Study on Quantity Value Transfer Relationship of *Triplostegia glandulifera* Wall. ex DC. Decoction Pieces-Standard Decoction-Finished Product

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Abstract [**Objectives**] In order to develop *Triplostegia glandulifera* Wall. ex DC. into foromula granules, a high performance liquid chromatography (HPLC) determination method and a TLC identification method for loganic acid in decoction pieces, standard decoction and formula granules of *T. glandulifera* were established, and the quantity value transfer relationship of *T. glandulifera* pieces-standard decoction-formula granules was investigated. [**Methods**] An Agilent 1260 II high performance liquid chromatograph and a Waters Symmetry C_{18} column (4.6 mm × 250 mm, 5 μm) were used to perform gradient elution with acetonitrile-0.1% phosphoric acid solution as the mobile phase at a column temperature of 30 ℃ and a flow rate of 1 mL/min, and the detection wavelength was 240 nm. The TLC identification method of *T. glandulifera* was established using ethyl acetate-methanol-water (10∶2∶1) as the developer, and examination was carried out under a UV lamp (254 nm). The quantity value transfer law was analyzed by using the extract yield, the content of loganic acid and TLC chromatograms as the main evaluation indexes. [**Results**] The method for the determination and identification of loganic acid is stable, reproducible and reliable. The average yield, average loganic acid content and average loganic acid content transfer rate of 3 batches of *T. glandulifera* formula granules were, respectively, 18.7%, 41.1 mg/g and 43.1%, each of which was within corresponding range of mean ± 3SD of the 15 batches of standard decoction. [**Conclusions**] The content determination and TLC identification methods of loganic acid can be used to evaluate the quality of *T. glandulifera* formula granules. This study provides data basis for further research on *T. glandulifera* formula granules, and promotes the modernization of medicines for ethnic minorities.

Key words Pharmaceutics of Chinese medicine, *Triplostegia glandulifera* Wall. ex DC., Standard decoction, Loganic acid, Content determination

1 Introduction

Triplostegia glandulifera Wall. ex DC., also known as Luoboshen, Tongzishen, *etc.*, was first recorded in *Selected Chinese Herbal Medicines in Yunnan Province* (*Yunnan Zhongcaoyao Xuan*)[1]. The dried tuberous roots of *T. glandulifera* are used as a medicine, which has the effects of regulating menstruation, promoting blood circulation and tonifying the kidney, and is mainly used for treating amenorrhea, irregular menstruation, lumbago due to kidney deficiency, nocturnal emission, impotence and infertility. *T. glandulifera* is generally collected in autumn, and then washed and dried[2].

T. glandulifera is a medicinal plant for ethnic minorities in Yunnan Province, and there are few modern literatures on it. Existing literatures mainly focus on the isolation and identification of chemical components and pharmacological effects[1,3-17]. Studies have shown that the medicinal materials of *T. glandulifera* mainly contain iridoids, triterpenoids and alkaloids[1,3-11]. Zhi *et al.*[18] quickly identified the medicinal materials of *T. glandulifera* by UPLC-ESI-Q-TOF-MS/M, and found that they contain chemical components such as chlorogenic acid, cryptochlorogenic acid, isochlorogenic acid A, isochlorogenic acid B, isochlorogenic acid C, loganin, loganic acid and sweroside. Some studies proved that *T. glandulifera* has hypoglycemic, anti-tumor, anti-virus, anti-stress and anti-oxidation effects[12-17]. In addition, Shuangshen Granules[19-22], which is widely reported in the literature, is a compound Chinese patent medicine[23-24] prepared from several different Chinese herbal medicines, and does not contain *T. glandulifera*. The research of *T. glandulifera* formula granules is still in the blank stage.

