Selaginellae Doederleinii Herba

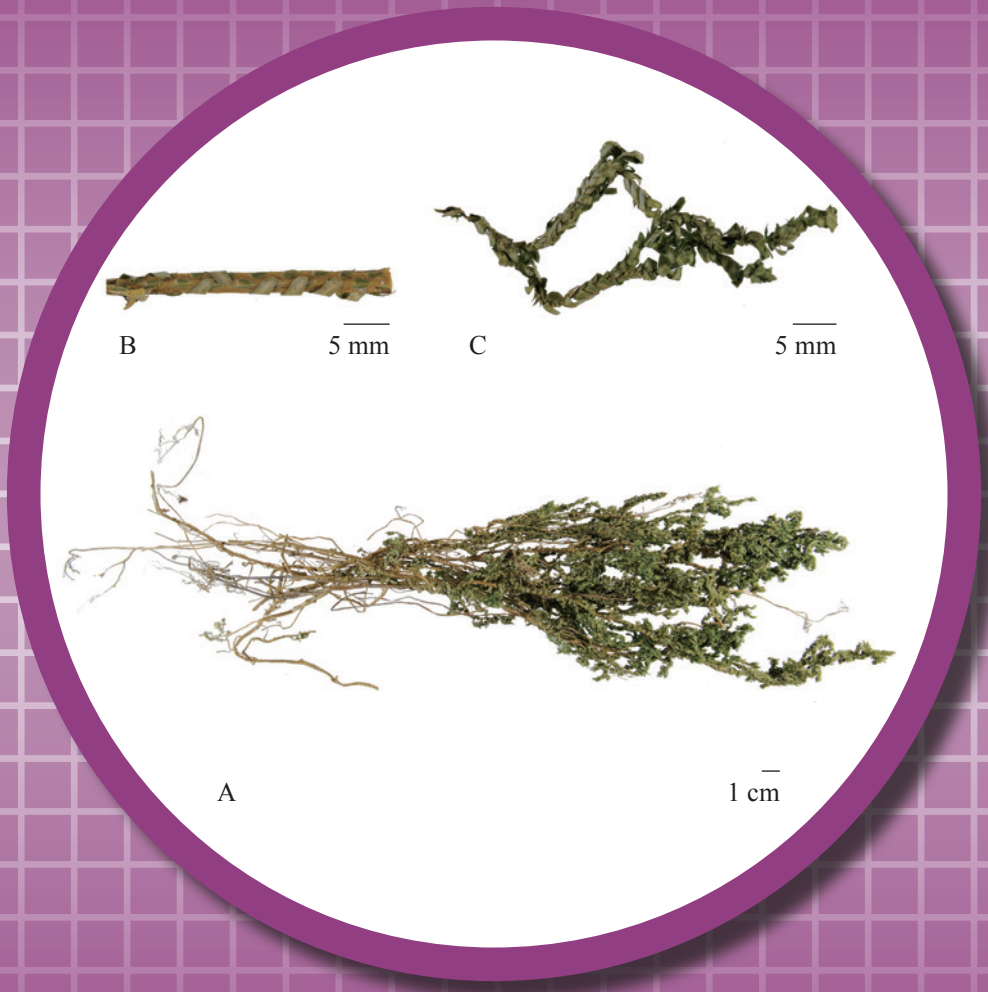

Figure 1 A photograph of Selaginellae Doederleinii Herba

A. Selaginellae Doederleinii Herba B. Magnified image of stem
C. Magnified image of small branch

1. NAMES

Official Name: Selaginellae Doederleinii Herba

Chinese Name: 石上柏

Chinese Phonetic Name: Shishangbai

2. SOURCE

Selaginellae Doederleinii Herba is the dried whole plant of *Selaginella doederleinii* Hieron. (Selaginellaceae). The whole plant containing adventitious roots is collected all year round, foreign matter removed, then dried under the sun to obtain Selaginellae Doederleinii Herba.

