Cynomorii Herba

Figure 1 A photograph of Cynomorii Herba

A. Cynomorii Herba B. Transverse section of stem

1. NAMES

Official Name: Cynomorii Herba

Chinese Name: 鎖陽

Chinese Phonetic Name: Suoyang

2. SOURCE

Cynomorii Herba is the dried fleshy stem of *Cynomorium songaricum* Rupr. (Cynomoriaceae). The fleshy stem is collected in spring, inflorescence removed, cut into sections, then dried under the sun to obtain Cynomorii Herba.

3. DESCRIPTION

Compressed-cylindrical, slightly curved, varying in length, up to 17 cm long, 15-50 mm in diameter. Externally brown, rough, with distinct longitudinal furrows and irregular pits, sometimes with remnants of triangular dark brown scales. Texture hard and heavy in weight, uneasily broken. Fracture pale brown to brown, with triangular yellow vascular bundles. Odour slight; taste sweet and astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork cells mostly decadent and deciduous, residual of cork cells occasionally found. Cortex consists of several layers of cells. Vascular bundles abundant, collateral, fan-shaped or semi-rounded, scattered in stele irregularly, small in the outermost layer, and relatively large inward (Fig. 2).

Powder

Colour brown. Starch granules fairly abundant, mainly simple starch granules, subrounded, hemispherical or polygonal, 3-30 μm in diameter, hilum pointed, cleft-like, stellate or V-shaped; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-3 units. Vessels mainly reticulate and sometimes spiral, 7-35 μm in diameter. Cork cells brown, subsquare, rectangular or polygonal, walls slightly thickened (Fig. 3).

