Commelinae Herba

Figure 1 A photograph of Commelinae Herba

A. Commelinae Herba
B. Magnified image of lower surface of leaf
C. Magnified image of upper surface of leaf
D. Magnified image of herb with fibrous roots
E. Magnified image of stem with fibrous roots and leaf

1. NAMES

Official name: Commelinae Herba

Chinese name: 鴨跖草

Chinese phonetic name: Yazhicao

2. SOURCE

Commelinae Herba is the dried aerial part of *Commelina communis* L. (Commelinaceae). The aerial part is collected in summer and autumn, foreign matter removed, then dried under the sun to obtain Commelinae Herba.

3. DESCRIPTION

Up to 60 cm long, yellowish-brown, yellowish-green or yellowish-white. Stems with longitudinal ridges, about 2 mm in diameter, frequently branched or sometimes with fibrous roots, nodes slightly swollen, internodes 3-11 cm long, texture soft, medullated in the centre. Leaves alternate, frequently crumpled and broken, when whole, lamina ovate-lanceolate or lanceolate, 3-9 cm long, 0.6-2.5 cm wide, apex acute, margin entire, base decurrent, forming membranous sheath and amplexicaul, veins parallel. Odour slight, taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermis consists of 1 layer of small, round or rectangular densely set cells. 1-3 layers of sclerenchymatous cells present under epidermis. Several layers of irregular shaped parenchymatous cells located under sclerenchyma. Fibres in bundles, arranged in a ring. Vascular bundles collateral, the outermost layer of vascular bundles relatively large and regularly arranged in an interrupted ring, surrounded by the fibre bundles. The inner side of vascular bundles small and irregular shaped, scattered irregularly in the parenchyma. Phloem consists of several layers of small cells. Xylem vessels sparse, arranged in a V-shaped, slightly lignified, two of vessels relatively large in the outmost layer of each vascular bundle [Fig. 2(i)].

Leaf: Sometimes clavate non-glandular hairs and short conical non-glandular hairs visible, and mostly fallen off after staining process. Upper and lower epidermis consists of 1 layer of cells. 1-6 layers of sclerenchymatous cells present on the inner side of the upper and lower epidermis of the midrib. Xylem vessels several in a group. Phloem narrow and shrunken, located beneath xylem. Raphides of calcium oxalate scattered or in bundles in parenchymatous cells; blue white or polychromatic under the polarized microscope [Fig. 2(ii)].

Powder

Colour brownish-green to pale brown. Lower epidermal cells with stomata and raphides of calcium oxalate; stomata parallel tetracytic type, slightly convex from the epidermis; subsidiary cells 4, left and right cells paralleled with guard cells, upper and lower cells vertical to guard cells. Raphides of calcium oxalate abundant, scattered or in bundle, length varying, up to 75 µm long; blue white or polychromatic under the polarized microscope. Non-glandular hairs two types, both bicelluar, one type is short conical non-glandular hairs, basal cell 30-60 µm long, 35-50 µm in diameter, walls relatively thick, apical cell short pointed. Another type is clavate non-glandular hairs, basal cell 45-60 µm long, walls slightly thick; apical cell relatively long, the apex obtusely rounded, the bottom slightly collapse, thin-walled, often fallen off. Vessels mainly reticulate type (Fig. 3).