3. DESCRIPTION

Herb usually in bundles, 15-83 cm long, externally green or yellowish-green; texture soft. Stem ribbed, 1-3 mm in diameter, multi-branched; yellow, long and thin, adventitious roots originated from the base of branches. Scaly leaves densely matted, imbricate, in 4 lines; each leaf ovate and rectangular-rounded, margin irregularly serrulated, 3-5 mm long, 1-2 mm wide. Sporophylls arranged into a spike disposed on terminal, the spike 4-angled. Odour slight; taste sweet and bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Main stem: Epidermis consists of 1 layer of cells, with thickened wall. Cortex consists of sclerenchymatous and parenchymatous cells; 3-7 layers of sclerenchymatous cells located on the outer side of the cortex, with thickened and heavily lignified wall; 8-12 layers of parenchymatous cells located on the inner part of the cortex, with slightly lignified wall. Endodermis consists of 1 layer of cells. Vascular bundle amphicribral; xylem consists of several layers of cells in the centre, surrounded by phloem, closely arranged, the cells relatively small, completely lignified. Leaf-trace vascular bundles 2-4, amphicribral, scattered on the outer side of cortex (Fig. 2).

Powder

Colour yellowish-green. Spores greyish-green, subrounded to subtriangular, 16-29 μm in diameter, with irregular protuberances on the surface. Epidermal cells of leaf greyish-yellow, walls undulate and sinuate, stomata anomocytic, rounded to oval, subsidiary cells 4-9. Non-glandular hairs unicellular, not easily broken. Sclerenchymatous cells numerous, subrectangular. Vessels scalariform, 5-34 μm in diameter (Fig. 3).

