Solidaginis Herba

Figure 1 A photograph of Solidaginis Herba

A. Solidaginis Herba B. A small flowering branch C. Upper surface of leaf
D. Lower surface of leaf E. Magnified capitulum

1. NAMES

Official Name: Solidaginis Herba

Chinese Name: 一枝黃花

Chinese Phonetic Name: Yizhihuanghua

2. SOURCE

Solidaginis Herba is the dried herb of *Solidago decurrens* Lour. (Asteraceae). The herb is collected on flowering and fruiting in autumn, soil removed, then dried under the sun to obtain Solidaginis Herba.

3. DESCRIPTION

30-100 cm long. Rhizome short and thick, pale yellow, with lateral roots and fine, fasciculate rootlets. Stem cylindrical, 2-5 mm in diameter; externally yellowish-green, greyish-brown to dark purplish-red, with a ridge line, pubescent on the upper part; texture fragile, easily broken; fracture fibrous, with pith. Leaves simple, alternate, usually crumpled and broken, when intact flattened out, ovate to lanceolate, 1-9 cm long, 0.3-1.5 cm wide; apex slightly acute or obtuse, margins entire or irregularly serrate; base attenuate-cuneate, merging with the petiole. Capitula about 7 mm in diameter, arranged into a raceme, occasionally with remnants of yellow ligulate florets, usually crumpled and twisted, involucre of 3 layers, ovate-lanceolate. Achenes petty, bearing yellowish-white pappus. Odour slightly aromatic; taste slightly bitter and pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermis consists of 1 layer of rectangular cells, covered with thin cuticle. Cortex relatively narrow; 3-6 layers of collenchymatous cells located in the outer side, cells irregular polygonal or subpolygonal; 2-5 layers of parenchymatous cells located in the inner side, cell shrunk, irregularly subpolygonal. Phloem extremely narrow, interrupted-annulated, fibre bundles present in the outer side, elliptical or crescent, mostly lignified. Xylem consists of lignified cells, vessels scattered singly or in groups. Pith large [Fig. 2 (i)].

Leaf: Upper and lower epidermal cells rectangular, relatively small. Non-glandular hairs located on both upper and lower epidermis. Mesophyll tissue consists of several layers of cells, arrange loosely. Collenchyma located in the inner side of upper and lower epidermis at the vascular bundle of midvein. Vascular bundle 1-3, the middle one relatively large [Fig. 2 (ii)].

Powder

Colour yellowish-brown. Pappus consists of multiseriate non-glandular hairs, margin cells slightly convex. Epidermal cells of leaf polygonal, anticlinal walls irregularly sinuous, stomata anomocytic. Non-glandular hairs consist of 2-3 cells, apical cells frequently atrophic rat-tailed, relatively small, walls thin. Non-glandular hairs on leaf margin consist of 3-7 cells, 180-500 μm long, walls slightly thickened. Fibres scattered or in bundles, relatively long, walls relatively thickened; polychromatic under the polarized microscope. Pollen grains subspherical, 22-33 μm in diameter, exine spiny, spines about 3 μm long, with 3 germinal pores. Vessels bordered-pitted and spiral, 25-72 μm in diameter (Fig. 3).

