Hyperici Ascyri Herba

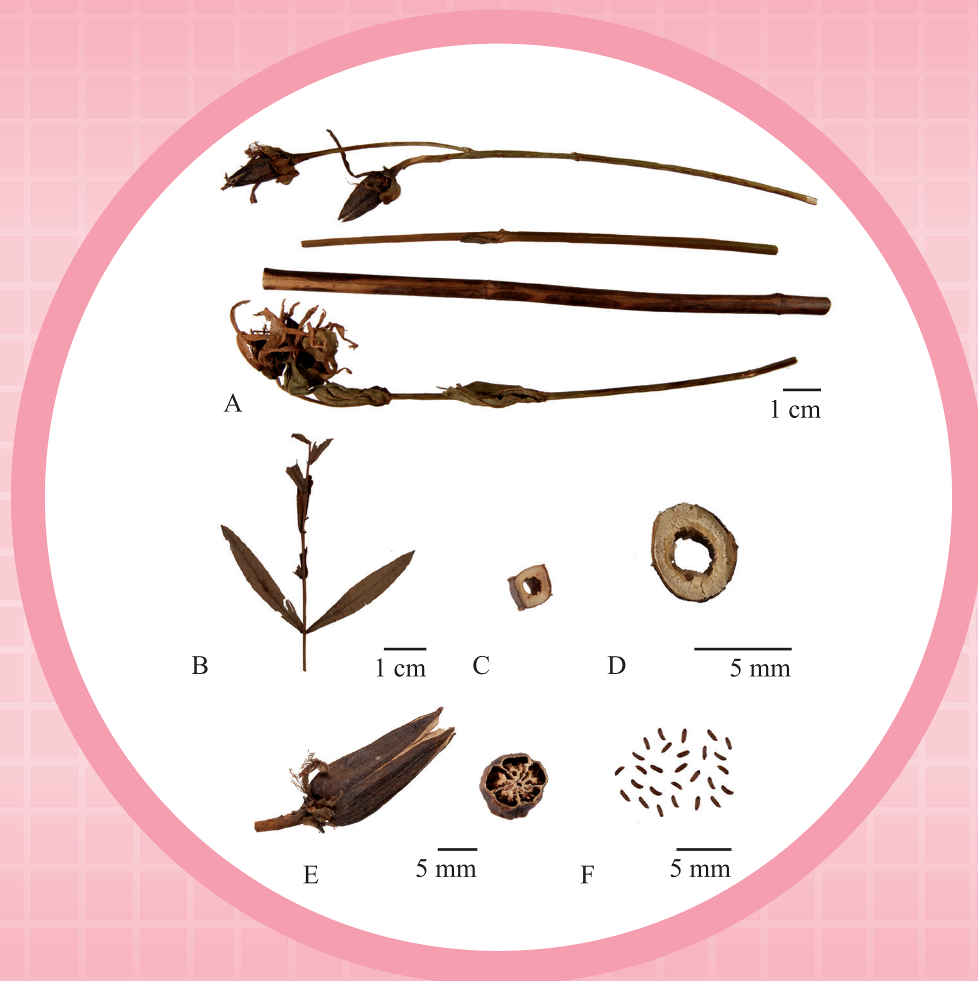


Figure 1 A photograph of Hyperici Ascyri Herba

A. Hyperici Ascyri Herba B. A branch with leaves
C. Magnified image of transverse section of upper part of stem
D. Magnified image of transverse section of lower part of stem
E. Magnified image of capsule and its transverse section F. Seeds

1. NAMES

Official name: Hyperici Ascyri Herba

Chinese name: 紅旱蓮

Chinese phonetic name: Honghanlian

2. SOURCE

Hyperici Ascyri Herba is the dried aerial part of *Hypericum ascyron* L. (Hypericaceae). The aerial part is collected in summer and autumn when the fruit is ripe, foreign matter removed, then dried under the sun to obtain Hyperici Ascyri Herba.

