Hyperici Japonici Herba

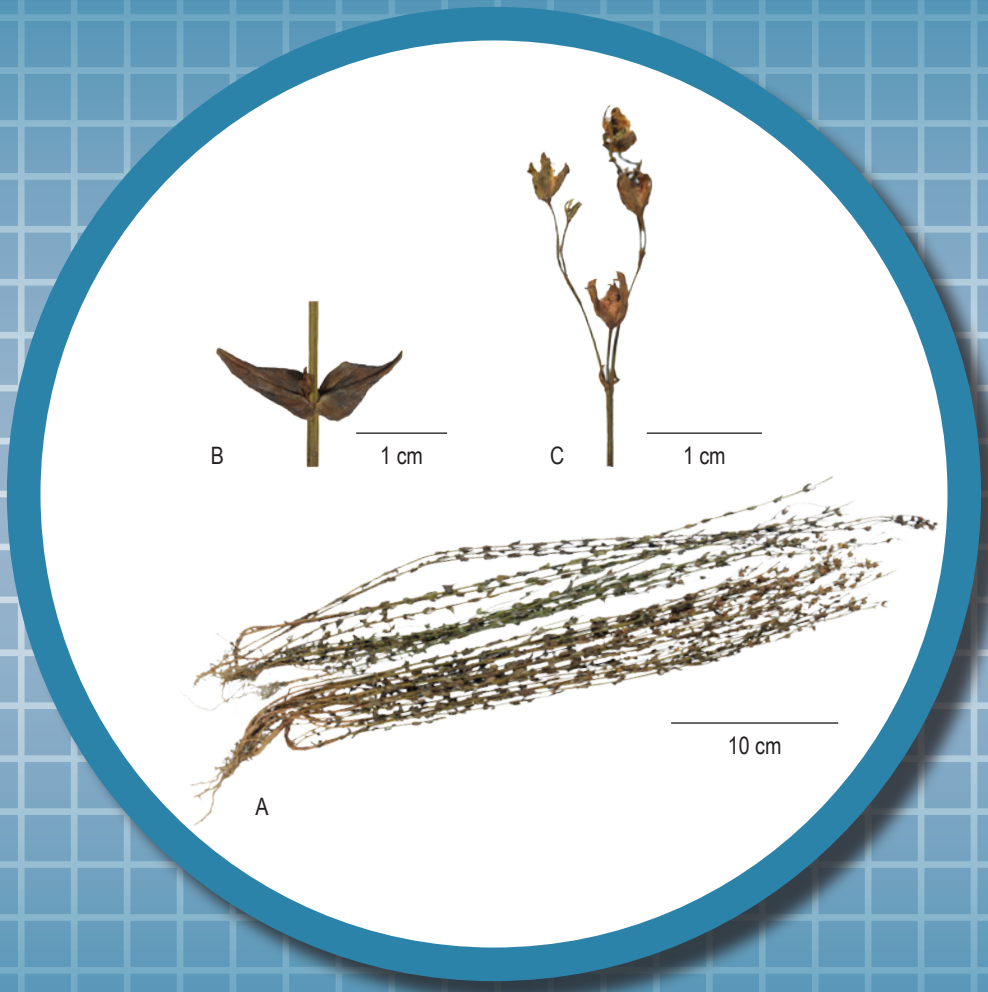


Figure 1 A photograph of Hyperici Japonici Herba

A. Hyperici Japonici Herba B. Magnified stem and leaf

C. Magnified flowers

1. NAMES

Official Name: Hyperici Japonici Herba

Chinese Name: 田基黃

Chinese Phonetic Name: Tianjihuang

2. SOURCE

Hyperici Japonici Herba is the dried herb of *Hypericum japonicum* Thunb. ex Murray (Clusiaceae). The whole plant is collected at flowering stage in spring and summer, washed clean, dried under the sun to obtain Hyperici Japonici Herba.

