture	2 437	2021 – 08 – 19	_
	J3	Longdong Village, Fengyi Town, Maoxian County, Aba Prefecture	1 799	2021 – 08 – 18	103°51′12.57″ E, 31°40′10.74″ N
	J4	Lvziping, Fengyi Town, Maoxian County, Aba Prefecture	1 832	2021 – 08 – 18	103°50′07.78″ E, 31°41′30.18″ N
Berberis aggregata C. K.	D5	Yadu Village, Chibusu Town, Maoxian County, Aba Prefecture	2 619	2021 – 08 – 16	103°52′15.47″ E, 31°52′42.37″ N
Schneid. (D5-D8)	D6	Daserigou, Chibusu Town, Maoxian County, Aba Prefecture	2 544	2021 – 08 – 19	103°19′52.60″ E, 31°41′27.91″ N
	D7	Xiaogou, Fengyi Town, Maoxian County, Aba Prefecture	1 910	2021 – 08 – 17	103°53′20.52″ E, 31°42′44.92″ N
	D8	Yadu Village, Chibusu Town, Maoxian County, Aba Prefecture	2 566	2021 – 08 – 17	103°52′15.42″ E, 31°52′42.45″ N

gray, while young branches are dark red and ribbed, with scattered black warts. Stem spines are slender, triforked, pale yellow or mauve red, and sometimes simple or absent. Leaves are leathery, obovate, obovate spatulate or oblanceolate, and have an entire margin, or the margin has 1 – 2 fine spines occasionally; they are nearly sessile. 4 – 7 flowers grow in thick clusters; pedicels are brown, and flowers are golden yellow; bracteoles are ovate; there are two kinds of sepals, of which outer sepals are ovate, and the inner sepals are obovate orbicular or obovate. Petals are obovate, and have a cleft apex and subacute lobe. Berries are nearly spherical and pink, and the apex has a distinct persistent style with slight white or pink. The flowering period is from June to September, and the fruiting period is from January to February in the following year.

B. aggregata C. K. Schneid. is a semi-evergreen or deciduous shrub, 2 – 3 m high. Old branches are dark brown, glabrous, and angulated. Young branches are light brown and puberulent, with sparse black warts. Stem spines are triforked and pale yellow. Leaves are nearly leathery, obovate-oblong or obovate, and obtuse, with one spiny tip. The leaf margin is spreading, and there are 2 – 8 spines on each side. It is sometimes entire. Petiole is short, or they are nearly sessile. Berries are nearly spherical or ovoid and red. The apex has a distinct persistent style and no white or pink. The flowering period is from May to June, and the fruiting period is from July to September.

3.1.2 Character identification. *B. wilsonae* Hemsley branches are 1 – 7 cm long. Old branches are brown gray, while young branches are dark red and ribbed. Stem spines are slender, triforked, 0.2 – 2.0 cm long, and pale yellow or mauve red. Leaves are leathery, obovate, obovate spatulate or oblanceolate, 0.1 – 1.5 long, and 0.2 – 0.6 cm wide, and have an entire margin, or the margin has 1 – 2 fine spines occasionally. Flowers are sometimes golden yellow, and bracteoles are ovate. Fruit is sometimes visible and nearly spherical. They are slightly bitter.

Different from *B. wilsonae* Hemsley branches, the stem thorns of *B. aggregata* C. K. Schneid. are thicker. Leaves are nearly leathery, 0.3 – 2.5 cm long, 0.4 – 1.5 cm wide, and obtuse, with one spiny tip, and the margin is spiny. Fruit is sometimes visible and ovoid.

