Cibotii Rhizoma

Figure 1 A photograph of Cibotii Rhizoma

A. Cibotii Rhizoma B. Transverse section
C. Raw slices of Cibotii Rhizoma (“Shenggoujipian”)
D. Steamed slices of Cibotii Rhizoma (“Shugoujipian”)

1. NAMES

Official Name: Cibotii Rhizoma

Chinese Name: 狗脊

Chinese Phonetic Name: Gouji

2. SOURCE

Cibotii Rhizoma is the dried rhizome of *Cibotium barometz* (L.) J. Sm. (Dicksoniaceae). The rhizome is collected in autumn and winter, soil removed and dried; or hard roots, petioles and hairs removed, then cut into thick slices and dried under the sun to obtain "Shenggoujipian" (Raw slice of Cibotii Rhizoma); or after steaming the rhizome, dried under the sun until almost dry, then cut into thick slices and dried thoroughly to obtain "Shugoujipian" (Steamed slice of Cibotii Rhizoma).

3. DESCRIPTION

Irregular long lump-shaped, up to 56 cm long, 15-120 mm in diameter. Externally dark brown, covered with remnants of golden hairs; upper part with several reddish-brown remnants base of woody petiole; lower part showing remnants of black fibrous roots. Texture hard. Odourless; taste weak and slightly astringent. "Shenggoujipian" (Raw slice of Cibotii Rhizoma) irregular long slat-shaped or rounded, 4-19 cm long, 15-80 mm in diameter, 1.5-5 mm thick, cut surface pale brown, relatively smooth, with a protuberant brownish-yellow xylem annulation or stria at a distance of 1-7 mm from the edges; edges uneven, occasionally with remnants of golden hairs; texture fragile; easily broken, starchy. "Shugoujipian" (Steamed slice of Cibotii Rhizoma) blackish-brown; texture hard (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Non-glandular hairs present outside epidermis, mostly fragmented. Epidermis consists of 1-3 layer(s) of cells. Sclerenchyma locate beneath epidermis, consists of 10 or more layers of cells. Xylem consists of tracheids, with the presence of phloem and endodermis at both inner and outer parts of the xylem. Cortex and pith composed of parenchymatous cells, filled with starch granules in raw sample or with starch gelatinous masses in steamed rhizome, some filled with yellowish-brown contents (Fig. 2).

Powder

Colour yellowish-brown to reddish-brown. Non-glandular hairs golden or yellowish-brown, mostly fragmented, 10-128 μm in diameter, wall of middle cell thin, apical cell long and pointed, wall slightly thickened. Starch granules numerous in raw rhizome, simple starch granules ovoid to ellipsoid, 5-49 μm in diameter, hilum pointed, cleft-like, V-shaped or Y-shaped, striations visible in the large ones; black and cruciate-shaped under the polarized microscope; compound starch granules few, composed of 2-3 units. Starch gelatinous masses colourless, present in steamed rhizome. Tracheids reticulate, commonly found, 22-73 μm in diameter. Sclerenchymatous cells yellowish-brown to reddish-brown, subrectangular or spindle-shaped, wall thickened, pits distinct. Endodermal cells yellow to yellowish-brown, subsquare, subrectangular or polygonal, wall slightly thickened and slightly sinuous (Fig. 3).