3. DESCRIPTION

Herb 17-67 cm long. Root fibrous, yellowish-brown. Stems solitary or basally branched, glabrous, with 4 ribs, externally yellowish-green or yellowish-brown. Texture fragile, easily broken; fracture hollow at centre. Leaf simple, opposite, sessile; lamina ovate or rounded-ovate, margin entire, 3-5-basinerved, the translucent glandular dots small. Cymes terminal; flowers small, orangish-yellow. Odourless; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Cork consists of 6-8 layers of cells. Cortex narrow, consisting of 3-4 layers of parenchymatous cells. Phloem narrow, cells mostly crumpled. Xylem occupies the major portion of the root, consisting of vessels and fibres; xylem rays consist 1 row of cells [Fig. 2 (i)].

Stem: Epidermis consists of 1 layer of epidermal cells. Cortex narrow, consisting of 3-4 layers of parenchymatous cells, arranged loosely; oil cavities scattered in the cortex, subrounded or elliptical. Endodermis distinct. Phloem narrow, cells mostly crumpled. Xylem broad, with vessels, fibres and rays; xylem rays consists 1 row of cells. Pith generally hollow [Fig. 2 (ii)].

Leaf: Upper epidermis consists of 1 layer relatively large cells, rectangular. Palisade tissue consists of 1 layer of cells. Spongy tissue arranged loosely, containing numerous and rounded oil cavities. Vascular bundles of midrib collateral and wedge-shaped. Lower epidermis consists of 1 layer rectangular, relatively large cells [Fig. 2 (iii)].

Powder

Colour greyish-green. Anticlinal walls of upper and lower epidermis of leaf sinuate to undulate, stomata anisocytic. Epidermal cells of young stem polygonal, anticlinal wall straight, stomata anomocytic. Pollen grains spherical, 14-42 μm in diameter, exine smooth. Secretory cells often broken, intact cells subrounded or elliptical, 26-115 μm in diameter, wall somewhat thick, pits present, lumen containing orangish-red or brownish-red resinous secretions. Some oil cavities broken, intact cavities 20-112 μm in diameter, surrounded by 10-12 secretory cells, lumen usually containing grey secretions or oil droplets. Epidermal cells of testa finely linear-scalariform, anticlinal wall sinuate, lignified. Cork cells pale yellow, subpolygonal or subsquare in surface view, wall thick. Vessels mainly spiral, 3-23 μm in diameter (Fig. 3).

