Polygalae Japonicae Herba

Figure 1 A photograph of Polygalae Japonicae Herba

A. Polygalae Japonicae Herba

B. Magnified image of upper surface of leaf

C. Magnified image of lower surface of leaf

D. Magnified image of flowers

E. Magnified image of root

1. NAMES

Official Name: Polygalae Japonicae Herba

Chinese Name: 瓜子金

Chinese Phonetic Name: Guazijin

2. SOURCE

Polygalae Japonicae Herba is the dried whole plant of *Polygala japonica* Houtt. (Polygalaceae). The whole plant is collected at flowering period in late spring, foreign matter removed, then dried under the sun to obtain Polygalae Japonicae Herba.

3. DESCRIPTION

Roots cylindrical, slightly curved, up to 4 mm in diameter; externally yellowish-brown, with longitudinal wrinkles; texture hard, fracture yellowish-white. Stems a few branched, 9-30 cm long, pale brown, pubescent. Leaves alternate, greyish-green on both surface, when whole ovate to ovate-lanceolate, 1-3 cm long, 5-10 mm wide; lateral veins distinct, apex short-acute, base rounded or cuneate, margin entire; petiole short or sessile, pubescent. Racemes axillary, the uppermost racemes lower than the top of stems; flowers butterfly-shaped. Odour slight; taste slightly pungent and bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Cork consists several layers of cells. Cortex consists of several layers of parenchymatous cells. Phloem relatively broad. Cambium consists of 3-5 layers of cells, arranged in a ring. Xylem well developed, lignified; xylem rays consist of 1-3 rows of cells; vessels single or 2-3 in groups [Fig. 2 (i)].

Stem: Epidermis consists of 1 layer of cells, covered with cuticle, unicellular non-glandular hairs occasionally found. Cortex consists of 5-7 layers of cells, the cells subrounded, arranged loosely. Endodermis consists of 1 layer of cells, the cells long-rounded, arranged densely, wall thickened, lignified. Phloem relatively narrow, the cells relatively small. Cambium indistinct. Xylem vessels mostly 2-5 in groups; xylem fibres in bundles, lignified. Pith consists of subrounded parenchymatous cells, arranged loosely, usually hollow in the centre [Fig. 2 (ii)].

Leaf: Upper epidermal cells oblong, lower epidermal cells subsquare, both covered with cuticle. Palisade tissue consists of 1 layer of oblong parenchymatous cells, arranged densely. Spongy tissue consists of several layers of subrounded parenchymatous cells, arranged loosely, with intercellular spaces. Clusters of calcium oxalate in rosette aggregates, scattered in mesophyll. Vascular bundle of midrib collateral. Collenchymatous cells located on the inner side of lower epidermis of midrib. Unicellular non-glandular hairs found on both surfaces [Fig. 2 (iii)].

Powder

Colour yellowish-green. Upper epidermal cell polygonal, anticlinal walls relatively straight, stomata anomocytic or anisocytic. Lower epidermal cells polygonal or irregular in shape, anticlinal wall sinuate, with dense stomata, most of the stomata anomocytic, anisocytic occasionally found. Non-glandular hairs numerous, unicellular, walls thin, with fine warty surface. Vessels mostly reticulate and spiral, 5-50 μm in diameter. Clusters of calcium oxalate few, in rosette aggregates with obtuse angles, 8-30 μm m in diameter, bright yellowish-white under the polarized microscope. Pollen grains subspherical or oblong, 30-65 μm in diameter, with striated sculptures. Fibres in bundle; polychromatic under the polarized microscope (Fig. 3).

