Identification of Tibetan and Qiang Edible and Medicinal Herbs *Cardamine tangutorum* and *Cardamine macrophylla*

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Abstract [**Objectives**] To study the original plants, characters, tissue structure, powder characteristics and thin-layer chromatography (TLC) characteristics of *Cardamine tangutorum* and *Cardamine macrophylla* as Tibetan and Qiang edible and medicinal herbs, and to provide the basis for the identification of *C. tangutorum* and *C. macrophylla*. [**Methods**] The identification of *C. macrophyll* and *C. tangutorum* was carried out by original plant identification, character identification, microscopic identification and TLC identification. [**Results**] *C. tangutorum* and *C. macrophylla* can be distinguished according to the shape of rhizome and stem, the difference of stem leaves and leaflets, and the difference of flower color; there is no obvious difference between the characteristics of the shape and the powder; the thin layer chromatography shows that in the thin layer chromatography of *C. tangutorum* and *C. macrophylla*, spots with the same color are shown on the corresponding positions of the ground part and the reference substance quercetin; the underground part and the position corresponding to the reference substance β-sitosterol all show the same color spots. [**Conclusions**] This study provides a reference for the identification of *C. tangutorum* and *C. macrophylla*.

Key words *Cardamine tangutorum*, *Cardamine macrophylla*, Original plant, Microscopic identification, Thin layer chromatography (TLC)

1 Introduction

Cardamine tangutorum O. E. Schulz and *Cardamine macrophylla* Willd of Cruciferae have a long history of being eaten by Tibetan, Qiang and Han people in Sichuan Province, also known as Shigecai. As a polybasic original plant, there is little difference in the plant species recorded in the literature. It has been proved that the efficacy of *C. macrophylla* and *C. tangutorum* is basically the same, but there are differences in different medicinal parts, and there is a habit of using them in different parts clinically; Tibetan medicine, Qiang medicine, Lisu medicine and other ethnic medicine have recorded and used Shigecai[1-6]. *C. macrophylla* is distributed in Sichuan, Gansu, Qinghai, Guizhou, Yunnan, Tibet, Inner Mongolia, Ningxia, Henan and other places, and grows under shrubs on hillsides, beside ditches, in stone crevices, and in wet places on alpine grass slopes at altitudes of 1 600 - 4 200 m. *C. tangutorum* is distributed in Henan, Shanxi, Shaanxi, Gansu, Qinghai, Sichuan, Yunnan and other places, and grows in grasslands in high mountains and valleys at an altitude of 2 100 - 4 400 m and in damp places under forests. Their distribution areas are basically the same.

At present, the pharmacognosy study of *C. tangutorum* and *C. macrophylla* is less, and mainly focused on the chemical components. In this paper, the original plants and medicinal materials of *C. tangutorum* and *C. macrophylla* were systematically studied from the shape and size of roots, stems, leaves, transverse section, the characteristics of the medicinal materials, and the thin layer chromatographic characteristics of the ground and underground parts of the original plants by means of character identification, microscopic identification and thin layer chromatographic identification. It provides a reference for the research ideas and methods of *C. tangutorum* and *C. macrophylla*.

Received: April 15, 2024 Accepted: June 25, 2024
Supported by Scientific Research Project for School-level Teachers of Sichuan College of Traditional Chinese Medicine in 2023 (23ZRYB08); Tibetan Plateau Ethnic Medicinal Resources Protection and Utilization Key Laboratory Open Fund Project of Southwest Minzu University (QTPEM2305).
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2 Materials

2.1 Instruments OLYMPUS BX41 microscope; RE-2000 A rotary evaporator (Shanghai Yarong Biochemical Instrument Factory); TIGER3000 image processing imaging software (Shanghai Xingxing Industrial Co., Ltd.); HW-12 electric thermostatic water bath (Shanghai Yiheng Scientific Instrument Co., Ltd.).

2.2 Materials and reagents *C. tangutorum* and *C. macrophylla* were collected from Aba Tibetan and Qiang Autonomous Prefecture, Ganzi Tibetan Autonomous Prefecture and Tibet in Sichuan Province. After the medicinal materials were collected, the medicinal materials were soaked in FAA fixing solution for later use. The TLC Silica gel was 60 (EMD Millipore Corporation), and all reagents were analytically pure.