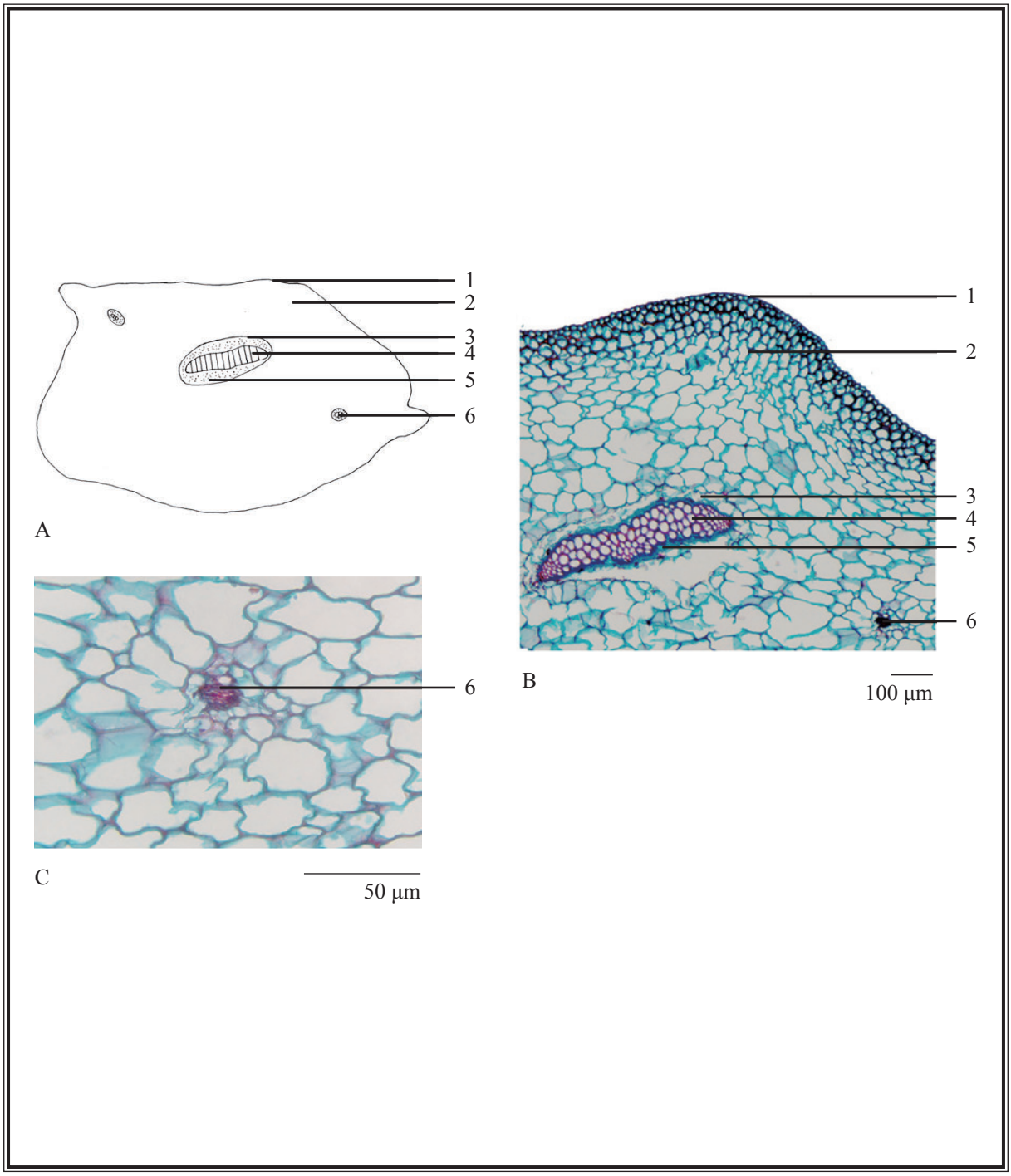

Figure 2 Microscopic features of transverse section of main stem of Selaginellae Doederleinii Herba

