Sedi Herba

Figure 1 A photograph of Sedi Herba

A. Sedi Herba B. Magnified image of herb
C. Magnified image of leaves and adventitious roots D. Magnified image of leaf

1. NAMES

Official name: Sedi Herba

Chinese name: 垂盆草

Chinese phonetic name: Chuipencao

2. SOURCE

Sedi Herba is the dried whole plant of *Sedum sarmentosum* Bunge (Crassulaceae). The whole plant is collected in summer and autumn, foreign matter removed, then dried under the sun to obtain Sedi Herba.

3. DESCRIPTION

Stems slender, length varies up to over 20 cm long, some nodes bearing slender adventitious roots. Roots small and short. Leaves whorled in 3, blade oblanceolate to oblong, yellowish-green to green, 0.8-2.7 cm long, 3-9 mm wide, nearly acute at the apex, sharply narrow at the base. Odour slight; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermal cells rectangular, the outer walls thickened. Cortex broad and consists of 10 layers of parenchymatous cells, some of cells containing brown to reddish-brown masses. Vascular cylinder small. Vascular bundles collateral. Phloem located outside xylem, arranged in a ring. Xylem vessels subrounded. Pith triangular, cells polygonal, walls thickened [Fig. 2 (i)].

Leaf: Blade cells usually shrunken. Upper and lower epidermis consist of 1 layer of cells, walls thickened. Mesophyll cells usually shrunken. Vascular bundles collateral, with the phloem cells usually shrunken, vessels subrounded. Stomata sometimes visible on the epidermis [Fig. 2 (ii)].

Powder

Colour pale brown. Upper and lower epidermal cells show a wavy and curved anticlinal wall, with stomata present on both surfaces, mostly of anisocytic type. Cells containing brown to reddish-brown masses occasionally found. Microcrystals of calcium oxalate scattered in parenchymatous cells; bright white under the polarized microscope. Vessels mainly spiral and bordered-pitted vessels (Fig. 3).