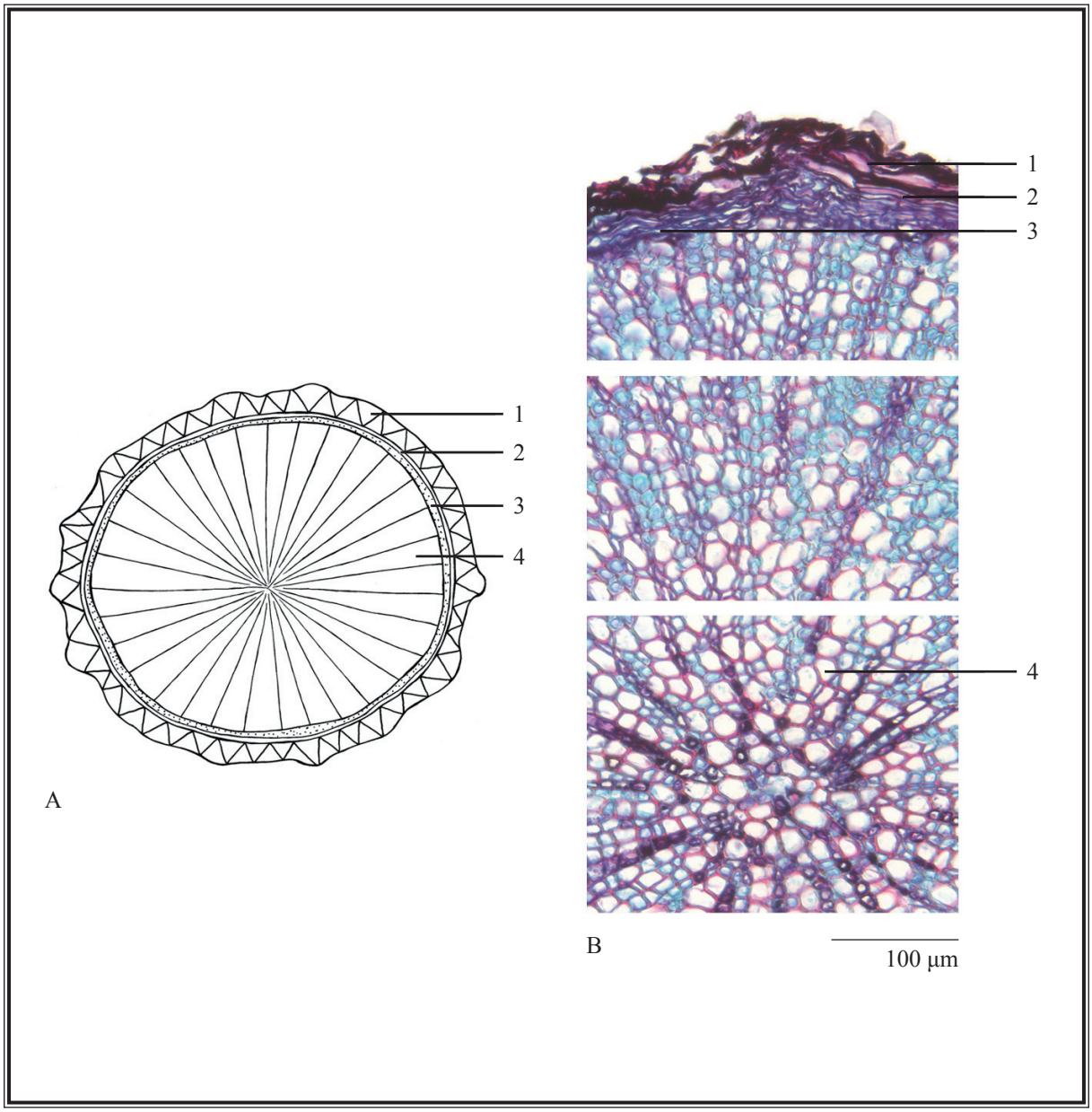


Figure 2 (i) Microscopic features of transverse section of root of Hyperici Japonici Herba

