Selaginellae Herba

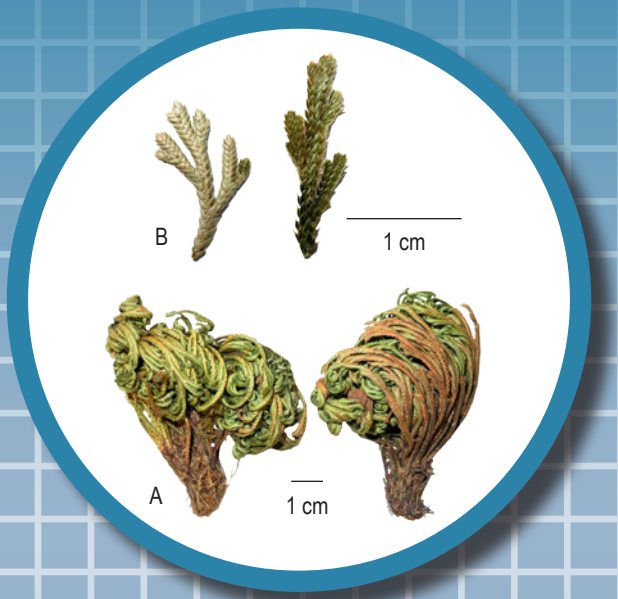

Figure 1 (i) A photograph of dried herb of *Selaginella tamariscina* (Beauv.) Spring

A. Selaginellae Herba B. Small branches

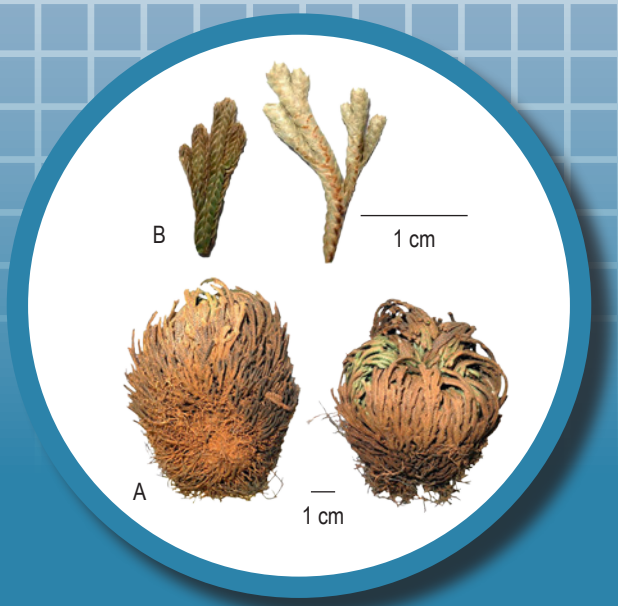

Figure 1 (ii) A photograph of dried herb of *Selaginella pulvinata* (Hook. et Grev.) Maxim.

A. Selaginellae Herba B. Small branches

1. NAMES

Official Name: Selaginellae Herba

Chinese Name: 卷柏

Chinese Phonetic Name: Juanbai

2. SOURCE

Selaginellae Herba is the dried herb of *Selaginella tamariscina* (Beauv.) Spring or *Selaginella pulvinata* (Hook. et Grev.) Maxim. (Selaginellaceae). The whole plant is collected throughout the year, fibrous root and soil removed, then dried under the sun to obtain Selaginellae Herba.

3. DESCRIPTION

***Selaginella tamariscina* (Beauv.) Spring:** Crumpled into a fisted mass, 3-10 cm long. Branches fascicled, flat and branched, green or brownish-yellow, curved inward, scaly leaflets densely matted, each leaflet long-aristate at the apex. Central leaves (ventral leaves) bear 2 lines, ovate-oblong, arranged obliquely upward, margins membranous, irregularly serrulate. In dorsal leaves (lateral leaves), membranous margin of dorsal surface frequently brownish-black. Fibrous roots remain attached at the base, brown to dark brown, scattered or clustered in short-rod shape. Odour slight; taste weak [Fig. 1 (i)].

