Lophatheri Herba

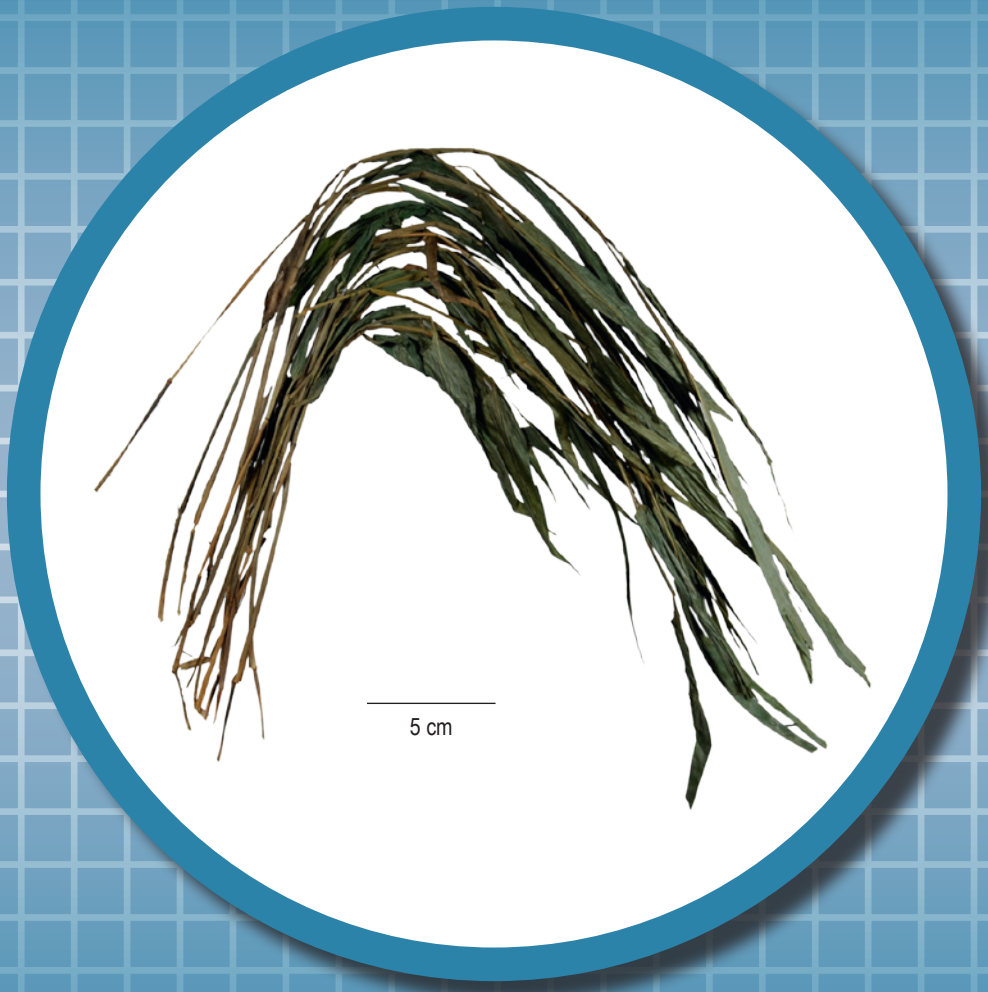

Figure 1 A photograph of Lophatheri Herba

1. NAMES

Official Name: Lophatheri Herba

Chinese Name: 淡竹葉

Chinese Phonetic Name: Danzhuye

2. SOURCE

Lophatheri Herba is the dried stem and leaf of *Lophatherum gracile* Brongn. (Poaceae). The stem and leaf are collected before the rachis emerges in summer, dried under the sun, then tied up in small bundles to obtain Lophatheri Herba.