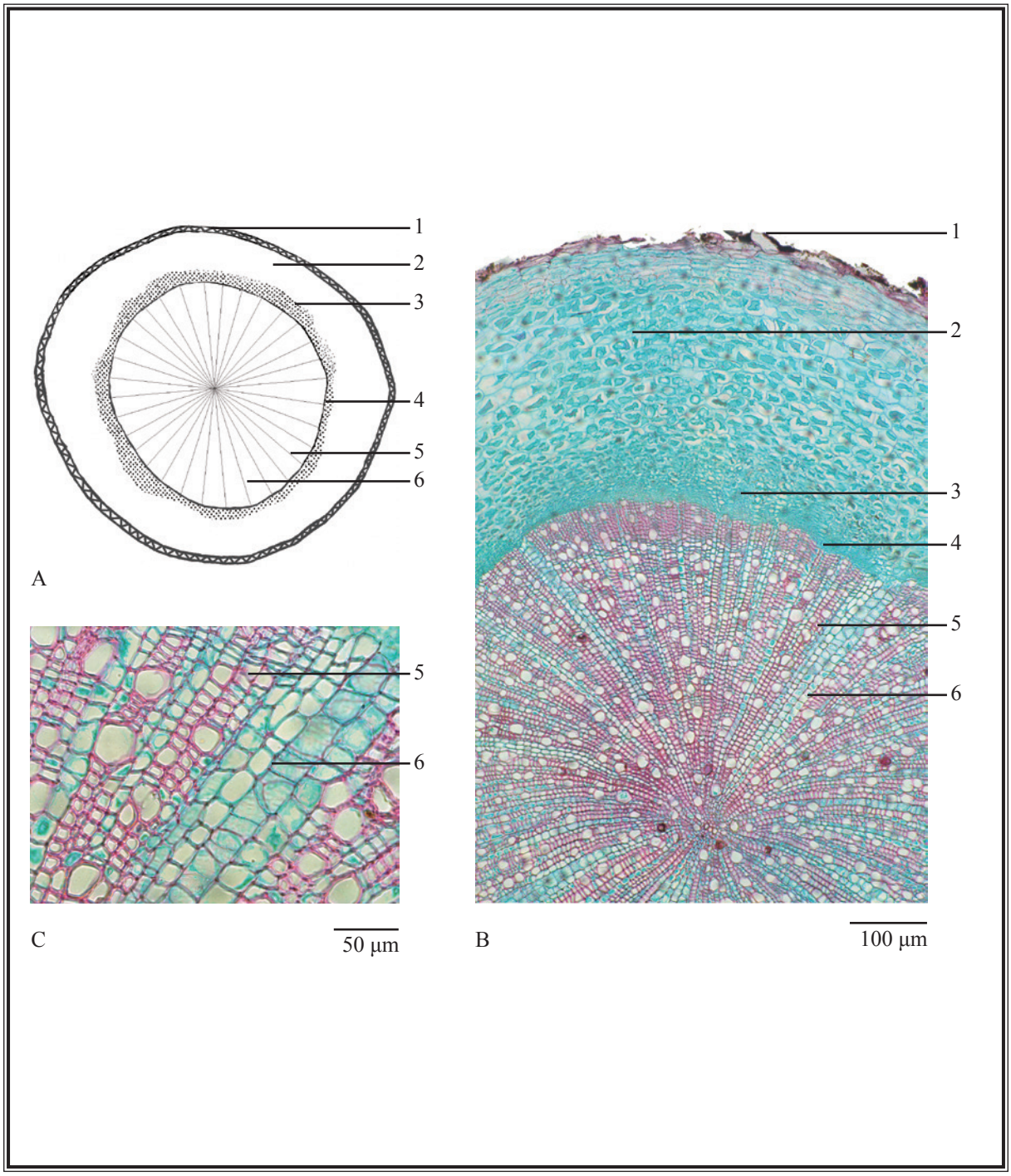

Figure 2 (i) Microscopic features of transverse section of root of Polygalae Japonicae Herba