3. DESCRIPTION

Stems quadrangular, gradually showing cylindrical near base, 1.1-9.0 mm in diameter; externally reddish-brown to brownish-green, nodes conspicuous; texture hard, fracture yellowish-white, with pith or hollow in the centre. Leaves simple, opposite; usually fallen off, crumpled and broken, ovate-lanceolate when intact and flattened out. Capsules conical, 0.6-2.6 cm long, 2.9-10.3 mm in diameter, reddish-brown, 5-valved at the apex; texture hard, with numerous seeds in axis. Seeds tiny, oblong, reddish-brown. Odour slightly aromatic; taste bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Upper Part of Stem: Epidermis consists of 1 layer of subsquare or subrectangular cells, covered with cuticle. Collenchyma present in the angular region, with cells subrounded or irregular in shape. Cortex narrow. Secretory canals arranged in a ring at the outer side of phloem. Phloem narrow. Cambium in a ring. Xylem broad. Pith broad, sometimes broken or hollow, mostly occupying about 1/2 of the stem, sometimes containing clusters of calcium oxalate [Fig. 2(i)].

Lower Part of Stem: Epidermis consists of 1 layer of subsquare or subrectangular cells, covered with cuticle. Collenchyma present in the angular region, obviously lignified. Cortex narrow. Secretory canals arranged in a ring at the outer side of phloem. Phloem narrow. Cambium in a ring. Xylem broad. Pith broad, almost hollowd [Fig. 2(ii)].

Leaf: Upper epidermis consists of 1 layer of subsquare cells. Palisade tissue consists of 1 layer of palisade cells. Spongy tissue with cells arranged loosely. Collenchyma located in the inner side of upper and lower epidermis at the midrib. Vascular bundles collateral, xylem vessels arranged radially. Secretory canals arranged at the outer side of phloem, forming a semicircle. Lower epidermis consists of 1 layer of irregular cells, relatively small [Fig. 2(iii)].

Powder

Colour reddish-brown to brownish-green. Secretory canals usually contain pale yellow to yellowish-brown secretion. Lower epidermal cells of leaf with walls sinuous; stomata anomocytic, arranged densely. Collenchymatous cells subrounded to subrectangular, with thickened walls. Epidermal cells of testa pale yellow to yellowish-brown, stellate in shape, outer wall striations thickened. Clusters of calcium oxalate sometimes visible; polychromatic under the polarized microscope. Vessels mostly spiral and bordered-pitted vessels, respectively 10-48 μm and 6-40 μm in diameter. Epidermal cells of stem in fragments, subsquare to subrectangular, pale yellow to pale yellowish-brown. Fibres usually in bundles, 3-15 μm in diameter, walls relatively thickened; bright white to yellowish-brown under the polarized microscope (Fig. 3).

