Polygoni Orientalis Fructus

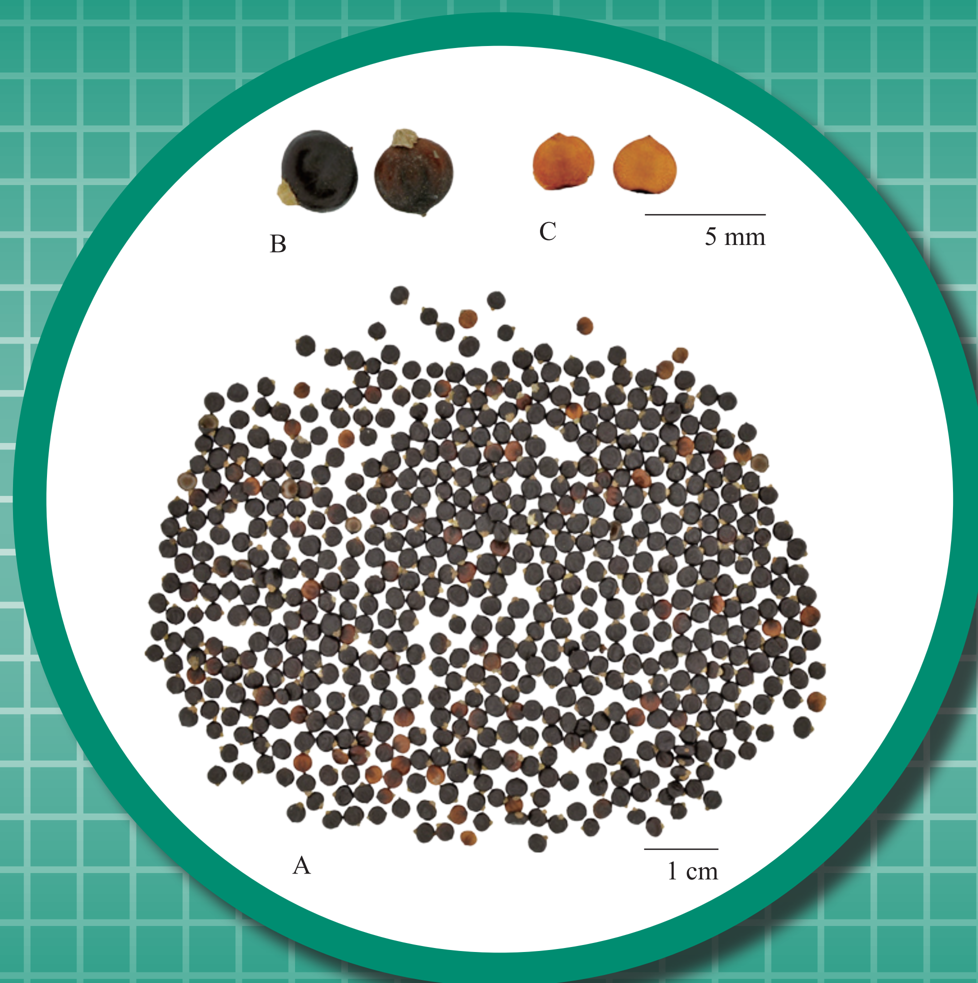

Figure 1 A photograph of Polygoni Orientalis Fructus

A. Polygoni Orientalis Fructus B. Magnified fruits C. Magnified seeds

1. NAMES

Official Name: Polygoni Orientalis Fructus

Chinese Name: 水紅花子

Chinese Phonetic Name: Shuihonghuazi

2. SOURCE

Polygoni Orientalis Fructus is the dried ripe fruit of *Polygonum orientale* L. (Polygonaceae). The fruit spike is collected in autumn when ripe, then dried under the sun, thresh to gather the fruits and foreign matter removed to obtain Polygoni Orientalis Fructus.

