

Caulis Clematidis Armandii



Figure 1 A photograph of Caulis Clematidis Armandii

1. NAMES

Official Name: *Caulis Clematidis Armandii*

Chinese Name: 川木通

Chinese Phonetic Name: Chuanmutong

2. SOURCE

Caulis Clematidis Armandii is the dried stem of the liana *Clematis armandii* Franch. (Ranunculaceae). The stem is collected in the spring and autumn and, after removal of the coarse bark, the stripped stem is dried whole under the sun or, while fresh, cut into thin slices, then the slices dried under the sun to obtain *Caulis Clematidis Armandii*.

3. DESCRIPTION

The stem is long and cylindrical, slightly twisted, 1-5 cm in diameter. Externally yellowish-brown or dull yellowish-brown, marked with longitudinal grooves and ridges. Nodes usually swollen, with leaf scars and branch scars. Any bark that remains is easily torn off. The texture is hard, not easily broken, the slices 2-5 mm thick, uneven along the margin, the bark that remains yellowish-brown, the xylem pale yellowish-brown or pale yellow, with yellowish-white radial striations and cracks, vessels scattered. Pith relatively small, whitish or yellowish-brown, occasionally hollowed. Odourless with a faint taste (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

The cork layer and cortex are usually rubbed off, and the stele surrounded by curved fibre bundles. 1-2 rows of lignified fibre bundles in the phloem, and stone cells, are visible, but some groups of sieve elements are obliterated and compressed. The fascicular cambium is obvious. The xylem bundles consists of vessel elements, tracheids, xylem fibres and xylem parenchyma cells, all having

cell wall lignified; the diameter of the vessels vary in size. Medullary rays 21-26 cells high, 6-8 cells wide, thin-walled, lignified, usually with small pits; secondary rays smaller. Parenchyma cells in the pith somewhat rounded, with a slightly lignified wall and having small pits (Fig. 2).

Powder

Colour yellowish-brown. Phloem fibres elongated, fusiform, with two ends relatively sharp, 287-882 μm long or even longer occasionally, 21-28 μm in diameter, with thick lignified wall and small lumina, few phloem fibres have larger lumina and possess septa or simple pits. Stone cells subrectangular or fusiform, walls thick, pit canals and pits obvious, 38-119 μm long, 28-39 μm wide. Most bordered pitted vessels 28-152 μm in diameter. Bordered pitted tracheids also present, 17-25 μm in diameter, the wall lignified. The xylem fibres, with thickened wall, lignified, 267-493 μm long, 24-29 μm in diameter, possessing simple pits, bordered pits, and aggregated reticulated pits. Xylem parenchyma cells rectangular, with the slightly slender end connected with fibres, 89-191 μm long, 23-37 μm in diameter, the wall thick and lignified, with simple pits (Fig. 3).

4.2 Physicochemical Identification

Procedure

Weigh 1.0 g of the powdered sample and put into a test tube, then add 5 mL of dichloromethane. Sonicate (490 W) the mixture for 30 min. Filter and transfer 1 mL of filtrate to another test tube. Cautiously add about 1 mL of sulphuric acid along the inner wall of the test tube. Allow to stand for 30 min. A red or reddish-brown ring is observed at the interface of the two solvent layers.

4.3 Thin-Layer Chromatographic Identification [Appendix IV(A)]

Standard solution

Oleanolic acid standard solution

Weigh 1.0 mg of oleanolic acid CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of cyclohexane and acetone (4:1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