***Selaginella pulvinata* (Hook. et Grev.) Maxim.:** Crumpled into a globular or fisted mass, 2-9.5 cm long. Central leaves (ventral leaves) bear 2 lines, ovate-lanceolate. Lamina asymmetrical, the inner side relatively straight, the outer side frequently thickened by inward folding, margins entire. Fibrous roots scattered and remain attached at the base. Odour slight; taste weak [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

***Selaginella tamariscina* (Beauv.) Spring:** Epidermis consists of 1 layer of cells, with thickened wall, covered with cuticle. Cortex mainly consists of sclerenchyma and parenchyma; 2-3 layers of sclerenchymatous cells located on the outer side of the cortex, with unlignified wall; more than 10 layers of sclerechymatous cells located in the middle part, with thickened wall, heavily lignified; several layers of parenchymatous cells located on the inner part, arranged densely. 2-4 leaf-trace vascular bundles scattered in cortex. 1 or 2 long-ellipsoid or ovoid-rounded air cavities present in the inner part of cortex, with elongated-ellipsoid or elongated strip-shaped stele in the centre of air cavity; all (of these) connected with the endodermis through several transverse bridges, transverse bridges arranged radially. Stele sheath distinct, xylem cells completely lignified [Fig. 2 (i)].

***Selaginella pulvinata* (Hook. et Grev.) Maxim.:** Air cavity 1, ovate-triangular [Fig. 2 (ii)].

Powder

Colour yellowish-brown or brown. Margin cells of leaf long and narrow, convex, denticulate or long hairy. Epidermal cells of leaf subsquare or subrectangular, stomata anomocytic, subsidiary cells 5-7. Spores yellowish-brown, subrounded, subtriangular or subellipsoid, 17-77 μm in diameter, with irregular protuberance in the surface, exine smooth after protuberance falling off. Scalariform tracheids yellowish-brown or grey, 4-21 μm in diameter [Fig. 3 (i) and (ii)].