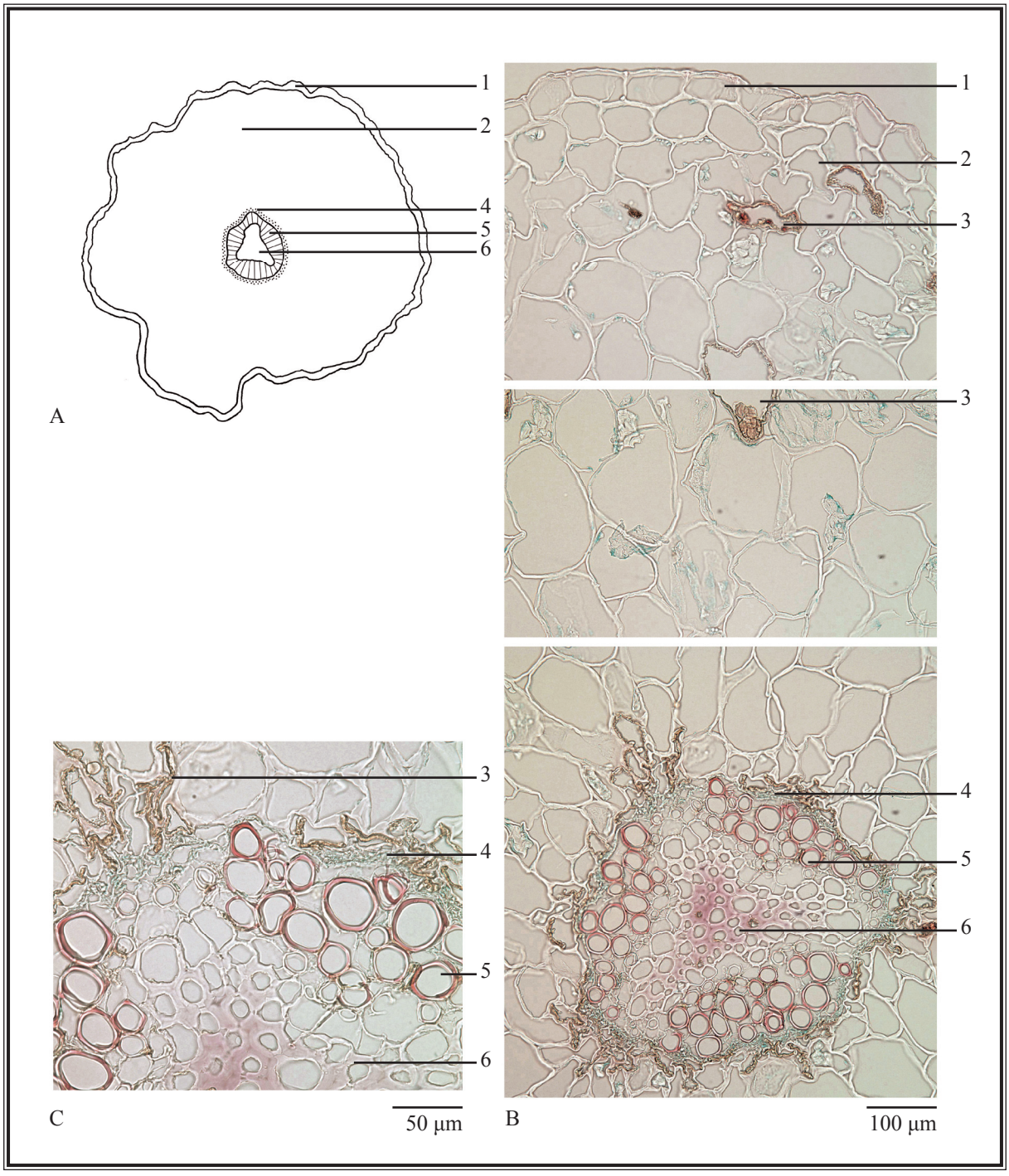

Figure 2 (i) Microscopic features of transverse section of stem of Sedi Herba

A. Sketch B. Section illustration C. Section magnified

1. Epidermis 2. Cortex 3. Cells containing brown to reddish-brown masses
4. Phloem 5. Xylem 6. Pith

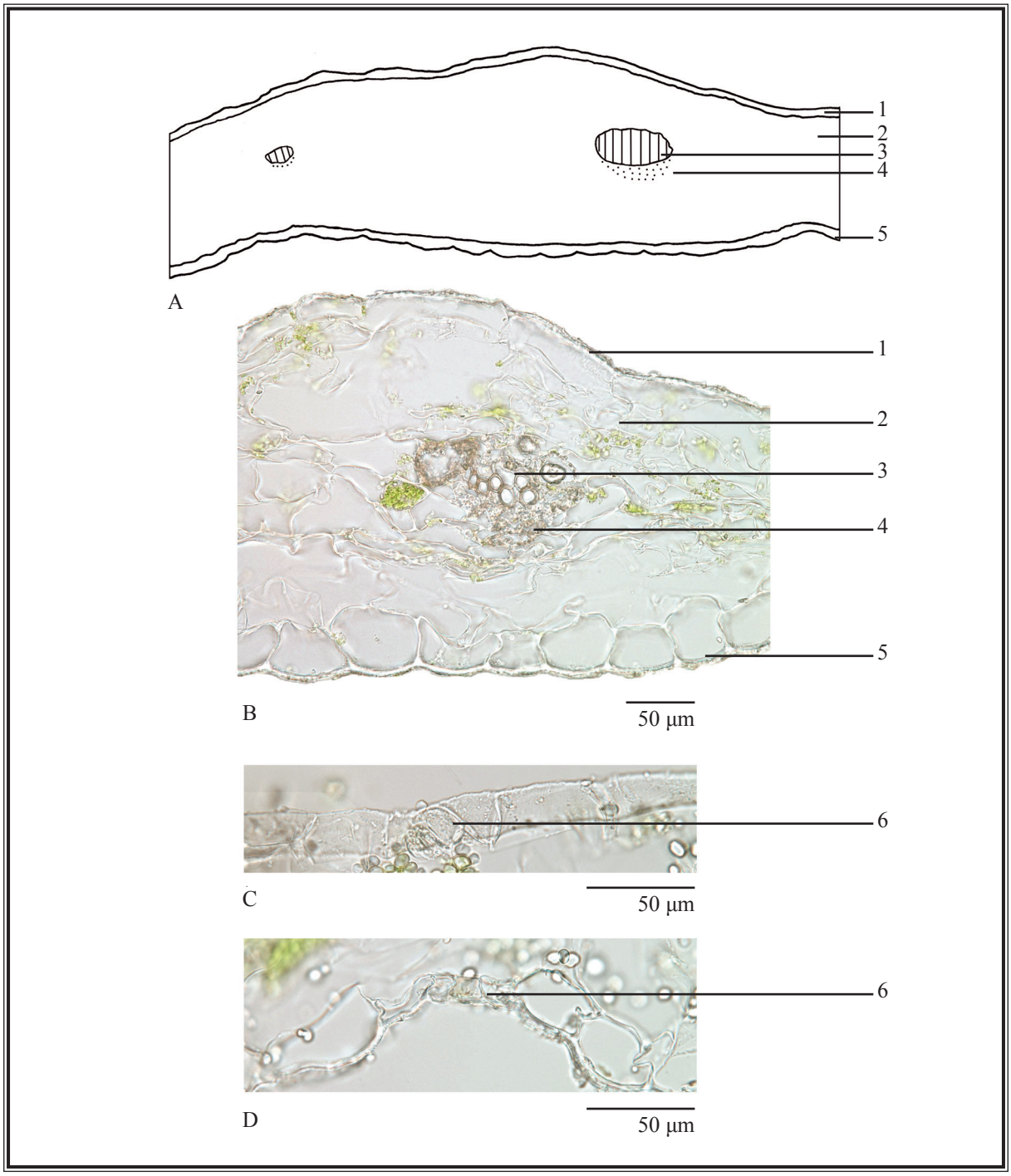

Figure 2 (ii) Microscopic features of transverse section of leaf of Sedi Herba

A. Sketch B. Section illustration (midrib with blade) C. Upper epidermis with stoma
D. Lower epidermis with stoma

1. Upper epidermis 2. Mesophyll 3. Vessel 4. Phloem 5. Lower epidermis
6. Stoma

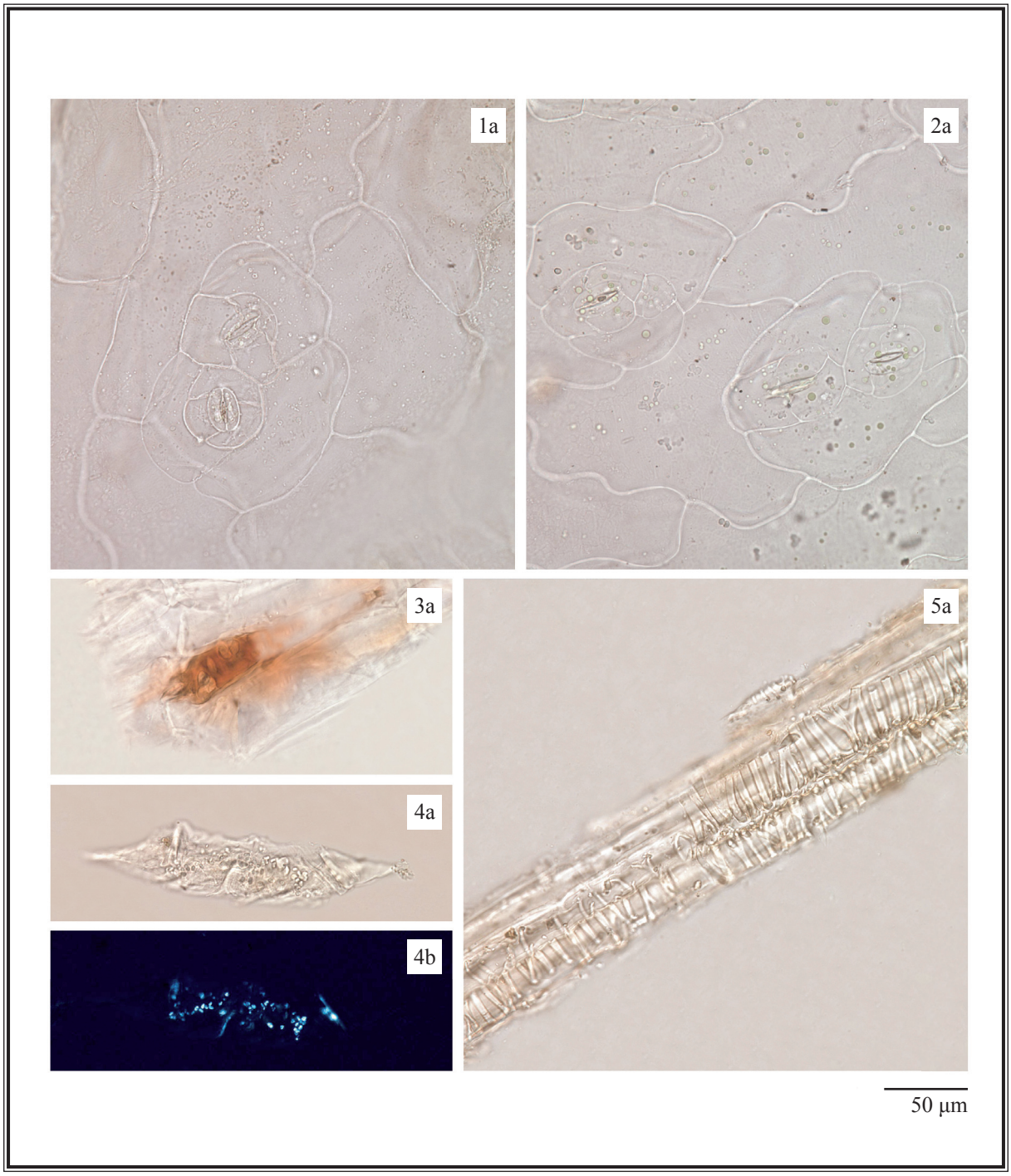

