Establishment of Thin-layer Chromatography Identification Method for Vicatia thibetica de Boiss. and Preliminary Study on its Quality

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Abstract: *Objective:* To establish a thin-layer chromatography (TLC) identification method for *Vicatia thibetica* and provide an experimental basis for its quality control. *Methods:* With ferulic acid as the reference standard, the effects of extraction conditions, developing solvents, sample application volumes and color development conditions on TLC identification were investigated. After determining the optimal conditions, qualitative identification was carried out on 11 batches of *Vicatia thibetica* de Boiss. samples from different sources. *Results:* The optimal conditions were as follows: ultrasonic extraction of the sample with 95% ethanol for 15 min, chloroform-methanol (10:1) as the developing solvent, sample application volume of 6 μL, color development with 5% sulfuric acid-ethanol solution, and visualization under 365 nm ultraviolet (UV) light. 11 batches of Vicatia thibetica de Boiss. from different sources showed basically consistent TLC spot types, while the relative content of characteristic component ferulic acid varied significantly. *Conclusion:* This method has clear spots, excellent resolution and simple operation, and can be used for the rapid preliminary screening of *Vicatia thibetica* medicinal materials.

Keywords: Jiawa; *Vicatia thibetica* de Boiss.; thin-layer chromatography; quality control

1. Introduction

Tibetan medicine "(ཇྭ་བ)Jiawa", namely *Vicatia thibetica* de Boiss.[1]of the Vicatia genus in the Apiaceae family, is a commonly used medicinal material in Tibetan clinical practice and also one of the components of the basic Tibetan medicine prescription "Wugen San", with both medicinal and edible properties. It tastes sweet, pungent and bitter, and is warm in nature. It has the effects of dispelling cold, tonifying the body and drying yellow water, and is used for the treatment of diseases such as lumbago due to deficiency-cold and stomach diseases[2]. This medicinal material is mainly distributed in plateau areas such as India, Pakistan, Nepal, and Sichuan, Yunnan, Xizang of China[3]. However, Tibetan medicine "Jiawa" has long had problems such as confused origin and inconsistent varieties actually used in producing areas[4]. In addition, the lack of unified specifications in harvesting, processing, storage and other links directly leads to uneven quality and difficult guarantee of clinical efficacy. At present, studies on the quality control methods of *Vicatia*

thibetica de Boiss. are mostly focused on precise instrumental analysis methods such as HPLC and UPLC-Q-TOF-MS[5-7], which have limitations such as high instrument cost, complex operation and difficulty in popularization in primary medical institutions. Thin layer chromatography (TLC) has the advantages of simple operation, strong specificity and low cost, and there is no report on the TLC identification method of this medicinal material yet. Based on this, this study took the active component ferulic acid as the reference standard, optimized the extraction and TLC conditions, established a TLC identification method for *Vicatia thibetica de Boiss.*, and carried out a preliminary study on the quality through 11 batches of test samples from different sources, in order to provide experimental evidence for the improvement of the quality control system of this medicinal material.

2. Instruments and materials

2.1. Instruments

Multifunctional grinder (Dongguan Fangtai Electric Appliance Co., Ltd.); HZT-A+200 electronic analytical balance (0.1 mg, Huazhi (Fujian) Electronic Technology Co., Ltd.); ZF-7 digital ultrasonic cleaner (300 W, 40 kHz, Kunshan Meimei Ultrasonic Instrument Co., Ltd.); electric constant temperature water bath (Shanghai Libang Instrument Technology Co., Ltd.); silica gel G TLC plates (100 mm×200 mm, Yantai Jiangyou Silica Gel Co., Ltd.); TLC spotting capillaries (0.3 mm×100 mm, Nantong Surui Experimental Equipment Co., Ltd.).

2.2. Materials

Ferulic acid reference standard (batch number: 23031013, purity ≥ 98%) was purchased from Chengdu Master Biotech Co., Ltd.; a total of 11 batches of *Vicatia thibetica de Boiss.* medicinal materials, including self-collected and commercially purchased ones, were collected from Xizang and Sichuan, as detailed in **Table 1**. All chemical reagents used in the experiment were of analytical grade and purchased from Tianjin Aopu Shenghua Chemical Co., Ltd.