3. DESCRIPTION

Oblate-spheroidal, 2-3.5 mm in diameter, 0.5-1.5 mm thick. Externally brownish-black or reddish-brown, lustrous, slightly dented on both surfaces, and slightly with longitudinal protuberance in the middle. Apex with a protuberant remnant of style; base with a pale brown, slightly protuberant scar of fruit stalk; some with the remnants of membranous perianth. Seed pale brown. Texture hard. Odour slight; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Exocarp consists of 1 layer of palisade cells, yellowish-brown to reddish-brown, arranged orderly, cell interspaces indistinct, cell wall sinous and graudually thickened from base to top, lumen small, containing reddish-brown contents. Mesocarp consists of 3 layers of parenchymatous cells with reddish-brown to dark reddish contents. Endocarp consists of 1 layer of parenchymatous cells. Epidermal cells of testa 1 layer, thin, indistinct. Testa consists of 1 layer of cells, flattened, cells interspace distinct, covered with cuticle layer. Cuticle layer underneath testa, colourless. Endosperm consists of parenchymatous cells, polygonal, containing numerous starch granules. Embryo small (Fig. 2).

Powder

Colour greyish-brown. Palisade cells of exocarp mostly in groups, yellowish-brown to reddish-brown, cell interspace indistinct, lumens small, containing yellowish-brown to reddish-brown contents; 1 layer of cells in lateral view, 105-214 µm long, 13-31 µm wide, walls 4-14 µm thick, irregularly thickened; cells polygonal, stellate or subrounded in top view. Testa cells connected with cuticle, edge of cuticle often curled, testa cells flattened, subrectangular or irregular, anticlinal walls sinuate, arranged loosely, intercellular space large. Epidermal cells of testa long, anticlinal walls sinuate. Mesocarp cells reddish-brown to dark red, subrounded. Endocarp cells colourless to pale brown, rectangular. Starch granules numerous, simple starch granules subrounded, sometimes polygonal, 2-9 µm in diameter, hilum dotted; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-4 units (Fig. 3).