Figure 3 Microscopic features of powder of Sedi Herba

1. Upper epidermal cells of leaf with stomata 2. Lower epidermal cells of leaf with stomata
3. Cells containing reddish-brown to brown masses 4. Microcrystals of calcium oxalate 5. Spiral vessels

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

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

Standard solution

Luteolin 7-O-β-D-glucoside standard solution

Weigh 1.0 mg of luteolin 7-*O*-β-D-glucoside CRS (Fig. 4) and dissolve in 2 mL of methanol (75%).

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 (75%). 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 (75%).

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 luteolin 7-*O*-β-D-glucoside standard solution (1 μL) and the test solution (2 μL) 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 105ºC (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).

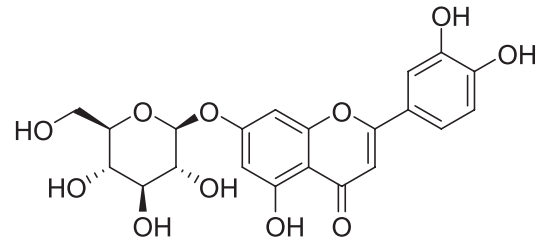

Figure 4 Chemical structure of luteolin 7-*O*-β-D-glucoside

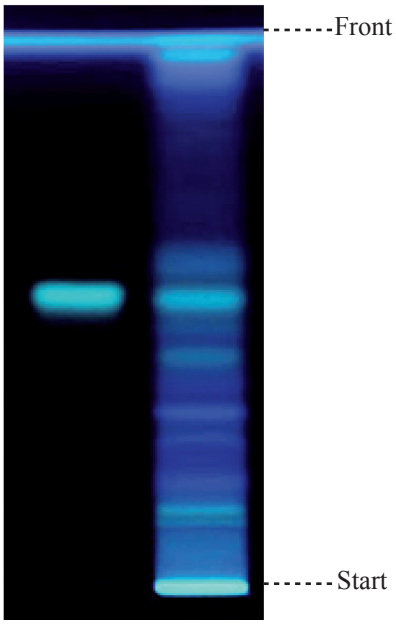

Figure 5 A reference HPTLC chromatogram of Sedi Herba extract observed under UV light (366 nm) after staining

1. Luteolin 7-*O*-β-D-glucoside 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 luteolin 7-*O*-β-D-glucoside (Fig. 5).