A. Sketch B. Section illustration

1. Cork 2. Cortex 3. Phloem 4. Xylem

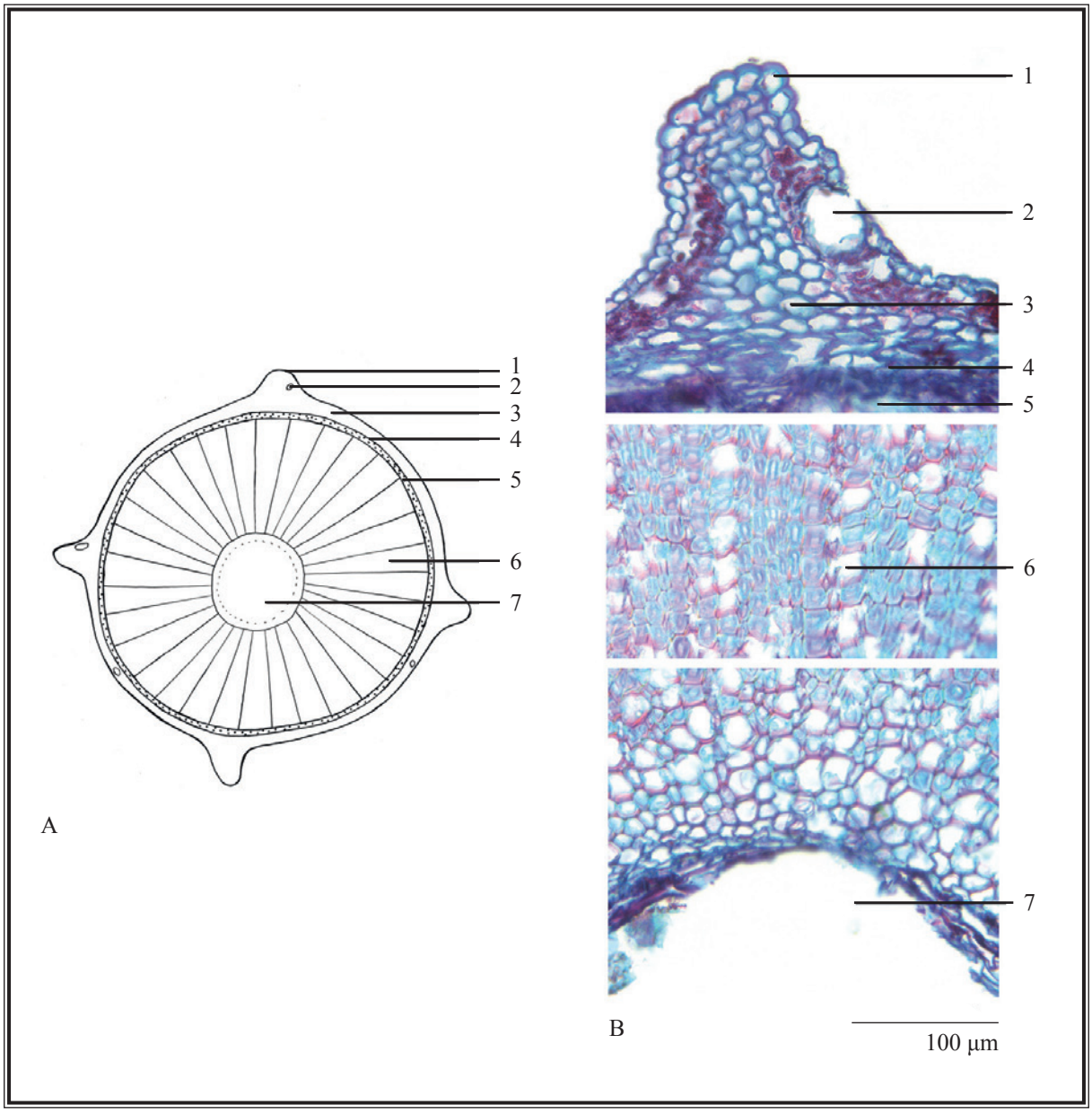


Figure 2 (ii) Microscopic features of transverse section of stem of Hyperici Japonici Herba