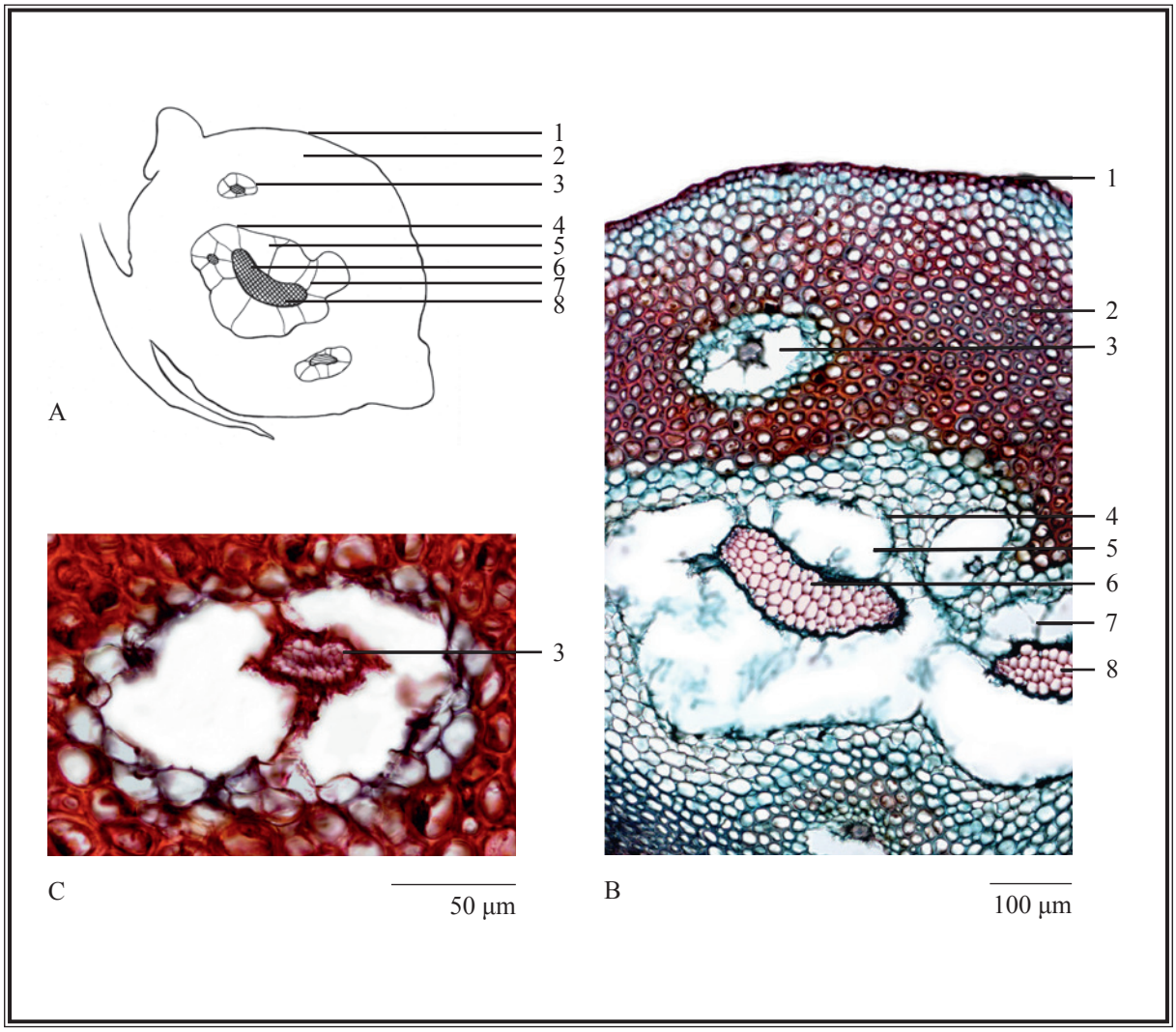

Figure 2 (i) Microscopic features of transverse section of main stem of *Selaginella tamariscina* (Beauv.) Spring