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

Standard solution

Luteolin 7-O-β-D-glucoside standard solution for fingerprinting, Std-FP (25 mg/L)

Weigh 2.5 mg of luteolin 7-*O*-β-D-glucoside CRS and dissolve in 100 mL of methanol (75%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (75%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction one more time. Combine the filtrates and make up to the mark with methanol (75%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (348 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.5 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	95 → 86	5 → 14	linear gradient
35 – 55	86 → 77	14 → 23	linear gradient
55 – 60	77 → 55	23 → 45	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of luteolin 7-*O*-β-D-glucoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of luteolin 7-*O*-β-D-glucoside should not be more than 5.0%; the RSD of the retention time of luteolin 7-*O*-β-D-glucoside peak should not be more than 2.0%; the column efficiency determined from luteolin 7-*O*-β-D-glucoside peak should not be less than 40000 theoretical plates.

The *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject luteolin 7-*O*-β-D-glucoside Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of luteolin 7-*O*-β-D-glucoside peak in the chromatogram of luteolin 7-*O*-β-D-glucoside Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify luteolin 7-*O*-β-D-glucoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of luteolin 7-*O*-β-D-glucoside Std-FP. The retention times of luteolin 7-*O*-β-D-glucoside 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 five characteristic peaks of Sedi Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Sedi Herba extract

Peak No.	RRT	Acceptable Range
1	0.44	± 0.03
2	0.65	± 0.03
3	0.81	± 0.03
4	0.92	± 0.03
5 (marker, luteolin 7-*O*-β-D-glucoside)	1.00	-

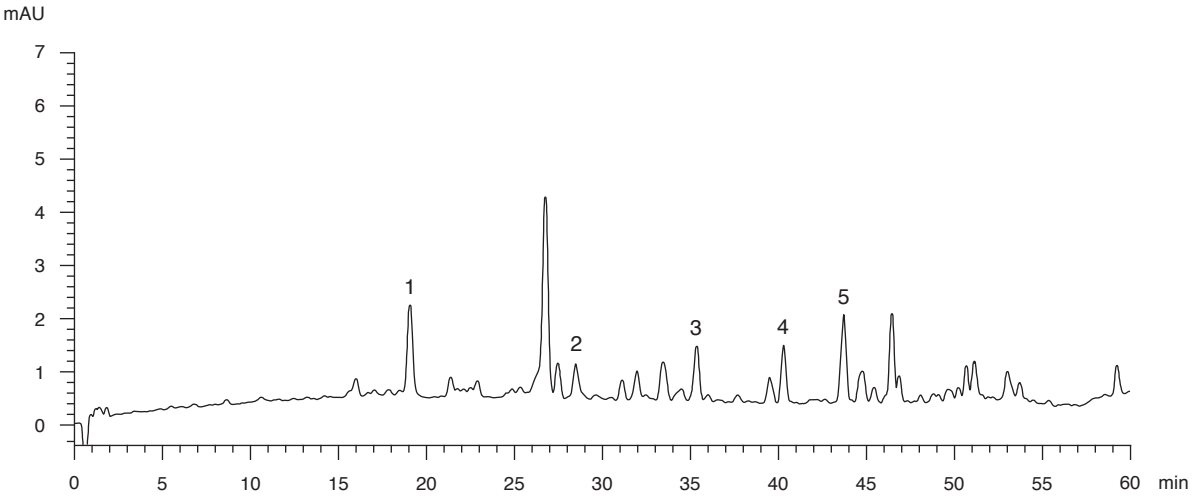

Figure 6 A reference fingerprint chromatogram of Sedi Herba extract

For positive identification, the sample must give the above five 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 5.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 17.5%.
Acid-insoluble ash: not more than 6.0%.

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 19.0%.
Ethanol-soluble extractives (cold extraction method): not less than 10.0%.