A. Sketch B. Section illustration

1. Epidermis 2. Secretory cells 3. Cortex 4. Endodermis 5. Phloem
6. Xylem 7. Pith

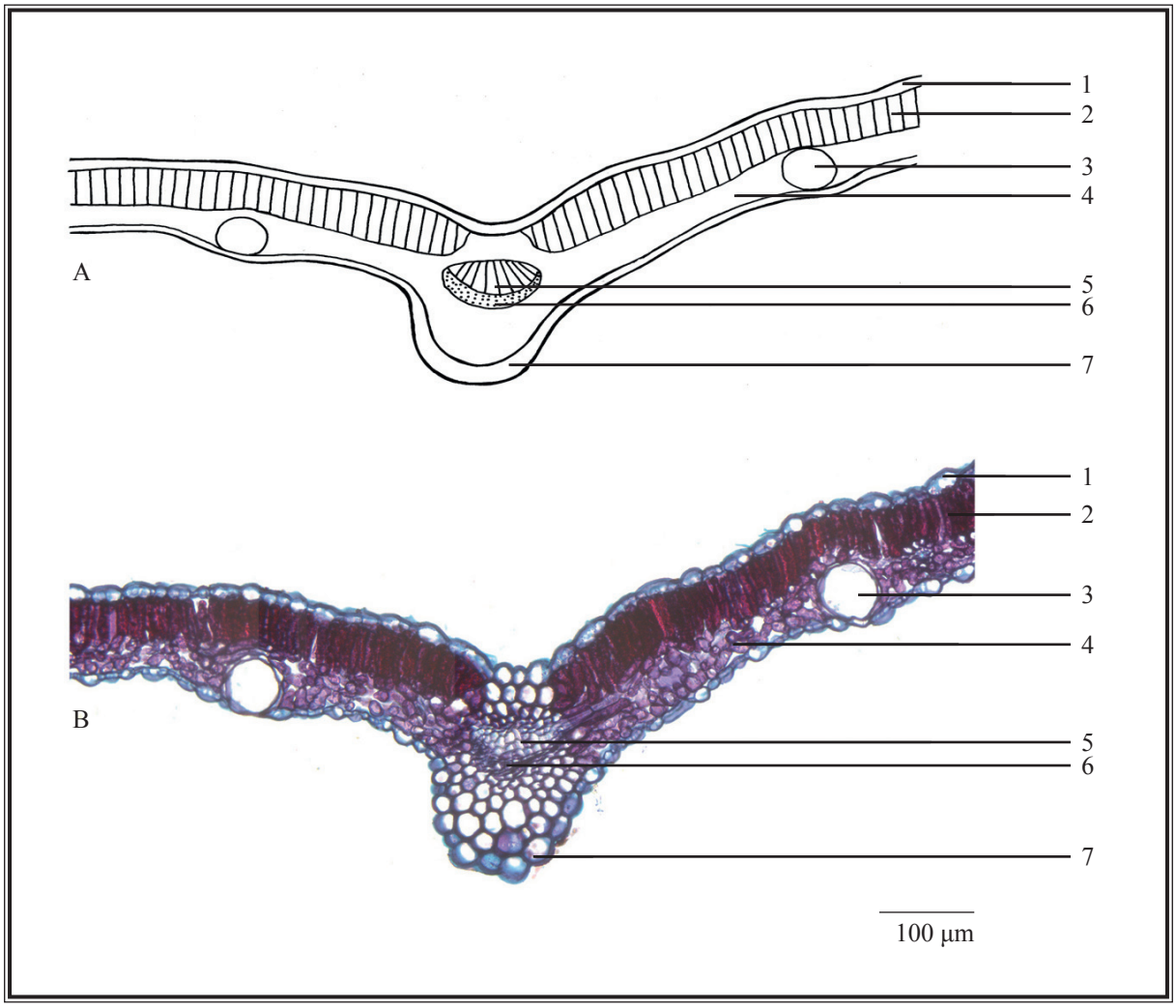


Figure 2 (iii) Microscopic features of transverse section of leaf of Hyperici Japonici Herba

