

Preliminary Study on Quality Analysis of Yao Medicine *Pileostegia tomentella* Hand.-Mazz.

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Abstract [Objectives] To establish a method for quality analysis of Medicine *Pileostegia tomentella* Hand.-Mazz. [Methods] According to the testing method of *Chinese Pharmacopoeia* (2020 Edition), the water content, total ash and alcohol-soluble extract of *P. tomentella* Hand.-Mazz. were determined. [Results] 10 batches of *P. tomentella* Hand.-Mazz. from different habitats and in different collection time in Guangxi all had the same plant morphology and medicinal properties. The experimental results showed that the water content was 7.92%–10.35%, the total ash content was 5.53%–6.62%, the acid-insoluble ash content was 0.59%–0.76%, and the extract content was 11.63%–16.12%. [Conclusions] The identification method in this study can be used for the quality analysis of *P. tomentella* Hand.-Mazz., and the experimental results can provide a scientific basis for the development and utilization of *P. tomentella* Hand.-Mazz. and the establishment of its quality standard.

Key words *Pileostegia tomentella* Hand.-Mazz., Character identification, Quality analysis

1 Introduction

Pileostegia tomentella Hand.-Mazz., a Yao medicine, is the vine and stem of *Pileostegia* Hook. f. et Thoms. in the Saxifragaceae family, and it is mainly distributed in Guangxi, Guangdong and other places. The whole plant has the effect of promoting blood circulation and dispelling blood stasis^[1-2]. *P. tomentella* Hand.-Mazz. has the effects of dispelling wind and dampness, removing blood stasis and relieving pain, and setting bones. It is often used clinically for treating soreness of waist and legs, rheumatism, numbness, and it is externally applied for treating traumatic injury, fracture, traumatic bleeding, etc.^[1-2] It is recorded in *Chinese Materia Medica*^[3], *Guangxi Medicinal Plants List*^[4], *Guangxi Flora*^[5] and other books. *P. tomentella* Hand.-Mazz. has anti-tumor effect and can be used to treat a variety of malignant tumors, so it is called Zhongliuteng, also known as Xiaoliuteng^[6]. Studies have shown that *P. tomentella* Hand.-Mazz. has significant anti-tumor effect^[7]. It is reported in the literature that the main effective components of *P. tomentella* Hand.-Mazz.

are flavonoids^[7-8], coumarins^[9-10], iridoids^[10] and so on. *P. tomentella* Hand.-Mazz., a Yao medicine, is a commonly used Chinese herbal medicine. At present, there is little research on its medicinal resources, and there is no perfect quality standard control method. In order to develop the medicinal resources of *P. tomentella* Hand.-Mazz. and establish a scientific quality control method of medicinal materials, the characteristics, tests and extracts of *P. tomentella* Hand.-Mazz. were systematically studied in this paper.

2 Materials

2.1 Instruments and reagents 101A-3E electric air blast drying oven (Shanghai Experimental Instrument Factory Co., Ltd.); SX2-4-10 box resistance furnace-muffle furnace (Shanghai Experimental Instrument Factory Co., Ltd.); HWSZ6 electric thermostatic water bath (Shanghai Yiheng Scientific Instrument Co., Ltd.); SQP electronic analytical balance [Sartorius Scientific Instrument (Beijing) Co., Ltd.]. Ethanol (analytically pure, Sinopharm Chemical Reagent Co., Ltd.).

2.2 Medicinal materials A total of 10 batches of medicinal materials used in the experiment were collected from different areas in Guangxi, and were identified as *P. tomentella* Hand.-Mazz. by Professor Tan Yong from College of Pharmacy of Guangxi University of Chinese Medicine, which was the vine and stem of *P. Hook. f. et Thoms.* in the Saxifragaceae family. See Table 1 for specific sources.