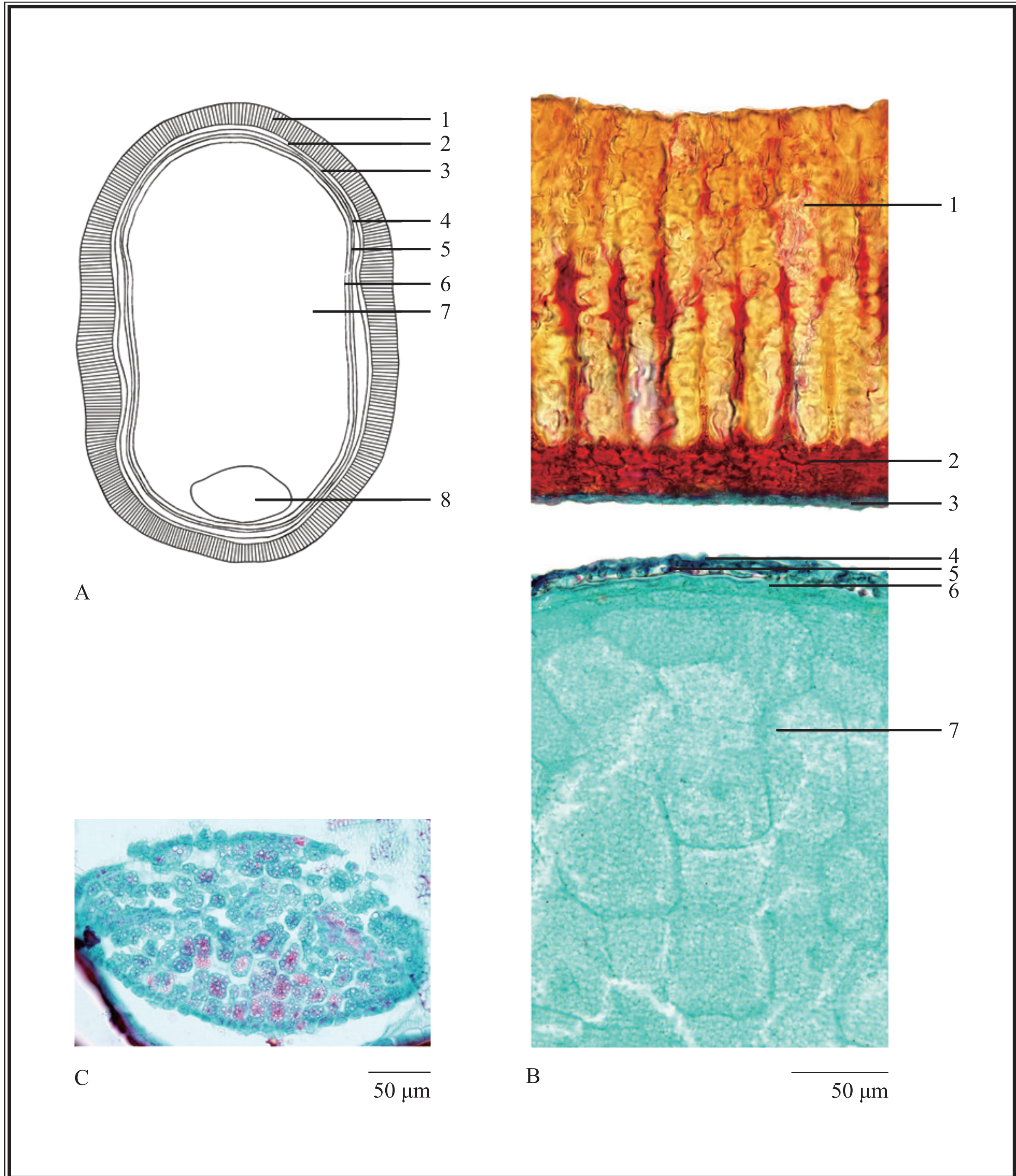

Figure 2 Microscopic features of transverse section of Polygoni Orientalis Fructus