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

1. Epidermis 2. Cortex 3. Leaf-trace vascular bundle 4. Endodermis 5. Air cavity 6. Stele 7. Transverse bridge 8. Xylem

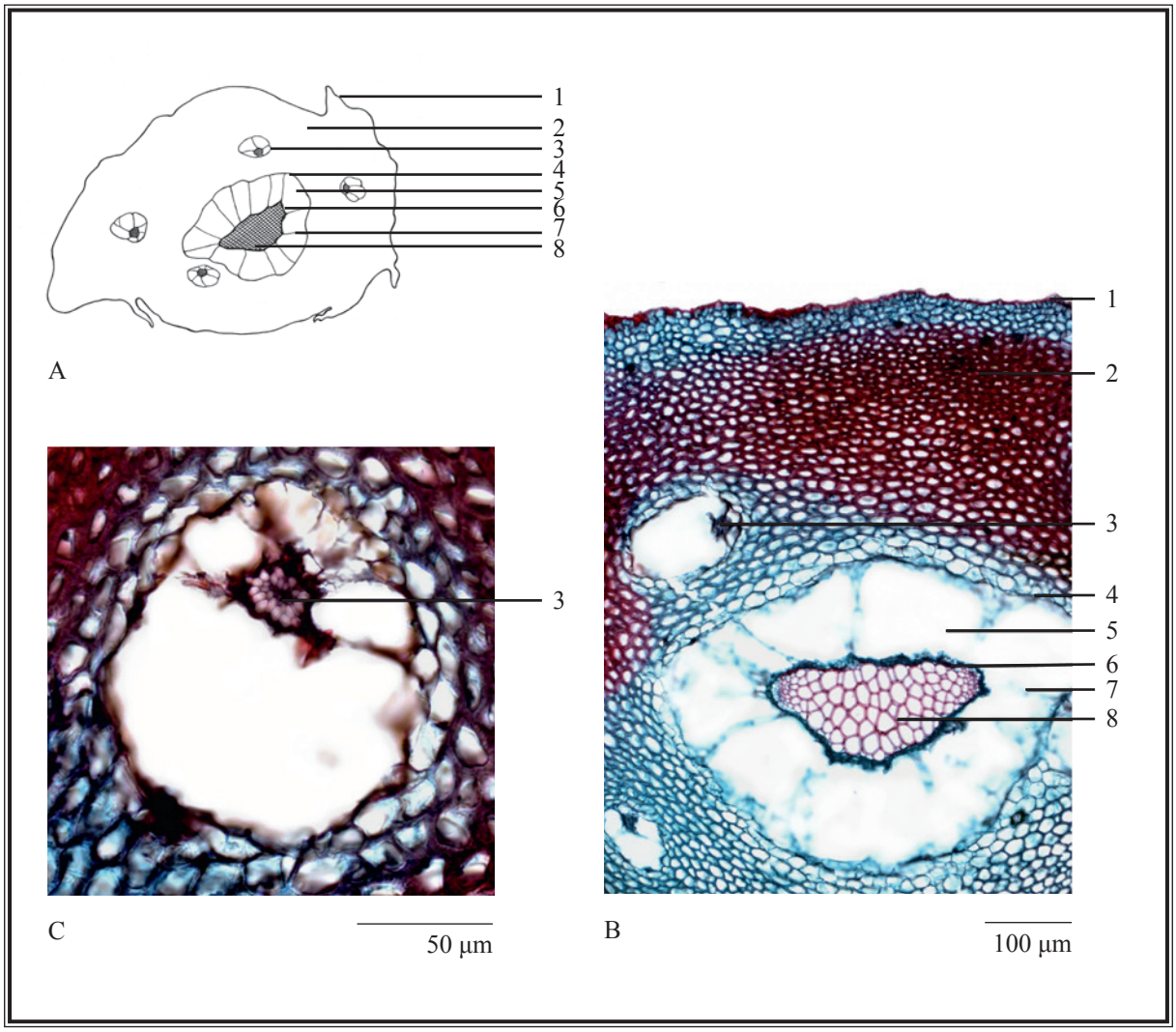