A. Sketch B. Section illustration C. Section magnified

1. Cork 2. Cortex 3. Phloem 4. Cambium 5. Xylem 6. Xylem ray

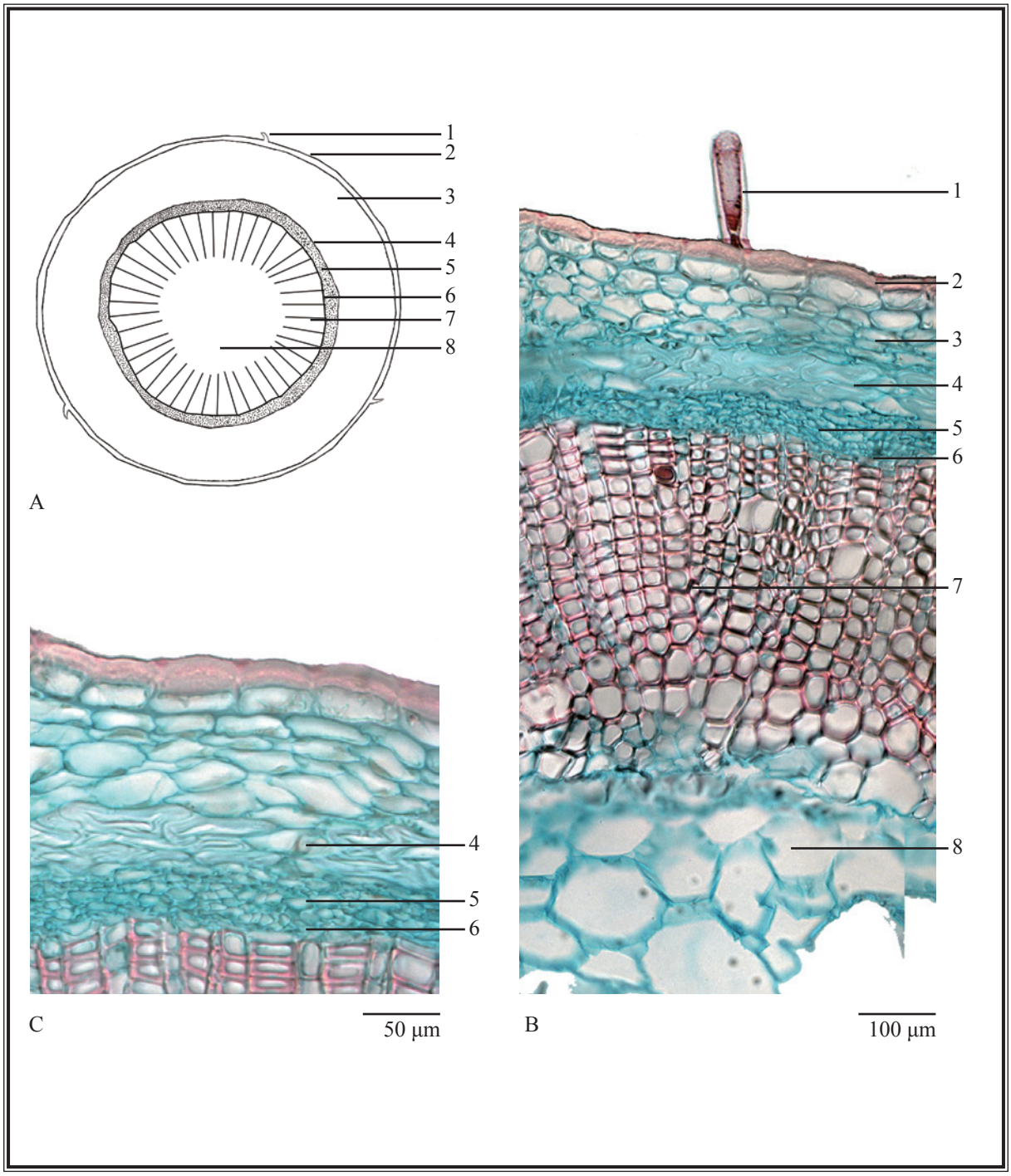

Figure 2 (ii) Microscopic features of transverse section of stem of Polygalae Japonicae Herba