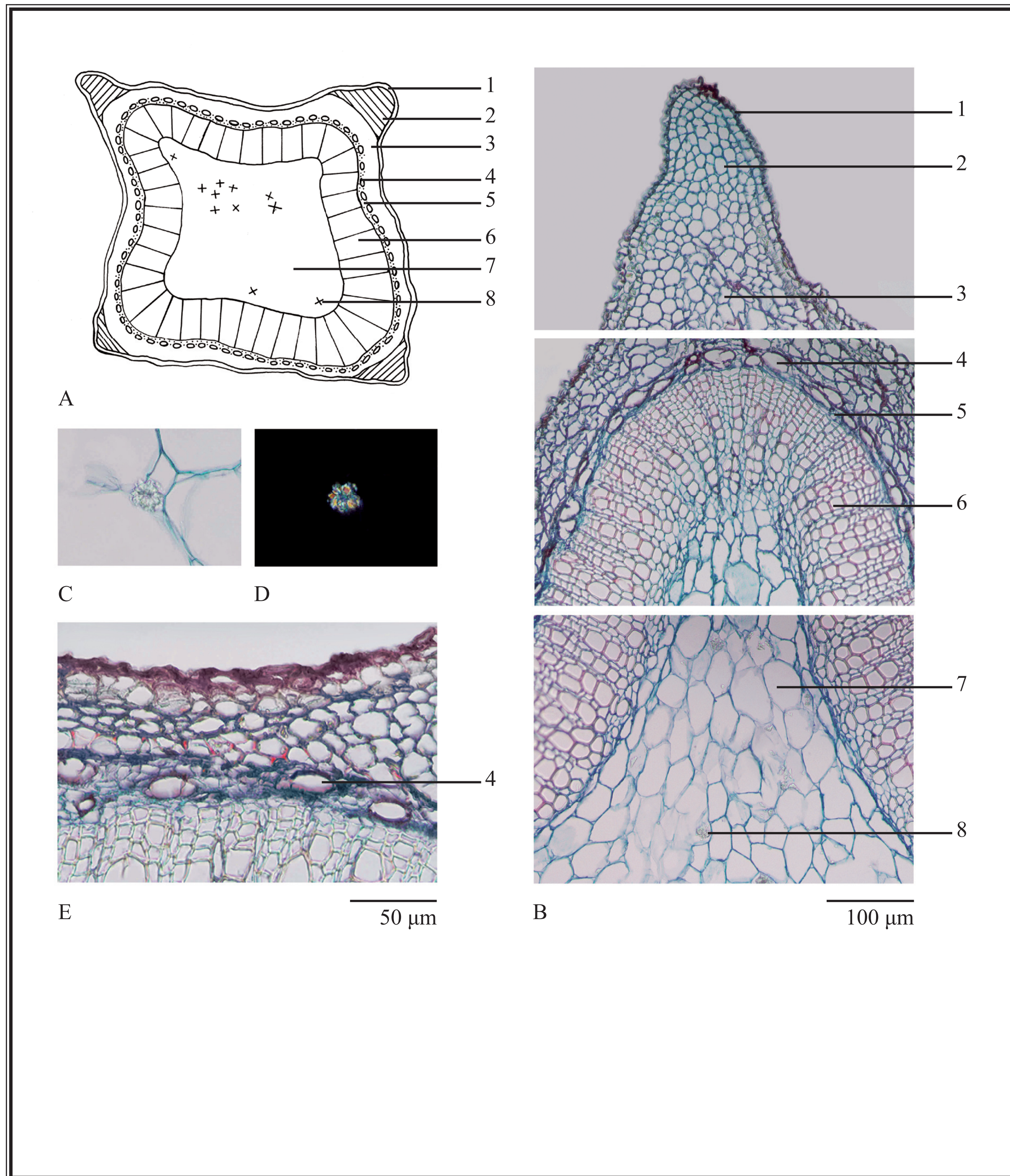


Figure 2 (i) Microscopic features of transverse section of upper part of stem of Hyperici Ascyri Herba

A. Sketch B. Section illustration C. Cluster of calcium oxalate (under the light microscope) D. Cluster of calcium oxalate (under the polarized microscope) E. Secretory canal