3 Methods and results

3.1 Identification of plant morphology and traits It is evergreen climbing shrub, 16 m long; twigs, leaves and inflorescences were densely covered with light brown or rust-colored stellate pilose, often with 3–6 radial lines; old branches were cylindrical,

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subglabrous, grayish brown. Leaves were leathery, oblong or obovate-oblong, sparsely oblanceolate, 5 – 10 (18) cm long and 2.5 – 5.0 (8) cm wide. Apex was acute or broadly acute, with pointed end protruding. The base was round or slightly concave near petiole and heart-shaped, and the top two leaves of sparse branchlets were broadly cuneate. Edge was subentire or subapical-

ly triangular coarsely toothed or irregularly undulate. Young leaves were abaxially curly, sparsely covered with stellate hairs, later glabrescent, grayish green or yellowish green when dry. Its lower part was densely hairy, with 8 – 13 hairs on each side of leaf veins; petiole was 1.2 – 1.5 cm long.

Table 1 Sample information of *Pileostegia tomentella* Hand. -Mazz.

Medicinal material No.	Source of medicinal material	Collection time	Remarks
XMGGT1	Jingxi City, Guangxi Zhuang Autonomous Region	October 2022	Collection
XMGGT2	Napo County, Baise City	November 2022	Collection
XMGGT3	Lingyun County, Guangxi Zhuang Autonomous Region	November 2022	Market of Traditional Chinese Medicine in Yulin City
XMGGT4	Du'an County, Guangxi Zhuang Autonomous Region	September 2022	Collection
XMGGT5	Hezhou City, Guangxi Zhuang Autonomous Region	October 2022	Collection
XMGGT6	Tian'e County, Hechi City	November 2022	Market of Traditional Chinese Medicine in Yulin City
XMGGT7	Bama County, Guangxi Zhuang Autonomous Region	October 2022	Market of Traditional Chinese Medicine in Yulin City
XMGGT8	Debao County, Guangxi Zhuang Autonomous Region	August 2021	Market of Traditional Chinese Medicine in Yulin City
XMGGT9	Gongcheng County, Guilin City	October 2022	Collection
XMGGT10	Xing'an County, Guangxi Zhuang Autonomous Region	November 2021	Market of Traditional Chinese Medicine in Yulin City

Corymbose panicles were terminal, 10 – 25 cm long and wide; bracts were linear or subulate, 5 – 10 mm long, 1 – 2 mm wide, stellate hairy; flowers were white; pedicel was 2 mm long; calyx was tube cup-shaped, 2 mm high, and lobes were triangular, sparsely stellate hairy; petals were ovate, 2 mm long, caducous, glabrous; there were 8 – 10 stamens, filaments were 5 – 6 mm long; style was 1.5 mm long, stigma was paniculate, 4 – 6-lobed, hairy. Capsule was turbinate, flat-topped, 4 mm in diameter. It was sparsely stellate hairy, with persistent style and stigma, angulate, dark brown; seeds were small, 2 mm long, brown. The flowering period was from March to August, and the fruiting period was from September to December.

The root of this product was cylindrical, with yellowish brown to grayish brown surface, longitudinal wrinkles and marks, 0.2 – 2.0 cm in diameter; it was hard and difficult to break, with brown skin of the section and grayish white wood. The stem was cylindrical, with yellowish brown to grayish brown surface, longitudinal wrinkles and small marks. There were obvious irregular grooves in the cork of old stems, with a diameter of 0.6 – 3.0 cm. It was hard and difficult to break. The skin of the section was brown, the wood was gray-white, with fine radial texture. The pith was grayish yellow, or hollow. It had a slight smell and tasted slightly bitter.

3.2 Water content determination

3.2.1 Determination method. According to the water content determination method in General Rule 0832, Volume IV of *Chinese Pharmacopoeia* (2020 Edition), 2.5 g of medicinal powder (sifted by a 24-mesh sieve) was laid flat in a flat weighing bottle dried to a constant weight, with a thickness of less than 5 mm and a thickness of loose test sample of less than 10 mm, which was accurately weighed. After drying medicinal material at 105 °C for 5 h, and the bottle cap was closed, the medicinal material was moved to the dryer, cooled 30 min, and weighed precisely. It was dried at 105 °C for 1 h, cooled and weighed until the difference between the weights measured twice in a row did not exceed 5 mg.

According to the weight loss, the water content (%) in medicinal materials was calculated.

3.2.2 Water content determination results. According to the determination method in Section 3.2.1, the water content of 10 batches of *P. tomentella* Hand. -Mazz. from different producing areas in Guangxi and at different collection time was determined, and the results are shown in Table 2.