A. Sketch B. Section illustration C. Leaf-trace vascular bundle

1. Epidermis 2. Cortex 3. Endodermis 4. Xylem 5. Phloem 6. Leaf-trace vascular bundle

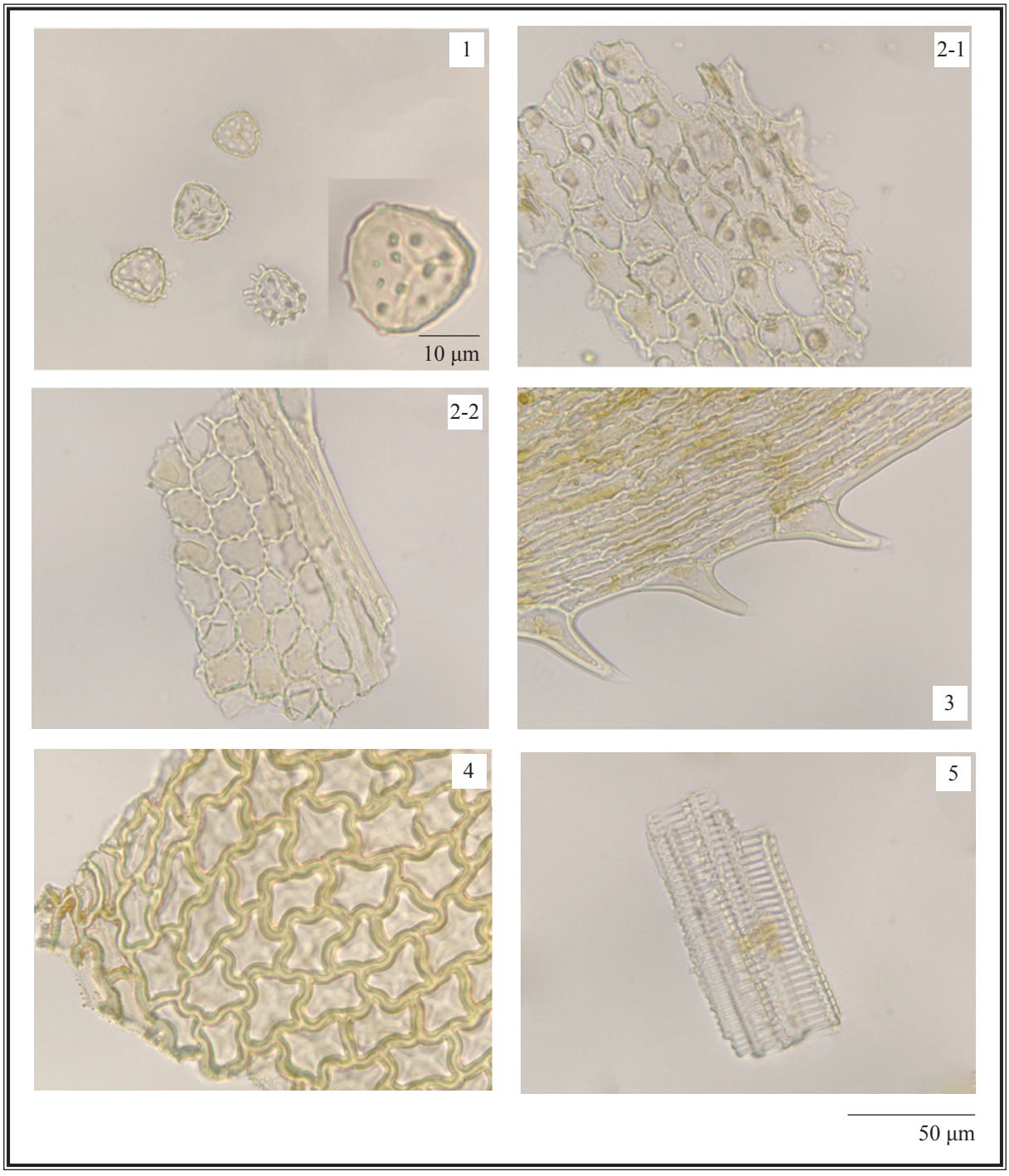

Figure 3 Microscopic features of powder of Selaginellae Doederleinii Herba (under the light microscope)

1. Spores 2. Epidermal cells of leaf 3. Non-glandular hairs 4. Sclerenchymatous cells
5. Scalariform vessels

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

Standard solution

Amentoflavone standard solution

Weigh 2.0 mg of amentoflavone CRS (Fig. 4) and dissolve in 10 mL of ethanol (50%). Place it in a water bath at about 60ºC for 5 min.

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC), formic acid and ethyl acetate (1:1.5:8, v/v).

Spray reagent

Weigh 5 g of aluminium trichloride and dissolve in 100 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 ethanol (50%). Sonicate (150 W) the mixture for 30 min. Filter the mixture.

Procedure

Carry out the method by using a TLC polyamide plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately amentoflavone standard solution (1 μL) and the test solution (3 μL) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the TLC 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 TLC plate for development. 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 heat at about 105ºC (about 5 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

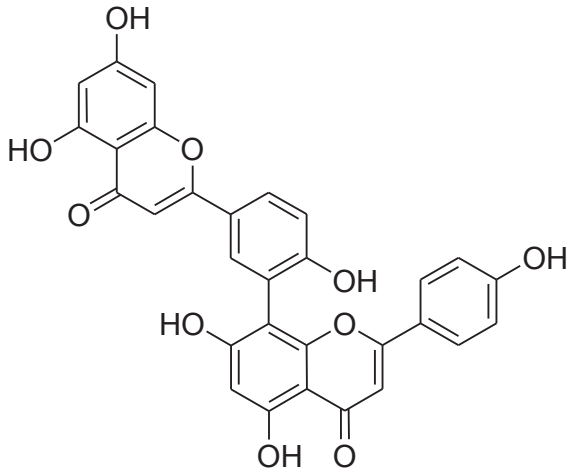

Figure 4 Chemical structure of amentoflavone

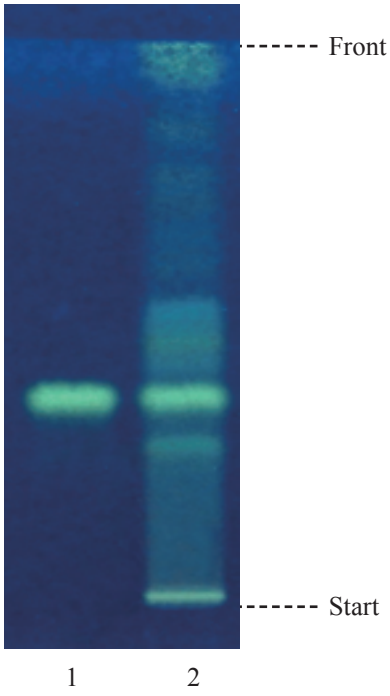

Figure 5 A reference TLC chromatogram of Selaginellae Doederleinii Herba extract observed under UV light (366 nm) after staining

1. Amentoflavone 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 amentoflavone (Fig. 5).

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

Standard solution

Amentoflavone standard solution for fingerprinting, Std-FP (40 mg/L)

Weigh 0.4 mg of amentoflavone CRS and dissolve in 10 mL of ethanol (50%). Place it in a water bath at about 60ºC for 5 min.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 25 mL of ethanol (50%). Reflux the mixture for 1 h. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 3000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (330 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. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.5% Formic acid (%, v/v)	Elution
0 – 20	35 → 42	65 → 58	linear gradient
20 – 25	42 → 50	58 → 50	linear gradient
25 – 40	50 → 60	50 → 40	linear gradient
40 – 45	60 → 65	40 → 35	linear gradient
45 – 50	65 → 100	35 → 0	linear gradient
50 – 60	100	0	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 μL of amentoflavone Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of amentoflavone should not be more than 5.0%; the RSD of the retention time of amentoflavone peak should not be more than 2.0%; the column efficiency determined from amentoflavone peak should not be less than 15000 theoretical plates.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject amentoflavone Std-FP and the test solution (5 µL each) into the HPLC system and record the chromatograms. Measure the retention time of amentoflavone peak in the chromatogram of amentoflavone Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify amentoflavone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of amentoflavone Std-FP. The retention times of amentoflavone 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 Selaginellae Doederleinii Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Selaginellae Doederleinii Herba extract