Figure 2 (ii) Microscopic features of transverse section of main stem of *Selaginella pulvinata* (Hook. et Grev.) Maxim.

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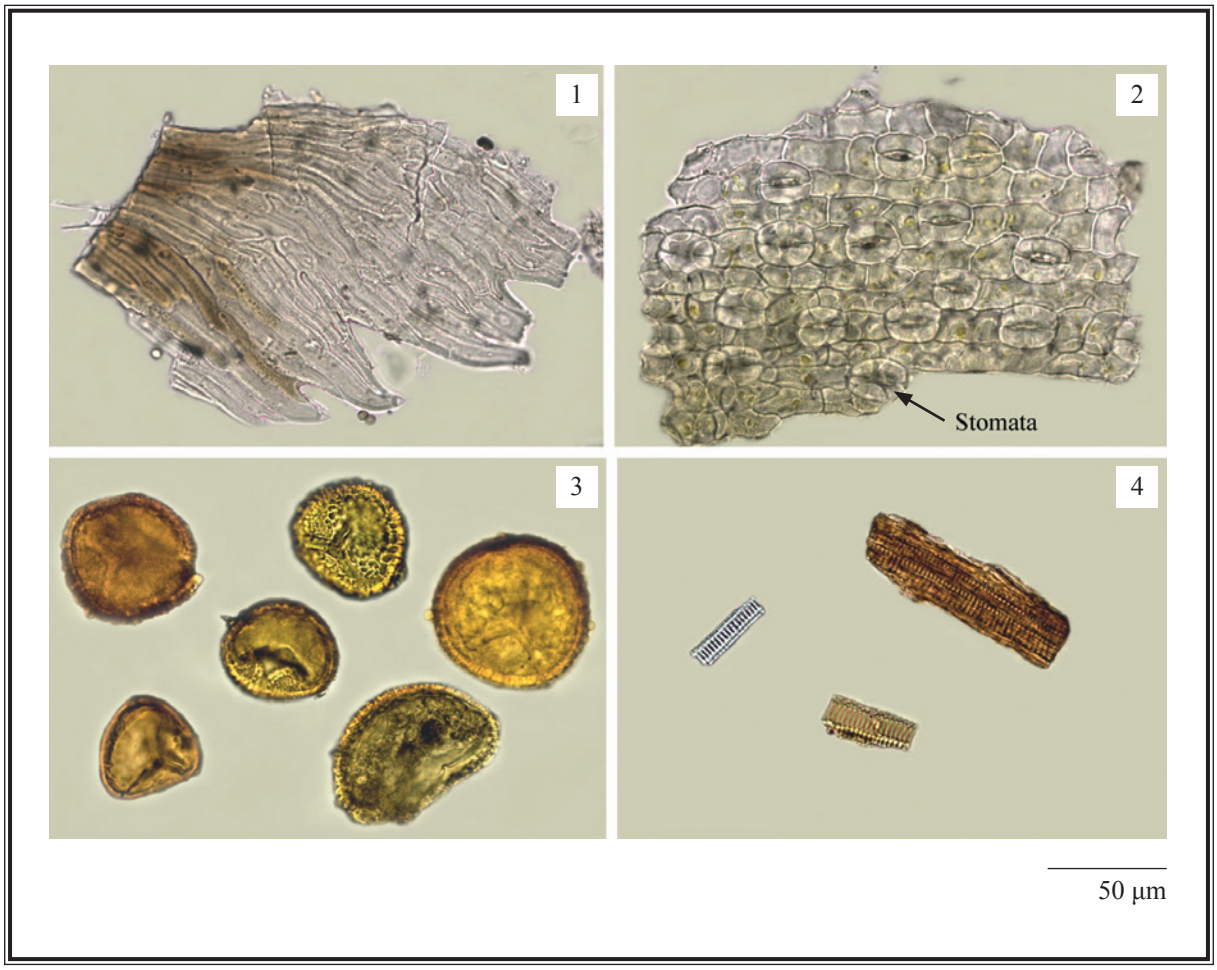