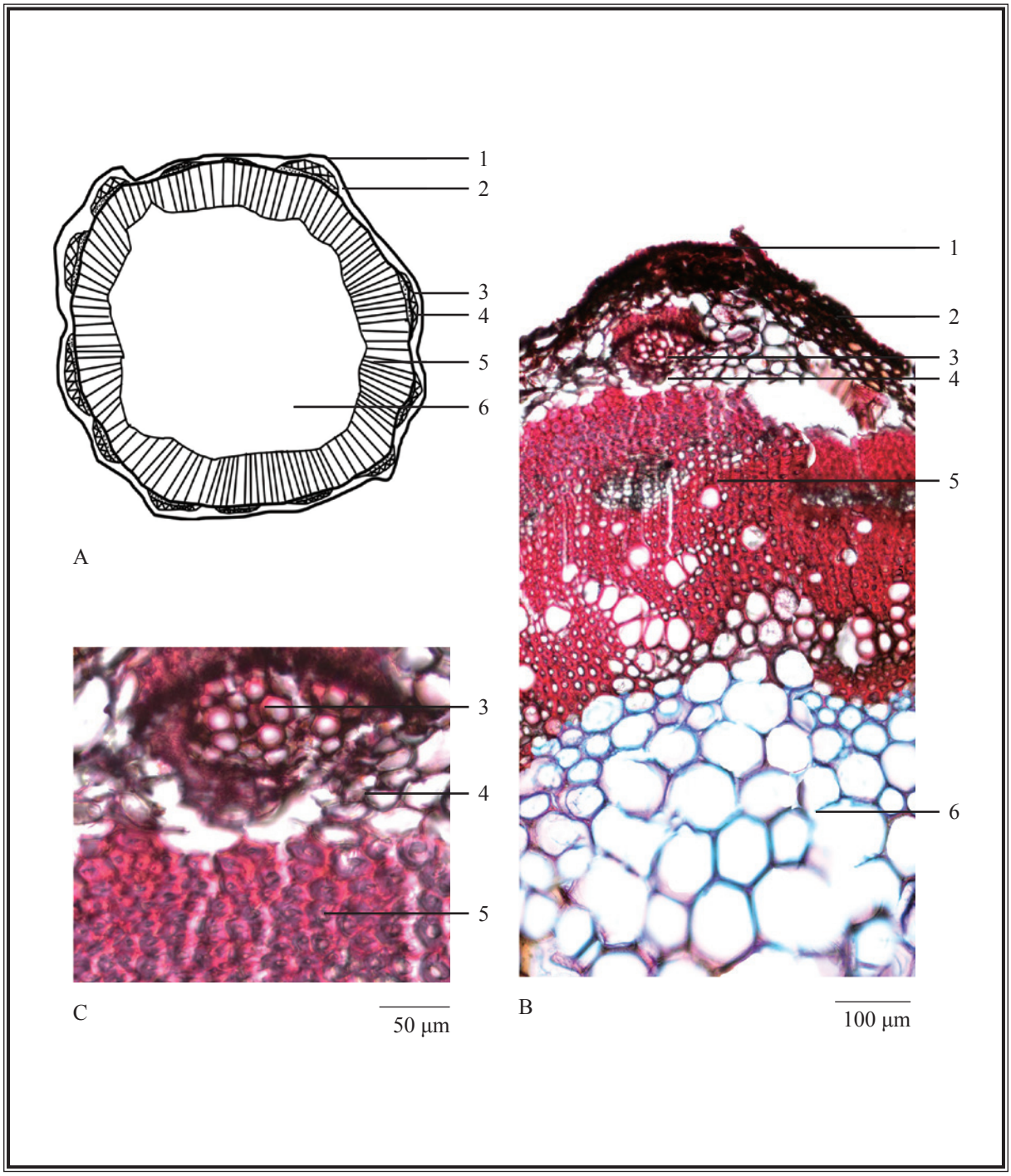

Figure 2 (i) Microscopic features of transverse section of stem of Solidaginis Herba