In this study, 15 batches of *T. glandulifera* decoction pieces from different producing areas were collected to prepare standard decoction and formula granules of *T. glandulifera* and establish the content determination and TLC identification methods of loganic acid, and with the standard decoction as a bridge[25-32], the transfer law of loganic acid content and TLC identification quantity of *T. glandulifera* decoction pieces-standard decoction-finished product was studied. This study provides a technical basis for further establishing the quality standard of *T. glandulifera* formula granules and better developing *T. glandulifera* formula granules.

2 Materials and methods

2.1 Instruments High performance liquid chromatograph: Waters Alliance e2695, provided by Waters Corporation; LC-15C, provided by Shimadzu Corporation; 1260 Infinity II, provided by Agilent Technologies Inc.; chromatographic column:

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Waters Symmetry C_{18} (4.6 mm × 250 mm, 5 μm), provided by Waters Corporation; Agilent Eclipse Plus C_{18} (4.6 mm × 250 mm, 5 μm), provided by Agilent Technologies Inc.; Thermoscientific Acclaim™ 120 C_{18} (4.6 mm × 250 mm, 5 μm), provided by Thermo Fisher Scientific Inc.

Analytical balance, provided by Sartorius Scientific Instruments (Beijing) Co., Ltd.; JM-A5002 electronic balance (d = 0.01 g), provided by Yuyao Jiming Weighing and Calibration Equipment Co., Ltd.; water-circulation vacuum pump, provided by Shanghai Senco Technology Co., Ltd.; ultrasonic cleaner (KQ-250, power 250 W, frequency 40 kHz), provided by Kun Shan Ultrasonic Instruments Co., Ltd.; three-purpose UV analyzer (ZF-2 model), provided by Shanghai Anting Electronic Instrument Factory; healthy medicated diet pot, provided by Hefei Royalstar Household Appliances Co., Ltd.; rotary evaporator (RE-3000 model), provided by Shanghai Yarong Biochemical Instrument Factory; freeze dryer (FD-1C-50 model), provided by Beijing Biocool Experimental Instrument Co., Ltd.; UPR-II-20L ultra-pure water system, provided by Sichuan ULUPURE Ultrapure Technology Co., Ltd.

2.2 Reagents Loganic acid reference substance (batch No.: 111865-202005, purity: 97.5%), provided by National Institutes for Food and Drug Control; acetonitrile (chromatographically pure), provided by Fisher Company, USA; phosphoric acid (chromatographically pure), provided by ACS company, USA; ultra-pure water, homemade.

2.3 Decoction pieces Fifteen batches of medicinal materials of *T. glandulifera* were identified as dried tuberous roots of *T. glandulifera* by Sun Baohui, director of Hebei Institute for Drug Control. Impurities were removed from the medicinal materials of *T. glandulifera* to obtain *T. glandulifera* decoction pieces[2]. Table 1 shows details of the samples.

Table 1 Information of 15 batches of *Triplostegia glandulifera* decoction pieces

No.	Batch number of decoction pieces	Producing area
1	2010101	Shuangbai County
2	2010102	Shuangbai County
3	2010103	Shuangbai County
4	2011101	Eryuan County
5	2011102	Eryuan County
6	2011103	Eryuan County
7	2011131	Weishan Yi and Hui Autonomous County
8	2011132	Weishan Yi and Hui Autonomous County
9	2011133	Weishan Yi and Hui Autonomous County
10	2011134	Yulong Naxi Autonomous County
11	2011135	Yulong Naxi Autonomous County
12	2011136	Yulong Naxi Autonomous County
13	2011141	Luquan Yi and Miao Autonomous County
14	2011142	Luquan Yi and Miao Autonomous County
15	2011143	Luquan Yi and Miao Autonomous County

2.4 Preparation of standard decoction *T. glandulifera* has the effects of tonifying the kidney, nourishing the liver, tonifying the spleen and calming heart, and belongs to nourishing medicines. Combined with the guiding principle of standard decoction preparation in *Technical Requirements for Quality Control and Standard Formulation of Traditional Chinese Medicine Formula Granules*[33], *T. glandulifera* decoction pieces were decocted twice, and the appropriate decocting time was 60 and 40 min respectively. Meanwhile, it is advisable to add water to a level 3 cm higher than the decoction pieces of *T. glandulifera*.