3. DESCRIPTION

Herb 13-68.9 cm long. Stems cylindrical, nodiferous, externally yellowish-green, fracture hollow. Leaf sheaths dehiscent. Lamina lanceolate, some crumpled and rolled, 5.6-25.5 cm long, 0.8-3.8 cm wide, externally pale green or yellowish-green; veins parallel, bearing lateral veinlets, forming rectangular reticulate venation, more distinct at the lower surfaces. Texture pliable and light in weight. Odour slight; taste weak (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermis consists of 1 layer of small, round-rectangular densely set cells, with outer thickened wall, laminated; small and short unicellular non-glandular cells, stomata and cuticles present on the surface. Vascular bundles collateral, surrounded by a sheath of fibres, arranged in an interrupted ring beneath epidermis. Further inside, larger vascular bundles embedded in the ground parenchyma arranged in a second interrupted ring. Pith always hollow and broken [Fig. 2 (i)].

Leaf: Upper epidermal cells vary in size; the cells located above the mesophyll tissue between veins relative large, the cells located on the vein or above mechanical tissue relative minute. Palisade tissue consists of 1 layer of short, cylindrical cells. Spongy tissue consists of 2-4 layers of cells. Vessels sparse, arranged in a V-shaped. Phloem located beneath xylem, with 2-3 layers of fibres between the phloem and xylem. Cells of lower epidermis rectangular, relatively small, arranged in order, covered with thick cuticle. Layer of fibre bundles present on the inner side of the upper and lower epidermis of the vein [Fig. 2 (ii)].

Powder

Colour pale greyish-green. Non-glandular hairs unicellular, long or short conical. Lower epidermal cells of sheath subrectangular in surface view, anticlinal walls undulate, beaded, lignified, long and short cells arranged alternately. Upper epidermal cells of leaf rectangular or subsquare, anticlinal wall thin, sinuous. Long and short cells of lower epidermis of leaf arranged alternately or several connected, long cell rectangular, anticlinal wall sinuous, short cell is silica cell, dumbbell-shaped arranged along the veins; stomata mainly presented in lower epidermis of leaf, numerous, guard cells dumbbell-shaped, subsidiary cells nearly round-triangular. Epidermal cells of stem long subrectangular, stomata occasionally found (Fig. 3).