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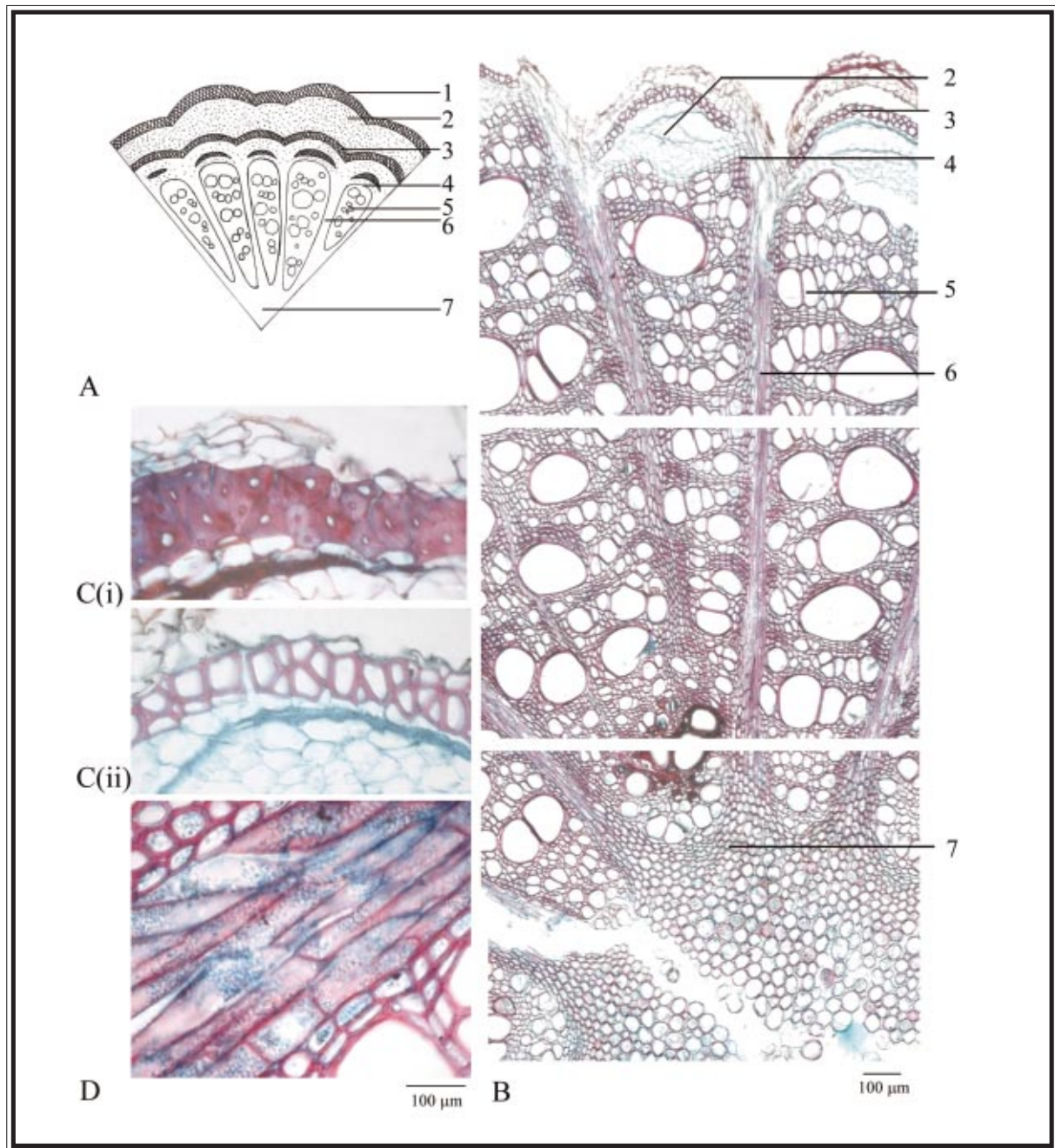


Figure 2 Microscopic features of transverse section of *Caulis Clematidis Armandii*