The 15 batches of *T. glandulifera* decoction pieces were prepared into standard decoctions, respectively. Specifically, 100 g of *T. glandulifera* was weighed each time, and the weighed material was added in a healthy medicated diet pot and soaked with water 9 times the mass of the pieces for the first time for 30 min. The liquid in the pot was heated to boiling with strong fire and kept at a state of slightly boiling for 60 min with slow fire. Next, the liquid medicine was filtered with a piece of 200-mesh (0.075 mm, the same below) filter cloth while it was hot, and the filtrate was added in a container for later use. Subsequently, water was added for the second time at an amount 7 times the mass of the decoction pieces, and the liquid in the pot was heated to boiling with strong fire and kept at a state of slightly boiling for 40 min. Next, the liquid medicine was filtered with a piece of 200-mesh filter cloth while it was hot. The two decoctions were combined, and the combined decoction was quickly cooled, and vacuum concentrated at low temperature (the temperature should not exceed 65 ℃) until the ratio of the mass (g) of the decoction pieces to the volume (mL) of the liquid medicine was about 1∶3. Finally, the concentrate was freeze-dried to obtain the standard decoction of *T. glandulifera*.

2.5 Preparation of formula granules Through the investigation of different technical conditions, the pilot-scale process of *T. glandulifera* extraction preparation was determined. In specific, a proper amount of *T. glandulifera* decoction pieces was decocted in water twice. For the first time, the medicinal material was decocted in water with an amount 10 times of the material for 90 min. For the second time, the decoction was obtained by decocting in water with an amount 8 times of the material for 60 min. Filtration was performed to obtained decoctions, which were combined, concentrated and spray-dried. Finally, granulation was performed after adding a proper amount of dextrin. Three batches of *T. glandulifera* decoction pieces with serial numbers 1 – 3 were taken to prepare *T. glandulifera* formula granules, respectively.

2.6 Content determination method

2.6.1 Chromatographic conditions. The chromatographic column used was Waters Symmetry C_{18} column (4.6 mm × 250 mm, 5 μm), and acetonitrile-0.1% (volume fraction, the same below) phosphoric acid was used as the mobile phase. Gradient elution was carried out according to the procedure in Table 2. The flow rate was 1.0 mL/min; the detection wavelength was 240 nm; the column temperature was 30 ℃; and the sample volume was 10 μL. The number of theoretical plates should be no less than 5 000 according to the peak of loganic acid.

Table 2 Gradient elution procedure

Time//min	φ (acetonitrile)//%	φ (0.1% phosphoric acid)//%
0 – 12	9	91
12 – 13	9→85	91→15
13 – 18	85	15
18 – 19	85→9	15→91
19 – 25	9	91

2.6.2 Preparation of reference solution. A proper amount of loganic acid reference substance was weighed accurately. Methanol was added to prepare a solution containing 0.2 mg per 1 mL, that is, the reference solution.

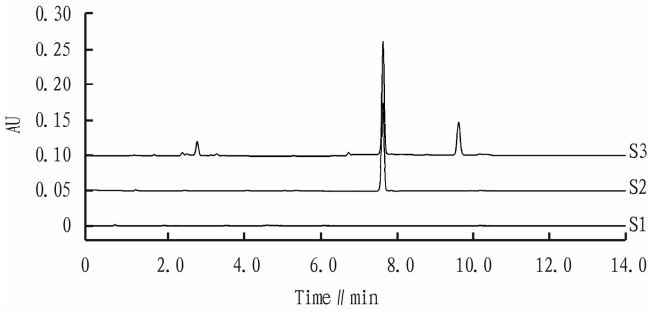
2.6.3 Preparation of test solutions. (i) Preparation of test solution of standard decoction: A proper amount of standard decoction of *T. glandulifera* was ground, and about 0.1 g was weighed accurately, and added into a conical flask with a stopper. Next, 25 mL of 75% (volume fraction, the same below) methanol solution was added accurately, and the conical flask was weighed for its mass. Next, ultrasonic treatment (power 250 W, frequency 40 kHz) was performed for 20 min. Subsequently, the conical flask was weighed again, and the lost mass was made up with 75% methanol solution. Finally, the extraction system was mixed well and filtered to obtain the filtrate.