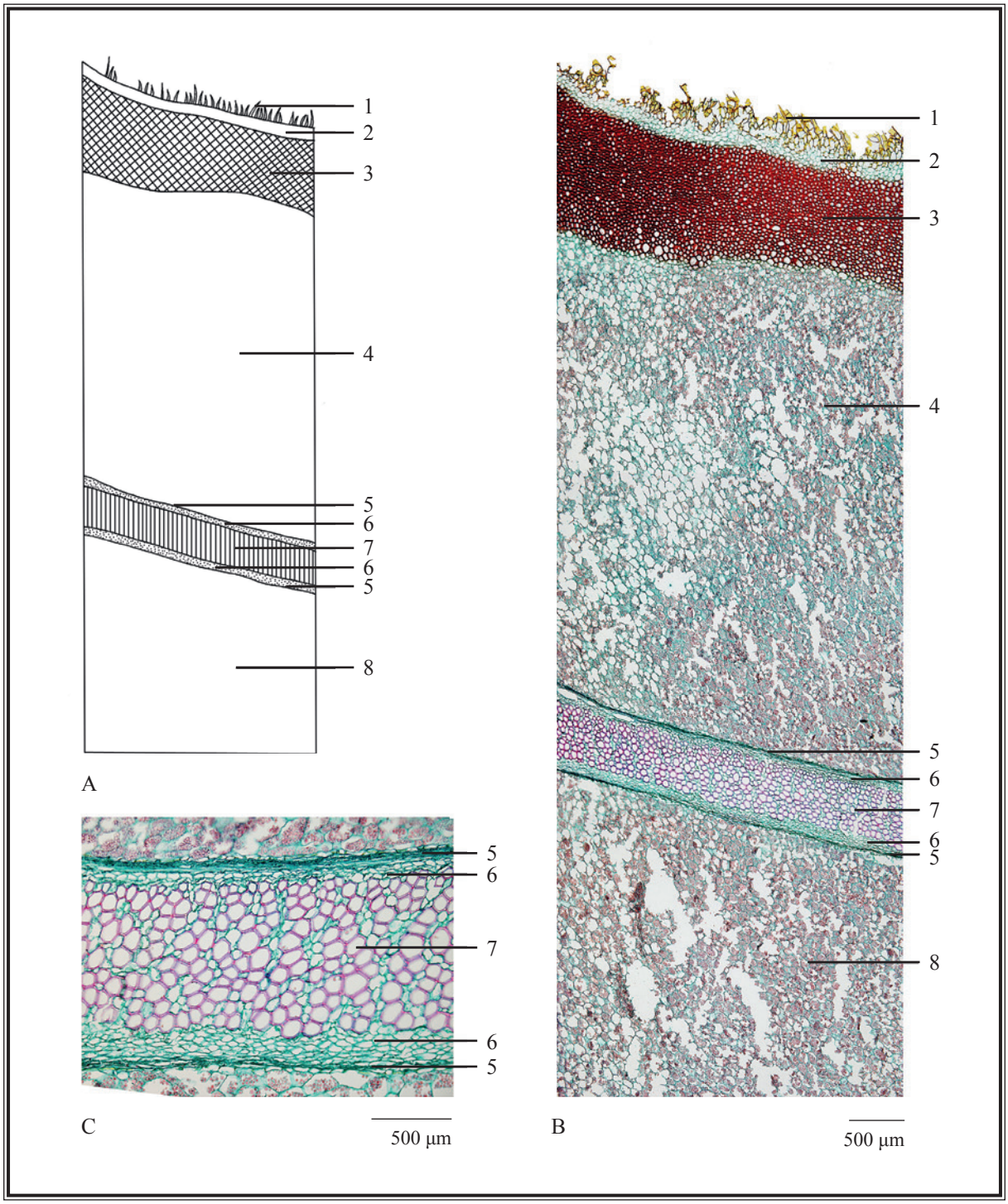


Figure 2 Microscopic features of transverse section of Cibotii Rhizoma

A. Sketch B. Section illustration C. Section magnified

1. Non-glandular hair 2. Epidermis 3.Sclerenchyma 4. Cortex
5. Endodermis 6. Phloem 7. Xylem 8. Pith

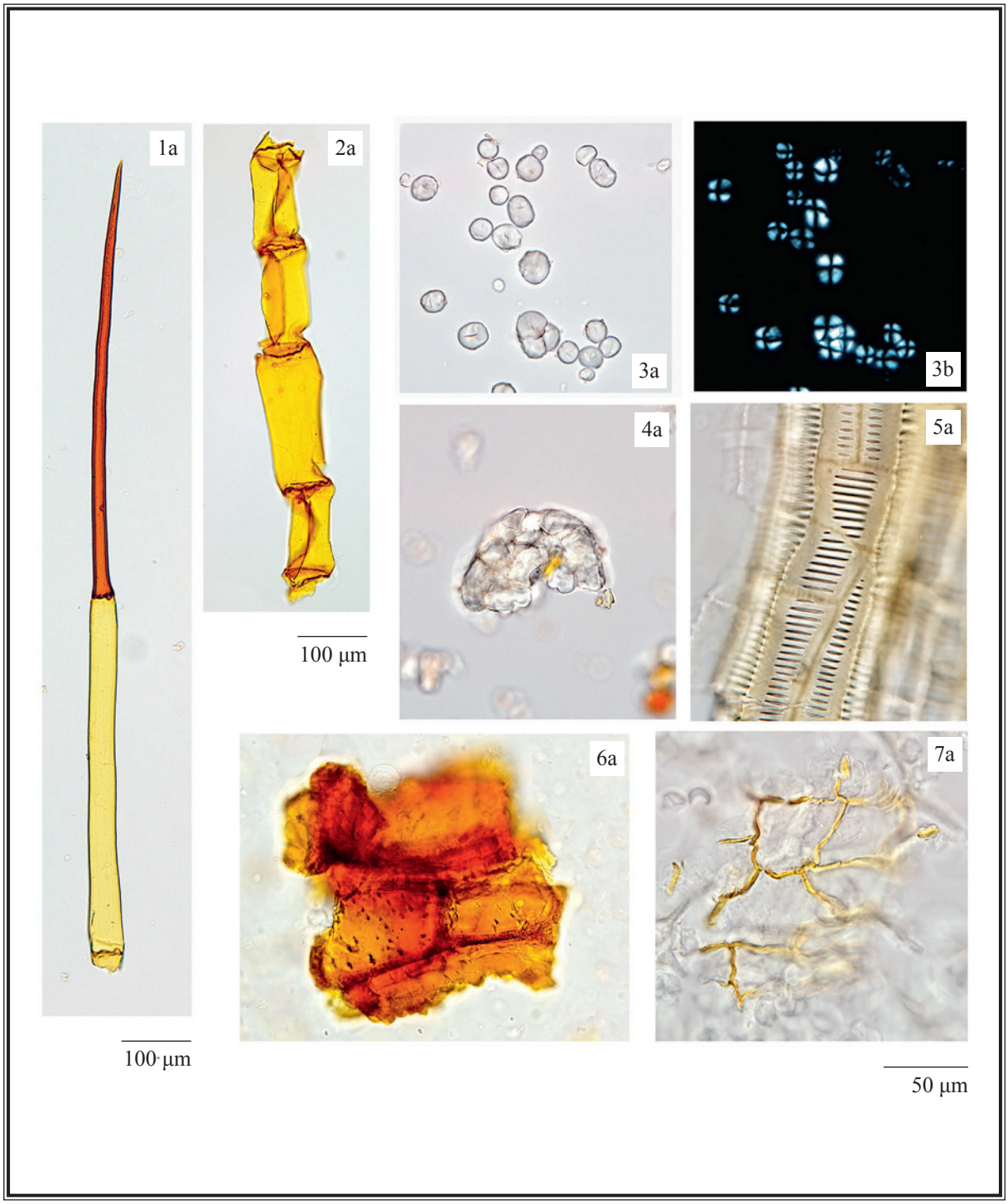


Figure 3 Microscopic features of powder of Cibotii Rhizoma

1, 2. Non-glandular hairs 3. Starch granules
4. Starch gelatinous mass (in steamed rhizome) 5. Reticulate tracheids
6. Sclerenchymatous cells 7. Endodermal cells

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

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

Standard solutions

Protocatechuic acid standard solution

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

Protocatechuic aldehyde standard solution

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

Developing solvent system

Prepare a mixture of n-hexane, ethyl acetate and glacial acetic acid (5:5:0.1, v/v).

Spray reagent

Weigh 2.5 g of ferric trichloride and dissolve in 50 mL of ethanol.

Test solution

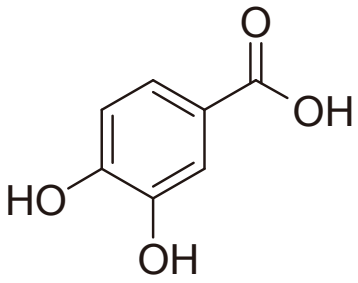
Weigh 2.0 g of the powdered sample and place it in a 100-mL conical flask, then add 50 mL of methanol. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 30 mL of water and 5 mL of hydrochloric acid (22.4%, w/v). Transfer the solution to a 250-mL separating funnel. Extract for three times each with 20 mL of diethyl ether. Combine the diethyl ether extracts and transfer to a 250-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate (2-10 µm) and a freshly prepared developing solvent system as described above. Apply separately protocatechuic acid standard solution (2 µL), protocatechuic aldehyde standard solution (2 µL) and the test solution (20 µL) to the plate. Develop over a path of about 8 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 dry it in air until the spots or bands become visible. Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of protocatechuic acid and protocatechuic aldehyde.