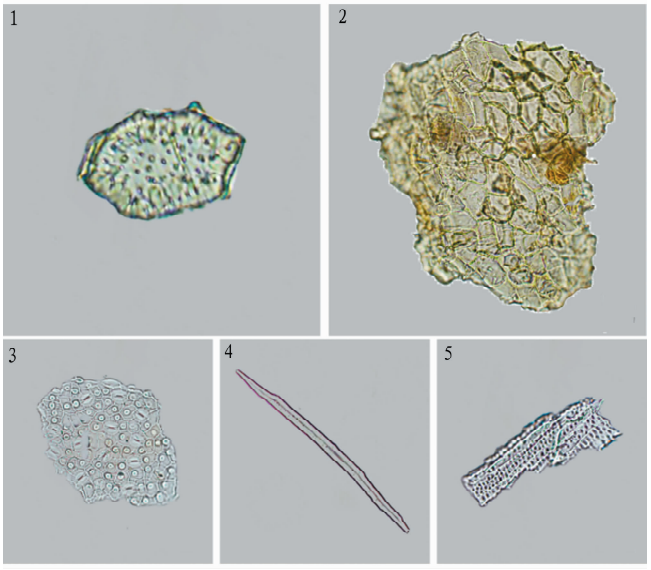
3.1.3 Microscopic identification. The powder is grayish brown to brownish yellow. Stone cells are yellowish green to yellowish brown and present in all sizes and shapes, and often exist in sheets, with the diameter of 8 – 46 μm. Cork cells are oblong and yellowish or yellowish brown, with thick walls. Leaf epidermal cells are irregular, and have circular protrusions. Stomata are infinitive, and there are 3 – 6 accessory cells. Fibers are scattered individually and long prismatic, and the edges are sometimes curved like microwaves. Pores and furrows are distinct and 12 – 30 μm in diameter. Catheters with reticulated pores are common, with the diameter of 15 – 28 μm (Fig. 1).

3.1.4 Identification by thin layer chromatography. Firstly, 1.0 g of the powder of barberry branches (passing through a No. 3 sieve), to which 20 mL of methanol was added. After being treated in ultrasonic wave for 20 min, it was filtered, and the filtrate was dried. 5 mL of methanol was added to the residue to dissolve it, and it was as the test product solution. Berberine hydrochloride control substance was added with methanol to prepare 0.1 mg/mL solution as the control solution. According to the 0502 thin layer chromatography in the General Rules of Volume IV of the *Pharmacopoeia of the People's Republic of China* (2020 edition) (hereinafter referred to as the *Chinese Pharmacopoeia*), 2 – 5 μL of the above two solutions were absorbed, respectively, and a silica gel G thin layer plate was dotted with them. The upper solution of n-butanol-acetoic-water (16 ∶ 4 ∶ 7) was as the development agent to make them unfolded. It was taken, dried, and viewed under UV lamp (365 nm). In the chromatogram of the test product, fluorescence spots of the same color were displayed at corresponding positions to the chromatogram of the control product (Fig. 2).

3.2 Determination of moisture, ash and extract content In accordance with the General Rules of Volume IV of the *Chinese Pharmacopoeia*[14], the content of moisture (the fourth method of General Rules 0832), total ash (General Rules 2302), acid insoluble ash (General Rules 2302), and extract (General Rules 2201) were detected (Table 2).

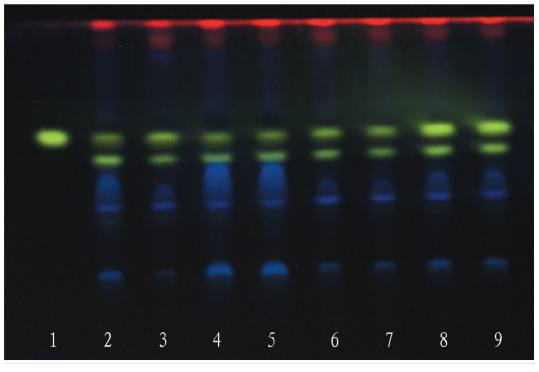
Table 2 Water, ash and extract content in the samples %

Sources of the samples	Moisture	Total ash	Acid insoluble ash	Extract
J1	5.81	3.70	0.71	17.56
J2	7.16	5.97	1.66	16.40
J3	7.01	3.66	0.54	24.43
J4	7.27	2.82	0.40	21.83
D5	5.89	4.92	0.42	17.64
D6	7.42	4.60	0.22	14.26
D7	7.38	4.63	0.95	16.00
D8	6.03	3.73	0.63	18.04
Average	6.75	4.29	0.69	18.27



Note: 1. Stone cells; 2. Cork cells; 3. Epidermal cells and stomata; 4. Fiber; 5. Catheter with reticulated pores.

Fig. 1 Microscopic identification of barberry branches



Note: 1. Berberine hydrochloride control substance; 2 – 9. 8 batches of barberry branch samples (2 – 5. J1 – J4; 6 – 9. D1 – D4).