(ii) Preparation of test solution of formula granules: Because the content of dextrin in formula granules was less, the sampling amount of formula granules after conversion was close to that of standard decoction, and the material basis of formula granules was consistent with that of standard decoction, the preparation method for the test solution of formula granules was the same as that of standard decoction.

2.7 Methodological investigation of content determination

2.7.1 Investigation of specificity. An appropriate amount of dextrin was weighed and prepared into a negative sample solution according to the preparation method of test solution in Section **2.6.3**. The reference solution, negative sample solution and test solution were determined according to the content determination conditions. The results are shown in Fig. 1. The results showed that dextrin did not interfere with the content determination, and the content determination method established in this study had good specificity and could be used to determine the content of loganic acid in standard decoction and formula granules of *T. glandulifera*.

2.7.2 Investigation of linearity. The reference solution of loganic acid with the mass concentration of 0.201 3 mg/mL was accurately injected into the liquid chromatograph according to the volumes of 4, 6, 10, 15 and 20 μL, respectively, and determined according to the chromatographic conditions in Section **2.6.1**. A standard curve was drawn with the peak area value of loganic acid as the *y*-axis and the sample volume of loganic acid as the *x*-axis. The regression equation was obtained as $y = 1.395\ 9 \times 10^6 x - 2.924\ 9 \times 10^3$, $R^2 = 0.999\ 1$, indicating a good linear relationship within the mass range of 0.885 2 – 4.026 μg for loganic acid.



NOTE S1. Negative control; S2. loganic acid control; S3. *T. glandulifera* standard decoction control.

Fig. 1 Assay chromatogram of *Triplostegia glandulifera* standard decoction

2.7.3 Investigation of precision. The standard decoction prepared from *T. glandulifera* decoction pieces with the batch number of 2010101 was prepared into a test solution according to the preparation method of test solution in Section **2.6.3**. Next, 10 μL of the loganic acid reference solution and test solution were accurately pipetted and continuously injected for 6 times according to the determined chromatographic conditions, respectively, and the *RSD* values of loganic acid peak area were calculate to be 0.98% and 0.64%. The results showed that the precision of the instruments was good.

2.7.4 Investigation of repeatability. The standard decoction prepared from *T. glandulifera* decoction pieces with the batch number of 2010101 was prepared into six parallel samples according to the preparation method of test solution in Section **2.6.3**. The samples were injected and determined according to the determined chromatographic conditions, and the *RSD* of loganic acid content was calculated to be 0.98%, which met the requirement of ≤1.5% stipulated by *Pharmacopoeia of the People's Republic of China* (2020 edition, Volume IV)[34] (hereinafter referred to as *Chinese Pharmacopoeia*) ≤1.5%.

2.7.5 Investigation of stability. The standard decoction prepared from *T. glandulifera* decoction pieces with the batch number of 2010101 was prepared into a test solution according to the preparation method of test solution in Section **2.6.3**. The solution was determined at 0, 6, 12, 18 and 24 h, respectively. The *RSD* of loganic acid peak area was 1.13%, indicating that the sample was stable within 24 h.