3 Methods and results

3.1 Original plant identification *C. macrophylla* is perennial herb and its rhizome is prostrate, densely covered with fibrous roots. Stems are erect. Cauline leaves usually 4 - 5; leaflets 4 - 5 pairs, leaflets elliptic or ovate-lanceolate, 4 - 9 cm long, 1 - 2.5 cm wide, base of terminal leaflet cuneate, petiolules absent, base of uppermost pair of leaflets often decurrent. Racemes flowery; petals lilac, purplish red, rarely white. Long siliques flat, 35 - 45 mm long. Flowering from May to June and fruiting from July to August.

By comparison, *C. tangutorum* has a creeping scaly rhizome; the stem is short and simple, the lower part is naked and leafless, and the upper part usually has three small pinnately compound leaves; the flower is purple (Fig. 1).

NOTE A. B are ground and underground parts of *Cardamine macrophylla*; C. D are ground and underground parts of *C. tangutorum*.

Fig. 1 Original plants

3.2 Character identification The ground parts of this plant are mostly wrinkled and curled, often forming a ball. The stem is cylindrical, with yellow-green or yellow-brown surface and obvious longitudinal grooves. The leaves are mostly broken, and the intact ones are elliptic or ovate-lanceolate after flattening, 1 – 9 cm long and 0. 5 – 3. 0 cm wide; surface yellow-green to dark green, sometimes lilac, sometimes scattered or sparsely pubescent, apex obtuse or shortly acuminate, margin sharply serrate or obtusely serrate. Racemes are terminal and very small. It smells fragrant and tastes slightly bitter. The underground part (rhizome) of this plant is long cylindrical, with branches, complete 5 – 25 cm long, 0. 1 – 2. 0 cm in diameter, with few or dense fibrous roots. The surface is yellowish brown, slightly shiny, smooth or with different depths of longitudinal wrinkles. The texture is slightly brittle, the cortex of the cross section is yellowish white with many fissures, and the vascular bundles are yellowish white and arranged radially. It smells and tastes slightly sweet, as shown in Fig. 2.

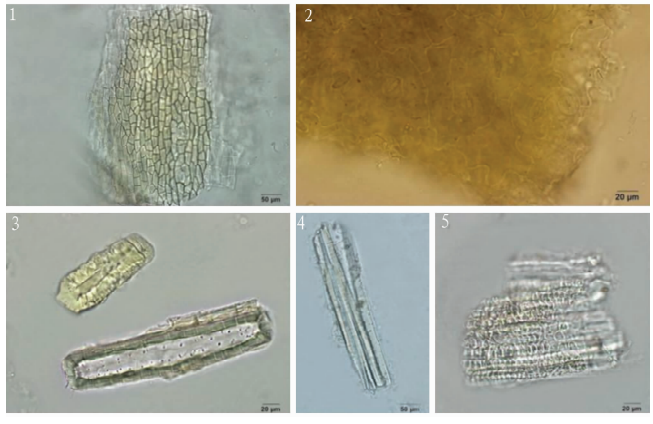
3.3 Identification of tissue structure

3.3.1 Powder identification. Took powder of ground and underground parts of *C. tangutorum* and *C. macrophylla*, dilute glycerol, chloral hydrate and nitric acid-chromic acid dissociation methods were used for observation. It was found that there was no significant difference in powder identification between the two, so they were described uniformly.

NOTE A. B are ground and underground parts of *Cardamine macrophylla*; C. D are ground and underground parts of *C. tangutorum*.