A. Sketch B. Section illustration C. Section magnified

1. Non-glandular hair 2. Epidermis 3. Cortex 4. Endodermis 5. Phloem 6. Cambium 7. Xylem 8. Pith

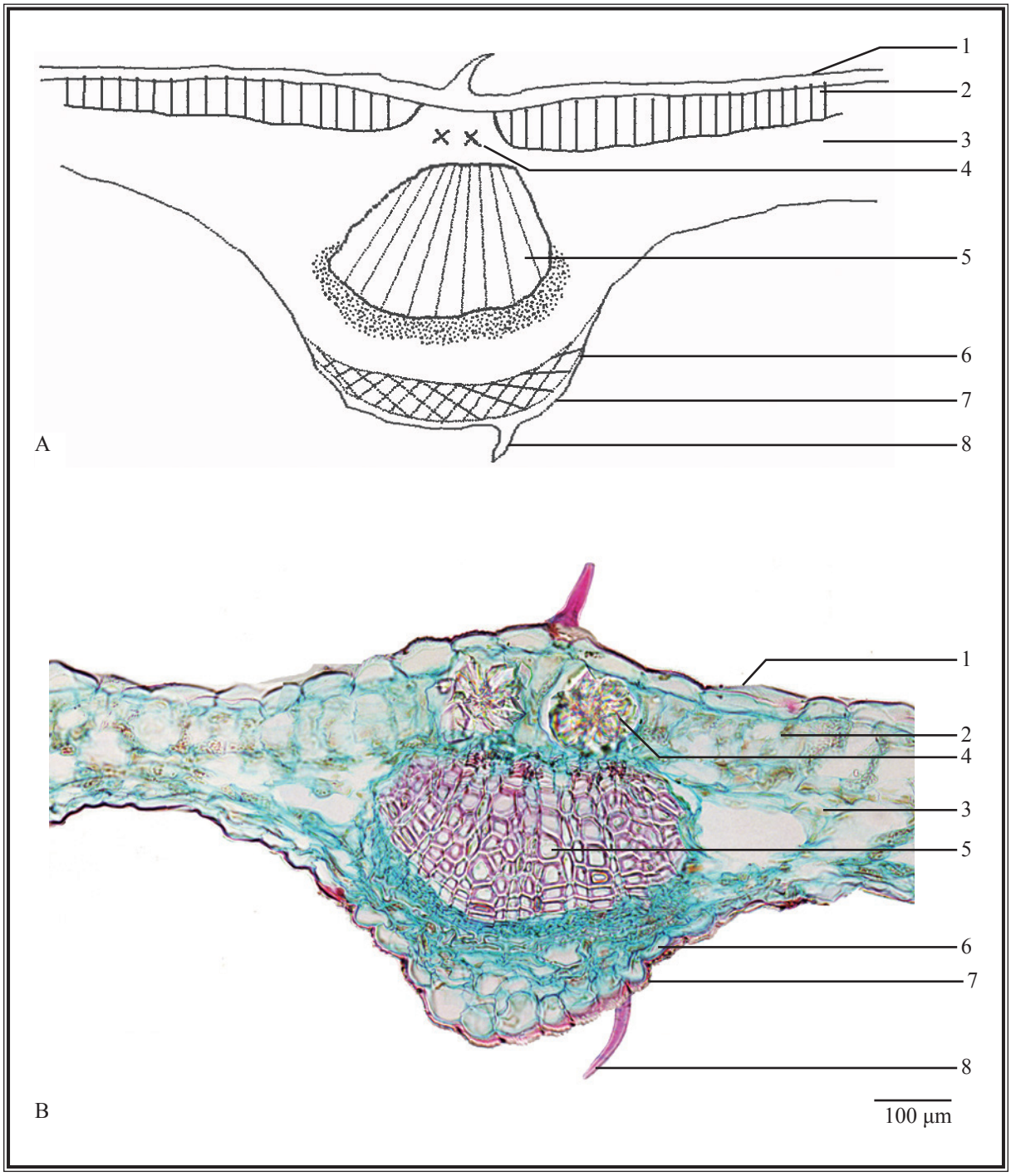

Figure 2 (iii) Microscopic features of transverse section of leaf of Polygalae Japonicae Herba