Peak No.	RRT	Acceptable Range
1 (marker, amentoflavone)	1.00	-
2	1.16	± 0.03
3	1.67	± 0.03
4	2.61	± 0.08
5	2.76	± 0.06

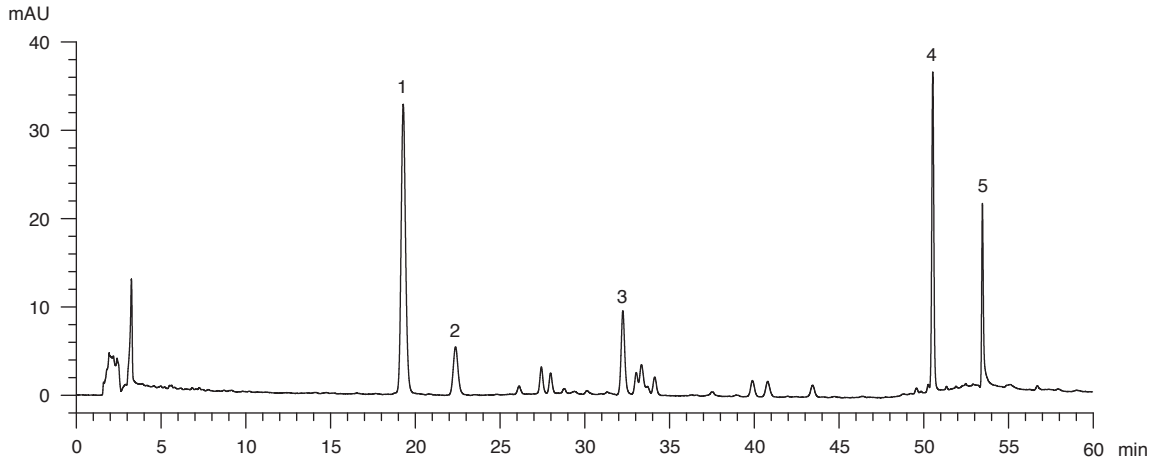

Figure 6 A reference fingerprint chromatogram of Selaginellae Doederleinii Herba extract

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. 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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 11.5%.
Acid-insoluble ash: not more than 6.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 16.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 9.0%.
Ethanol-soluble extractives (cold extraction method): not less than 5.0%.

7. ASSAY

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

Standard solution

Amentoflavone standard stock solution, Std-Stock (500 mg/L)

Weigh accurately 5.0 mg of amentoflavone CRS and dissolve in 10 mL of ethanol (50%). Place it in a water bath at about 60ºC for 5 min.

Amentoflavone standard solution for assay, Std-AS

Measure accurately the volume of the amentoflavone Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 1, 5, 10, 40, 80 mg/L for amentoflavone.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of ethanol (50%). Reflux the mixture for 1 h. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with ethanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (330 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. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.5% Formic acid (%, v/v)	Elution
0 – 10	38	62	isocratic
10 – 15	38 → 45	62 → 55	linear gradient
15 – 20	45 → 90	55 → 10	linear gradient
20 – 30	90	10	isocratic
30 – 35	90 → 38	10 → 62	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of amentoflavone Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of amentoflavone should not be more than 5.0%; the RSD of the retention time of amentoflavone peak should not be more than 2.0%; the column efficiency determined from amentoflavone peak should not be less than 8000 theoretical plates.

The *R* value between amentoflavone 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 amentoflavone Std-AS (5 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of amentoflavone against the corresponding concentrations of amentoflavone Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify amentoflavone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of amentoflavone Std-AS. The retention times of amentoflavone 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 amentoflavone in the test solution, and calculate the percentage content of amentoflavone in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.086% of amentoflavone ($C_{30}H_{18}O_{10}$), calculated with reference to the dried substance.