From Table 2, it can be seen that the water content of 10 batches of *P. tomentella* Hand. -Mazz. in Guangxi was different, and the average water content was 9.29%. In October, 2022, the water content of *P. tomentella* Hand. -Mazz. collected in Hezhou City, Guangxi Zhuang Autonomous Region was the highest among 10 batches of medicinal materials, reaching 10.35%, so the limit was set at 120% of the highest value^[11–14], and it was tentatively determined that the water content of *P. tomentella* Hand. -Mazz., a Yao medicine in Guangxi, was not higher than 12.42%.

Table 2 Determination results of water content in *Pileostegia tomentella* Hand. -Mazz. ($n=10$, %)

Medicinal material No.	Water content	Average content
XMGGT1	9.27	9.29
XMGGT2	8.43	
XMGGT3	10.05	
XMGGT4	9.71	
XMGGT5	10.20	
XMGGT6	7.92	
XMGGT7	8.70	
XMGGT8	10.35	
XMGGT9	9.62	
XMGGT10	8.65	

3.3 Ash determination

3.3.1 Determination of total ash. According to the ash determination method in General Rule 2302, Volume IV of *Chinese Pharmacopoeia* (2020 Edition), 4 g of medicinal powder (sifted by a

24-mesh sieve) was taken into a crucible dried to a constant weight, and weighed (accurate to 0.01 g). It was slowly heated to avoid burning the medicinal materials. When it was completely carbonized, the crucible was placed in a muffle furnace, and the temperature was gradually raised to 600 °C, so that it completely turned into ashes and reached a constant weight. According to the weight of residue, the content of total ash (%) in medicinal materials was calculated.

3.3.2 Determination of acid-insoluble ash. The ash obtained above was taken, and about 10 mL of dilute hydrochloric acid was slowly added to the crucible. The crucible was covered with a surface dish and heated in a water bath for 10 min. The surface dish was washed with 5 mL of hot water, and the washing liquid was transferred to the crucible and filtered with ashless filter paper. The residue in the crucible was washed on the filter paper with water until the washing solution did not show chloride reaction. The filter paper and the filter residue were moved to the same crucible and weighed. It was slowly heated, and when it was completely carbonized, the crucible was placed in a muffle furnace, and the temperature was gradually raised to 600 °C, so that it completely turned into ashes and reached a constant weight. According to the weight of residue, the content of acid-insoluble ash (%) in medicinal materials was calculated.

3.3.3 Ash determination results. According to the determination methods in Section 3.3.1 and 3.3.2, the ash content of 10 batches of *P. tomentella* Hand. -Mazz. from different producing areas in Guangxi and at different collection time were determined. The results are shown in Table 3.

Table 3 Determination results of ash content in *Pileostegia tomentella* Hand. -Mazz. ($n = 10$, %)

Medicinal material No.	Total ash		Acid-insoluble ash	
	Average content	RSD	Average content	RSD
XMGGT1	6.52	0.15	0.59	0.15
XMGGT2	6.30	0.20	0.62	0.20
XMGGT3	5.53	0.16	0.68	0.21
XMGGT4	6.31	0.24	0.67	0.13
XMGGT5	6.62	0.21	0.65	0.21
XMGGT6	6.39	0.24	0.70	0.18
XMGGT7	6.54	0.12	0.74	0.12
XMGGT8	5.78	0.15	0.69	0.16
XMGGT9	6.24	0.20	0.76	0.21
XMGGT10	6.48	0.16	0.72	0.10

It can be seen from Table 3 that there were some differences in the total ash and acid-insoluble ash content of 10 batches of *P. tomentella* Hand. -Mazz. in Guangxi. The highest content of total ash was 6.62% for *P. tomentella* Hand. -Mazz. collected in Hezhou City, Guangxi in October 2022, so the limit was set at 120% of the highest value^[7-10], and the total ash content of *P. tomentella* Hand. -Mazz. was tentatively determined to be not higher than 7.94%. In the determination of acid-insoluble ash, the

highest content was 0.76% for *P. tomentella* Hand. -Mazz. collected in Gongcheng County, Guilin City in October 2022, so the limit was set at 120% of the highest value^[11-14], and the acid-insoluble ash of *P. tomentella* Hand. -Mazz. was tentatively determined to be not higher than 0.91%.