A. Sketch B. Section illustration

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Cluster of calcium oxalate
5. Vascular bundle 6. Collenchyma 7. Lower epidermis 8. Non-glandular hair

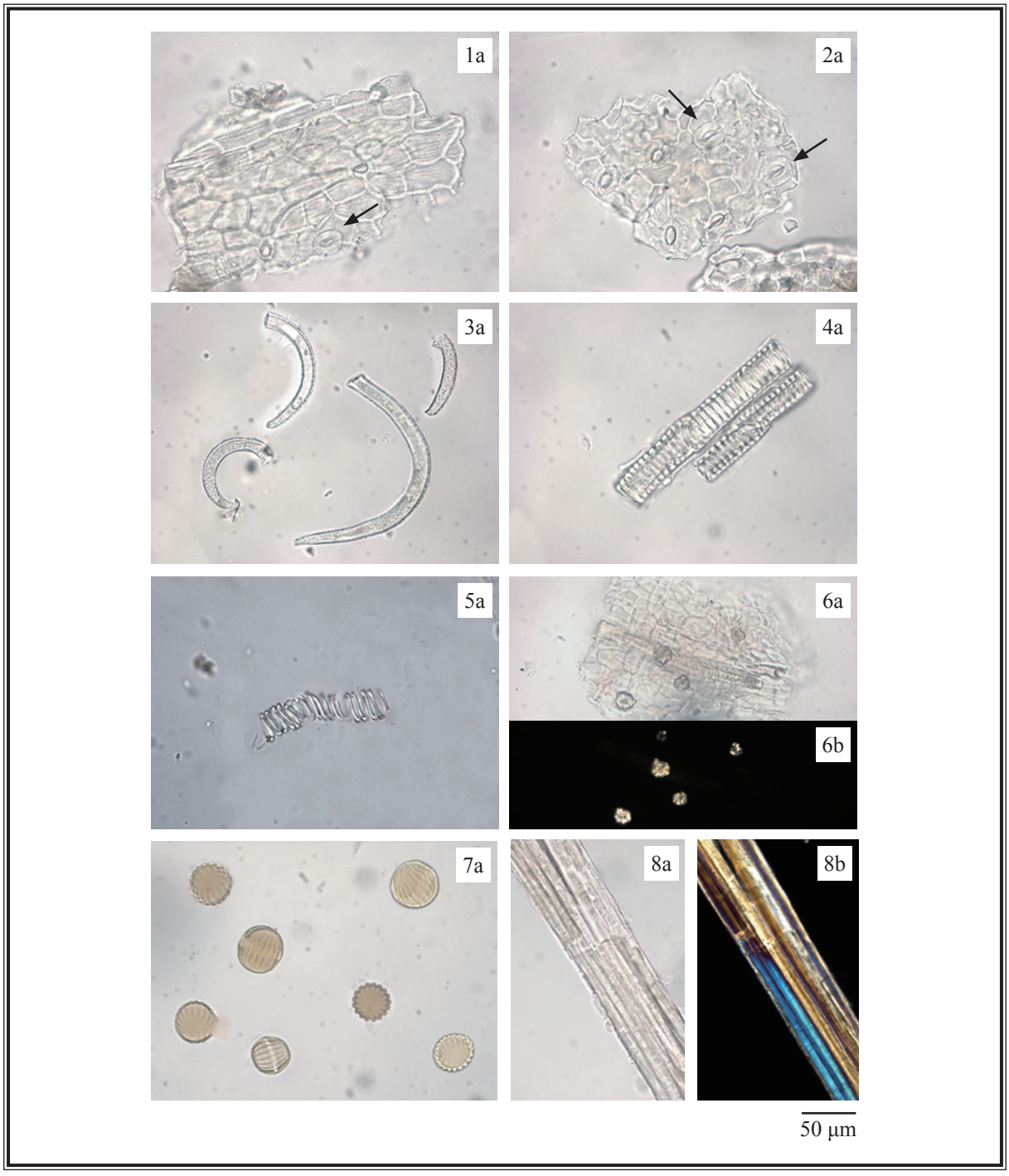

Figure 3 Microscopic features of powder of Polygalae Japonicae Herba

1. Upper epidermal cell with stomata (⟶) 2. Lower epidermal cells with stomata (⟶)
3. Non-glandular hairs 4. Reticulate vessels 5. Spiral vessel 6. Clusters of calcium oxalate
7. Pollen grains 8. Fibres

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

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

Standard solution

Polygalasaponin F standard solution

Weigh 2.0 mg of polygalasaponin F CRS (Fig. 4) and dissolve in 2 mL of methanol (70%).

Developing solvent system

Prepare a mixture of water, *n*-butanol and glacial acetic acid (5:4:1, v/v). Shake well and use the upper layer.

Spray reagent

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