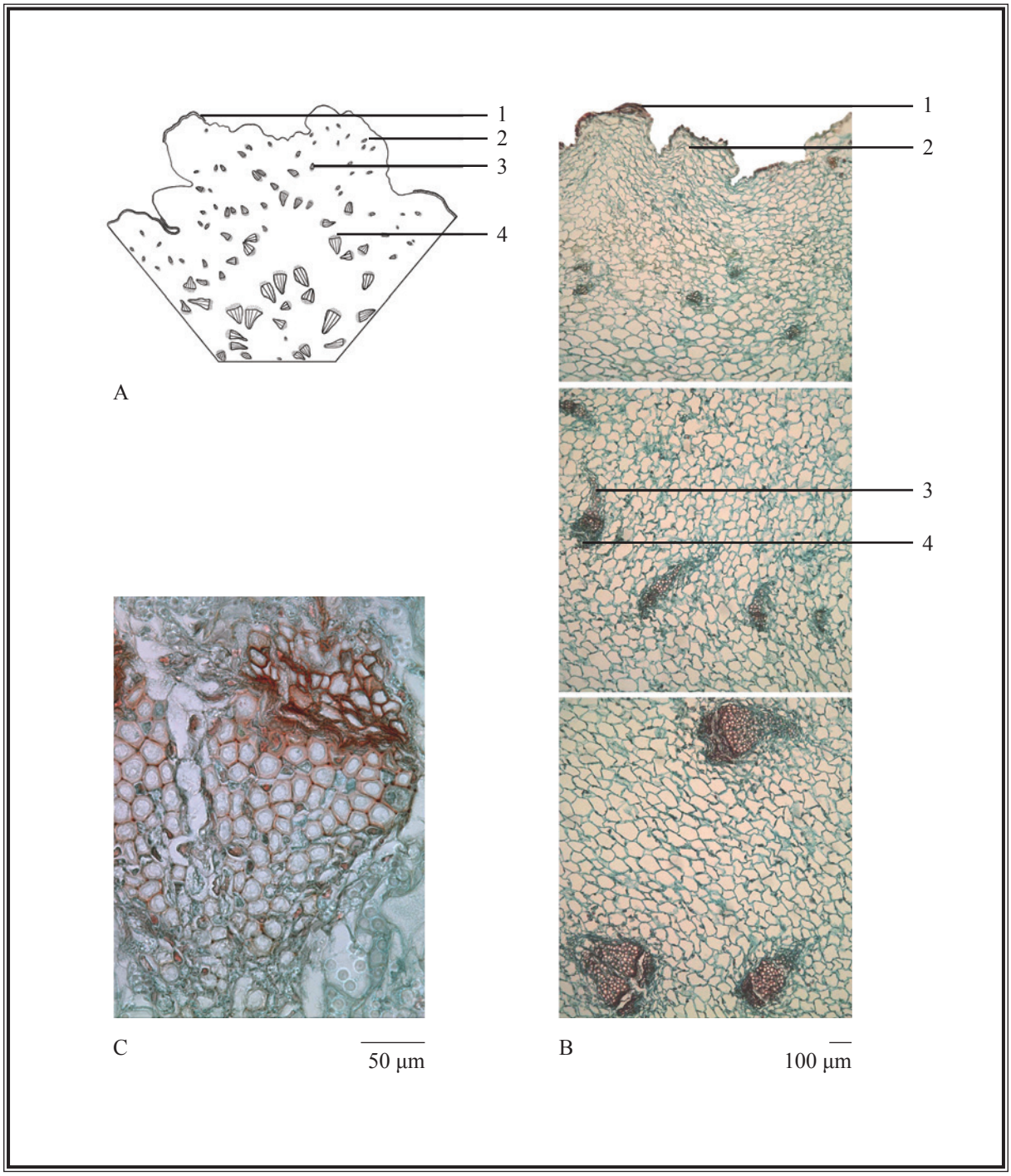


Figure 2 Microscopic features of transverse section of Cynomorii Herba

A. Sketch B. Section illustration C. Vascular bundle

1. Cork 2. Cortex 3. Xylem 4. Phloem

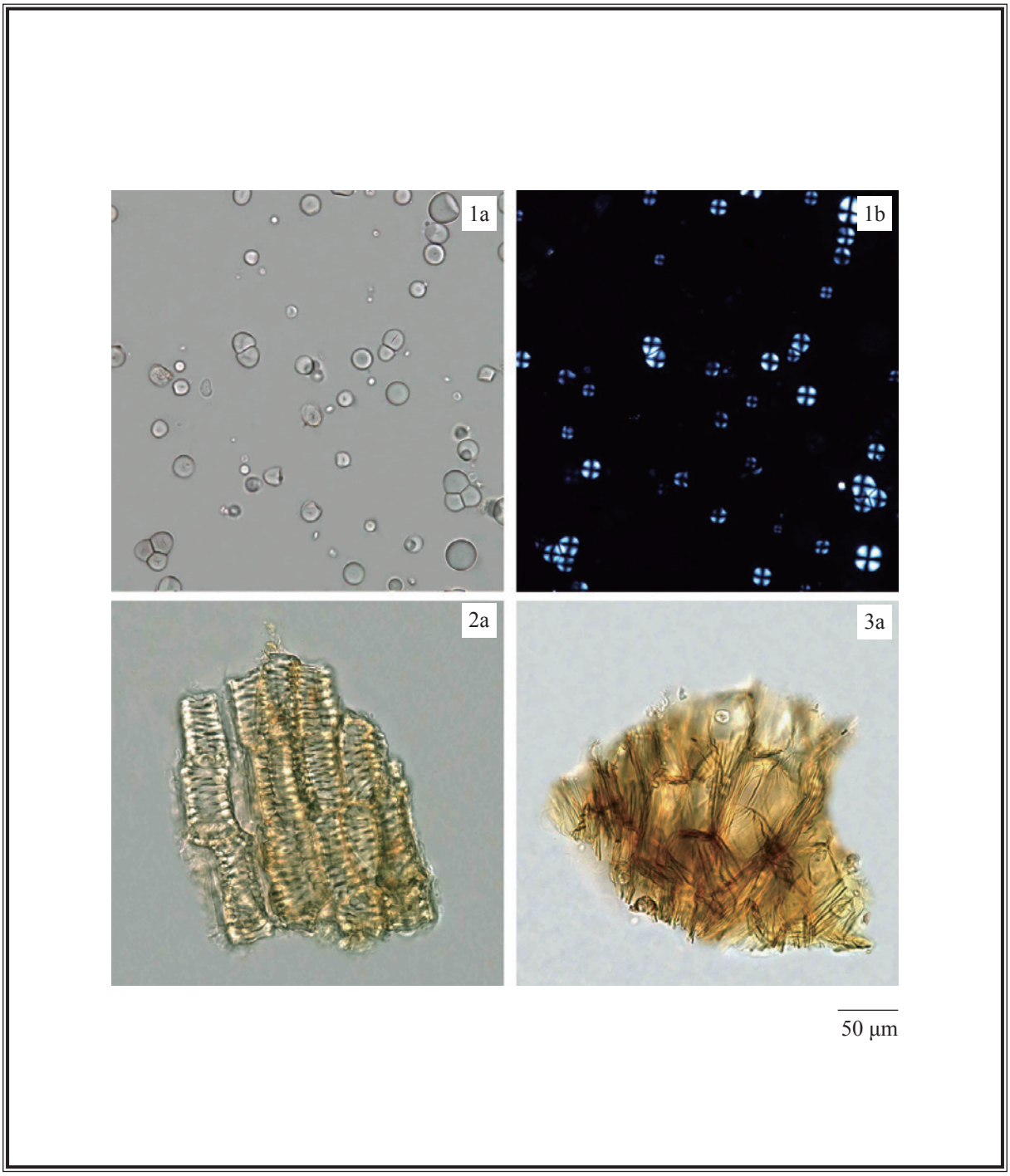


Figure 3 Microscopic features of powder of Cynomorii Herba

1. Starch granules 2. Vessels 3. Cork cells

a. Features under the light microscope b. Features under the polarized microscope

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

Standard solution

L-Proline standard solution

Weigh 1.0 mg of L-proline CRS (Fig. 4) and dissolve in 1 mL of water.

Developing solvent system

Prepare a mixture of *n*-propanol, water, glacial acetic acid and ethanol (4:2:1:1, v/v).

Spray reagent

Weigh 1 g of ninhydrin and dissolve in 50 mL of ethanol.

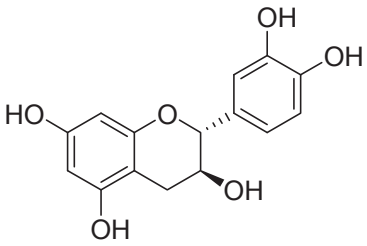