2.7.6 Investigation of accuracy. Six portions of standard *T. glandulifera* decoction were accurately weighed, 0.05 g each. The reference substance of loganic acid was added accurately according to 50%, 100% and 150% of the mass fraction of loganic acid in the sample, and test solutions were prepared according to the preparation method of test solution. After determination, the recovery was in the range of 98.7% – 101.1%, with an average value of 99.50%, and the *RSD* was 0.98%, which was in line with the provisions of *Chinese Pharmacopoeia*[34] on the recovery limit of 95% – 105%.

2.7.7 Study on durability. (i) Investigation of different column temperatures. The content of loganic acid was determined at column temperatures of 30, 35 and 40 ℃ respectively, and the effects of different column temperatures on the content deter-

mination were investigated. The results showed that under different column temperature conditions, the quantitative detection of the same sample showed good separation of loganic acid, and the results were basically consistent, indicating that the durability meets the requirements within the column temperature range of (35 ± 5) ℃.

(ii) Investigation of different flow rates. The content of loganic acid was determined at the flow rates of 0.9, 1.0 and 1.1 mL/min, respectively, and the effects of different flow rates on the content determination were investigated. The results showed that the quantitative detection of the same sample at different flow rates resulted in good separation of loganic acid, and the results were basically consistent, indicating that the durability met the requirements within the flow rate range of (1.0 ± 0.1) mL/min.

(iii) Investigation of different detection wavelengths. The content of loganic acid was determined at the detection wavelengths of 235, 240 and 245 nm, respectively, and the effects of different detection wavelengths on the content determination were investigated. The results showed that the quantitative detection of the same sample using different detection wavelengths resulted in good separation of loganic acid, and the results were basically consistent, indicating that the durability met the requirements within the wavelength range of (240 ± 5) nm.

(iv) Investigation of different initial ratios of mobile phases. The content of loganic acid was determined with the initial ratios of mobile phases (acetonitrile-0.1% phosphoric acid solution) at 8∶92, 9∶91 and 10∶90 (volume ratio, the same below), respectively, and the effects of different initial ratios of mobile phases on the content determination were investigated. The results showed that when different initial ratios of mobile phases were used for quantitative detection of the same sample, loganic acid was well separated and the results were basically consistent. It indicated that the durability met the requirements when the initial volume fraction of acetonitrile in the mobile phase solution was in the range of (9 ± 1)%.

(v) Investigation of different chromatographic columns. Different chromatographic columns (Agilent Eclipse Plus C_{18}, 4.6 mm × 250 mm, 5 μm; Waters Symmetry C_{18}, 4.6 mm × 250 mm, 5 μm; Thermoscientific Acclaim™ 120 C_{18}, 4.6 mm × 250 mm, 5 μm) were used to determine the content of loganic acid, and the effects of different chromatographic columns on the content determination were investigated. The results showed that when different chromatographic columns were used for quantitative detection of the same sample, loganic acid was well separated, and the results were basically consistent, indicating that the durability met the requirements.

(vi) Investigation of different chromatographs. Different liquid chromatographs (Waters Alliance E 2695, LC-15C, 1260 Infinity II) were employed to determine the content of loganic acid, and the effects of different chromatographs on the content determination was investigated. The results showed that different chromatograms performed well in the quantitative detection of the same sample, and loganic acid was well separated, and the results were basically consistent, indicating that the durability met the requirements.

2.8 Identification method

2.8.1 Preparation of reference solution. The reference substance of sweroside was added with methanol to prepare a solution containing 0.5 mg per 1 mL as the reference solution.

2.8.2 Preparation of test solution. A 0.2 g of standard decoction sample was weighed and ground, and added with 5 mL of methanol. The mixture was ultrasonically treated for 15 min, and then filtered to obtain the filtrate as a test solution.

2.8.3 Thin layer chromatography conditions. A silica gel GF_{254} TLC plate was selected, and ethyl acetate-methanol-water (volume ratio: 10∶2∶1) was used as the developer. The sample amount was 2 – 4 μL, and it was examined under an ultraviolet lamp at a wavelength of 254 nm. In the chromatogram of the test sample, a spot with the same color appeared at the position corresponding to the chromatogram of the control sample.