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (70%). Sonicate (100 W) the mixture for 30 min. Centrifuge at about 2000 × *g* for 5 min. Collect the supernatant.

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 polygalasaponin F standard solution and the test solution (3 μL each) 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 7 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 105°C until the spots or bands become visible (about 5 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

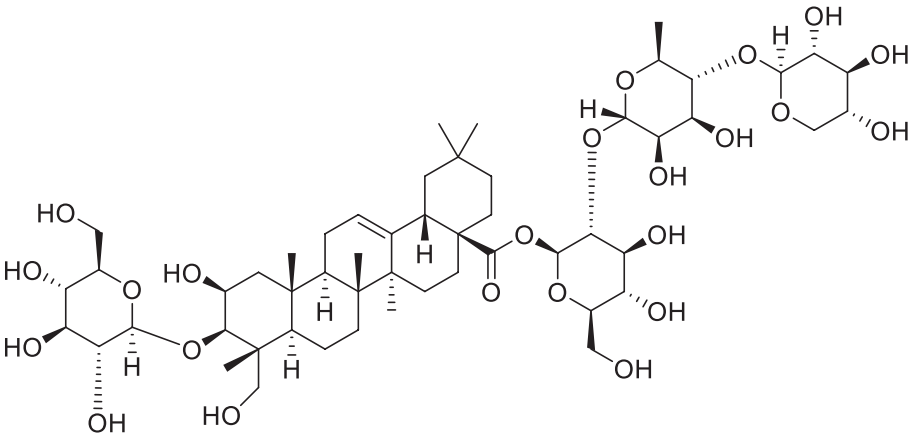

Figure 4 Chemical structure of polygalasaponin F

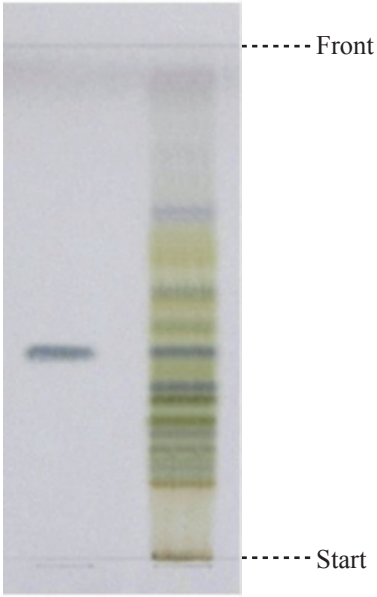

Figure 5 A reference HPTLC chromatogram of Polygalae Japonicae Herba extract observed under visible light after staining

1. Polygalasaponin F 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 polygalasaponin F (Fig. 5).

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

Standard solution

Polygalasaponin F standard solution for fingerprinting, Std-FP (300 mg/L)