Figure 3 (i) Microscopic features of powder of dried herb of *Selaginella tamariscina* (Beauv.) Spring (under the light microscope)

1. Margin cells of leaf 2. Epidermal cells of leaf with stomata 3. Spores 4. Scalariform tracheids

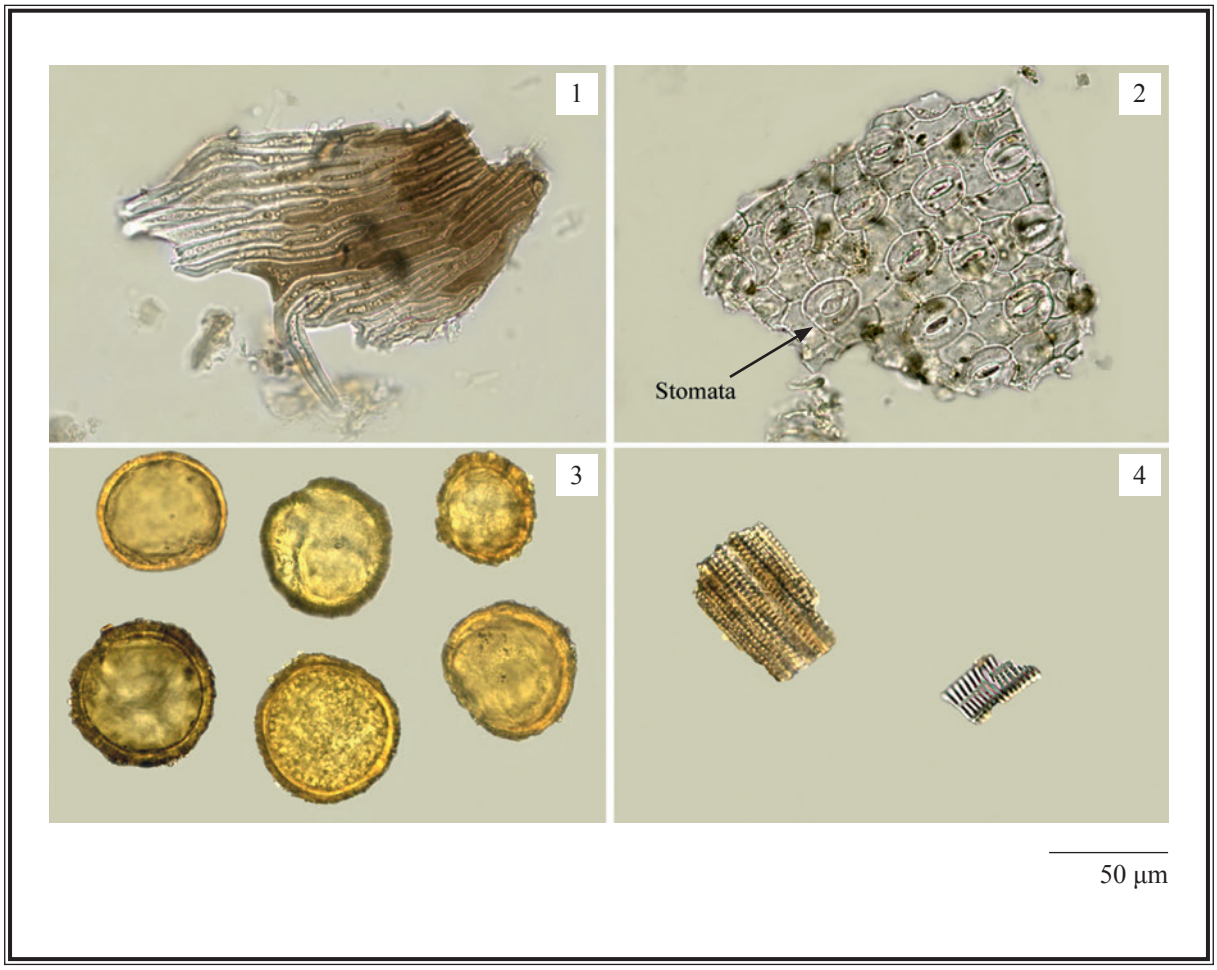

Figure 3 (ii) Microscopic features of powder of dried herb of *Selaginella pulvinata* (Hook. et Grev.) Maxim. (under the light microscope)

1. Margin cells of leaf 2. Epidermal cells of leaf with stomata 3. Spores 4. Scalariform tracheids

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

Standard solution

Amentoflavone standard solution

Weigh 0.5 mg of amentoflavone CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent systems

Developing solvent system 1

Prepare a mixture of ethyl acetate, cyclohexane and formic acid (5:2:0.5, v/v).

Developing solvent system 2

Prepare a mixture of cyclohexane and ethyl acetate (5:2, v/v).

Spray reagent

Weigh 2 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 methanol. Sonicate (100 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-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, two twin trough chambers and the freshly prepared developing solvent systems as described above. Apply separately amentoflavone standard solution and the test solution (4 µL each) to the plate. Before the development, add the developing solvent system 1 to one of the troughs of the first 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 3 cm. After the development, remove the plate from the chamber and dry in air. Add the developing solvent system 2 to one of the troughs of the second 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 7 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 UV light (366 nm). 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 amentoflavone.

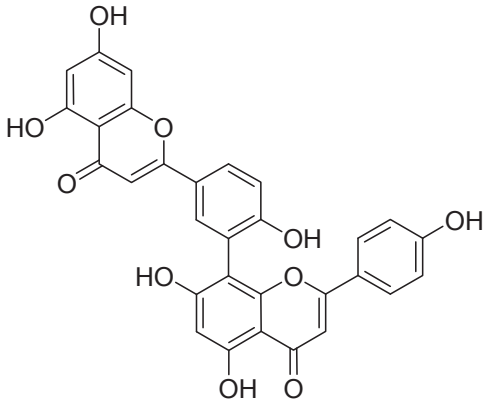

Figure 4 Chemical structure of amentoflavone

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 methanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 50 mL of methanol. Reflux the mixture for 5 h. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL volumetric flask and make up to the mark with methanol. 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)	0.2% Phosphoric acid (%, v/v)	Elution
0 – 20	40 → 44	60 → 56	linear gradient
20 – 45	44 → 65	56 → 35	linear gradient
45 – 60	65 → 70	35 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 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 12000 theoretical plates.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.0 [Fig. 5 (i) or (ii)].