2.9 Methodological investigation of identification

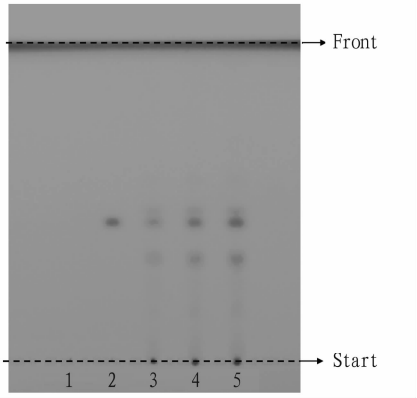
2.9.1 Investigation of specificity. First, 0.2 g of dextrin was weighed and prepared into a negative sample solution according to the preparation method of test solution in Section **2.6.3**. Next, 3 μL of negative sample solution, sweroside reference solution and test solution were pipetted, developed and examined according to the above TLC conditions, as shown in Fig. 2. The results showed that the negative sample solution did not show the same spot at the corresponding positions of the control solution and the test solution. Therefore, the excipient did not interfere with the thin-layer chromatography identification of *T. glandulifera* standard decoction. The method has good specificity, and can be used for the identification of standard decoction and formula granules.

2.9.2 Investigation of identification methodology. (i) Different brands of silica gel plates. Different brands of silica gel GF_{254} thin-layer chromatography plates (provided by Yantai Institute of Chemical Industry, Qingdao Ocean Chemical Co., Ltd. and Merck of Germany) were used to investigate the development of *T. glandulifera* standard decoction. The results showed that different brands of TLC plates had no effect on the identification of *T. glandulifera* standard decoction.

(ii) Different temperatures. The development of *T. glandulifera* standard decoction was investigated at the development temperatures of 20, 25 and 30 ℃, respectively. The results showed that the temperature in the range of 20 – 30 ℃ had no effect on the identification of *T. glandulifera* standard decoction.

(iii) Different developer ratios. Ethyl acetate-methanol-water was used as the developer with the ratios of 9∶3∶1, 10∶2∶1 and 11∶1∶1, respectively, and the development conditions of *T. glandulifera* standard decoction were investigated. The results showed that the fine adjustment of developer ratio had no effect on the identification of *T. glandulifera* standard decoction.

(iv) Different sample volumes. The sample volumes of 2, 3 and 4 μL were selected to investigate the development of *T. glandulifera* standard decoction. The results showed that the slight change of sample size had no effect on the identification of *T. glandulifera* standard decoction.



NOTE 1. Negative sample solution; 2. Sweroside reference solution; 3,4,5. *T. glandulifera* standard decoction.

Fig. 2 Thin layer chromatogram of standard *Triplostegia glandulifera* decoction

3 Results and analysis

3.1 Determination results of loganic acid content Fifteen batches of *T. glandulifera* standard decoction powder were prepared into test solutions. The determination results are shown in Table 3.

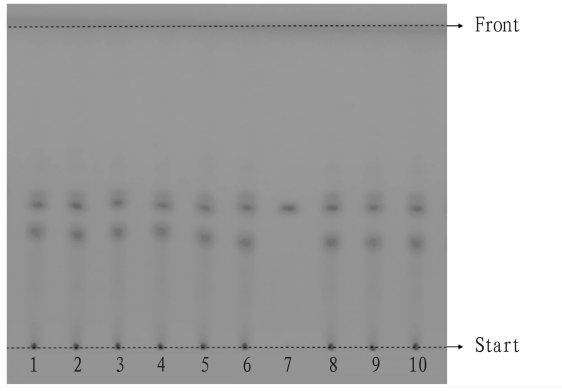
Table 3 Loganic acid content and content transfer rate in 15 batches of standard decoction ($n=2$)