1. Epidermis 2. Collenchyma 3. Cortex 4. Secretory canal 5. Phloem 6. Xylem 7. Pith 8. Cluster of calcium oxalate

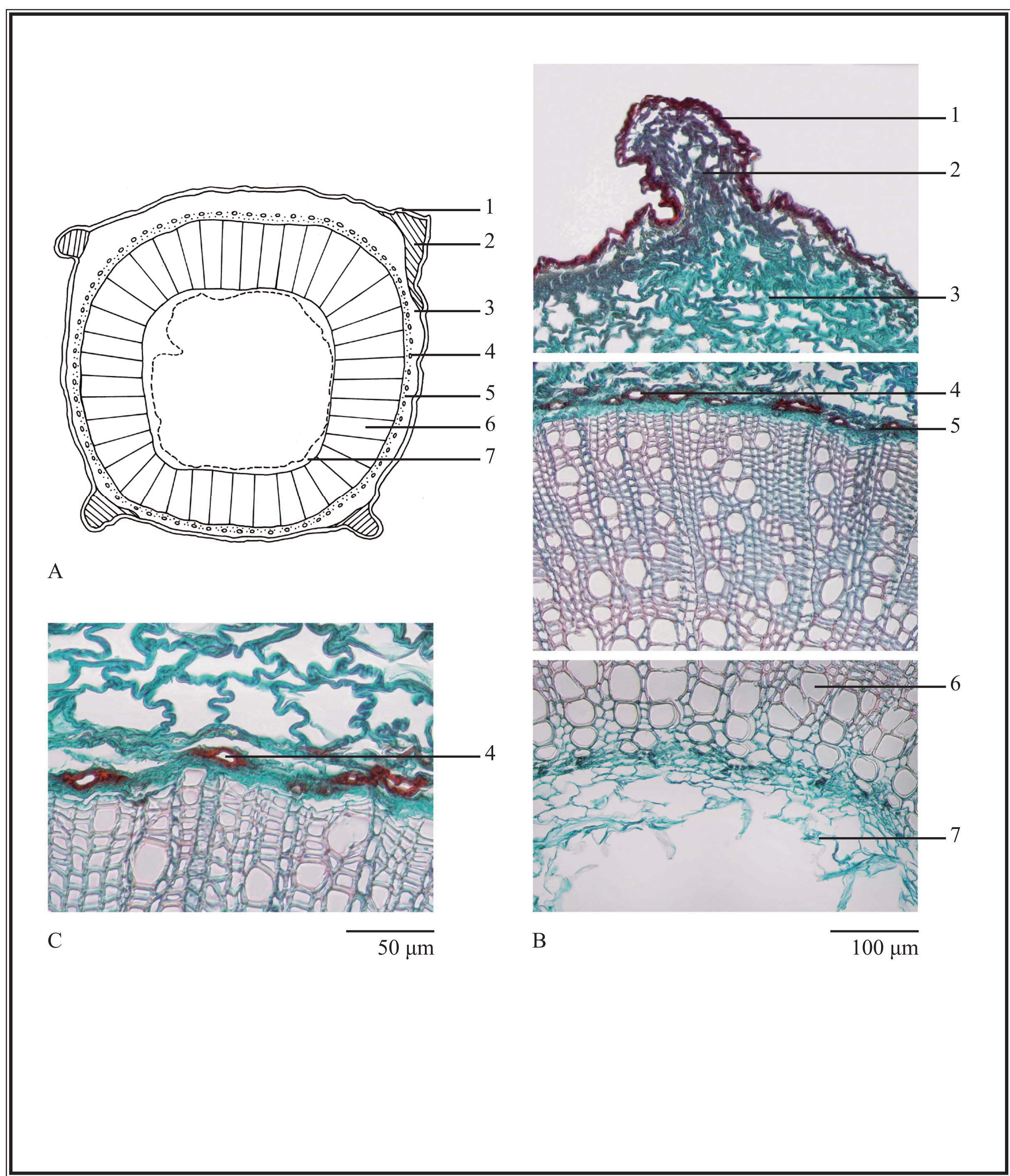


Figure 2 (ii) Microscopic features of transverse section of lower part of stem of Hyperici Ascyri Herba