A. Sketch B. Section illustration

1. Upper epidermis 2. Palisade tissue 3. Oil cavities 4. Spongy tissue
5. Xylem 6. Phloem 7. Lower epidermis

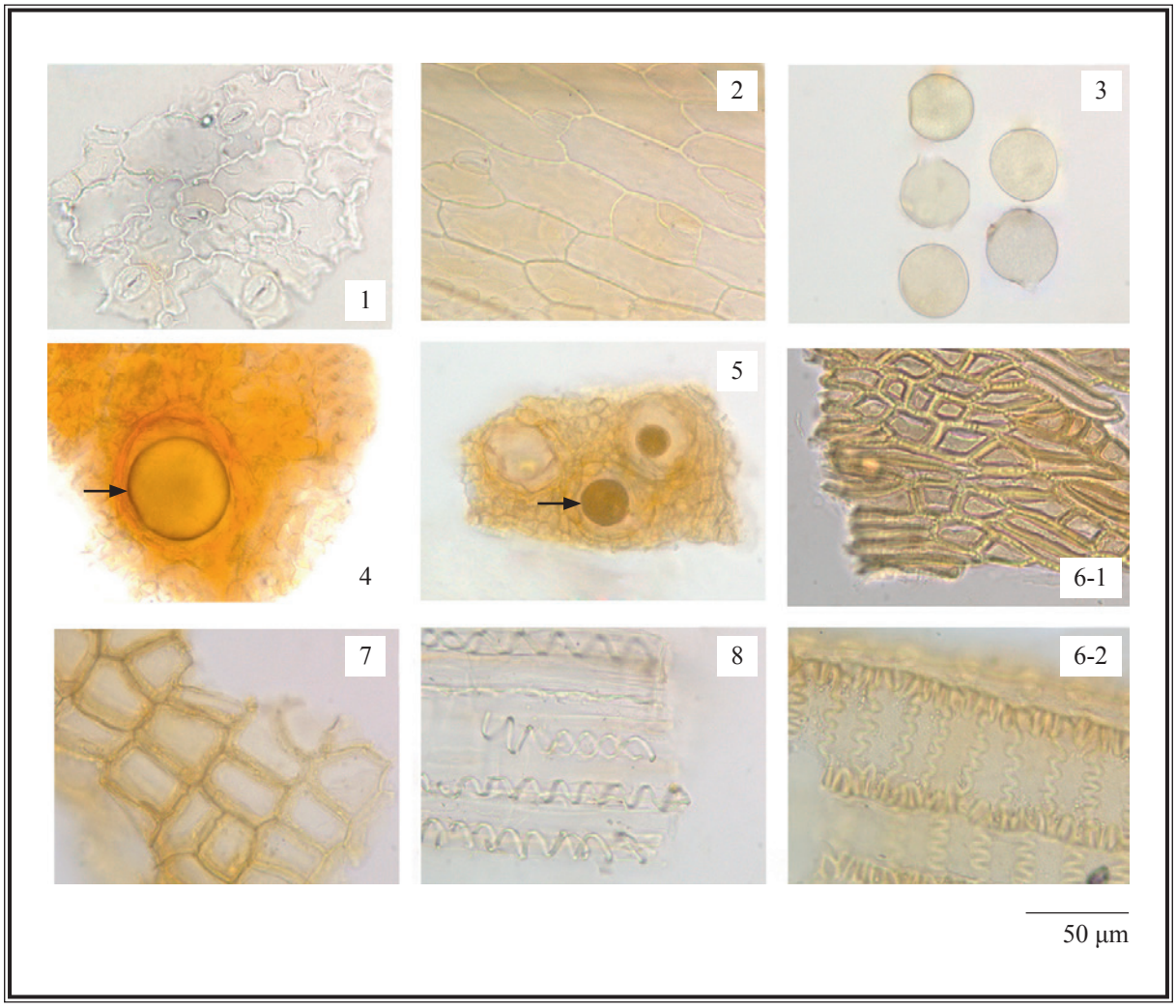


Figure 3 Microscopic features of powder of Hyperici Japonici Herba (under the light microscope)

1. Epidermal cells of leaf 2. Epidermal cells of young stem 3. Pollen grains 4. Secretory cell
5. Oil cavities 6. Epidermal cells of testa (6-1 in lower surface view, 6-2 in upper surface view)
7. Cork cells 8. Spiral vessels

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

Standard solutions

Isoquercitrin standard solution

Weigh 2.0 mg of isoquercitrin CRS (Fig. 4) and dissolve in 2 mL of ethanol (50%).

Quercitrin standard solution

Weigh 2.0 mg of quercitrin CRS (Fig. 4) and dissolve in 2 mL of ethanol (50%).

Developing solvent system

Prepare a mixture of ethyl acetate, formic acid and water (10:0.8:0.6, v/v).