Weigh 3.0 mg of polygalasaponin F CRS and dissolve in 10 mL of methanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (70%). Sonicate (100 W) the mixture for 1 h. Centrifuge at about 1200 × *g* for 10 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 60°C; nebulizer gas (N_2) pressure: 3.5 bar] 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)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 60	80 → 60	20 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of polygalasaponin F Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of polygalasaponin F should not be more than 5.0%; the RSD of the retention time of polygalasaponin F peak should not be more than 2.0%; the column efficiency determined from polygalasaponin F peak should not be less than 90000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 6).

Procedure

Separately inject polygalasaponin F Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of polygalasaponin F peak in the chromatogram of polygalasaponin F Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify polygalasaponin F peak in the

chromatogram of the test solution by comparing its retention time with that in the chromatogram of polygalasaponin F Std-FP. The retention times of polygalasaponin F 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 three characteristic peaks of Polygalae Japonicae Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Polygalae Japonicae Herba extract

Peak No.	RRT	Acceptable Range
1	0.85	± 0.03
2	0.98	± 0.03
3 (marker, polygalasaponin F)	1.00	-

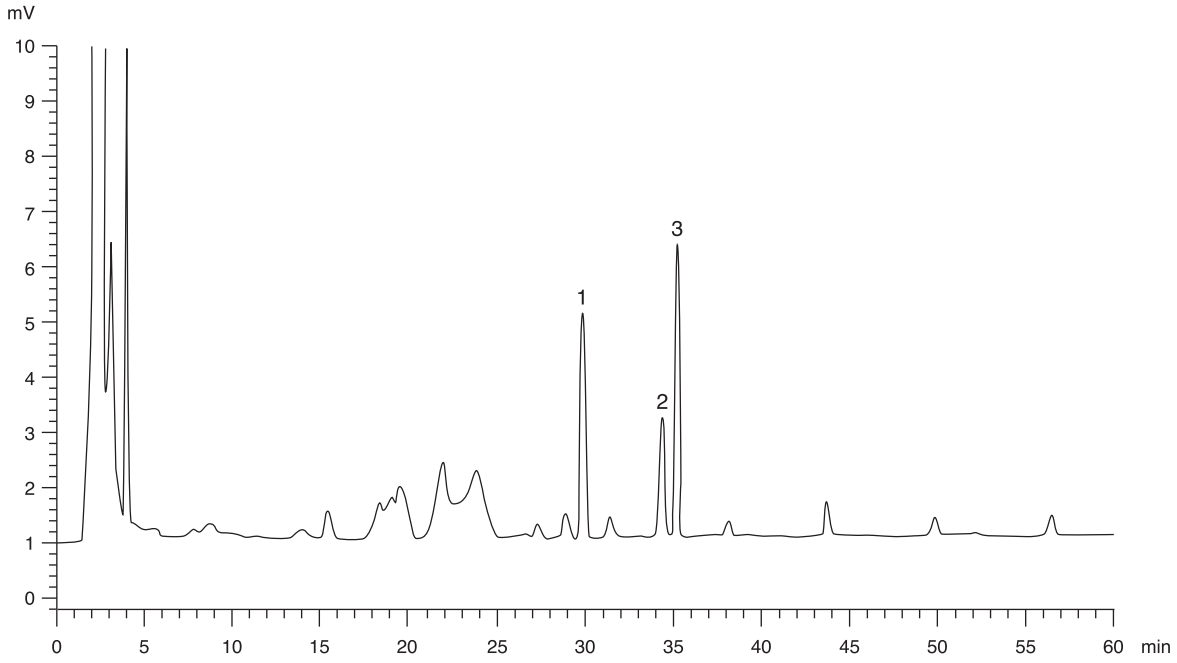

Figure 6 A reference fingerprint chromatogram of Polygalae Japonicae Herba extract

For positive identification, the sample must give the above three 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 – Aflatoxins *(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 9.0%.
Acid-insoluble ash: not more than 5.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 9.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 12.0%.
Ethanol-soluble extractives (cold extraction method): not less than 14.0%.