Fig. 2 Medicinal materials

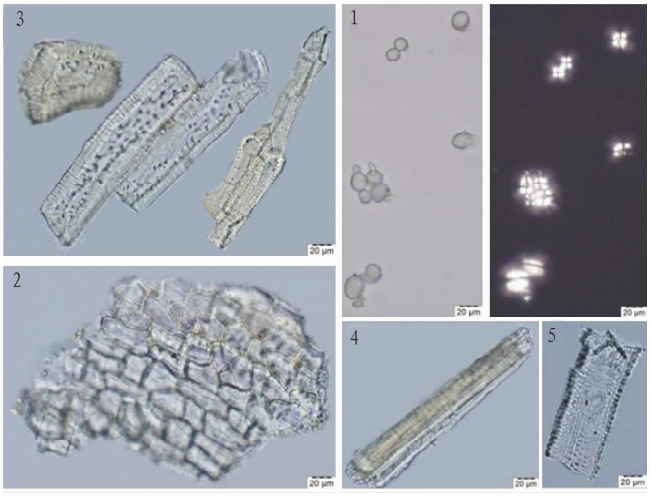
The powder of the ground part of this plant is yellowish green or brownish yellow. The epidermal cells of the stem are square or polygonal, and the anticlinal walls are thickened or beaded. The leaf epidermal cells are irregular in shape and the stomata are irregular. Sclereids (stone cells) are more common, single scattered or several groups, light yellow, polygonal, round or square, 20 – 80 μm in diameter, with dense laminae and obvious pore grooves. The fibers are mostly bundled, elongated, and have thick walls. The vessels are mostly reticulated vessels and spiral vessels, as shown in Fig. 3.



NOTE 1. Stem epidermal cells; 2. Leaf epidermal cells; 3. Stone cells; 4. Fibers; 5. Vessels.

Fig. 3 Powder of ground parts of medicinal materials

The powder of the underground part of the product is light gray or greyish white. Starch grains are single, round, 5 – 15 μm in diameter, and the umbilicus is herringbone or cross-shaped. Stone cells are common and rectangular, fusiform and polygonal, with thick walls and fine pits, 15 – 120 μm in diameter. The bast fibers are mostly in bundles, with woody walls and pits, 20 – 60 μm in diameter. Vessels include reticulated vessel, bordered reticulated vessel, and spiral vessel, 15 – 65 μm in diameter, as shown in Fig. 4.