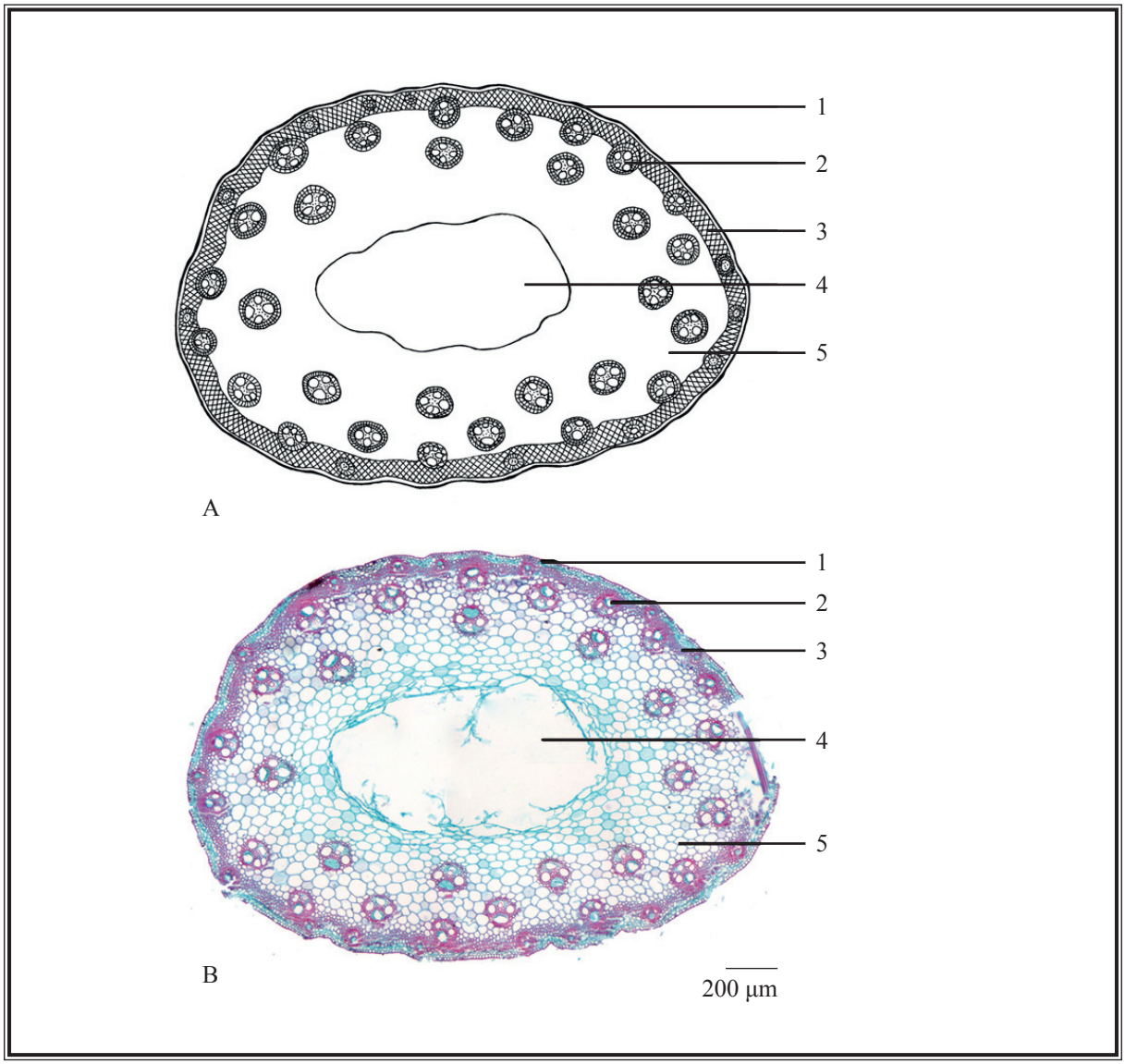

Figure 2 (i) Microscopic features of transverse section of stem of Lophatheri Herba