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of water. Allow to stand for 30 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately L-proline standard solution and the test solution (1 μL each) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the HPTLC plate in the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully tilt the chamber to allow sufficient solvent to pass from the trough containing the solvent to the other containing the HPTLC plate for development. 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 105ºC until the spots or bands become visible (about 5-10 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

(i)



(ii)

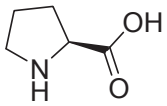


Figure 4 Chemical structures of (i) (+)-catechin and (ii) L-proline

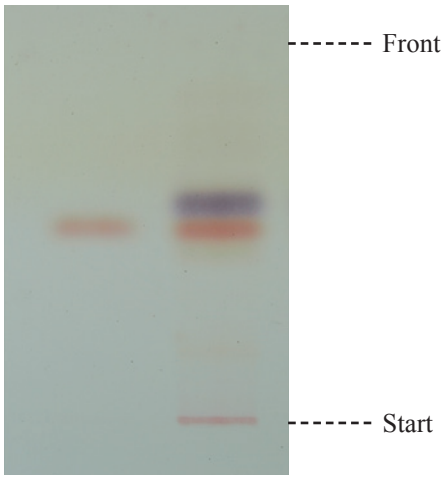


Figure 5 A reference HPTLC chromatogram of Cynomorii Herba extract observed under visible light after staining

1. L-Proline standard solution 2. Test solution

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 L-proline (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting *(Appendix XII)*