Fig. 2 Thin layer identification map of barberry branches

3.3 Determination of berberine content[14]

3.3.1 Chromatographic conditions. Shimadzu WondaSilTM C_{18} (4.6 mm × 150 mm, 5 μm) was adopted. Isometric elution was conducted in acetonitrile – 0.02 mol/L potassium dihydrogen phosphate solution (22 : 78). The detection wavelength was 265 nm. Column temperature was 30 ℃. The flow rate was 1.0 mL/min. The sampling size was 10 μL. The results are shown in Fig. 3.

3.3.2 Preparation of test product solution. Firstly, about 1 g of this powder (passing through No. 4 sieve) was put in a corked conical bottle, to which 50 mL of methanol was added, and it was weighed. It was extracted in ultrasonic wave for 1 h, cooled, and weighed again. Methanol was used to make up the weight lost. It was shaken well and filtered, and the filtrate was taken to obtain the test product solution.

3.3.3 Investigation of linear relationship. At first, 11.98 mg of berberine hydrochloride reference substance was placed in a 10 mL volumetric bottle, to which methanol was added to make 1.198 mg/mL of berberine hydrochloride reference substance as the reserve solution. 2 mL of the above reserve was put in a 10 mL volumetric bottle, to which methanol was added to make 0.240 mg/mL solution; 4 mL of 0.240 mg/mL solution was put in a 10 mL volumetric bottle, to which methanol was added to make 0.096 mg/mL solution; 4 mL of 0.096 mg/mL solution was put in a 10 mL volumetric bottle, to which methanol was added to make 0.038 mg/mL solution, and the concentrations 0.015 and 0.002 mg/mL were obtained. Taking the peak area (Y) as the ordinate and berberine hydrochloride content (X, mg/mL) as the abscissa, the regression equation of berberine hydrochloride was obtained: Y = 36 102X – 12.906, R^2 = 0.999 5. The results showed that berberine hydrochloride had good linearity in the range of 0.002 – 0.240 mg/mL.

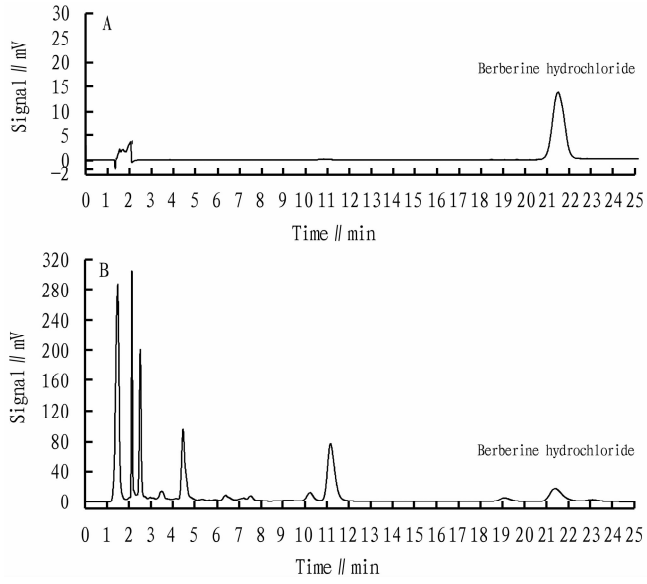


Fig. 3 HPLC spectrum of berberine content in the control (A) and test products (B)

3.3.4 Precision test. 10 μL of the reference solution of berberine hydrochloride was precisely taken and sampled for 6 times under the chromatographic conditions of Section **3.3.1**. The *RSD* of the peak area of berberine hydrochloride was 0.42%, showing that the instrument had good precision.

3.3.5 Stability test. An appropriate amount of test solution (D1) was sampled at 0, 4, 10, 14 and 24 h according to the chromatographic conditions of Section **3.3.1**. The *RSD* of the peak area of berberine hydrochloride was 0.44%, indicating that the control solution and the test solution had good stability within 24 h.

3.3.6 Repeatability test. 6 samples of fine powder (D1) were accurately weighed (each sample was about 1.0 g) to make 6 parallel test solutions. Under the chromatographic condition of Section **3.3.1**, 10 μL of the test solutions was sampled. The peak area was recorded, and the content was calculated. The *RSD* of berberine content was 1.13%, indicating that the method had good repeatability.