A. Sketch B. Section illustration

1. Epidermis 2. Vascular bundles 3. Fibre layer 4. Pith (hollow) 5. Cortex parenchyma

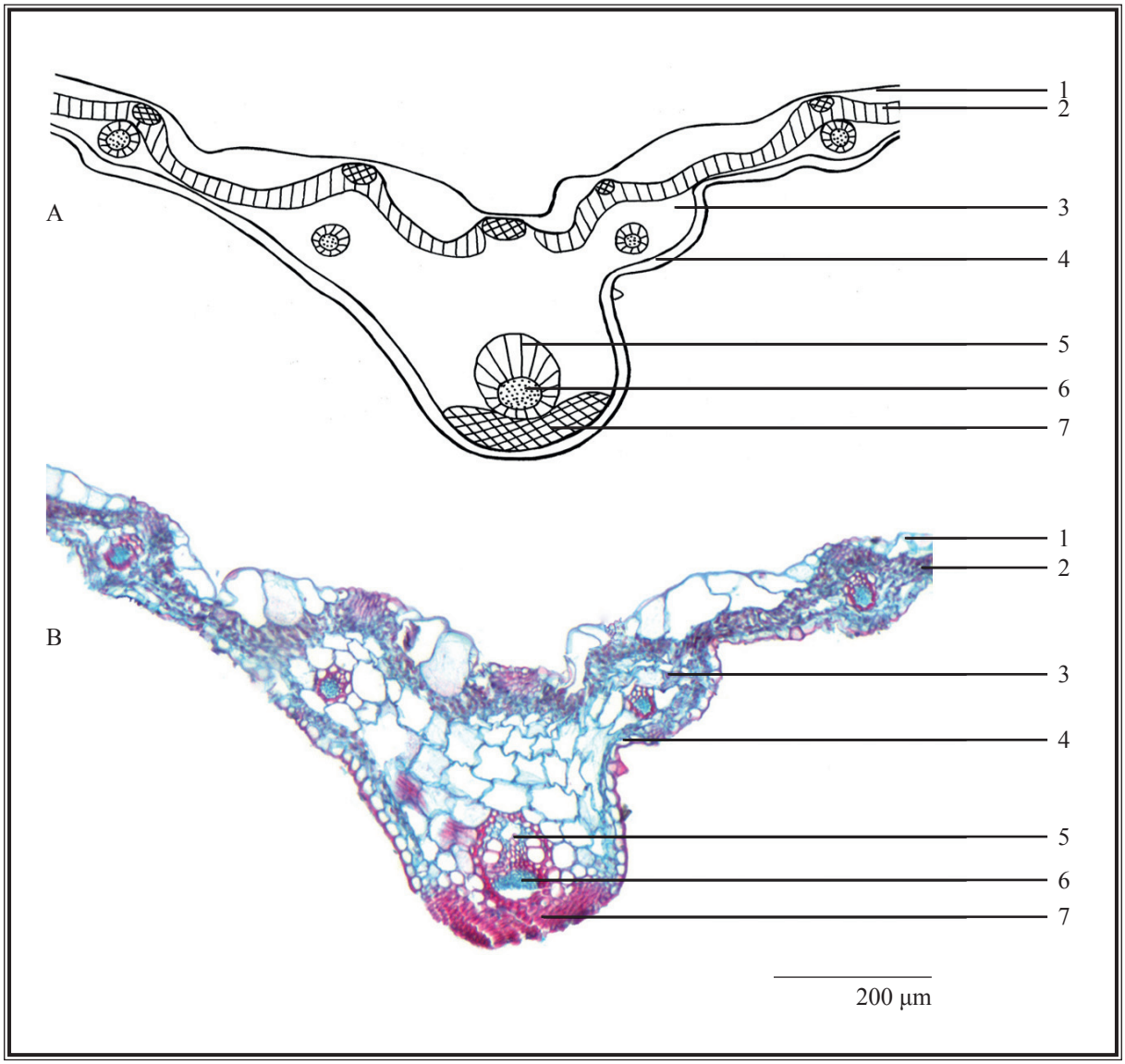

Figure 2 (ii) Microscopic features of transverse section of leaf of Lophatheri Herba

A. Sketch B. Section illustration

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Lower epidermis
5. Xylem 6.Phloem 7. Layer of fibre bundle