A. Sketch B. Section illustration C. Phloem fibre

1. Epidermis 2. Cortex 3. Phloem fibre 4. Phloem 5. Xylem 6. Pith

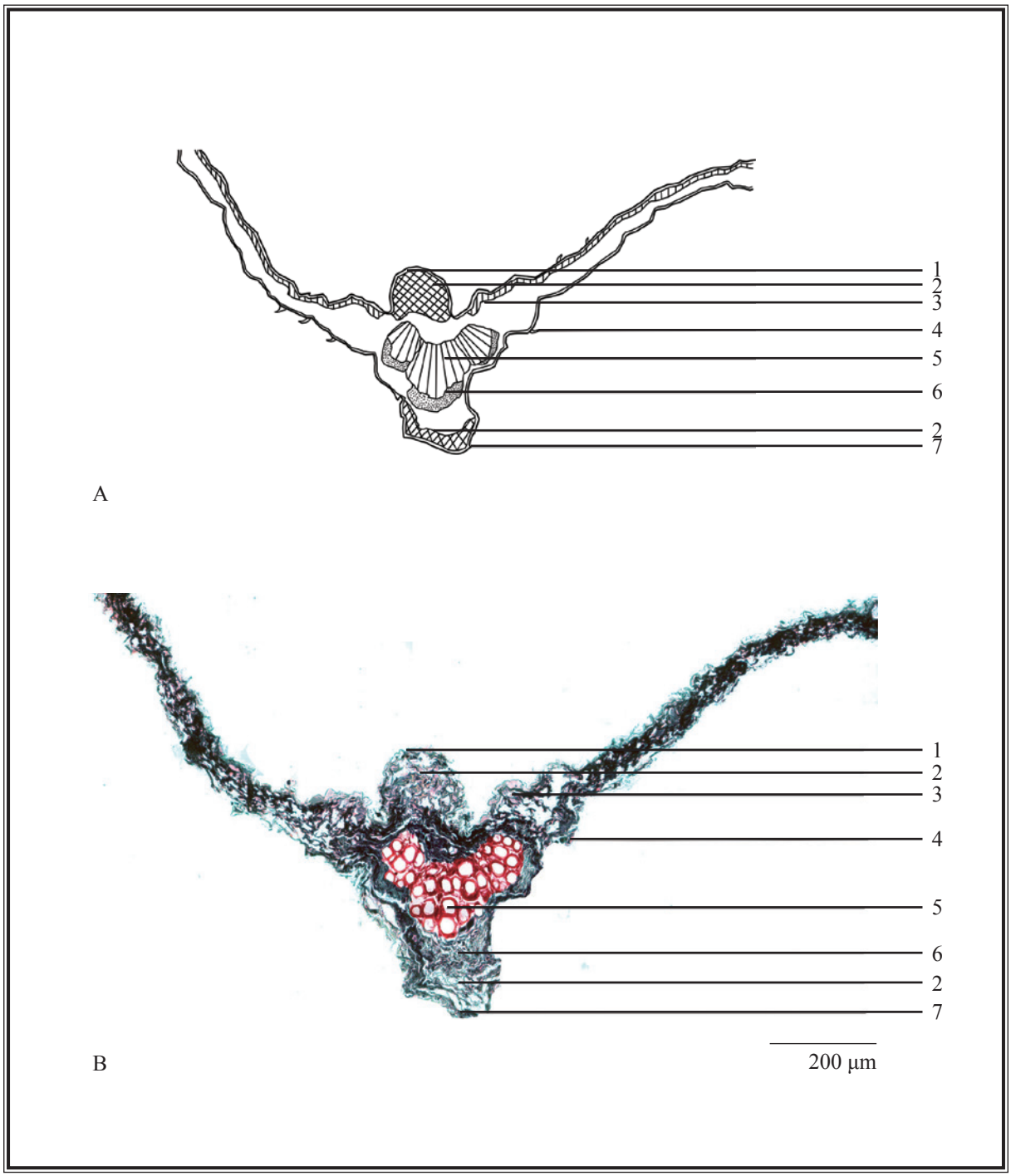

Figure 2 (ii) Microscopic features of transverse section of leaf of Solidaginis Herba