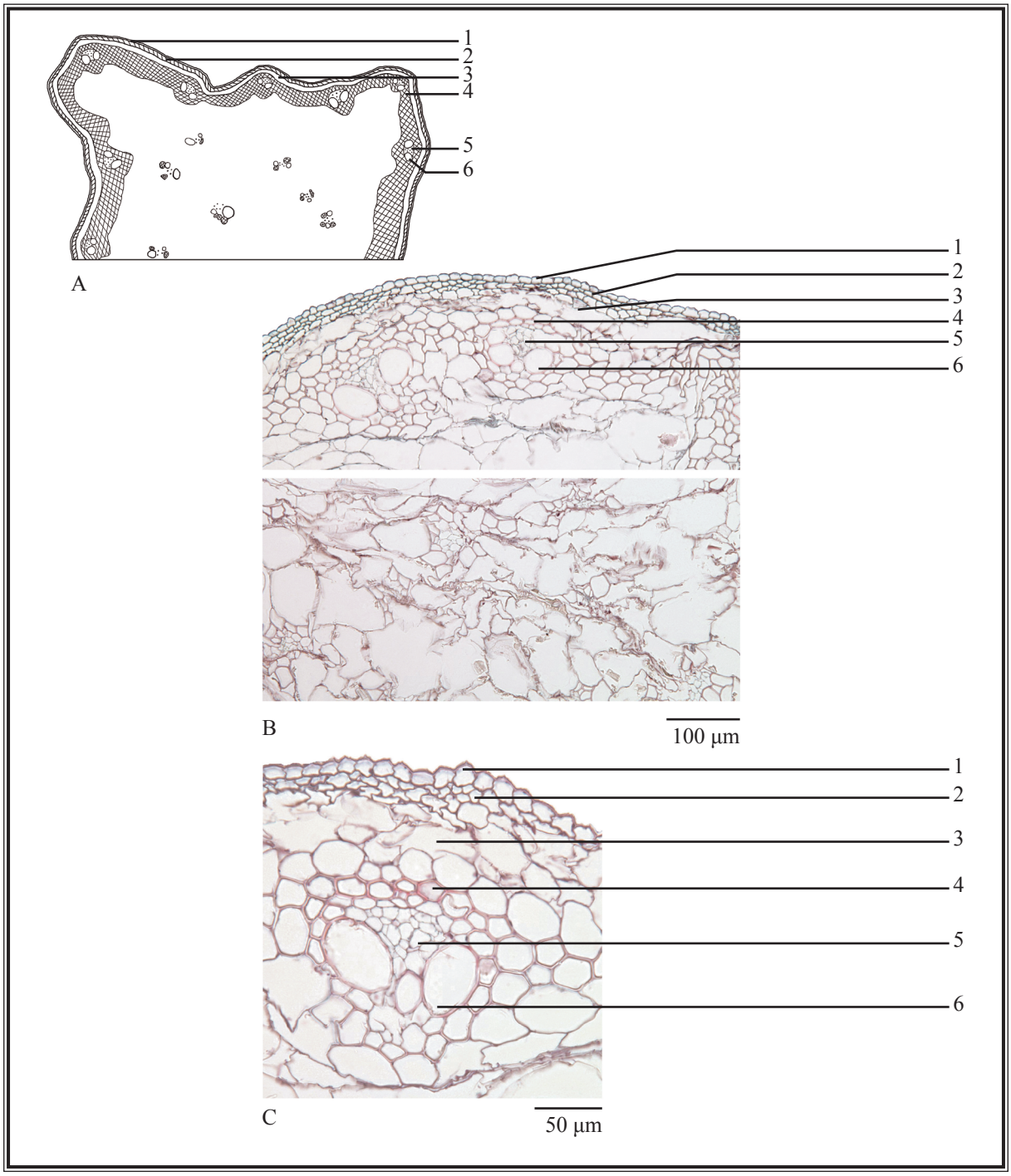

Figure 2 (i) Microscopic features of transverse section of stem of Commelinae Herba