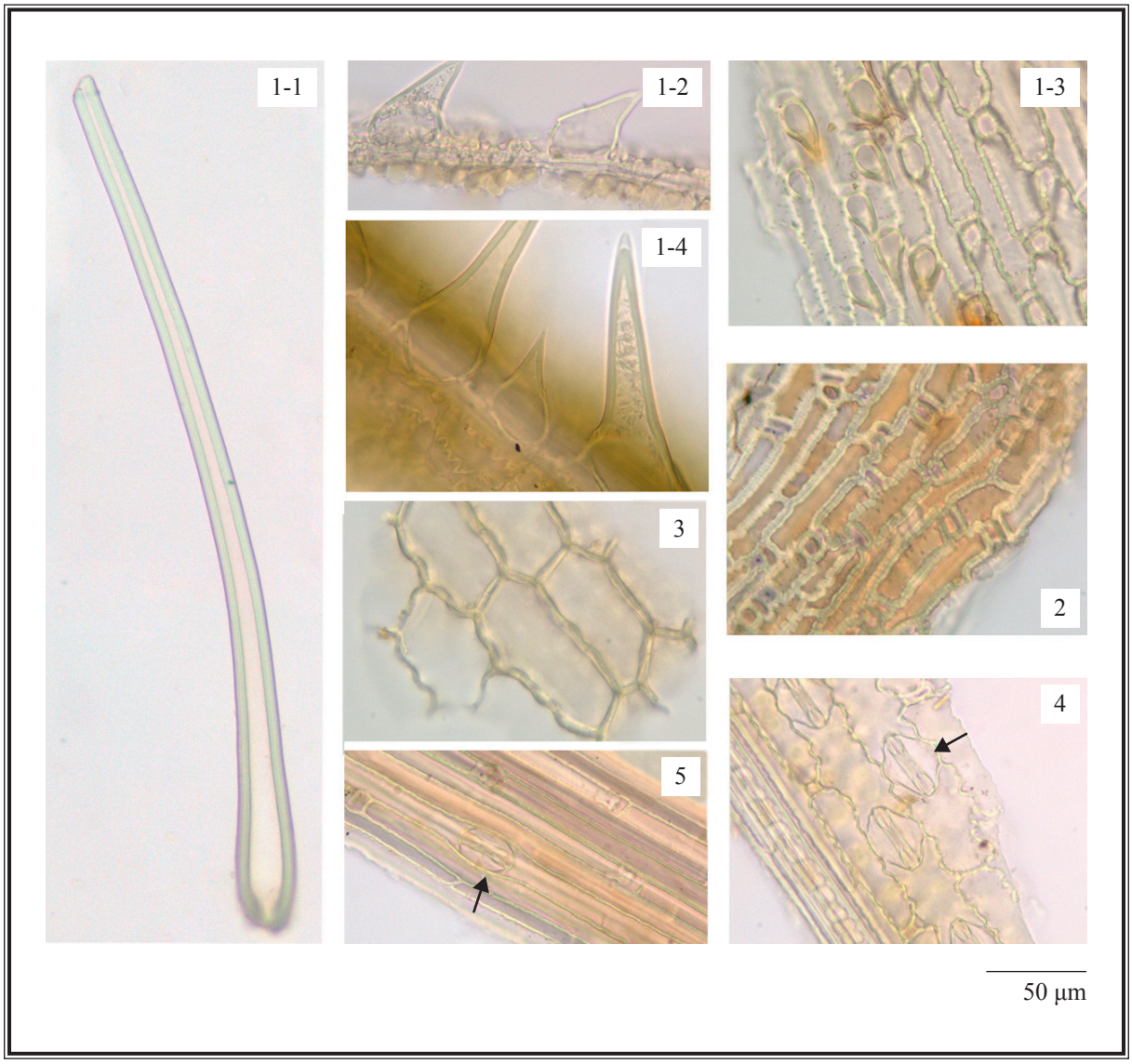

Figure 3 Microscopic features of powder of Lophatheri Herba (under the light microscope)

1. Non-glandular hairs (1-1 long unicellular, 1-2 short unicellular, 1-3 short unicellular in surface view, 1-4 short unicellular) 2. Lower epidermal cells of sheath
3. Upper epidermal cells of leaf 4. Lower epidermal cells of leaf with stomata
5. Epidermal cells of stem with stomata

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

Standard solution

Isoorientin standard solution

Weigh 0.9 mg of isoorientin CRS (Fig. 4) and dissolve in 10 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of ethyl acetate, formic acid and water (5:0.8:0.2, v/v).

Spray reagent

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

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 30 mL of ethanol (70%). Sonicate (150 W) the mixture for 10 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 20 mL of water. Transfer the aqueous solution to a separating funnel. Extract for three times each with 20 mL of ethyl acetate. Combine the ethyl acetate extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of ethanol (70%). Make appropriate dilution where necessary.

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 isoorientin standard solution (1.5 µL) and the test solution (4 µL) 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 5 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 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 for 30 s and heat at about 105 °C until the spots or bands become visible (about 2 min). 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 isoorientin.

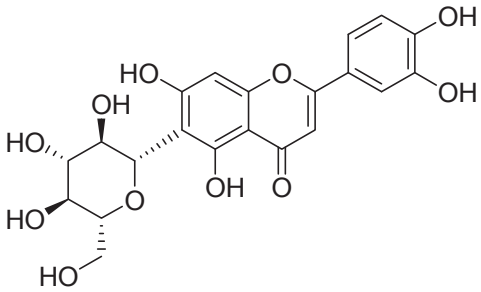

Figure 4 Chemical structure of isoorientin

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

Standard solution

Isoorientin standard solution for fingerprinting, Std-FP (15 mg/L)

Weigh 1.5 mg of isoorientin CRS and dissolve in 100 mL of ethanol (30%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 30 mL of ethanol (30%). Sonicate (200 W) the mixture for 30 min. 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 (360 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)	1% Acetic acid (%, v/v)	Elution
0 – 5	5	95	isocratic
5 – 10	5 → 13	95 → 87	linear gradient
10 – 30	13	87	isocratic
30 – 37	13 → 16	87 → 84	linear gradient
37 – 60	16	84	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of isoorientin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of isoorientin should not be more than 5.0%; the RSD of the retention time of isoorientin peak should not be more than 2.0%; the column efficiency determined from isoorientin peak should not be less than 14000 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. 5).