Standard solution

(+)-Catechin standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 2.5 mg of (+)-catechin CRS (Fig. 4) and dissolve in 25 mL of methanol (60%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (60%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol (60%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (205 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 35ºC during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.4% phosphoric acid and acetonitrile (90:10, v/v). The elution time is about 50 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of (+)-catechin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of (+)-catechin should not be more than 5.0%; the RSD of the retention time of (+)-catechin peak should not be more than 2.0%; the column efficiency determined from (+)-catechin peak should not be less than 4000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject (+)-catechin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of (+)-catechin peak in the chromatogram of (+)-catechin Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify (+)-catechin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of (+)-catechin Std-FP. The retention times of (+)-catechin 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 three characteristic peaks of Cynomorii Herba extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the three characteristic peaks of Cynomorii Herba extract

Peak No.	RRT	Acceptable Range
1	0.76	± 0.04
2	0.89	± 0.03
3 [marker, (+)-catechin]	1.00	-

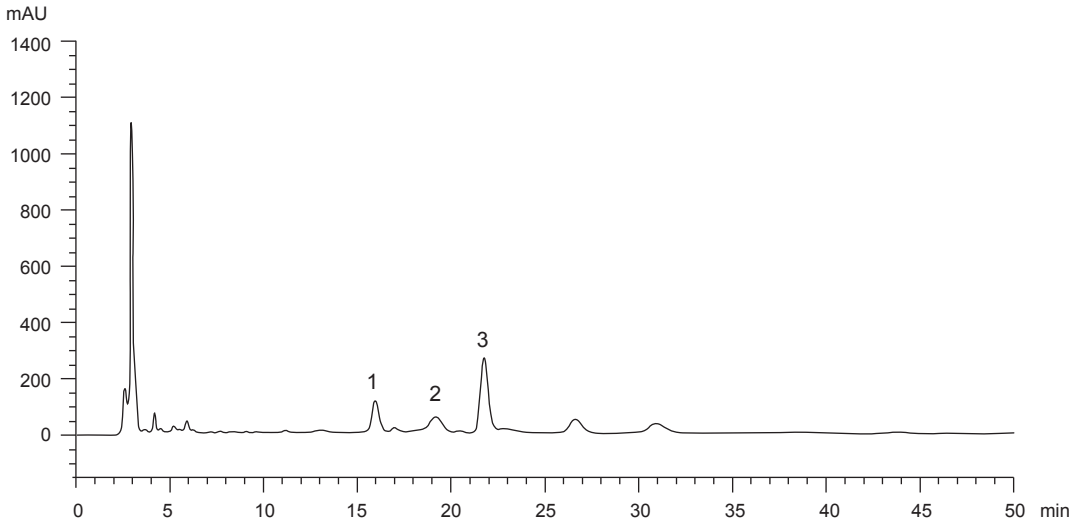


Figure 6 A reference fingerprint chromatogram of Cynomorii Herba extract

For positive identification, the sample must give the above three characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 6).

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 9.5%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 26.0%.
Ethanol-soluble extractives (cold extraction method): not less than 27.0%.

7. ASSAY

Carry out the method as directed in Appendix IV (B).

Standard solution
(+)-Catechin standard stock solution, Std-Stock (500 mg/L)
Weigh accurately 5.0 mg of (+)-catechin CRS and dissolve in 10 mL of methanol (60%).
(+)-Catechin standard solution for assay, Std-AS
Measure accurately the volume of the (+)-catechin Std-Stock, dilute with methanol (60%) to produce a series of solutions of 2, 10, 20, 40, 60 mg/L for (+)-catechin.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (60%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol (60%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (205 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 35ºC during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.4% phosphoric acid and acetonitrile (90:10, v/v). The elution time is about 50 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of (+)-catechin Std-AS (20 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of (+)-catechin should not be more than 5.0%; the RSD of the retention time of (+)-catechin peak should not be more than 2.0%; the column efficiency determined from (+)-catechin peak should not be less than 4000 theoretical plates.

The *R* value between (+)-catechin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curve

Inject a series of (+)-catechin Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of (+)-catechin against the corresponding concentrations of (+)-catechin Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify (+)-catechin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of (+)-catechin Std-AS. The retention times of (+)-catechin peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of (+)-catechin in the test solution, and calculate the percentage content of (+)-catechin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.025% of (+)-catechin ($C_{15}H_{14}O_6$), calculated with reference to the dried substance.