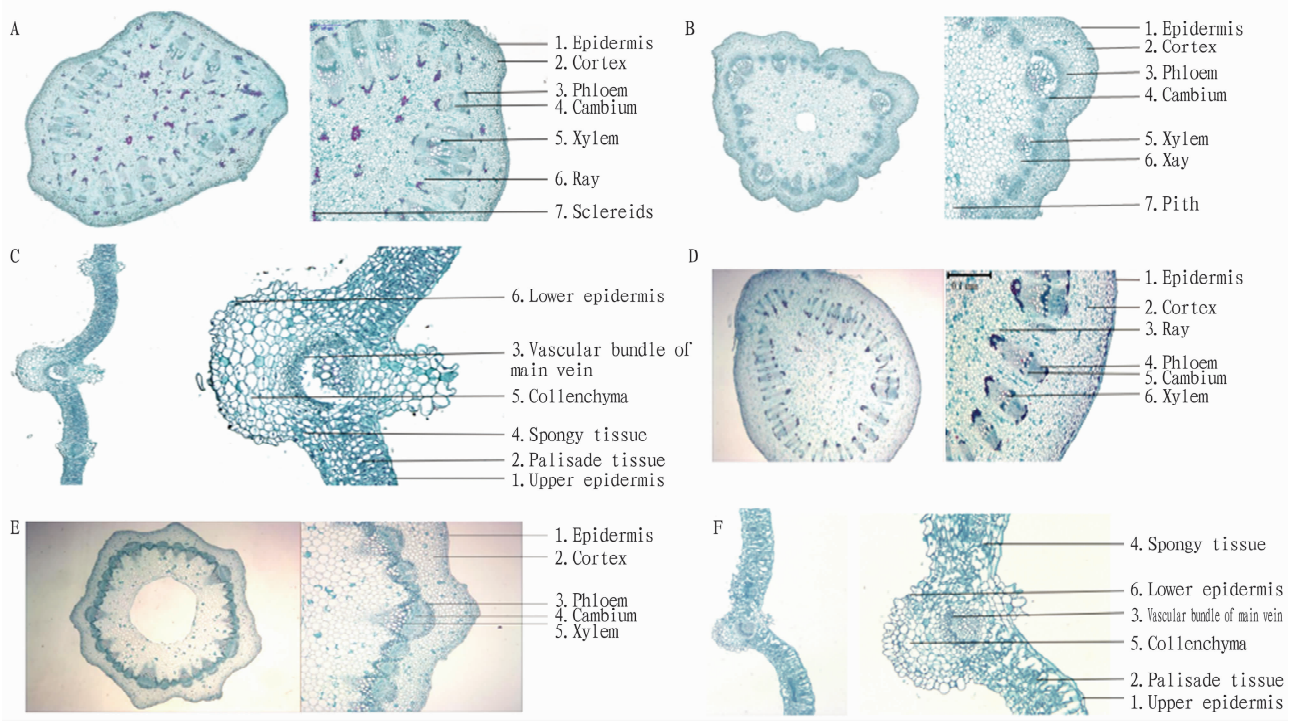


NOTE 1. Starch grains; 2. Cork cells; 3. Stone cells; 4. Bast fibers; 5. Vessels.

Fig. 4 Powder of underground parts of medicinal materials

3.3.2 Cross section identification. Took *C. macrophylla* and *C. tangutorum* medicinal materials soaked in FAA fixation solution, divided them into root, stem and leaf, and made paraffin sections according to the conventional paraffin section method (material selection→fixation→washing→dehydration→transparent→wax soaking→embedding→section→patch→baking→staining→sealing) for observation[7-8]. The root of *C. tangutorum* is oval in cross section, with one row of small cells in the epidermis, which are arranged neatly and closely; the parenchyma cells outside the cortex are small, closely arranged in 5 – 6 rows, and mostly irregular; endodermis not obvious, phloem compact but narrow; cambium 2 – 3 rows; xylem broad, vessels 3 – 8 rows radially arranged; rays are broad. A large number of sclereids were observed in the inner and outer vascular bundles, cortex and primary xylem. The epidermis of stem cross section is composed of round-like cells. The cortex consists of 8 – 12 rows of loosely arranged parenchyma cells. The vascular bundles are tenacious and arranged in a funnel-shaped ring. The cambium is 1 – 2 rows, closely arranged, and the pith is wide. The upper and lower epidermal cells of the cross section of the leaf are in one row separately, and the cells are square like. There is a row of cells in the upper and lower epidermis of the vein, which are closely arranged and round-like. There are 4 – 5 rows of parenchyma cells above the lower epidermis, and stomata can be seen in the lower epidermis. Palisade tissue in 2 rows, closely arranged, not through the main vein. Spongy tissue in 2 – 3 rows, loosely arranged. Vascular bundles are of external phloem.

The cross section of *C. macrophyla* root is oval or irregular. The parenchyma cells outside the cortex are closely arranged in 4 – 5 rows, and stone cells can be seen. Other characteristics are the same as those of *C. tangutorum*; the epidermis of the stem cross section is composed of one row of closely arranged rectangular cells, seen in the xylem and pith; other characteristics are the same as those of *C. tangutorum*. Most of the upper epidermal cells in the transverse section of the leaf are elongated, while most of the lower epidermal cells are round or irregular, closely arranged; other characteristics are the same as *C. tangutorum*, as shown in Fig. 5.



NOTE A, B, C are the cross sections of the root, stem and leaf of *Cardamine macrophylla*; D, E, F are the cross sections of the root, stem and leaf of *C. tangutorum*.

Fig. 5 Tissue structure of medicinal materials

3.4 TLC identification

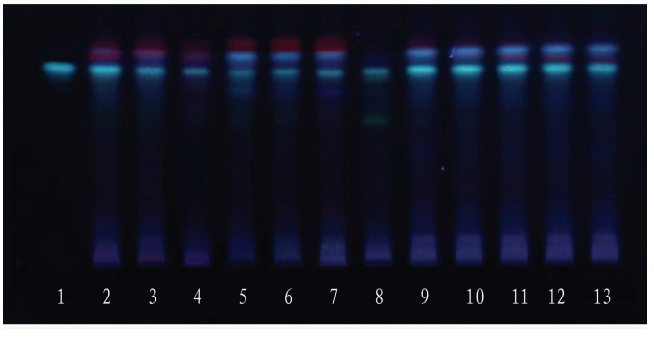
3.4.1 Preparation of reference solution. Took an appropriate amount of quercetin and added methanol to prepare a solution containing 0.4 mg/mL as the reference solution of quercetin. Then, took an appropriate amount of β-sitosterol and added methanol to prepare a solution containing 1 mg per 1 as the reference solution of β-sitosterol.