A. Sketch B. Section illustration C. Secretory canal

1. Epidermis 2. Collenchyma 3. Cortex 4. Secretory canal 5. Phloem 6. Xylem 7. Pith

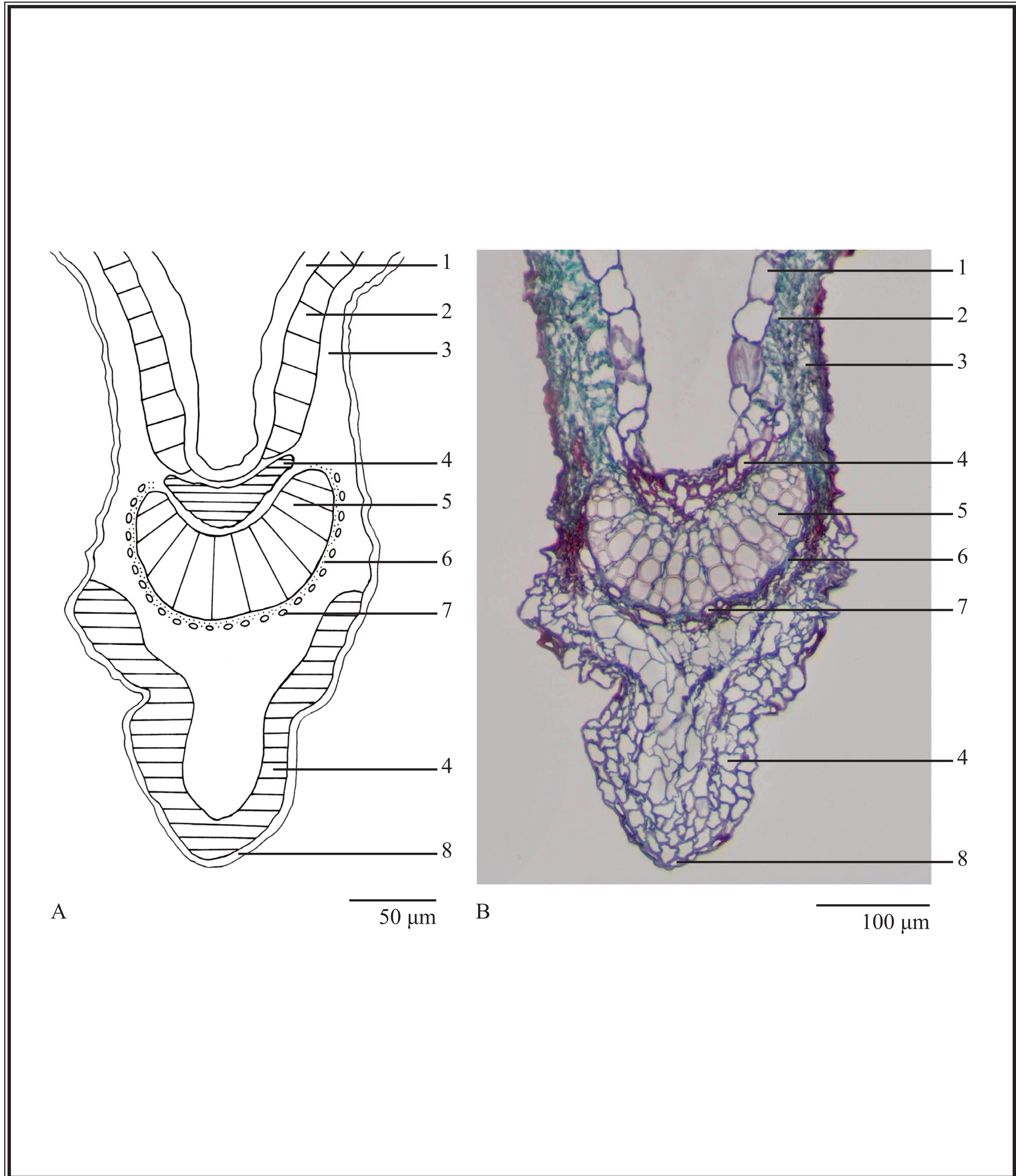


Figure 2 (iii) Microscopic features of transverse section of leaf of Hyperici Ascyri Herba

A. Sketch B. Section illustration

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Collenchyma 5. Xylem 6. Phloem
7. Secretory canal 8. Lower epidermis