A. Sketch B. Section illustration C. Embryo

1. Exocarp 2. Mesocarp 3. Endocarp 4. Epidermal cell of testa 5. Testa
6. Cuticle layer 7. Endosperm 8. Embryo

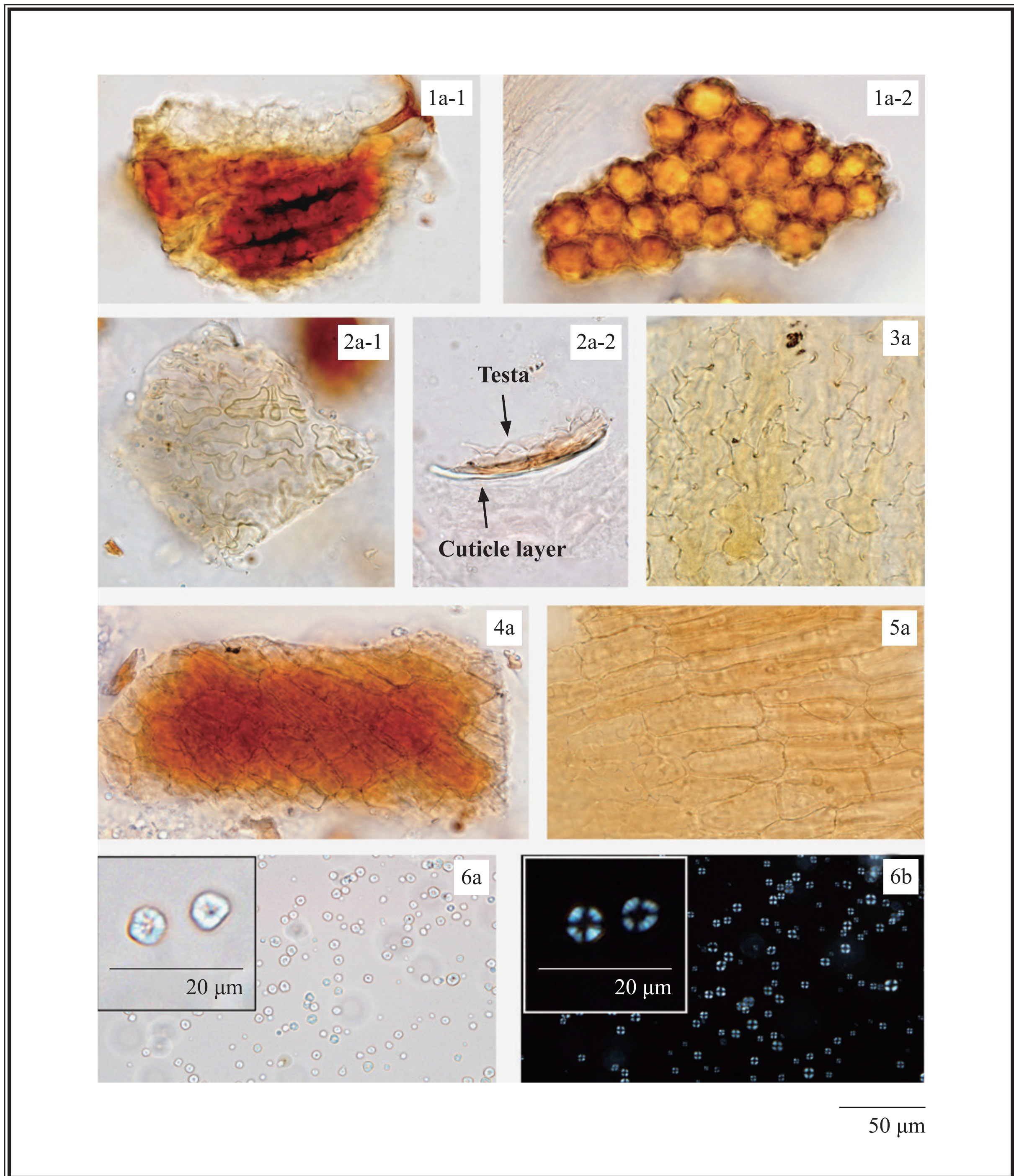

Figure 3 Microscopic features of powder of Polygoni Orientalis Fructus

1. Palisade cells of exocarp (1-1 in lateral view, 1-2 in top view)
2. Testa cells with cuticle layer (2-1 in top view, 2-2 in lateral view)
3. Epidermal cell of testa　4. Mesocarp cells　5. Endocarp cells　6. Starch granules

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

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

Standard solution

Taxifolin standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, *n*-hexane and glacial acetic acid (15:10:1, v/v).

Spray reagent

Weigh 1 g of ferric trichloride and dissolve in 100 mL of ethanol.

Test solution

Weigh 5.0 g of the powdered sample and place it in a 50-mL conical flask, then add 30 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 1 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 taxifolin standard solution (2 µL) and the test solution (6 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 in air. Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

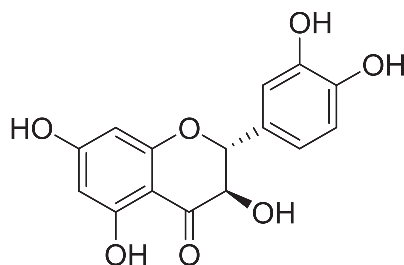

Figure 4 Chemical structure of taxifolin

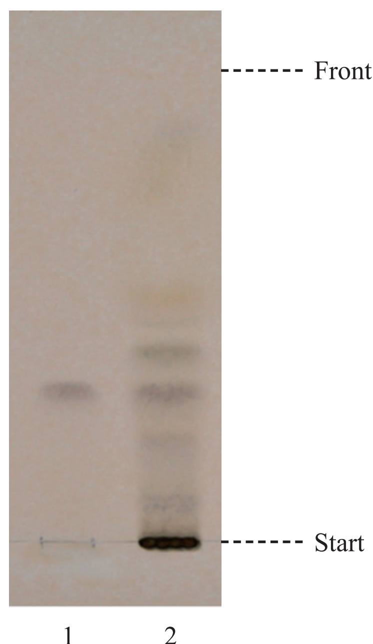

Figure 5 A reference HPTLC chromatogram of Polygoni Orientalis Fructus extract observed under visible light after staining