Table 1. Sample information of *Vicatia thibetica de Boiss.*

No.	Producing area	Sample type
1	Maizhokunggar County, Xizang	Wild
2	Nyingchi, Xizang	Wild
3	Qamdo, Xizang	Wild
4	Nyingchi (purchased), Xizang	Cultivated
5	Aba, Sichuan	Wild
6	Lhasa, Xizang	Wild
7	Aba (purchased), Sichuan	Wild
8	Nyingchi (purchased), Xizang	Cultivated
9	Maizhokunggar County, Xizang	Wild
10	Nyingchi, Xizang	Wild
11	Maizhokunggar County, Xizang	Wild

3. Methods and results

3.1. Methodology investigation of TLC for Vicatia thibetica de Boiss.

3.1.1. Selection of extraction solvent

Wild *Vicatia thibetica de Boiss.* medicinal materials (dried roots) produced in Maizhokunggar County, Lhasa City, Xizang were selected, and 3 g of powder was accurately weighed for four portions, which were respectively placed in 100 mL

stoppered Erlenmeyer flasks. 60 mL of petroleum ether, chloroform, 95% ethanol and methanol were added in turn. After sealing, the flasks were weighed, ultrasonically treated for 15 min, and cooled to room temperature. The lost weight was made up with the original extraction solvent, followed by filtration. The successive filtrate was concentrated to dryness under reduced pressure at 65 °C, and the residue was dissolved with 1 mL of the corresponding solvent to obtain four kinds of test solutions.

According to the thin layer chromatography test (General Rule 0502, Chinese Pharmacopoeia 2020 Edition), 6 μL of each of the above test solutions was accurately pipetted and applied to the same silica gel G TLC plate. Ascending development was carried out with chloroform-methanol (10:1) as the developing solvent. After the solvent was volatilized completely, 5% sulfuric acid ethanol solution was evenly sprayed, and the plate was heated at 105 °C until the spots were clearly colored. Visualization under daylight showed that the spots of all test samples were blurred; visualization under ultraviolet lamp (365 nm) showed that the spot characteristics of test samples obtained by extraction with different solvents were significantly different (see **Figure 1**). **Results**: The sample extracted with chloroform (SL) had serious tailing spots and poor resolution; the sample extracted with petroleum ether (S) had light-colored characteristic spots and insufficient specificity; the sample extracted with 95% ethanol (Y) had clear spots and good resolution; the sample extracted with methanol (J) had obvious impurity interference and was easy to block the capillary during sample application. Therefore, 95% ethanol was determined as the suitable extraction solvent.
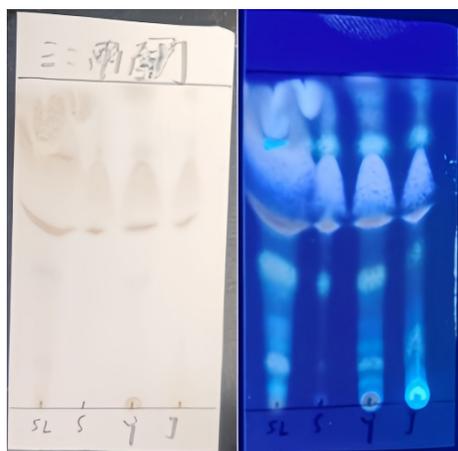

Figure 1. Investigation of extraction solvents for TLC identification of *Vicatia thibetica de Boiss.*
Note: SL: Chloroform extract, S: Petroleum ether extract, Y: 95% ethanol extract, J: Methanol extract

3.1.2. Selection of visualization method

6 μL of each of the four test solutions prepared in Section 3.1.1 was pipetted and applied to four silica gel G TLC plates. After development with chloroform-methanol (10:1) as the developing solvent, four visualization methods were adopted respectively: ① Spraying with 5% phosphomolybdic acid ethanol solution and heating at 105 °C (visualization under daylight); ② Placing in a sealed developing tank containing iodine powder and fumigating with iodine vapor (visualization under daylight); ③ Spraying with 5% vanillin concentrated sulfuric acid solution and heating at 105 °C (visualization under daylight); ④ Spraying with 5% sulfuric acid ethanol solution, heating at 105 °C and visualization under ultraviolet light at 365 nm **(Figure 2)**. **Results**: The 4th visualization method had the optimal spot clarity and resolution. Therefore, method ④ was selected as the visualization method for TLC identification of *Vicatia thibetica de Boiss*.
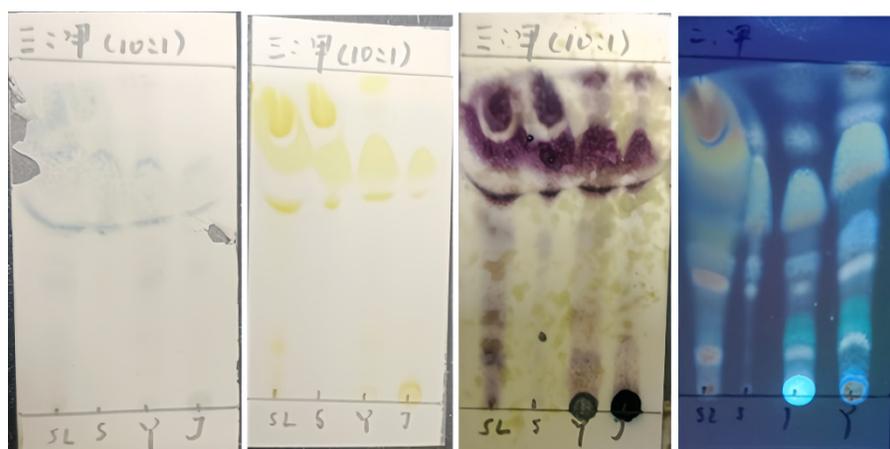
Figure 2. Investigation of visualization methods for TLC identification of *Vicatia thibetica de Boiss.*
Note: (1) Color development with 5% phosphomolybdic acid ethanol solution, (2) Color development with iodine vapor, (3) Color development with 5% vanillin concentrated sulfuric acid solution, (4) Color development with 5% sulfuric acid ethanol solution (365 nm ultraviolet light); 1. SL: Chloroform extract, 2. S: Petroleum ether extract, 3. Y: 95% ethanol extract, 4. J: Methanol extract