A. Sketch B. Section illustration C. Section magnified

1. Epidermis 2. Sclerenchyma 3. Parenchyma 4. Fibre bundle 5. Phloem
6. Xylem

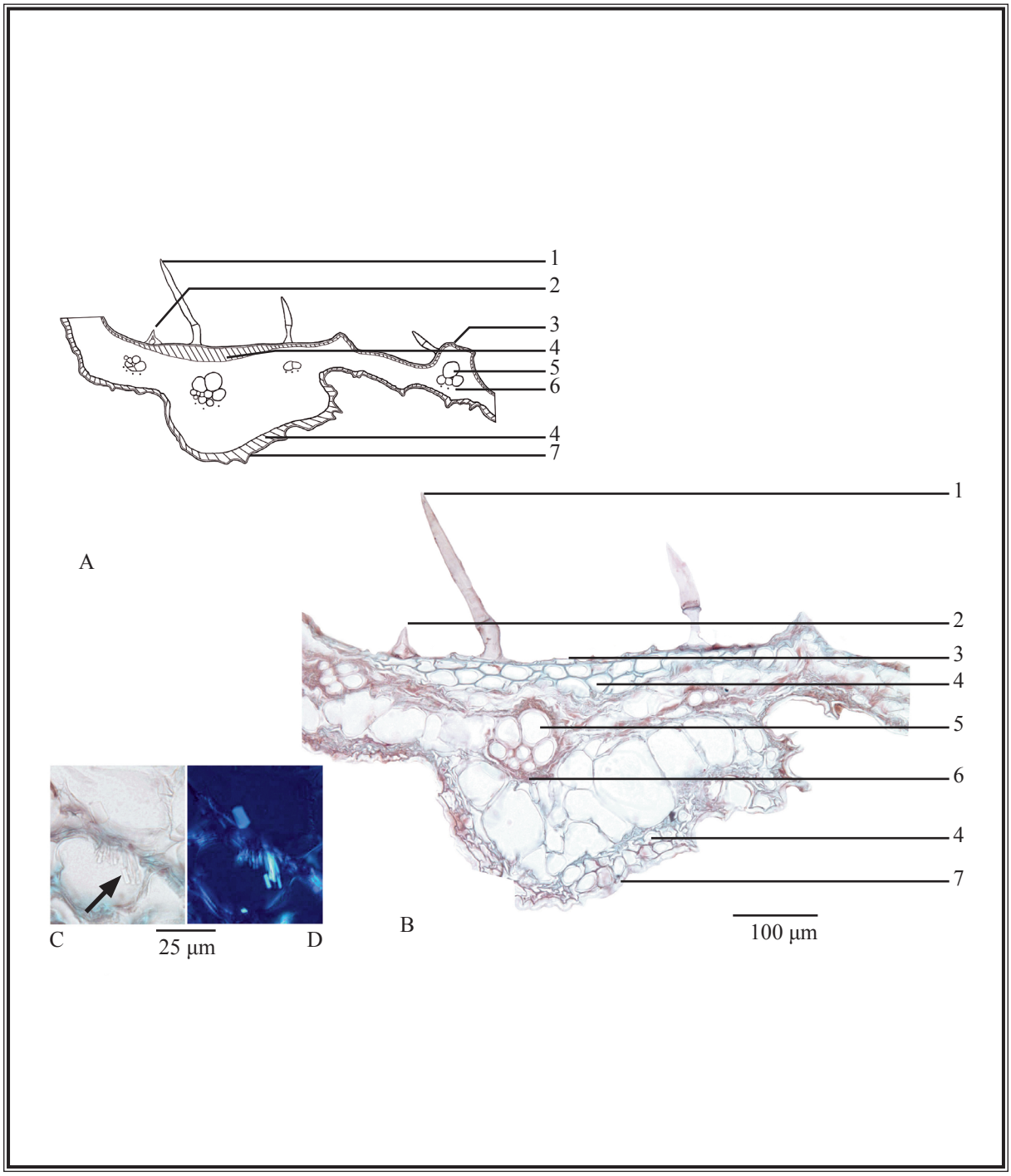

Figure 2 (ii) Microscopic features of transverse section of leaf of Commelinae Herba