Procedure

Separately inject amentoflavone Std-FP and the test solution (10 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 four characteristic peaks [Fig. 5 (i) or (ii)] 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 four characteristic peaks of Selaginellae Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (marker, amentoflavone)	1.00	-
2	1.16	± 0.03
3	2.15	± 0.06
4	2.78	± 0.11

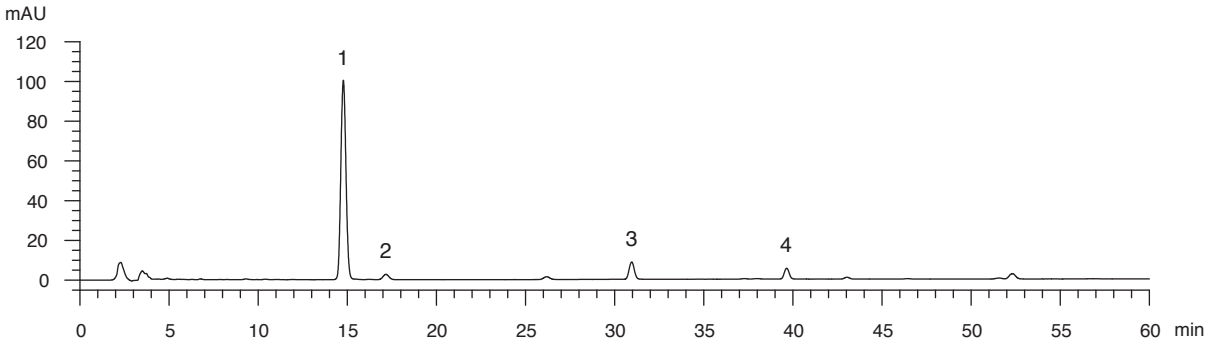

Figure 5 (i) A reference fingerprint chromatogram of dried herb of *Selaginella tamariscina* (Beauv.) Spring extract

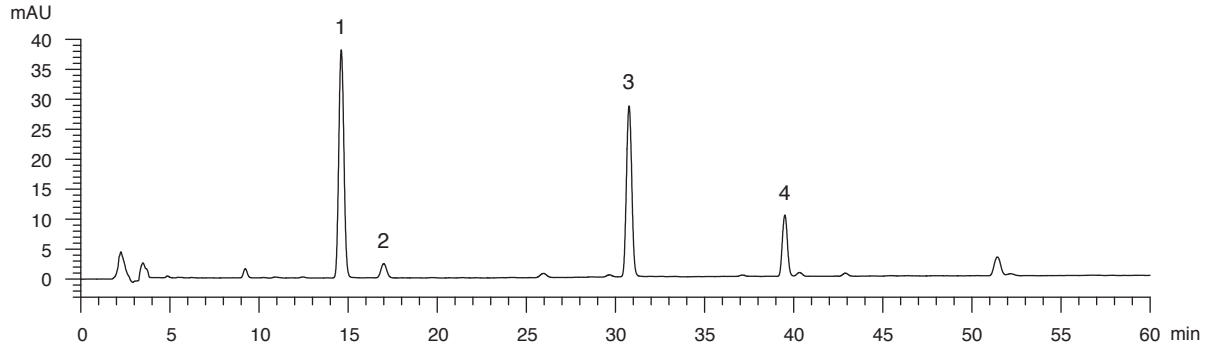

Figure 5 (ii) A reference fingerprint chromatogram of dried herb of *Selaginella pulvinata* (Hook. et Grev.) Maxim. extract

For positive identification, the sample must give the above four characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the respective reference fingerprint chromatograms [Fig. 5 (i) or (ii)].

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 4.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 13.0%.
Acid-insoluble ash: not more than 2.5 %.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 3.0%.
Ethanol-soluble extractives (cold extraction method): not less than 2.0%.

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 1.0 mg of amentoflavone CRS and dissolve in 2 mL of methanol.

Amentoflavone standard solution for assay, Std-AS

Measure accurately the volume of the amentoflavone Std-Stock, dilute with methanol to produce a series of solutions of 1, 8, 20, 40, 60 mg/L for amentoflavone.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 50 mL of methanol. Reflux the mixture for 5 h. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL volumetric flask and make up to the mark with methanol. 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. The mobile phase is a mixture of 0.2% phosphoric acid and acetonitrile (58:42, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 µL of amentoflavone Std-AS (20 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 (10 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 10 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 2.0%. 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 0.30% of amentoflavone ($C_{30}H_{18}O_{10}$), calculated with reference to the dried substance.