A. Sketch B. Section illustration (the part of pericycle fibres has rubbed off)

C(i) and (ii). Phloem fibre bundles D. Ray cells

1. Pericycle fibres 2. Phloem 3. Phloem fibres 4. Cambium 5. Xylem 6. Ray 7. Pith

Caulis Clematidis Armandii

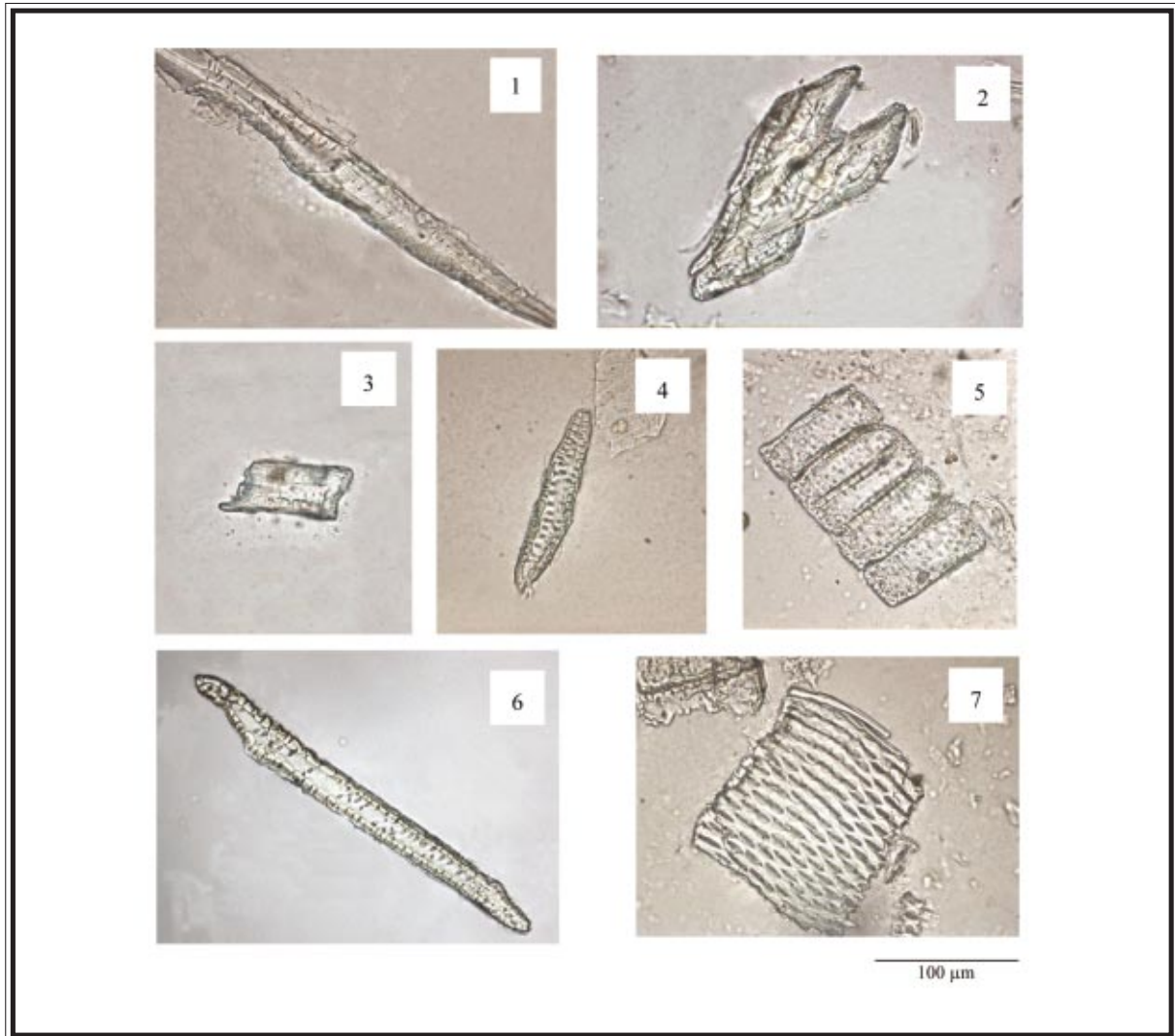


Figure 3 Microscopic features of powder of *Caulis Clematidis Armandii* (under the light microscope)

1. Phloem fibre
2. A group of stone cells
3. Subrectangular stone cells
4. Tracheid
5. Xylem parenchyma cells
6. Xylem fibre
7. Bordered pitted vessels

Test solution

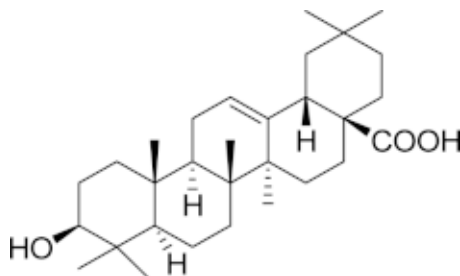
Weigh 10.0 g of the powdered sample and put into a 150-mL conical flask, then add 50 mL of methanol. Sonicate (490 W) the mixture for 60 min. Centrifuge at about $1800 \times g$ for 10 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask, then add 5 mL of 12 M hydrochloric acid. Reflux the mixture for 3 h. Cool down to room temperature. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1.5 mL of methanol.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately oleanolic acid standard solution (2 μL) and the test solution (3 μL) to the plate. Develop over a path of about 5 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Spray the plate evenly with the spray reagent and heat at about 70°C until the spots or bands become visible (about 10 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV(A).

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the R_f value, corresponding to that of oleanolic acid.

(i)



(ii)

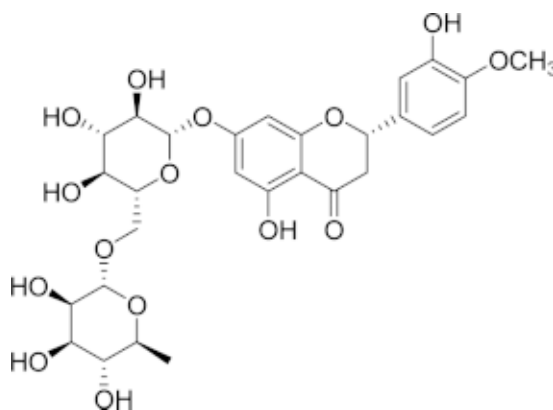


Figure 4 Chemical structures of (i) oleanolic acid and (ii) hesperidin

4.4 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

Hesperidin standard solution for fingerprinting, Std-FP (80 mg/L) [Hesperidin is added as a marker (Internal standard)].