A. Sketch B. Section illustration

C. Raphides of calcium oxalate (under the light microscope)

D. Raphides of calcium oxalate (under the polarized microscope)

1. Clavate non-glandular hair 2. Short conical non-glandular hair 3. Upper epidermis

4. Sclerenchyma 5. Xylem 6. Phloem 7. Lower epidermis

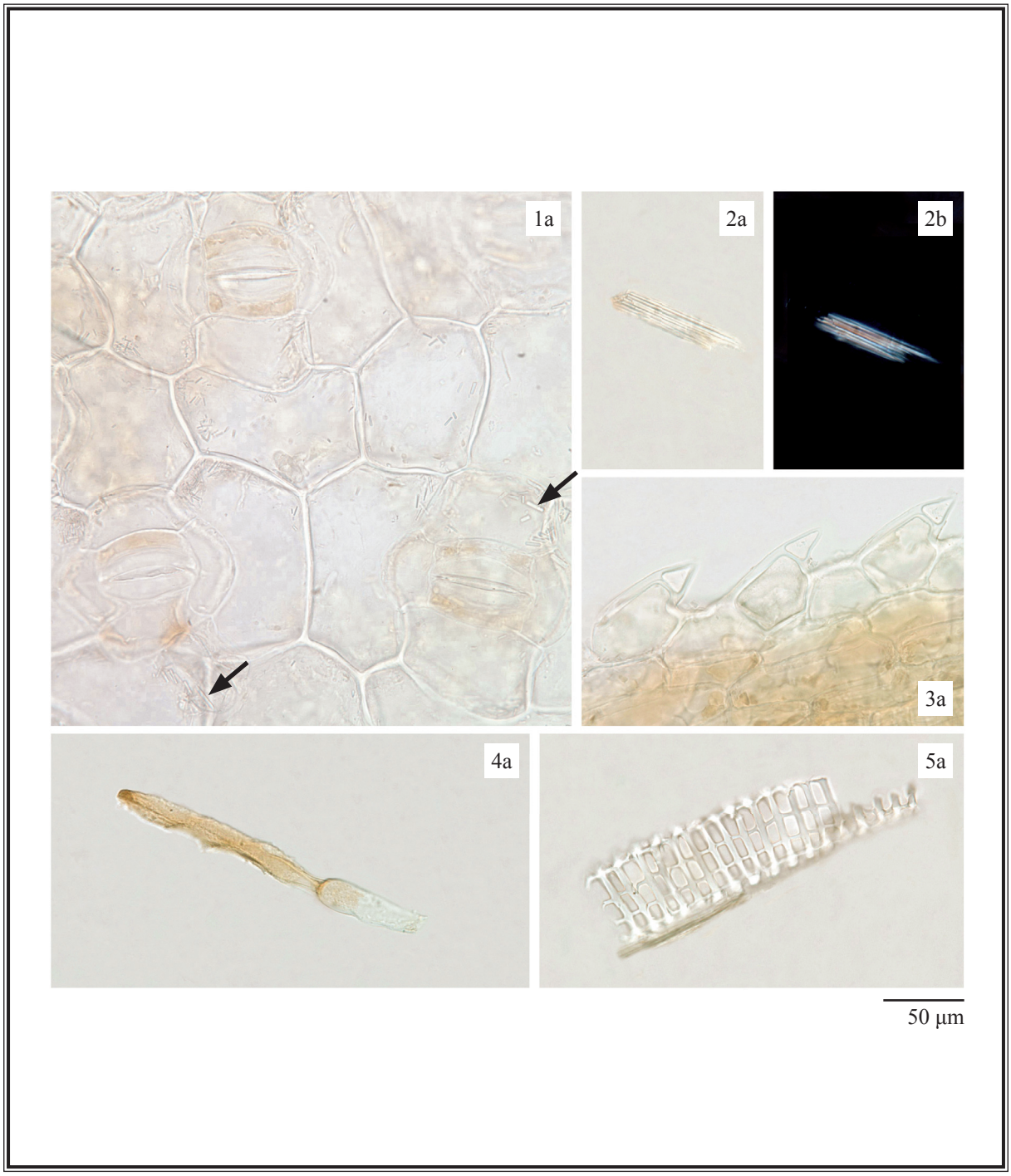

Figure 3 Microscopic features of powder of Commelinae Herba

1. Lower epidermal cells with tetracytic stomata (→ scattered raphides of calcium oxalate)
2. Raphides of calcium oxalate in bundle 3. Short conical non-glandular hair
4. Clavate non-glandular hair 5. Reticulate vessel

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

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

Standard solution

Isoorientin standard solution

Weigh 1.0 mg of isoorientin CRS (Fig. 4) and dissolve in 4 mL of methanol (50%).

Developing solvent system

Prepare a mixture of ethyl acetate, acetone, formic acid and water (8: 1: 0.5: 0.5, v/v).

Spray reagent

Weigh 3 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 (50%). Sonicate (270 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol (50%).