3.4.2 Preparation of test solution. (i) Test solution of ground parts of the two medicinal materials: Took 0.5 g of ground part powder of two medicinal materials, added 50 mL of methanol-hydrochloric acid (4 : 1) mixed solution, heated and refluxed for 1 h, cooled, filtered, evaporated the filtrate to nearly dry, and dissolved the residue with 3 mL of methanol.

(ii) Test solution of underground parts of the two medicinal materials: took 1.0 g of the powder of the underground part of two medicinal materials, added 25 mL of ethyl acetate, sonicated for 30 min, filtered, evaporated the filtrate to dryness, and dissolved the residue with 2 mL of methanol.

3.4.3 Development of thin-layer chromatography. There was no significant difference between *C. macrophylla* and *C. tangutorum* in TLC identification, so they were described uniformly.

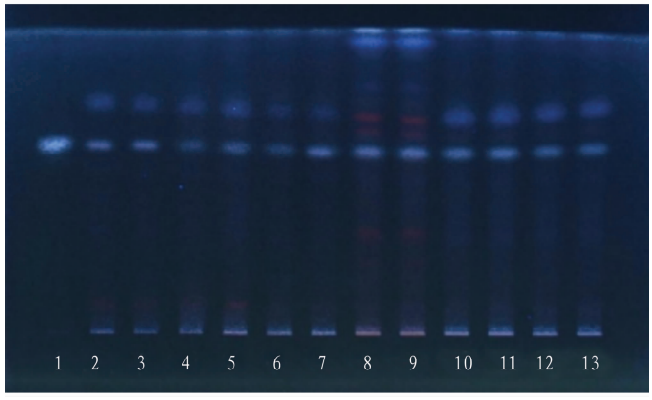
(i) The ground parts of the medicinal materials were tested by thin layer chromatography (General Rule 0502 in *Chinese Pharmacopoeia*)[9]. 3 – 5 μL of each of the reference solution and the test solution were taken and dropped on the same silica gel G thin layer plate, developed with toluene-ethyl acetate-formic acid (5 : 3 : 1) as the developing solvent, taken out, dried, and sprayed with 1% aluminum trichloride ethanol solution. The thin layer chromatography is shown in Fig. 6. It can be seen that at the corresponding position of the quercetin reference substance, the aerial part of the sample can show fluorescent spots of the same color.



NOTE 1. quercetin reference substance; 2 to 8. different batch of *C. tangutorum* medicinal materials; 9 to 13. different batch of *C. macrophylla* medicinal materials.

Fig. 6 Thin layer chromatography of ground parts of medicinal material

(ii) The underground parts of the medicinal materials were tested by thin layer chromatography (General Rule 0502 in *Chinese Pharmacopoeia*). Pipetted 5 – 10 μL of the reference solution and 5 – 10 μL of the test solution separately, dropped them on the same silica gel G thin layer plate, developed them with petroleum ether-ethyl acetate (3 : 1), took them out, dried them in the air, sprayed them with 10% sulfuric acid ethanol solution, and heated them at 105 ℃ until the spots became clear. The thin layer chromatography is shown in Fig. 7. It can be seen from Fig. 7 that at the corresponding position of the β-sitosterol reference substance, the underground part of the sample can show fluorescent spots of the same color.



NOTE 1. β-sitosterol reference substance; 2 to 8. different batch of *C. tangutorum* medicinal materials; 9 to 13. different batch of *C. macrophylla* medicinal materials.