7. ASSAY

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

Standard solution

Polygalasaponin F standard stock solution, Std-Stock (500 mg/L)
Weigh accurately 5.0 mg of polygalasaponin F CRS and dissolve in 10 mL of methanol (70%).
Polygalasaponin F standard solution for assay, Std-AS
Measure accurately the volume of the polygalasaponin F Std-Stock, dilute with methanol (70%) to produce a series of solutions of 50, 80, 100, 200, 300 mg/L for polygalasaponin F.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (100 W) the mixture for 1 h. Centrifuge at about 1200 × *g* for 10 min. Transfer the supernatant to a 20-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 60°C; nebulizer gas (N_2) pressure: 3.5 bar] 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 water and acetonitrile (70:30, v/v). The elution time is about 20 min.

System suitability requirements

Perform at least five replicate injections, each using 20 µL of polygalasaponin F Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of polygalasaponin F should not be more than 5.0%; the RSD of the retention time of polygalasaponin F peak should not be more than 2.0%; the column efficiency determined from polygalasaponin F peak should not be less than 4000 theoretical plates.

The *R* value between polygalasaponin F 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 polygalasaponin F Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of polygalasaponin F against the natural logarithm of the corresponding concentrations of polygalasaponin F Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify polygalasaponin F peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of polygalasaponin F Std-AS. The retention times of polygalasaponin F 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 polygalasaponin F in the test solution, and calculate the percentage content of polygalasaponin F in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.60% of polygalasaponin F ($C_{53}H_{86}O_{23}$), calculated with reference to the dried substance.

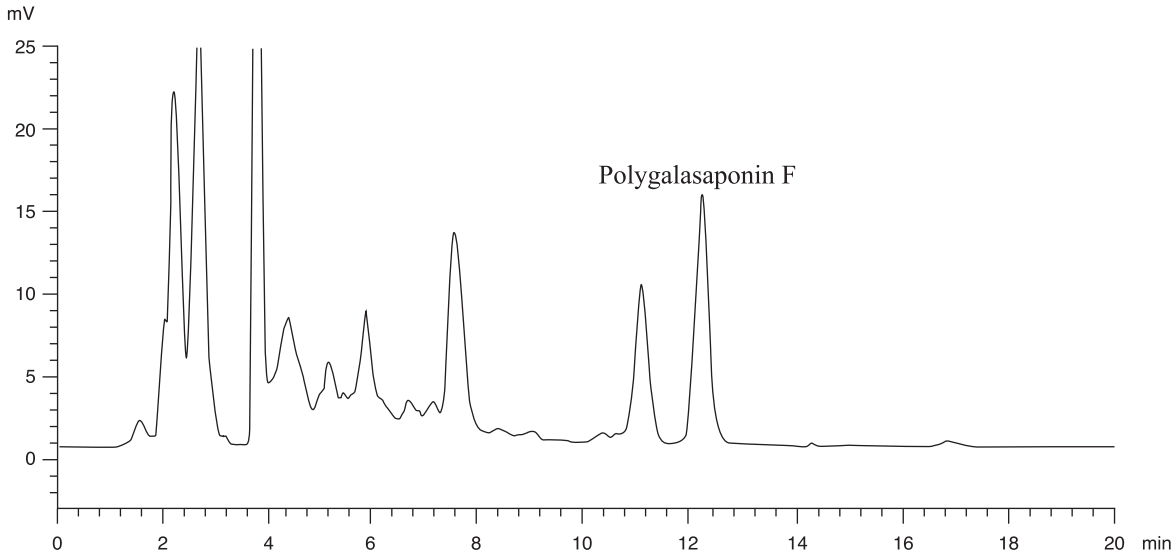

Figure 7 A reference assay chromatogram of Polygalae Japonicae Herba extract