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 isoorientin standard solution and the test solution (1 μL each) to the plate. Develop over a path of about 6 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 110˚C (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).

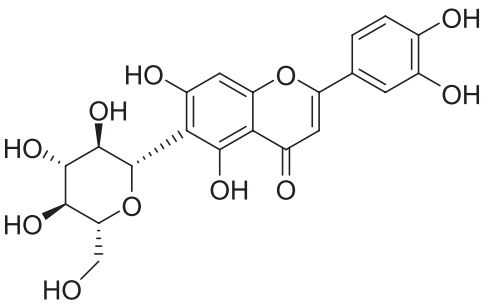

Figure 4 Chemical structure of isoorientin

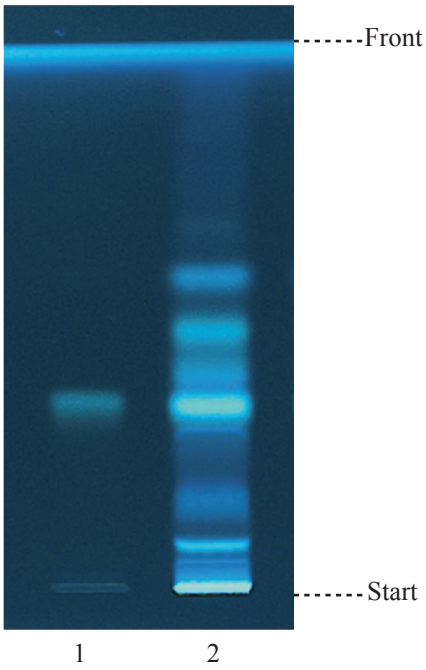

Figure 5 A reference HPTLC chromatogram of Commelinae Herba extract observed under UV light (366 nm) after staining

1. Isoorientin 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 isoorientin (Fig. 5).

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

Standard solution

Isoorientin standard solution for fingerprinting, Std-FP (25 mg/L)

Weigh 2.5 mg of isoorientin CRS and dissolve in 100 mL of methanol (50%).

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction one more time. Combine the filtrates and make up to the mark with methanol (50%). Filter through a 0.2-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (348 nm) and a column (2.1 × 100 mm) packed with ODS bonded silica gel (1.7 μm particle size, 130 Å pore size and 185 m^2/g surface area). The flow rate is about 0.5 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 12	90 → 82	10 → 18	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 3 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 10000 theoretical plates.

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject isoorientin Std-FP and the test solution (3 µL each) into the UHPLC 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. 6) 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 Commelinae Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.73	± 0.03
2 (marker, isoorientin)	1.00	-
3	1.35	± 0.04
4	1.45	± 0.04