Standard decoction No.	Extract yield//%	ω (standard decoction) mg/g	ω (decoction pieces)//%	Content transfer rate//%
S1	16.8	39.2	1.7	38.7
S2	16.3	45.3	1.9	38.9
S3	16.0	45.5	1.8	40.4
S4	15.7	34.6	1.7	32.0
S5	16.5	35.9	1.7	34.8
S6	15.9	34.9	1.7	32.6
S7	18.0	35.1	1.6	39.5
S8	17.8	35.8	1.6	39.8
S9	18.4	34.8	1.5	42.7
S10	17.9	38.3	1.7	40.3
S11	18.5	38.2	1.7	41.6
S12	18.1	37.3	1.7	39.7
S13	15.4	45.8	2.0	35.3
S14	16.3	45.2	2.0	36.8
S15	16.3	44.9	2.0	36.6
Mean	16.9	39.4	1.8	38.0
RSD/%	6.3	11.6	8.9	8.4
±3SD	13.7 – 20.1	25.7 – 53.1	1.3 – 2.2	28.4 – 47.5

NOTE Content transfer rate = ω(standard decoction)/ω(decoction pieces) × Extract yield × 100%.

As shown in Fig. 3, TLC identification chromatograms of *T. glandulifera* decoction pieces, standard decoction and formula granules exhibited consistent spots and positions, indicating that the substance transfer of TLC identification chromatograms from the decoction pieces to formula granules was consistent.

3.2 Identification Three batches of *T. glandulifera* decoction pieces, standard decoction and formula granules were subjected to thin-layer development, respectively. The determination results are shown in Fig. 3.



NOTE 1,2,3. *T. glandulifera* formula granules; 4,5,6. *T. glandulifera* standard decoction; 7. sweroside reference solution; 8,9,10. *T. glandulifera* decoction pieces.

Fig. 3 Thin layer chromatogram of *Triplostegia glandulifera* decoction pieces, standard decoction and formula granules

3.3 Value transfer relationship of *Triplostegia glandulifera* decoction pieces, standard decoction and formula granules The average extract yields, loganic acid contents and content transfer rates of 3 batches of *T. glandulifera* decoction pieces, 15 batches of standard decoction and 3 batches of formulated granules are shown in Table 4 and Table 5. According to the data analysis of the 15 batches of standard decoction in Table 4, there were great differences in the extract yield, loganic acid content and content transfer rate among *T. glandulifera* standard decoction from different producing areas. The extract yields, loganic acid contents and content transfer rates of the 3 batches of formula granules in Table 5 showed that the extract yield, loganic acid content and content transfer rate of *T. glandulifera* formula granules were stable, and the loganic acid content and content transfer rate of finished *T. glandulifera* product were higher than those of standard decoction.

Table 4 Value transfer of 3 batches of *Triplostegia glandulifera* decoction pieces-standard decoction-formula granules

No.	Item	Extract yield//%	ω (loganic acid)//mg/g	Content transfer rate//%
1	Decoction pieces		16.5	
	Standard decoction	16.8	39.2	39.9
	Formula granules	18.2	40.0	44.1
2	Decoction pieces		18.8	
	Standard decoction	16.3	45.3	39.3
	Decoction pieces	18.8	42.0	42.0
3	Decoction pieces		18.2	
	Standard decoction	16.0	45.5	40.0
	Decoction pieces	19.1	41.2	43.2

Table 5 Value transfer of standard decoction-formula granules

Item	Extract yield//%	ω (loganic acid)//mg/g	Content transfer rate//%
Mean value of formula granules	18.7	41.1	43.1
Mean value of standard decoction	16.9	39.4	38.0
Mean value ±3SD of standard decoction	13.7 – 20.1	25.7 – 53.1	28.8 – 47.3

It can be seen from Table 6 that the extract yields, loganic acid contents and content transfer rates of standard decoction prepared from different batches of *T. glandulifera* decoction pieces from the same producing area were stable except for Shuangbai County, Yunnan Province. The extract yield of standard decoction prepared from different batches of *T. glandulifera* decoction pieces produced in Shuangbai County, Yunnan Province was stable, but the content and content transfer rate of loganic acid in decoction pieces and standard decoction were quite different.