3.4 Determination of extracts The medicinal materials used for determination need to be crushed so that they can be sifted by a 24-mesh sieve and mixed evenly.

3.4.1 Selection of determination conditions. 10% ethanol, 30% ethanol, 50% ethanol, 70% ethanol and 90% ethanol were used as solvents for *P. tomentella* Hand. -Mazz produced in Jingxi City, Guangxi, and the content of extracts in the medicinal materials was determined according to the alcohol-soluble extract determination method (cold-dipping method and hot-dipping method, using ethanol as solvent) in General Rule 2201, Volume IV of *Chinese Pharmacopoeia (2020 Edition)*.

The results showed that the extract content of *P. tomentella* Hand. -Mazz. determined by 70% ethanol solvent (hot-dipping method) was the highest, so 70% ethanol was used as the extraction solvent (Table 4).

Table 4 Investigation of ethanol cold-dipping method and hot-dipping method (%)

Solvent	Cold-dipping method		Hot-dipping method	
	Average percentage of extract	RSD	Average percentage of extract	RSD
10% ethanol	9.39	1.81	10.26	1.85
30% ethanol	11.08	1.73	12.61	1.72
50% ethanol	12.26	1.58	14.08	1.68
70% ethanol	14.52	1.56	15.92	1.80
90% ethanol	12.65	1.73	13.50	1.64

3.4.2 Determination method. According to the determination method of alcohol-soluble extracts in General Rule 2201, Volume IV of *Chinese Pharmacopoeia (2020 Edition)*, 2.5 g of medicinal materials were accurately weighed, placed in a 100 mL conical flask, 60 mL of water was accurately measured, weighed, and refluxed for 1 h after standing for 1 h. After cooling, the conical flask was taken, corked, and it was replenished with ethanol, shaken, and filtered with a drying filter. 25 mL of filtrate was precisely taken and placed in an evaporation dish dried to a constant weight. After evaporation in water bath, it was dried at 105 °C for 3 h, cooled in a dryer to cool for 30 min, and weighed quickly and accurately. According to the weight of the extract, the content of the extract (%) from *P. tomentella* Hand. -Mazz. was calculated.

3.4.3 Extract determination results. According to the determination method in Section 3.4.2, 10 batches of *P. tomentella* Hand. -Mazz. from different producing areas in Guangxi and at different collection time were determined, and the results are shown in Table 5.

From Table 5, it can be seen that there were some differences in the extract content of 10 batches of *P. tomentella* Hand. -Mazz. in Guangxi, with an average content of 14.66%. In October, 2022, the extract content of *P. tomentella* Hand. -Mazz. collected in Gongcheng County, Guilin City was the lowest in the determi-

nation of 10 batches of medicinal material extracts, reaching 11.63%, so the limit was set at 80% of the lowest value^[11-14], and the extract of *P. tomentella* Hand.-Mazz. was tentatively determined to be not less than 9.30%.

Table 5 Determination results of extract content in *Pileostegia tomentella* Hand.-Mazz. ($n = 10$, %)

Medicinal material No.	Extract content	Average content of extract	RSD
XMGGT1	16.02	14.66	2.35
XMGGT2	15.17		2.14
XMGGT3	15.30		1.76
XMGGT4	14.75		2.05
XMGGT5	16.12		2.12
XMGGT6	15.08		1.80
XMGGT7	13.93		1.74
XMGGT8	14.51		2.20
XMGGT9	11.63		2.13
XMGGT10	14.12		2.18

4 Conclusion and discussion

In this experiment, the water, ash and extract content of *P. tomentella* Hand.-Mazz. from different producing areas in Guangxi and at different collection time was determined. The test results are as follows: for *P. tomentella* Hand.-Mazz., the water content was not more than 12.42%, the total ash content was not more than 7.94%, the acid-insoluble ash content was not more than 0.91%, and the extract content was not less than 9.30%. The test results were in accordance with the regulations. The results showed that different producing areas and different collection time had influence on the water content, ash and extract of medicinal materials. In this experiment, the water content, ash content and alcohol-soluble extract content of *P. tomentella* Hand.-Mazz. were determined, and the experimental results can provide a scientific basis for the development and utilization of *P. tomentella* Hand.-Mazz. and the formulation of its quality standards.

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