3.1.3. Investigation of extraction method

3 g of *Vicatia thibetica de Boiss.* medicinal material used in Section 3.1.1 was accurately weighed and divided into two portions: one portion was placed in a 100 mL stoppered Erlenmeyer flask, added with 20 mL of 95% ethanol, and ultrasonically treated for 15 min; the other portion was placed in a 500 mL round-bottom flask, added with 20 mL of 95% ethanol, and heated under reflux for 15 min. Both extracts were filtered and concentrated to dryness under reduced pressure, and the residues were dissolved with 1 mL of 95% ethanol to prepare test solutions. Development and visualization were carried out according to the optimal conditions (see **Figure 3**). **Results**: The ultrasonic extract (No.1 in **Figure 3**) had strong fluorescence and excellent resolution; the sample extracted by heating under reflux (No.2 in **Figure 3**) had inferior spot clarity and fluorescence intensity compared with the ultrasonic extract. Therefore, ultrasonic treatment was determined as the optimal extraction method.

Figure 3. Investigation of extraction methods for TLC identification of Vicatia thibetica de Boiss.
Note: 1: Ultrasonic extraction for 15 min, 2: Heating under reflux extraction for 15 min

3.1.4. Investigation of extraction time

3 g of *Vicatia thibetica de Boiss.* medicinal material used in Section 3.1.1 was accurately weighed and divided into four portions, each added with 20 mL of 95% ethanol, and ultrasonically treated for 10, 15, 30 and 60 min respectively; test solutions were prepared according to the method in Section 3.1.2. **Results** (see **Figure 4**): The sample ultrasonically treated for 10 min had light-colored spots and poor specificity; the sample extracted for 15 min had clear spots, good resolution and significant specificity; compared with 15 min, the samples extracted for 30 min and 60 min had no obvious improvement in the number of spots and fluorescence intensity, and the sample application capillary was easy to be blocked due to the high concentration of extract during sample application. Therefore, 15 min was determined as the optimal extraction time.

Figure 4. Investigation of extraction time for TLC identification of *Vicatia thibetica de Boiss.*

3.1.5. Selection of developing solvent

6 μL of each of the four test solutions in Section 3.1.1 was accurately pipetted and applied to four silica gel G TLC plates, and developed with four developing solvent systems in turn: ① n-hexane-chloroform-formic acid (10:4:1); ② petroleum ether-ethyl acetate-methanol-formic acid (10:4:2:1); ③ dichloromethane-methanol (10:1); ④ chloroform-methanol (10:1). The operation was carried out according to the visualization method in Section 3.1.2 (see **Figure 5**). **Results**: ① ~ ③ had problems such as spot tailing and overlapping, while developing solvent ④ had clear spots and good resolution. Therefore, chloroform-methanol (10:1) was selected as the developing solvent.
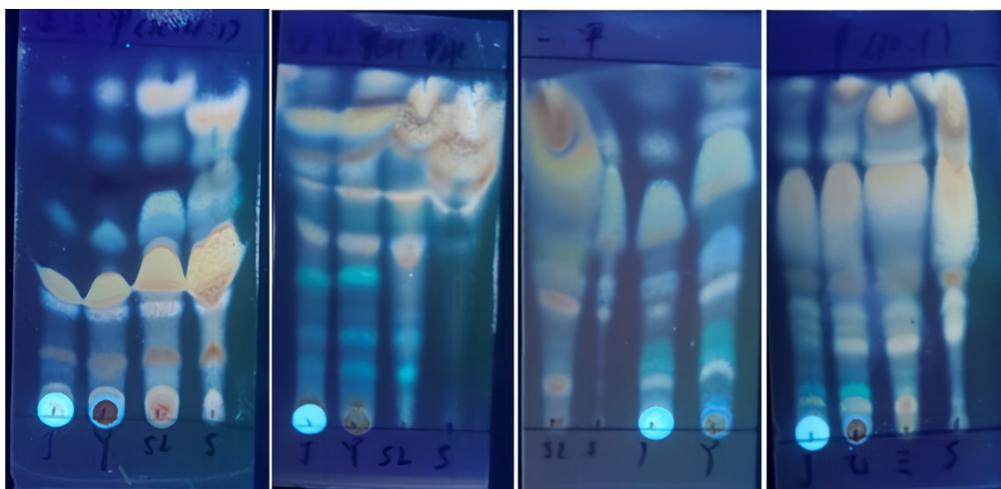