(i)



(ii)

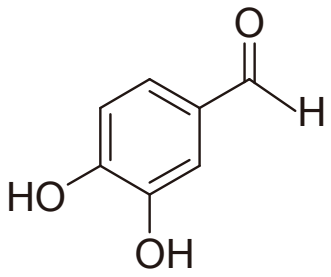


Figure 4 Chemical structures of (i) protocatechuic acid and (ii) protocatechuic aldehyde

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

Standard solutions

Protocatechuic acid standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 0.5 mg of protocatechuic acid CRS and dissolve in 10 mL of 1% acetic acid.

Protocatechuic aldehyde standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 0.5 mg of protocatechuic aldehyde CRS and dissolve in 10 mL of 1% acetic acid.

Test solution

Weigh 5.0 g of the powdered sample and place it in a 250-mL conical flask, then add 100 mL of methanol. Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 30 mL of water and 1 mL of hydrochloric acid. Transfer the solution to a 250-mL separating funnel. Extract for three times each with 50 mL of diethyl ether. Combine the diethyl ether extracts and transfer to a 250-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue for three times each with

1 mL of 1% acetic acid. Transfer the solution to a 5-mL volumetric flask and make up to the mark with 1% acetic acid. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 4% acetic acid and acetonitrile (95:5, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 20 μL of protocatechuic acid Std-FP and protocatechuic aldehyde Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of protocatechuic acid and protocatechuic aldehyde should not be more than 5.0%; the RSD of the retention times of protocatechuic acid and protocatechuic aldehyde peaks should not be more than 2.0%; the column efficiencies determined from protocatechuic acid and protocatechuic aldehyde peaks should not be less than 8000 theoretical plates.