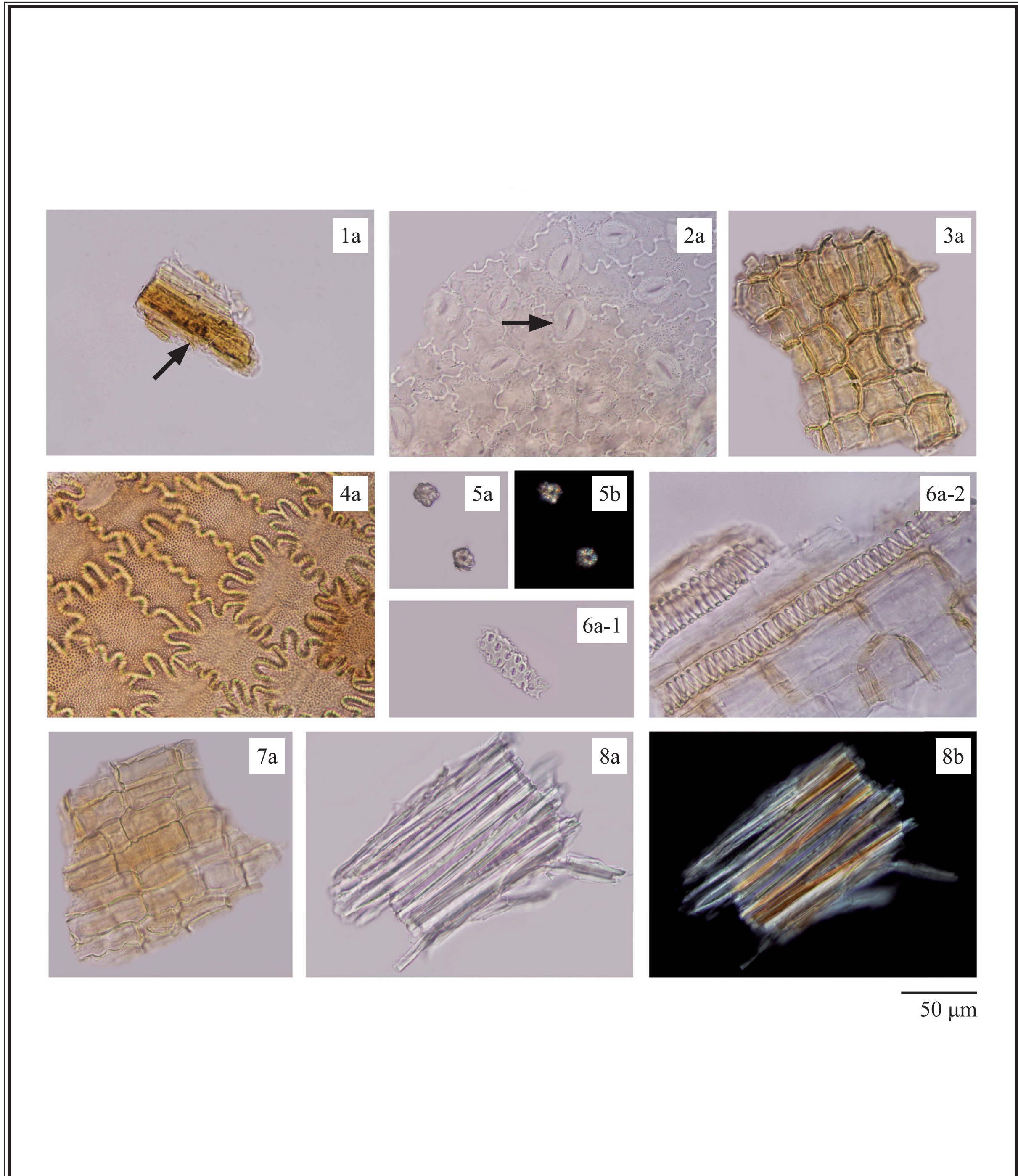


Figure 3 Microscopic features of the powder of Hyperici Ascyri Herba

1. Secretory canal (→) 2. Lower epidermal cells of leaf with anomocytic stomata (→)
3. Collenchymatous cells 4. Epidermal cells of testa 5. Clusters of calcium oxalate
6. Vessels (6-1 bordered-pitted vessel, 6-2 spiral vessels) 7. Epidermal cells of stem
8. Fibres

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

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

Standard solutions

Hyperoside standard solution

Weigh 0.25 mg of hyperoside CRS (Fig. 4) and dissolve in 1 mL of ethanol (95%).

Isoquercitrin standard solution

Weigh 0.25 mg of isoquercitrin CRS (Fig. 4) and dissolve in 1 mL of ethanol (95%).

Developing solvent system

Prepare a mixture of ethyl acetate, formic acid and water (12:2:1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

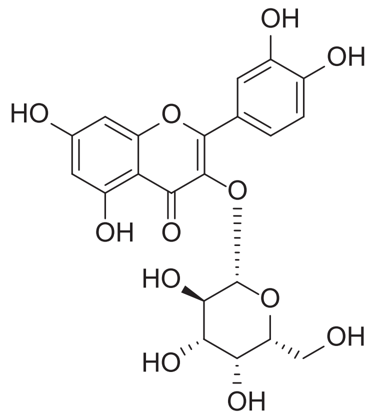
Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethanol (95%). Sonicate (400 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 ethanol (95%). Filter through a 0.45-μm nylon filter.

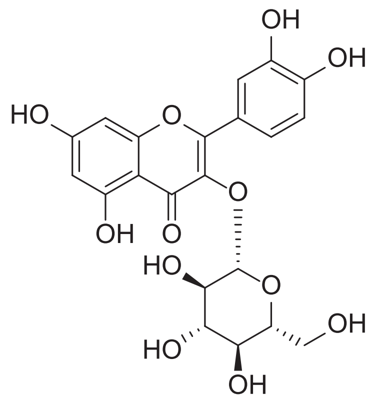
Procedure

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 hyperoside standard solution (1 μL), isoquercitrin standard solution (1 μL) and the test solution (2 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 and heat at about 105ºC (about 1 min). Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



(ii)



(iii)

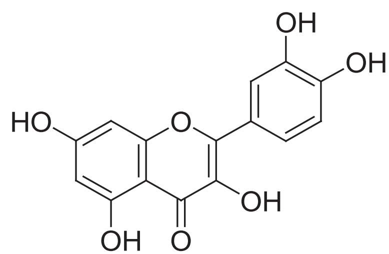


Figure 4 Chemical structures of (i) hyperoside (ii) isoquercitrin and (iii) quercetin