3.3.7 Sampling recovery test. Six samples of fine powder (D1) were accurately weighed (each sample was about 0.5 g) and placed in a corked conical bottle, to which an appropriate amount

of reference product was added. The test solution was prepared according to the method in Section **3.3.2**. The samples were injected under the chromatographic conditions of Section **3.3.1**. The average recovery rate of berberine was 89.63%, and *RSD* was 5.28% (Table 3).

Table 3 Results of sampling recovery test of berberine ($n=6$)

No.	Sample mass//g	Content of medicinal material//mg	Addition//mg	Detection//mg	Recovery rate//%	Average recovery rate//%	*RSD*//%
1	0.508 4	0.525 7	0.599 0	1.037 4	85.45	89.63	5.28
2	0.509 7	0.527 0	0.599 0	1.083 2	92.86		
3	0.501 6	0.518 7	0.599 0	1.028 0	85.03		
4	0.505 0	0.522 2	0.599 0	1.111 1	98.31		
5	0.500 7	0.517 7	0.599 0	1.032 1	85.88		
6	0.506 2	0.523 4	0.599 0	1.064 1	90.27		

3.3.8 Determination of sample content. The above 8 batches of barberry branches were taken to prepare test solutions according to the method in Section **3.3.2**, and the samples were injected under the chromatographic conditions of Section **3.3.1**. Peak area was measured and content was calculated by external standard method. The results were shown in Table 4.

Table 4 Berberine content in the 8 batches of barberry branches ($n=3$)

Sample	No.	Berberine content//%
Berberis wilsonae Hemsley (J1-J4)	J1	0.061
	J2	0.125
	J3	0.067
	J4	0.053
Berberis aggregata C. K. Schneid. (D5 – D8)	D5	0.103
	D6	0.066
	D7	0.297
	D8	0.103
	Average	0.109

4 Conclusions and discussion

4.1 Moisture, ash, and extract According to the related requirements in the General Rules of Volume IV of the *Chinese Pharmacopoeia* (2020 edition) and the "General Rules for the Verification of Medicinal Materials and Decoction Pieces", it is tentatively determined that the moisture content in the barberry branches shall not exceed 13%, and total ash shall not exceed 6%; acid insoluble ash shall not exceed 3%, and the extract shall not be less than 10%.

4.2 Thin-layer identification The development system of the upper solution of ethyl acetate-methanol-ammonia water (17∶4∶1)[15], ethyl acetate-butyl ketone-formic acid-water (10∶6∶2∶2)[16] and n-butanol-acetate-water (16∶4∶7) for barberry branches was investigated. The results showed that when the upper solution of n-butanol-acetic acid-water (16∶4∶7) was used as the developing agent, and the sample was developed, taken out, dried, and examined under an ultraviolet lamp (365 nm), *Rf* value was moderate, and the spots were clear, so the separation was the best.

4.3 Content and limit of berberine In this experiment, the mobile phase was acetonitrile – 0.02 mol/L potassium dihydrogen phosphate solution (24∶76), and it was found that the peak separation between berberine and the latter was not good, so the mobile phase ratio was adjusted; the separation of chromatographic peaks was better when the mobile phase ratio was 22∶78, so the ratio was selected as the mobile phase ratio.

The difference in berberine content measured in different producing areas and batches was relatively big, which may be related to the harvesting area, time and other factors. The method established in this experiment is simple and feasible, and can be used to accurately and scientifically evaluate the quality of barberry branches. Tentatively, Berberine hydrochloride ($C_{20}H_{18}ClNO_4$) should be calculated in barberry branches, and berberine ($C_{20}H_{17}NO_4$) content should not be less than 0.05%.

The quality control of medicinal materials is the key factor to guarantee the clinical effectiveness and safety of Chinese patent medicine. In this study, the quality standard of the Qiang medicinal material barberry branches was completed, and a comprehensive and specific quality control method was established to fill the gap that there is no standard for medicinal materials in Qiang medicine wine and Qiang ointment prescription for bone injury and bone disease in Qiang medicine and comprehensively utilize *B.* plants in Qiang nationality region of Sichuan.

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ingredients for the treatment of T2DM, and its specific targets need further research and experimental verification in the later stage, in order to ensure the accuracy of network pharmacological predictions. The discussion of capsicum network pharmacology can further promote the development and utilization of capsicum.

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