Procedure

Separately inject isoorientin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of isoorientin peak in the chromatogram of isoorientin Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify isoorientin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of isoorientin Std-FP. The retention times of isoorientin 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 Lophatheri Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.74	± 0.03
2	0.88	± 0.03
3 (marker, isoorientin)	1.00	-
4	1.36	± 0.03

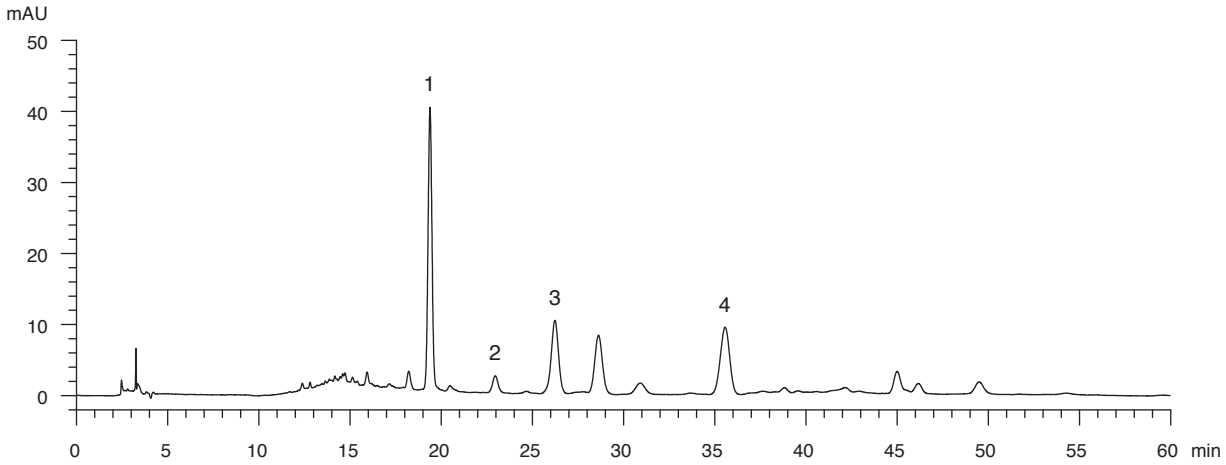

Figure 5 A reference fingerprint chromatogram of Lophatheri Herba extract

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 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 *(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 11.0%.
Acid-insoluble ash: not more than 5.5%.

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 10.0%.
Ethanol-soluble extractives (hot extraction method): not less than 8.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 2.0 mg of isoorientin CRS and dissolve in 10 mL of ethanol (30%).

Isoorientin standard solution for assay, Std-AS

Measure accurately the volume of the isoorientin Std-Stock, dilute with ethanol (30%) to produce a series of solutions of 2, 5, 10, 20, 30 mg/L for isoorientin.

Test solution

Weigh accurately 1.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (30%). Sonicate (200 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with ethanol (30%). Combine the solution and make up to the mark with ethanol (30%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (350 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)	1%Acetic acid (%, v/v)	Elution
0 – 5	5	95	isocratic
5 – 10	5 → 13	95 → 87	linear gradient
10 – 30	13	87	isocratic
30 – 37	13 → 16	87 → 84	linear gradient
37 – 40	16	84	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of isoorientin Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of isoorientin should not be more than 5.0%; the RSD of the retention time of isoorientin peak should not be more than 2.0%; the column efficiency determined from isoorientin peak should not be less than 9000 theoretical plates.

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

Limits

The sample contains not less than 0.022% of isoorientin ($C_{21}H_{20}O_{11}$), calculated with reference to the dried substance.