Spray reagent

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

Test solution

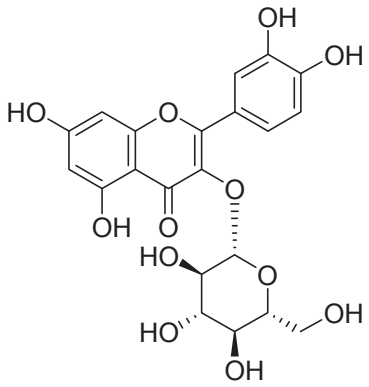
Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 30 mL of ethanol (50%). Sonicate (150 W) the mixture for 30 min. Filter the mixture.

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 isoquercitrin standard solution (0.3 µL), quercitrin standard solution (0.3 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 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 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 about 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 values by using the equation as indicated in Appendix IV (A).

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of isoquercitrin and quercitrin.

(i)



(ii)

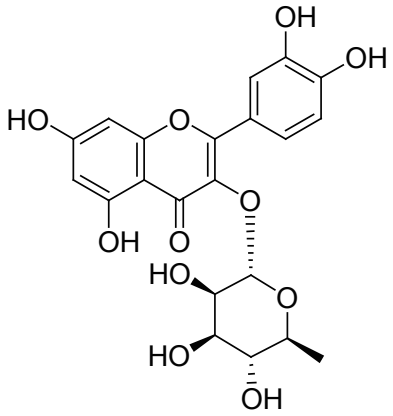


Figure 4 Chemical structures of (i) isoquercitrin and (ii) quercitrin

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

Standard solutions

Isoquercitrin standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 2.5 mg of isoquercitrin CRS and dissolve in 50 mL of ethanol (50%).

Quercitrin standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 2.5 mg of quercitrin CRS and dissolve in 50 mL of ethanol (50%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of ethanol (50%). 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 (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 1) –

Table 1 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.1% Acetic acid* (%, v/v)	Elution
0 – 5	10 → 20	90 → 80	linear gradient
5 – 7	20	80	isocratic
7 – 9	20 → 28	80 → 72	linear gradient
9 – 16	28	72	isocratic
16 – 24	28 → 32	72 → 68	linear gradient
24 – 35	32	68	isocratic

* Adjust the pH to 4 with triethylamine

System suitability requirements

Perform at least five replicate injections, each using 10 μL of isoquercitrin Std-FP and quercitrin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of isoquercitrin and quercitrin should not be more than 5.0%; the RSD of the retention times of isoquercitrin and quercitrin peaks should not be more than 2.0%; the column efficiencies determined from isoquercitrin and quercitrin peaks should not be less than 80000 and 35000 theoretical plates respectively.

The *R* value between peak 4 and the closest peak; and the *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 and 1.0 respectively (Fig. 5).

Procedure

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

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Hyperici Japonici Herba extract

Peak No.	RRT	Acceptable Range
1	0.47	± 0.03
2 (taxifolin-7-O- α -L-rhamnoside)	0.90	± 0.03
3	0.94	± 0.03
4 (marker, isoquercitrin)	1.00	-
5 (quercitrin)	1.11	± 0.03
6 (quercetin)	2.05	± 0.03