Figure 5. Investigation of developing solvents for TLC identification of Vicatia thibetica de Boiss.

Note: 1. n-hexane-chloroform-formic acid (10:4:1), 2. petroleum ether-ethyl acetate-methanol-formic acid (10:4:2:1), 3. dichloromethane-methanol (10:1), 4. chloroform-methanol (10:1); SL: Chloroform extract, S: Petroleum ether extract, J: Methanol extract, Y: 95% ethanol extract

3.1.6. Investigation of sample application volume

2, 6, 10 and 12 µL of the 95% ethanol test solution in Section 3.1.1 were respectively pipetted and applied to the same silica gel G TLC plate, and development and visualization were carried out according to the optimal conditions (Figure 6). Results: The spot signal was weak at 2 µL; the spots were clear and the intensity was moderate at 6 µL; the spots slightly diffused at 10 µL; the spots were overloaded at 12 µL. Therefore, 6 µL was determined as the optimal sample application volume.

Figure 6. Investigation of sample application volume for TLC identification of *Vicatia thibetica de Boiss.*

3.2. TLC identification of Vicatia thibetica de Boiss. medicinal materials

3.2.1. Preparation of test solutions

3 g of each of the 11 batches of *Vicatia thibetica de Boiss.* medicinal materials was accurately weighed and placed in 100 mL stoppered Erlenmeyer flasks respectively. Solvent was added at the ratio of 60 mL of 95% ethanol to 3 g of sample. After sealing, the flasks were ultrasonically treated for 15 min; after cooling, the lost weight was made up with 95% ethanol, followed by filtration. The filtrate was evaporated to dryness under reduced pressure at 65 °C, and the residue was dissolved with 1 mL of 95% ethanol to prepare the test solutions.

3.2.2. Preparation of reference standard solution

2 mg of ferulic acid reference standard was accurately weighed and dissolved with 1 mL of 95% methanol to prepare a reference standard solution with a mass concentration of 2 mg/mL.