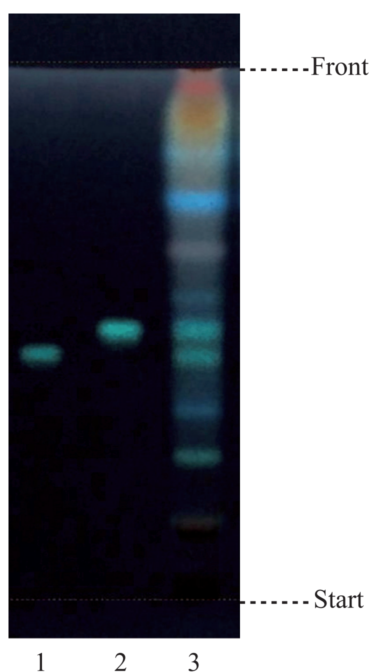


Figure 5 A reference HPTLC chromatogram of Hyperici Ascyri Herba extract observed under UV light (366 nm) after staining

1. Hyperoside standard solution 2. Isoquercitrin standard solution 3. Test solution

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 hyperoside and isoquercitrin (Fig. 5).

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

Standard solutions

Hyperoside standard solution for fingerprinting, Std-FP (5 mg/L)

Weigh 0.05 mg of hyperoside CRS and dissolve in 10 mL of ethanol (70%).

Isoquercitrin standard solution for fingerprinting, Std-FP (5 mg/L)

Weigh 0.05 mg of isoquercitrin CRS and dissolve in 10 mL of ethanol (70%).

Quercetin standard solution for fingerprinting, Std-FP (5 mg/L)

Weigh 0.05 mg of quercetin CRS (Fig. 4) and dissolve in 10 mL of ethanol (70%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol (70%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Filter and transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction two more times. Wash the residue with ethanol (70%). Combine the extracts 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 (355 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)	0.2% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	90	10	isocratic
5 – 15	90 → 85	10 → 15	linear gradient
15 – 45	85 → 70	15 → 30	linear gradient
45 – 60	70 → 40	30 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of hyperoside Std-FP, isoquercitrin Std-FP and quercetin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of hyperoside, isoquercitrin and quercetin should not be more than 5.0%; the RSD of the retention times of hyperoside, isoquercitrin and quercetin peaks should not be more than 2.0%; the column efficiencies determined from hyperoside, isoquercitrin and quercetin peaks should not be less than 100000, 100000 and 150000 theoretical plates respectively.

The *R* value between peak 2 and the closest peak; the *R* value between peak 3 and the closest peak; and the *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject hyperoside Std-FP, isoquercitrin Std-FP, quercetin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of hyperoside, isoquercitrin and quercetin peaks in the chromatograms of hyperoside Std-FP, isoquercitrin Std-FP, quercetin Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify hyperoside, isoquercitrin and quercetin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of hyperoside Std-FP, isoquercitrin Std-FP and quercetin Std-FP. The retention times of hyperoside, isoquercitrin and quercetin 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 four characteristic peaks of Hyperici Ascyri Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.48	± 0.03
2 (marker, hyperoside)	1.00	-
3 (isoquercitrin)	1.02	± 0.03
4 (quercetin)	1.65	± 0.04

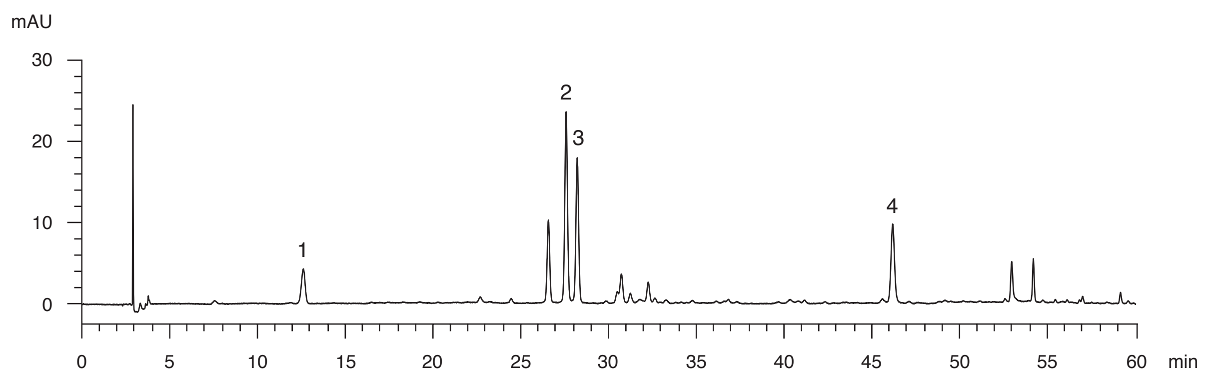


Figure 6 A reference fingerprint chromatogram of Hyperici Ascyri Herba extract

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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 3.5%.
Acid-insoluble ash: not more than 0.5%.