1. Taxifolin 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 taxifolin (Fig. 5).

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

Standard solution

Taxifolin standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.1 mg of taxifolin CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (70%). Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 10-mL volumetric flask. Make up to the mark with ethanol (70%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (275 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)	0.1% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 15	65 → 50	35 → 50	linear gradient
15 – 25	50	50	isocratic
25 – 40	50 → 40	50 → 60	linear gradient
40 – 50	40	60	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of taxifolin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of taxifolin should not be more than 5.0%; the RSD of the retention time of taxifolin peak should not be more than 2.0%; the column efficiency determined from taxifolin peak should not be less than 10000 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 taxifolin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of taxifolin peak in the chromatogram of taxifolin Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify taxifolin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of taxifolin Std-FP. The retention times of taxifolin 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 Polygoni Orientalis Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Polygoni Orientalis Fructus extract

Peak No.	RRT	Acceptable Range
1 (marker, taxifolin)	1.00	-
2	1.47	± 0.03
3	1.56	± 0.03
4	1.89	± 0.03
5	3.01	± 0.04

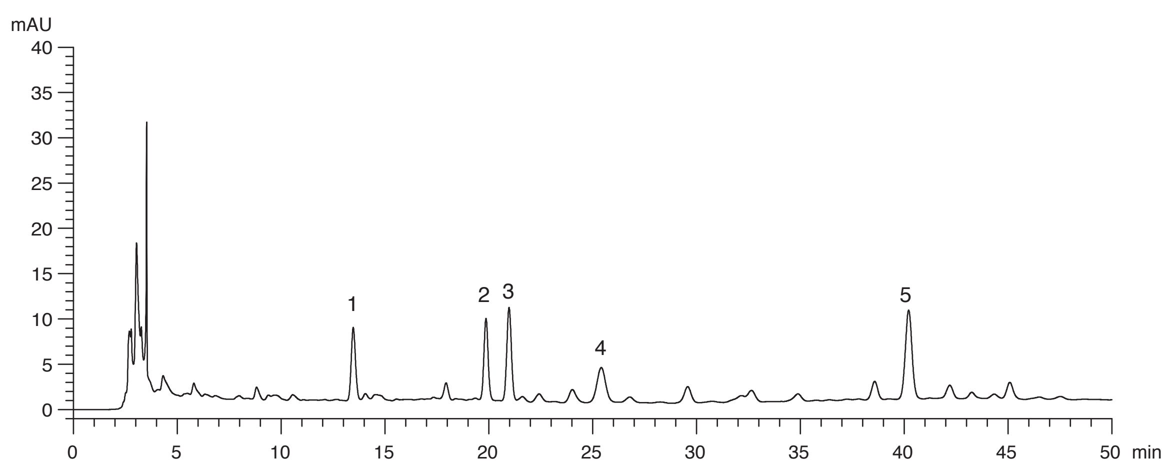

Figure 6 A reference fingerprint chromatogram of Polygoni Orientalis Fructus extract

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 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 3.5%.

Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 15.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 4.0%.

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

Taxifolin standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 2.0 mg of taxifolin CRS and dissolve in 10 mL of ethanol (70%).

Taxifolin standard solution for assay, Std-AS

Measure accurately the volume of the taxifolin Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 0.7, 1, 3, 5, 7 mg/L for taxifolin.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 90 mL of ethanol (70%). Reflux the mixture for 2 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL volumetric flask. Wash the residue for two times each with 5 mL of ethanol (70%). Combine the solutions and make up to the mark with ethanol (70%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (300 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)	0.1% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 15	65 → 50	35 → 50	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of taxifolin Std-AS (3 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of taxifolin should not be more than 5.0%; the RSD of the retention time of taxifolin peak should not be more than 2.0%; the column efficiency determined from taxifolin peak should not be less than 10000 theoretical plates.

The *R* value between taxifolin 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 taxifolin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of taxifolin against the corresponding concentrations of taxifolin 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 taxifolin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of taxifolin Std-AS. The retention times of taxifolin 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 taxifolin in the test solution, and calculate the percentage content of taxifolin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.16% of taxifolin ($C_{15}H_{12}O_7$), calculated with reference to the dried substance.