Weigh 2.0 mg of hesperidin CRS (Fig. 4) and dissolve in 25 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and put into a 50-mL conical flask, then add 2.5 mL of hesperidin Std-FP and 15 mL of methanol. Sonicate (490 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Repeat the extraction twice. Combine the filtrate. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45- μ m RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (205 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μ m particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows –

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	97	3	isocratic
5 – 20	97 \rightarrow 82	3 \rightarrow 18	linear gradient
20 – 60	82 \rightarrow 65	18 \rightarrow 35	linear gradient

System suitability requirements

Perform at least five replicate injections each with 10 μ L of hesperidin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of hesperidin should not be more than 5.0%; the RSD of the retention time of hesperidin peak should not be more than 2.0%; the column efficiency determined from hesperidin peak should not be less than 150000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject hesperidin Std-FP and the test solution (10 μ L each) into the HPLC system and record the chromatograms. Measure the retention time of hesperidin peak in the chromatogram of the hesperidin Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Under the same HPLC conditions, identify hesperidin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of hesperidin Std-FP. The retention times of hesperidin peaks from the two chromatograms should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

The RRTs and acceptable ranges of the five characteristic peaks of *Caulis Clematidis Armandii* extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the five characteristic peaks of *Caulis Clematidis Armandii* extract

Peak No.	RRT	Acceptable Range
1	0.60	± 0.03
2	0.75	± 0.03
3	0.81	± 0.03
4 (marker, hesperidin)	1.00	-
5	1.16	± 0.03

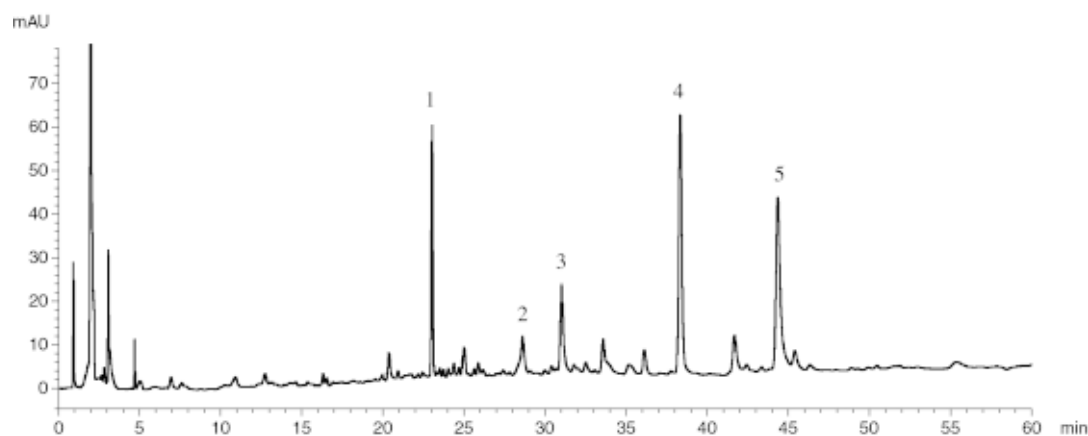


Figure 5 A reference fingerprint chromatogram of *Caulis Clematidis Armandii* extract

For positive identification, the sample must give the above five characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 5).

5. TESTS

5.1 Heavy Metals (*Appendix V*): meet the requirements.

5.2 Pesticide Residues (*Appendix VI*): meet the requirements.

5.3 Mycotoxins (*Appendix VII*): meet the requirements.

5.4 Sulphur Dioxide Residues (*Appendix XVII*): meet the requirements.

5.5 Foreign Matter (*Appendix VIII*): not more than 1.0%.

5.6 Ash (*Appendix IX*)

Total ash: not more than 2.5%.

Acid-insoluble ash: not more than 0.5%.

5.7 Water Content (*Appendix X*): not more than 12.0%.

5.8 Detection of Aristolochic Acid I (*Appendix XIII*): meet the requirements.

6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (cold extraction method): not less than 5.0%.

Ethanol-soluble extractives (cold extraction method): not less than 3.0%.