3.2.3. TLC identification of Vicatia thibetica de Boiss. medicinal materials from different batches

6 µL of each of the 11 batches of test solutions and ferulic acid reference standard solution was accurately pipetted and applied to the same silica gel G TLC plate. Development and visualization were carried out according to the optimal conditions (ultrasonic extraction with 95% ethanol for 15 min, chloroform-methanol (10:1) as developing solvent, color development with 5% sulfuric acid ethanol solution, visualization under 365 nm ultraviolet light, sample application volume of 6 µL) (see **Figure 7**). **Results**: At the position corresponding to the ferulic acid reference standard, clear brown spots were observed in the test samples No.4, 6-11, an extremely weak brown spot was only observed in test sample No.5, and no obvious spots were observed in test samples No.1-3; the size and fluorescence intensity of the spots at this position showed significant differences.
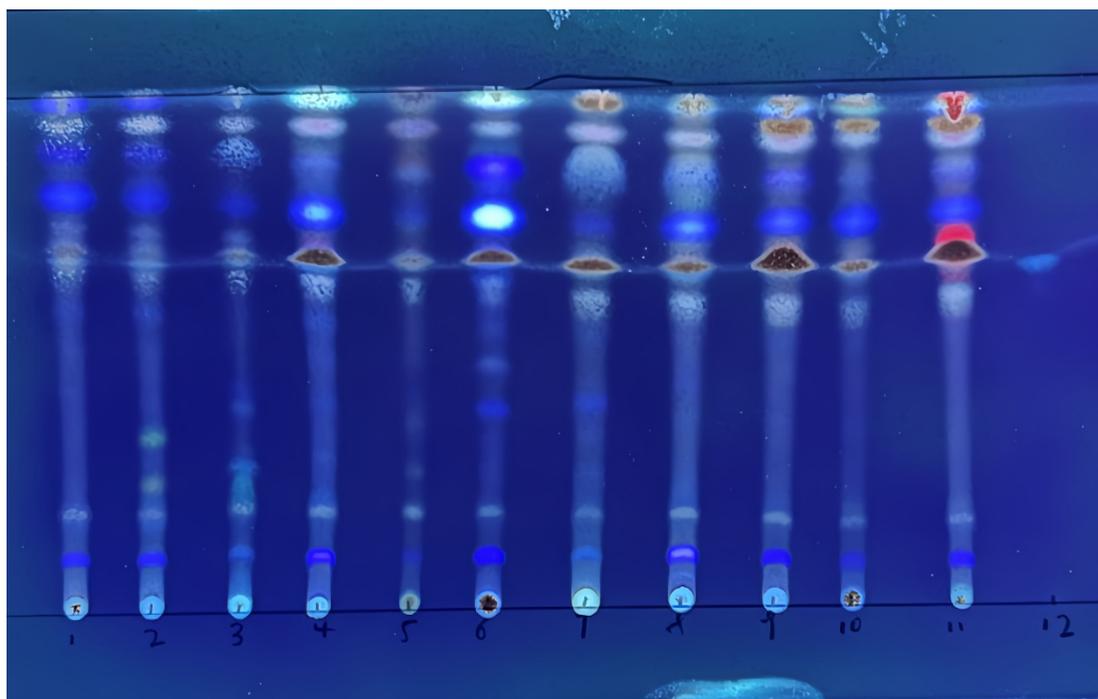
Figure 7. TLC chromatograms of Vicatia thibetica de Boiss. medicinal materials from different batches
1. Maizhokunggar County, Xizang, 2. Nyingchi, Xizang, 3. Qamdo, Xizang, 4. Nyingchi, Xizang, 5. Aba, Sichuan, 6. Lhasa, Xizang, 7. Aba, Sichuan, 8. Nyingchi, Xizang, 9. Maizhokunggar County, Xizang, 10. Nyingchi, Xizang, 11. Maizhokunggar County, Xizang, 12. Ferulic acid reference standard. (Information of No. 1-11 corresponds to Table 1)

4. Discussion

Modern studies have confirmed that *Vicatia thibetica de Boiss.* contains chemical components such as ferulic acid, chlorogenic acid, apigenin, amino acids and polysaccharides, and has pharmacological activities such as anti-fatigue, antioxidant and anti-aging[8]. The relative content of ferulic acid is significantly affected by the producing area[4]. This study established a TLC identification method for *Vicatia thibetica de Boiss.*, and the detection of 11 batches of test samples from different sources found that the characteristics between batches were basically consistent, but the relative content of ferulic acid showed significant differences. Even batches from the same producing area (such as No.1, 9 and 11 from Maizhokunggar County, Xizang) had significant differences in spots at the position corresponding to the ferulic acid reference standard, among which No.9 and 11 had the clearest and most significant spots at this position, suggesting that this producing area may be a dominant producing area for ferulic acid enrichment.

This study also has certain limitations: during the TLC identification, after the test sample was colored with sulfuric acid ethanol solution and heated, a brown spot appeared at the position corresponding to the ferulic acid reference standard, which was caused by the superposition of co-coloration of multiple coexisting components in the medicinal material, thus being different from the light blue color of the single ferulic acid reference standard; ferulic acid was not detected in 3 batches of test samples, which is speculated to be due to low relative content caused by differences in the growth environment of the producing areas, or degradation of ferulic acid caused by improper post-harvest processing and storage[9,10], resulting in the relative content being lower than the TLC detection limit; in addition, the experiment did not carry out a special investigation on temperature and humidity, the coverage of test samples was limited, only qualitative analysis of ferulic acid was carried out without quantitative research, leading to limited repeatability and representativeness of the results.

In summary, the TLC identification method established in this study can realize the rapid preliminary screening of *Vicatia thibetica de Boiss.* medicinal materials. In the follow-up, combined with quantitative technologies such as HPLC,

comparative studies on conditions such as producing areas and processing can be carried out to clarify the key influencing factors of its quality differences, further improve the quality evaluation system of *Vicatia thibetica de Boiss.*, and provide experimental support for the resource development and clinical application of this medicinal material.

Disclosure statement

The author declares no conflict of interest.

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