A. Sketch B. Section illustration

1. Upper epidermis 2. Collenchyma 3. Mesophyll tissue 4. Non-glandular hair
5. Xylem 6. Phloem 7. Lower epidermis

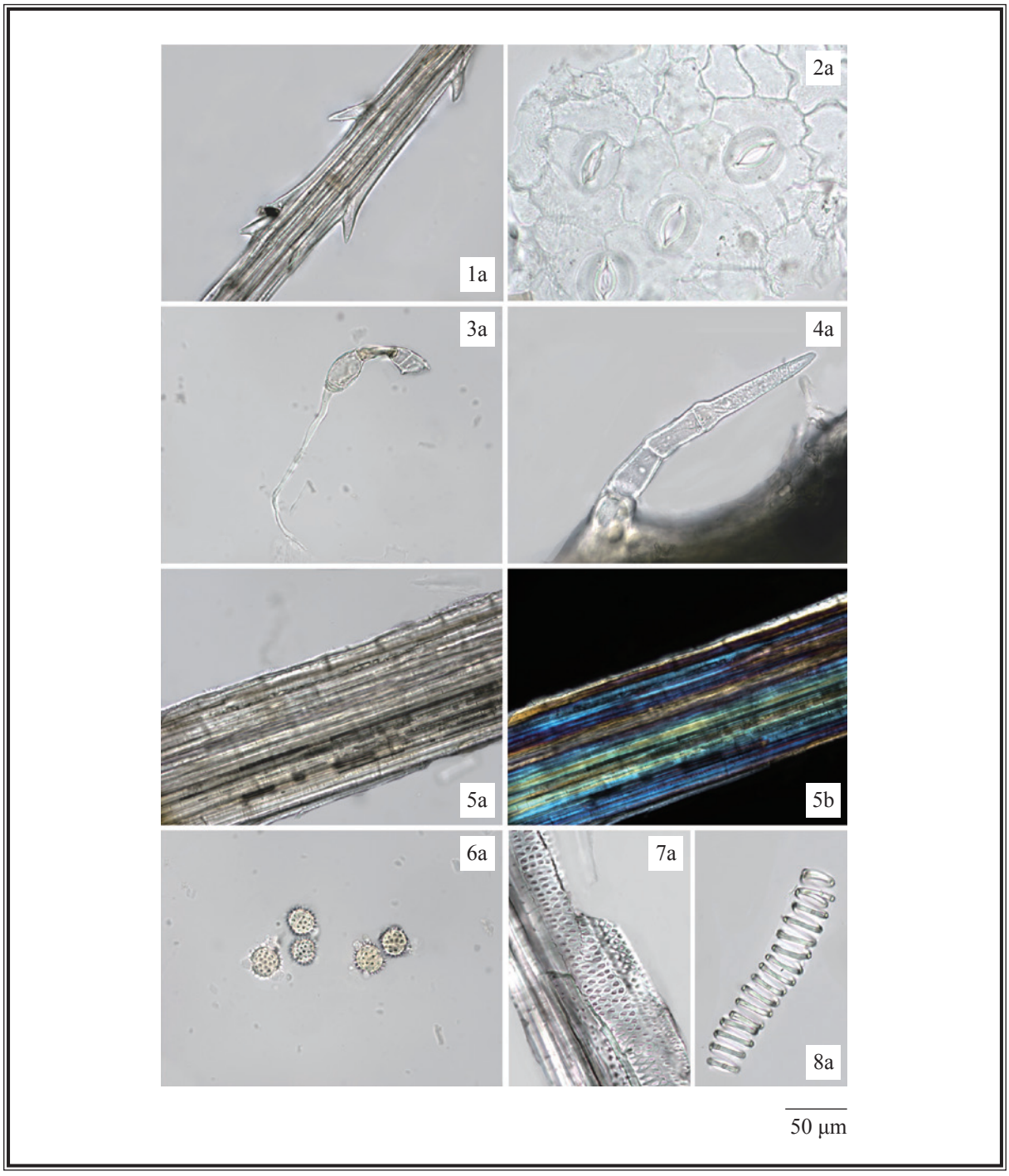

Figure 3 Microscopic features of powder of Solidaginis Herba

1. Pappus 2. Epidermal cells of leaf 3. Non-glandular hair
4. Non-glandular hair on the leaf margin 5. Fibre bundles 6. Pollen grains
7. Bordered-pitted vessels 8. Spiral vessel

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

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

Standard solutions

Chlorogenic acid standard solution

Weigh 1.0 mg of chlorogenic acid CRS (Fig. 4) and dissolve in 1 mL of methanol.