The *R* value between peak 2 and the closest peak; and the *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 and 1.0 respectively (Fig. 5).

Procedure

Separately inject protocatechuic acid Std-FP, protocatechuic aldehyde Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention times of protocatechuic acid and protocatechuic aldehyde peaks in the chromatograms of protocatechuic acid Std-FP, protocatechuic aldehyde Std-FP and the retention times of the three characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify protocatechuic acid and protocatechuic aldehyde peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of protocatechuic acid Std-FP and protocatechuic aldehyde Std-FP. The retention times of protocatechuic acid and protocatechuic aldehyde peaks in the chromatograms of the test solution and corresponding Std-FP 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 Cibotii Rhizoma extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the three characteristic peaks of Cibotii Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.49	± 0.03
2 (protocatechuic acid)	0.65	± 0.03
3 (marker, protocatechuic aldehyde)	1.00	-

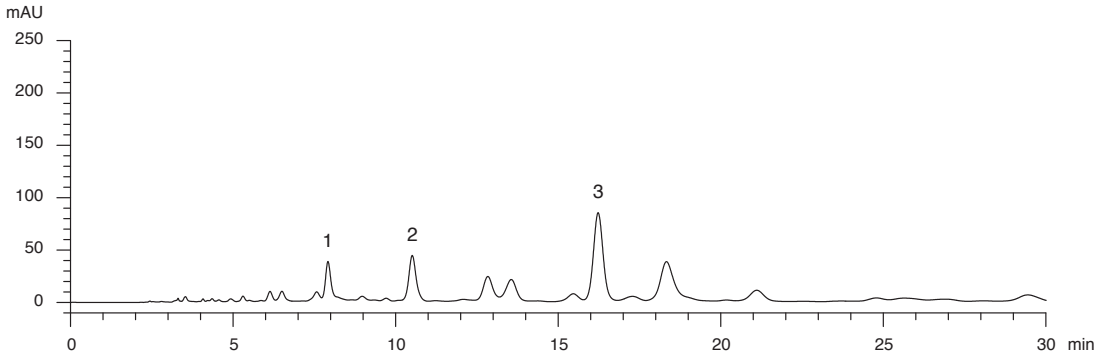


Figure 5 A reference fingerprint chromatogram of Cibotii Rhizoma extract

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. 5).

5. TESTS

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

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

5.3 Mycotoxins – Aflatoxins *(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)*

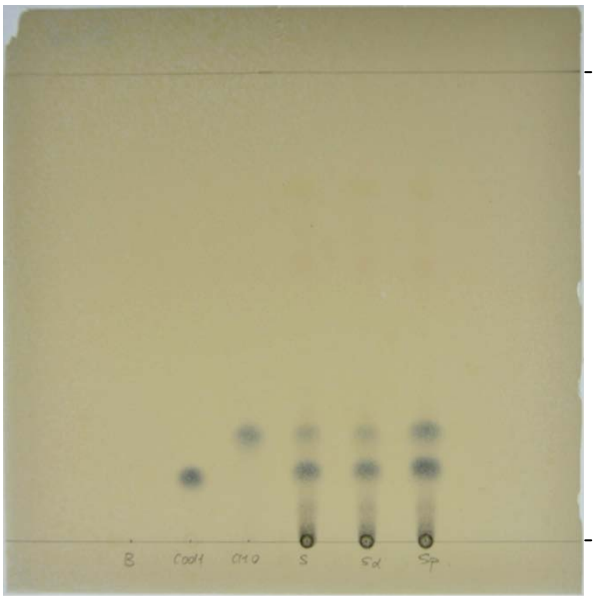
Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 32.0%.

Ethanol-soluble extractives (hot extraction method): not less than 20.0%.

Cibotii Rhizoma (狗脊)



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Standard (Protocatechuic acid)	Protocatechuic acid positive
3	Standard (Protocatechuic aldehyde)	Protocatechuic aldehyde positive
4	Sample (Cibotii Rhizoma)	Protocatechuic acid and protocatechuic aldehyde positive
5	Sample duplicate (Cibotii Rhizoma)	Protocatechuic acid and protocatechuic aldehyde positive
6	Spiked sample (Sample plus protocatechuic acid and protocatechuic aldehyde)	Protocatechuic acid and protocatechuic aldehyde positive

Figure 1 TLC results of Cibotii Rhizoma extract observed under visible light after staining