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 6.0%.
Ethanol-soluble extractives (cold extraction method): not less than 7.0%.

7. ASSAY

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

Standard solution
Mixed hyperoside, isoquercitrin and quercetin standard stock solution, Std-Stock (330 mg/L for hyperoside, 290 mg/L for isoquercitrin and 290 mg/L for quercetin)
Weigh accurately 8.25 mg of hyperoside CRS, 7.25 mg of isoquercitrin CRS and 7.25 mg of quercetin CRS, and dissolve in 25 mL of ethanol (70%).
Mixed hyperoside, isoquercitrin and quercetin standard solution for assay, Std-AS
Measure accurately the volume of the mixed hyperoside, isoquercitrin and quercetin Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 1, 2.5, 5, 10, 20 mg/L for hyperoside, 1, 2, 4.5, 9, 18 mg/L for isoquercitrin and 1, 2, 4.5, 9, 18 mg/L for quercetin.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol (70%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Filter and transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction two more times. Wash the residue with ethanol (70%). Combine the extracts 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 (355 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)	0.2% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	90	10	isocratic
5 – 15	90 → 85	10 → 15	linear gradient
15 – 45	85 → 70	15 → 30	linear gradient
45 – 60	70 → 40	30 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed hyperoside, isoquercitrin and quercetin Std-AS (5 mg/L for hyperoside, 4.5 mg/L for isoquercitrin and 4.5 mg/L for quercetin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of hyperoside, isoquercitrin and quercetin should not be more than 5.0%; the RSD of the retention times of hyperoside, isoquercitrin and quercetin peaks should not be more than 2.0%; the column efficiencies determined from hyperoside, isoquercitrin and quercetin peaks should not be less than 100000, 100000 and 150000 theoretical plates respectively.

The R value between hyperoside peak and the closest peak; the R value between isoquercitrin peak and the closest peak; and the R value between quercetin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curves

Inject a series of the mixed hyperoside, isoquercitrin and quercetin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of hyperoside, isoquercitrin and quercetin against the corresponding concentrations of the mixed hyperoside, isoquercitrin and quercetin 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 hyperoside, isoquercitrin and quercetin peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed hyperoside, isoquercitrin and quercetin Std-AS. The retention times of hyperoside, isoquercitrin and quercetin 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 hyperoside, isoquercitrin and quercetin in the test solution, and calculate the percentage contents of hyperoside, isoquercitrin and quercetin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.047% of the total content of hyperoside ($C_{21}H_{20}O_{12}$), isoquercitrin ($C_{21}H_{20}O_{12}$) and quercetin ($C_{15}H_{10}O_{7}$), calculated with reference to the dried substance.

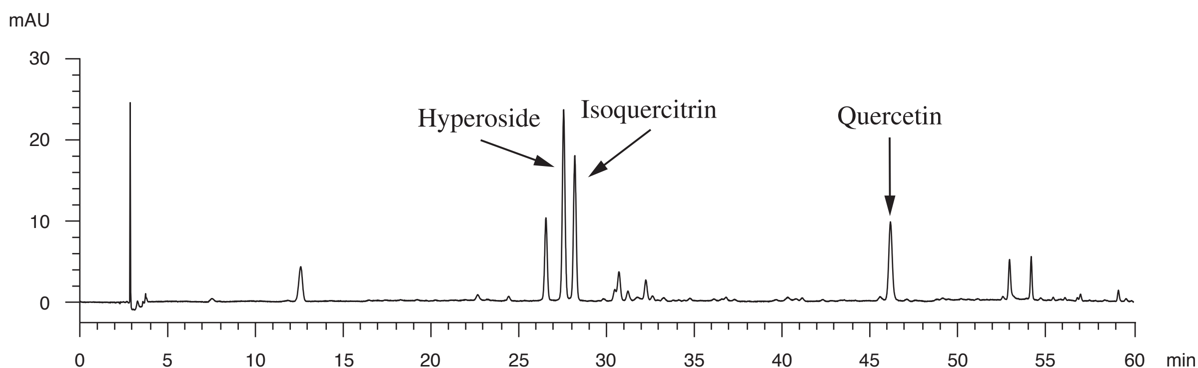


Figure 7 A reference assay chromatogram of Hyperici Ascyri Herba extract