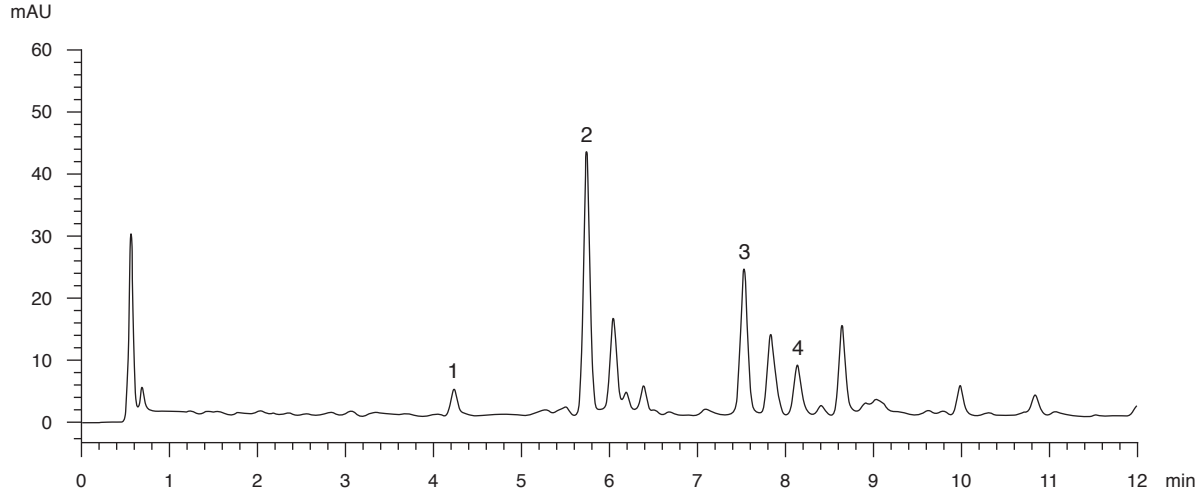

Figure 6 A reference fingerprint chromatogram of Commelinae Herba extract

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. 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 XVI)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 16.5%.
Acid-insoluble ash: not more than 5.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 10.0%.
Ethanol-soluble extractives (cold extraction method): not less than 4.0%.

7. ASSAY

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

Standard solution

Isoorientin standard stock solution, Std-Stock (250 mg/L)

Weigh accurately 2.5 mg of isoorientin CRS and dissolve in 10 mL of methanol (50%).

Isoorientin standard solution for assay, Std-AS

Measure accurately the volume of the isoorientin Std-Stock, dilute with methanol (50%) to produce a series of solutions of 0.5, 12.5, 25, 50, 75 mg/L for isoorientin.

Test solution

Weigh accurately 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction one more time. Combine the filtrates and make up to the mark with methanol (50%). Filter through a 0.2-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (348 nm) and a column (2.1 × 100 mm) packed with ODS bonded silica gel (1.7 μm particle size, 130 Å pore size and 185 m^2/g surface area). The flow rate is about 0.5 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 12	90 → 82	10 → 18	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 3 μL of isoorientin Std-AS (25 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 10000 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 (Fig. 7).

Calibration curve

Inject a series of isoorientin Std-AS (3 μL each) into the UHPLC 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 3 µL of the test solution into the UHPLC system and record the chromatogram. Identify isoorientin peak (Fig. 7) 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 5.0%. 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 0.011% of isoorientin ($C_{21}H_{20}O_{11}$), calculated with reference to the dried substance.

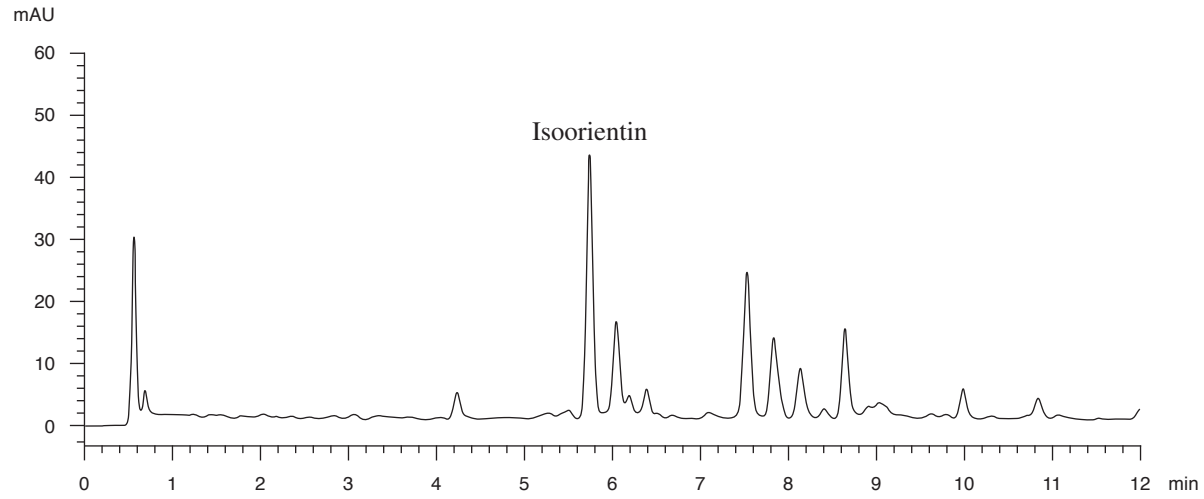

Figure 7 A reference assay chromatogram of Commelinae Herba extract