Rutoside standard solution

Weigh 1.0 mg of rutoside CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, butanone, formic acid and water (5:3:1:1, v/v).

Spray reagent

Weigh 1 g of ferric 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 30 mL of methanol. Sonicate (100 W) the mixture for 35 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol.

Procedure

Carry out the method by using a TLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately chlorogenic acid standard solution, rutoside standard solution and the test solution (5 µL each) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the TLC 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 TLC 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 until the spots or bands become visible (about 5-10 min). Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

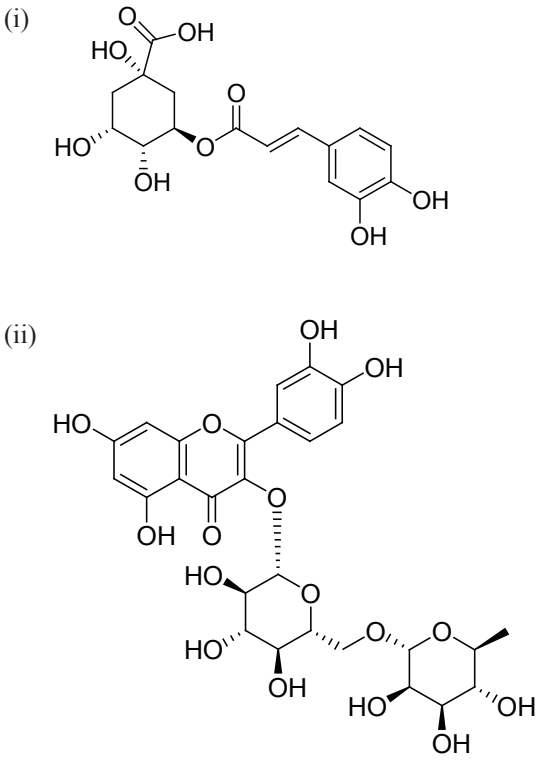

Figure 4 Chemical structures of (i) chlorogenic acid and (ii) rutoside

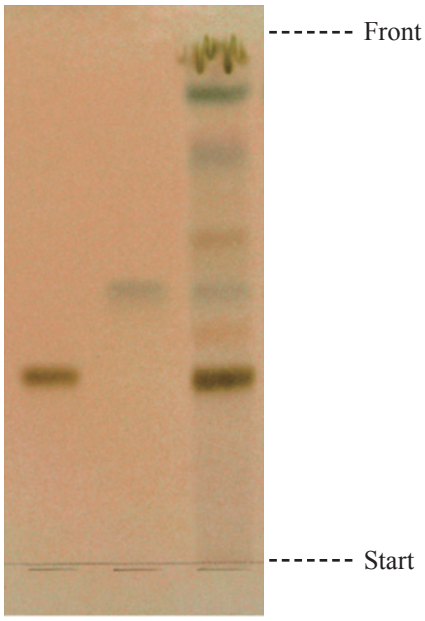

Figure 5 A reference TLC chromatogram of Solidaginis Herba extract observed under visible light after staining

1. Rutoside standard solution 2. Chlorogenic acid 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 chlorogenic acid and rutoside (Fig. 5).