7. ASSAY

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

Standard solution

Luteolin 7-O-β-D-glucoside standard stock solution, Std-Stock (250 mg/L)

Weigh accurately 2.5 mg of luteolin 7-*O*-β-D-glucoside CRS and dissolve in 10 mL of methanol (75%).

Luteolin 7-O-β-D-glucoside standard solution for assay, Std-AS

Measure accurately the volume of the luteolin 7-*O*-β-D-glucoside Std-Stock, dilute with methanol (75%) to produce a series of solutions of 2.5, 12.5, 25, 50, 100 mg/L for luteolin 7-*O*-β-D-glucoside.

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 (75%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction one more time. Combine the filtrates and make up to the mark with methanol (75%). Filter through a 0.45-µm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (348 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 1.5 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	95 → 86	5 → 14	linear gradient
35 – 55	86 → 77	14 → 23	linear gradient
55 – 60	77 → 55	23 → 45	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of luteolin 7-*O*-β-D-glucoside Std-AS (25 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of luteolin 7-*O*-β-D-glucoside should not be more than 5.0%; the RSD of the retention time of luteolin 7-*O*-β-D-glucoside peak should not be more than 2.0%; the column efficiency determined from luteolin 7-*O*-β-D-glucoside peak should not be less than 40000 theoretical plates.

The *R* value between luteolin 7-*O*-β-D-glucoside 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 luteolin 7-*O*-β-D-glucoside Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of luteolin 7-*O*-β-D-glucoside against the corresponding concentrations of luteolin 7-*O*-β-D-glucoside 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 luteolin 7-*O*-β-D-glucoside peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of luteolin 7-*O*-β-D-glucoside Std-AS. The retention times of luteolin 7-*O*-β-D-glucoside 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 luteolin 7-*O*-β-D-glucoside in the test solution, and calculate the percentage content of luteolin 7-*O*-β-D-glucoside in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.13% of luteolin 7-*O*-β-D-glucoside ($C_{21}H_{20}O_{11}$), calculated with reference to the dried substance.

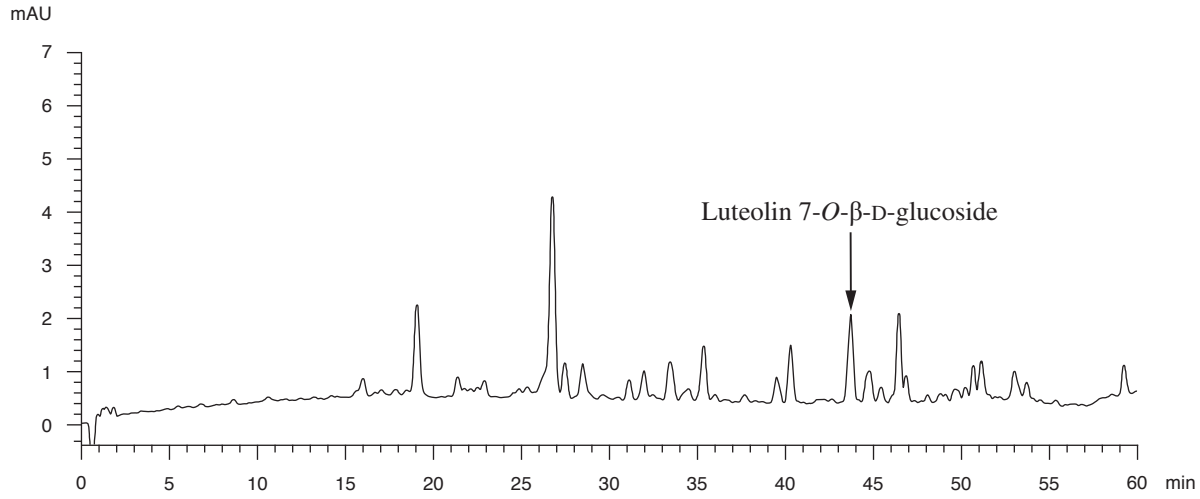

Figure 7 A reference assay chromatogram of Sedi Herba extract