Fig. 7 Thin layer chromatography of underground parts of medicinal material

4 Discussion

The main characteristics of *C. tangutorum* and *C. macrophylla* were obtained by systematic identification of the original plants, medicinal properties, tissue structure and thin layer chromatography of the ground and underground parts of *C. tangutorum* and *C. macrophylla*. It was found that there were no significant differences in the properties, powder characteristics and TLC characteristics between *C. tangutorum* and *C. macrophylla*. The characteristics of the original plant and the cross section are obviously different: the rhizome of *C. macrophylla* is creeping and extended, and is densely covered with fibrous roots. Stem erect, cauline leaves usually 4 – 5; leaflets 4 – 5 pairs, petals lilac, purplish red, rarely white. *C. tangutorum* differs from *C. macrophylla* in that the rhizome is creeping and scaly. The stem is short, simple, leafless and naked in the lower part, usually with 3 smaller pinnately compound leaves in the upper part. The flowers are purple, and the thin layer chromatography shows that in the thin layer chromatography of the test sample of the ground part of the medicinal material, the same color spots appear at the positions corresponding to the reference substance quercetin. In the thin layer chromatogram of the underground part of the test sample, spots of the same color appear at the positions corresponding to the reference substance β-sitosterol.

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Table 8 Comprehensive score of combined lyoprotectants

Dosage and type	Appearance score	Particle size increase//nm	Re-dissolution time//sec	PDI increase	Comprehensive score
5% Mannitol-sucrose	2	0	19.9	0	0.160 0
5% Mannitol-glucose	1	0	40.15	0	0.019 2
6% Mannitol-sucrose	3	0.4	26.1	0	0.241 6
6% Mannitol-glucose	4	0	37.3	0.003	0.323 0
7% Mannitol-sucrose	3	7.5	33.6	0.157	0.021 6
7% Mannitol-glucose	3	0	26.3	0	0.247 1
8% Mannitol-sucrose	3	10.3	39.4	0.117	−0.005 9
8% Mannitol-glucose	4	0	37.9	0	0.323 7
9% Mannitol-sucrose	4	0	40.13	0	0.319 3
9% Mannitol-glucose	4	12.8	42.1	0.129	0.044 1
10% Mannitol-sucrose	5	7.11	32.3	0	0.330 0
10% Mannitol-glucose	4	20.31	49.7	0.132	−0.084 1

The results demonstrated that the product obtained with a 10% mannitol-sucrose combination as the lyoprotectant exhibited the highest overall score. This was evidenced by its superior appearance, rapid re-dissolution, and the solution's enhanced clarity after re-dissolution, which exhibited a light blue milky hue. Additionally, the particle size and PDI after re-dissolution were comparable to those observed before lyophilization, as illustrated in Figs. 2 – 3.

Comprehensive score $= 0.5A/A_{max} - 0.3B/B_{max} - 0.1C/C_{max} - 0.1D/D_{max}$

4 Discussion

In order to enhance the stability of hydroxypropyl tetrahydropyrantriol liposomes, a preliminary study of their lyophilization process was carried out. The lyophilization process, type, and dosage of single lyoprotectant and combined lyoprotectants of hydroxypropyl tetrahydropyrantriol liposomes were evaluated using the appearance, re-dispersibility, particle size, and PDI of the products as indicators.

The use of a single lyoprotectant resulted in a gradual improvement in the appearance of the product with an increase in the dosage of mannitol. However, this was accompanied by an increase in particle size and PDI, a reduction in re-dispersability, and a slow re-dissolution rate. The increase in sucrose and glucose concentration did not result in an improvement in the appearance of the product. However, there was a significant reduction in particle size and PDI, and the re-dispersability was enhanced. The results of the screening for the type and dosage of single lyoprotectant were used to inform the examination of the type and dosage of combined lyoprotectants. The final lyophilization process was determined to be as follows: pre-freezing for 16 h at −80 ℃, with a total drying time of 36 h; hydroxypropyl tetrahydropyrantriol lyophilized liposomes were prepared by using additional 10% mannitol-sucrose as the lyoprotectant, and the resulting product had a fluffy and full appearance without shrinkage and collapse. The resulting products exhibited a fluffy and full appearance, with no shrinkage or collapse. There was no change in volume before and after lyophilization, and the products demonstrated good re-dispersibility, rapid re-dissolution, and minimal change in particle size and PDI before and after lyophilization.

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