Table 6 *RSD* summary for standard decoction prepared from different batches of *Triplostegia glandulifera* decoction pieces %

Producing area of decoction pieces	Extract yield	ω (standard decoction)	ω (decoction pieces)	Content transfer rate
Shuangbai County, Yunnan Province	2.6	8.2	6.7	1.2
Eryuan County, Yunnan Province	2.7	1.9	2.3	2.3
Weishan Yi and Hui Autonomous County, Yunnan Province	1.9	1.5	1.3	1.8
Yulong Naxi Autonomous County, Yunnan Province	1.7	1.4	1.0	1.4
Luquan Yi and Miao Autonomous County, Yunnan Province	3.3	1.0	0.8	1.9

The average extract yield, average loganic acid content and average content transfer rate of the 3 batches of *T. glandulifera* formula granules were, respectively, 18.7%, 41.1 mg/g and 43.1%, each of which was within corresponding range of mean ± 3SD of the 15 batches of standard decoction.

4 Conclusions

In this study, HPLC and TLC were used to establish an HPLC determination method and a TLC identification method of loganine acid content in *T. glandulifera*, a Yi medicine, for the first time, and the material basis transfer law of decoction pieces-standard decoction-formula granules was clarified, which provides a method for quality evaluation of *T. glandulifera* formula granules and technical support for the improvement and scientificity of quality control of *T. glandulifera* formula granules. The specific conclusions are as follows.

(i) An Agilent 1260 II HPLC and a Waters Symmetry C_{18} column (4.6 mm × 250 mm, 5 μm) were used to establish the method for the determination of loganic acid content. The method adopted gradient elution, which was carried out using acetonitrile-0.1% phosphoric acid solution as mobile phase with a flow rate of 1 mL/min and a column temperature of 30 ℃, and the detection wavelength was 240 nm. The TLC identification method was established using sweroside as the reference substance and ethyl acetate-methanol-water (volume ratio: 10 ∶ 2 ∶ 1) as the developer, and observation was carried out under an ultraviolet lamp (254 nm). Both methods had high stability and credibility.

(ii) Fifteen batches of *T. glandulifera* decoction pieces were prepared into standard decoction, and the extract yield (13.7% – 20.1%), loganine acid content (25.7 – 53.1 mg/g) and content transfer rate (28.8% – 47.3%) of *T. glandulifera* standard decoction were determined. Three batches of *T. glandulifera* formula granules were prepared in a pilot scale, and the average extract yield, loganine acid content and content transfer rate of the formula granules were, respectively, 18.7%, 41.1 mg/g and 43.1%, each of which was within corresponding range of mean ± 3SD of the 15 batches of standard decoction. The extract rate, loganine acid content and content transfer rate of the 3 batches of formula granules were basically the same as those of its standard decoction, and the TLC material transfer of decoction pieces-standard decoction-formula granules was consistent, suggesting that there is a reasonable material transfer law of *T. glandulifera* decoction pieces-standard decoction-formula granules.

(iii) This study makes up the blank of the research on the standard decoction and formula granules of *T. glandulifera*. Taking the standard decoction as a bridge, the feasibility of preparing *T. glandulifera* formula granules was preliminarily explored, providing a good example for the modernization of medicines for ethnic minorities.

There were great differences in the extract yield, loganic acid content and transfer rate among the 15 batches of *T. glandulifera* standard decoction, which shows that there are great differences among medicinal materials from different regions of Yunnan Province, which may be influenced by geographical environment and growth conditions[18 – 23]. In the future, the influencing factors that cause differences in different producing areas will be further explored. In addition, due to the limitation of time and conditions, we have not studied the fingerprints/characteristic spectra, which need further exploration.

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