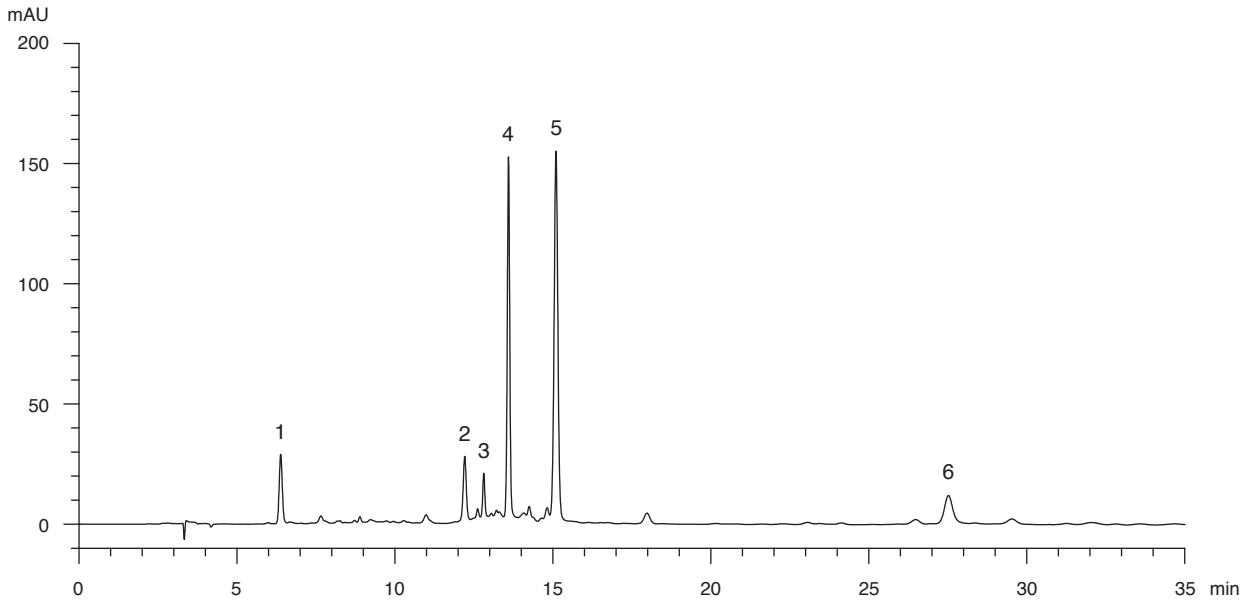


Figure 5 A reference fingerprint chromatogram of Hyperici Japonici Herba extract

For positive identification, the sample must give the above six 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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 7.5%.
Acid-insoluble ash: not more than 3.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 8.0%.
Ethanol-soluble extractives (cold extraction method): not less than 12.0%.

7. ASSAY

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

Standard solution

Mixed isoquercitrin and quercitrin standard stock solution, Std-Stock (200 mg/L each)

Weigh accurately 2.0 mg of isoquercitrin CRS and 2.0 mg of quercitrin CRS, and dissolve in 10 mL of ethanol (50%).

Mixed isoquercitrin and quercitrin standard solution for assay, Std-AS

Measure accurately the volume of the mixed isoquercitrin and quercitrin Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 5, 15, 35, 50, 70 mg/L for both isoquercitrin and quercitrin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (50%). 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. Combine the supernatants and make up to the mark with ethanol (50%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (256 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 0.1% acetic acid* and acetonitrile (74:26, v/v). The elution time is about 20 min.

* Adjust the pH to 4 with triethylamine

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed isoquercitrin and quercitrin Std-AS (35 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of isoquercitrin and quercitrin should not be more than 5.0%; the RSD of the retention times of isoquercitrin and quercitrin peaks should not be more than 2.0%; the column efficiencies determined from isoquercitrin and quercitrin peaks should not be less than 6500 theoretical plates.

The *R* value between isoquercitrin peak and the closest peak; and the *R* value between quercitrin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed isoquercitrin and quercitrin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of isoquercitrin and quercitrin against the corresponding concentrations of the mixed isoquercitrin and quercitrin Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify isoquercitrin and quercitrin peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed isoquercitrin and quercitrin Std-AS. The retention times of isoquercitrin and quercitrin peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of isoquercitrin and quercitrin in the test solution, and calculate the percentage contents of isoquercitrin and quercitrin in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.53% of the total content of isoquercitrin ($C_{21}H_{20}O_{12}$) and quercitrin ($C_{21}H_{20}O_{11}$), calculated with reference to the dried substance.