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

Standard solution

Rutoside standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of rutoside CRS and dissolve in 10 mL of ethanol (50%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 25 mL of ethanol (50%). Reflux the mixture for 1.5 h. Cool down to room temperature. Filter and transfer the filtrate to a 25-mL volumetric flask. Make up to the mark with ethanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 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% Phosphoric acid (%, v/v)	Elution
0 – 60	10 → 35	90 → 65	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of rutoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of rutoside should not be more than 5.0%; the RSD of the retention time of rutoside peak should not be more than 2.0%; the column efficiency determined from rutoside peak should not be less than 80000 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 rutoside Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of rutoside peak in the chromatogram of rutoside Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify rutoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of rutoside Std-FP. The retention times of rutoside 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 Solidaginis Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (chlorogenic acid)	0.45	± 0.03
2 (leiocarposide)	0.92	± 0.03
3 (marker, rutoside)	1.00	-
4	1.19	± 0.03

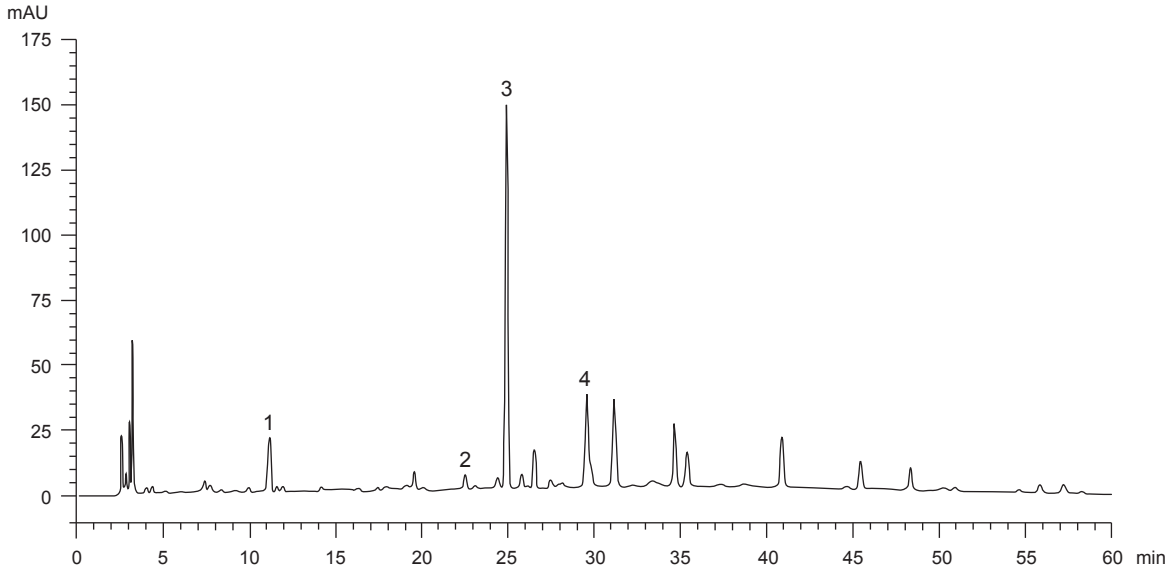

Figure 6 A reference fingerprint chromatogram of Solidaginis Herba extract

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 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 8.0%.
Acid-insoluble ash: not more than 2.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 18.0%.
Ethanol-soluble extractives (hot extraction method): not less than 19.0%.

7. ASSAY

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

Standard solution
Rutoside standard stock solution, Std-Stock (1000 mg/L)
Weigh accurately 2.0 mg of rutoside CRS and dissolve in 2 mL of ethanol (50%).
Rutoside standard solution for assay, Std-AS
Measure accurately the volume of the rutoside Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 5, 10, 25, 50, 75 mg/L for rutoside.

Test solution

Weigh accurately 0.3 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 20 mL of ethanol (50%). Reflux the mixture for 1.5 h. Cool down to room temperature. Filter and transfer the filtrate to a 25-mL volumetric flask. Make up to the mark with ethanol (50%). 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. The mobile phase is a mixture of 0.5% acetic acid and acetonitrile (85:15, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of rutoside Std-AS (25 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of rutoside should not be more than 5.0%; the RSD of the retention time of rutoside peak should not be more than 2.0%; the column efficiency determined from rutoside peak should not be less than 10000 theoretical plates.

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

Limits

The sample contains not less than 0.17% of rutoside ($C_{27}H_{30}O_{16}$), calculated with reference to the dried substance.