Plantaginis Herba

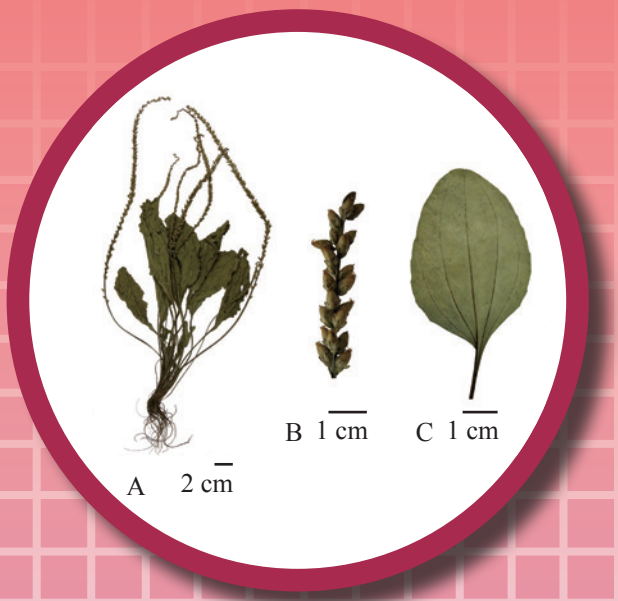

Figure 1 (i) A photograph of dried herb of *Plantago asiatica* L.

A. Whole plant B. Magnified image of capsules on spike
C. Magnified image of leaf

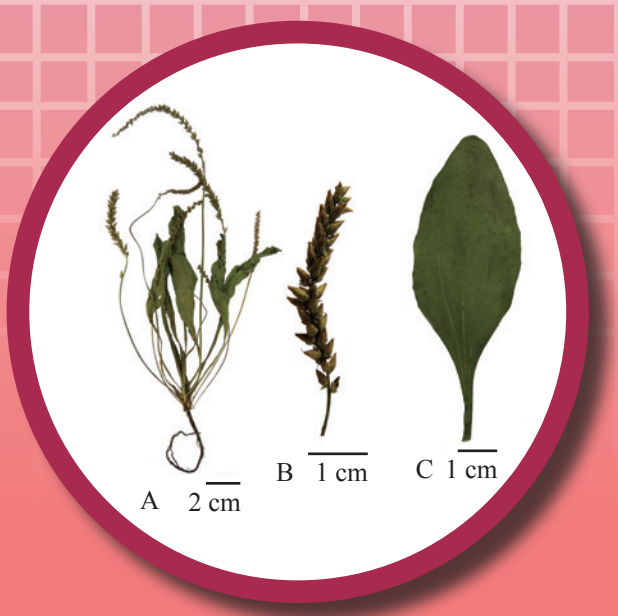

Figure 1 (ii) A photograph of dried herb of *Plantago depressa* Willd.

A. Whole plant B. Magnified image of capsules on spike
C. Magnified image of leaf

1. NAMES

Official Name: Plantaginis Herba

Chinese Name: 車前草

Chinese Phonetic Name: Cheqiancao

2. SOURCE

Plantaginis Herba is the dried herb of *Plantago asiatica* L. or *Plantago depressa* Willd. (Plantaginaceae). The whole plant is collected in summer, soil removed, then dried under the sun to obtain Plantaginis Herba.

3. DESCRIPTION

***Plantago asiatica* L.:** Roots fibrous, fascicled. Leaves basal, petiolate long; lamina crumpled, when whole, ovate-elliptic or broadly ovate, 3-13 cm long, 2.1-8 cm wide; externally greyish-green or blackish-green, with 5-7 distinct veins; obtuse or short acute at the apex, margin entire or irregularly sinuate-dentate. Spike several, continuous, with a long scape. Capsules ellipsoid, circumscissile near base, calyx persistent. Odour slightly aromatic; taste slightly bitter [Fig. 1 (i)].

***Plantago depressa* Willd.:** Main roots straight and long. Lamina relatively narrow, long-elliptic or elliptic-lanceolate, 5-10 cm long, 2-3 cm wide [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

Root

***Plantago asiatica* L.:** Epidermis consists of 1 layer of subrounded cells, covered with cuticle. Cortex broad, cells subrounded to elongated. Endodermis distinct. Phloem narrow, surrounding the xylem. Xylem broad, vessels arranged radially [Fig. 2 (i)].

***Plantago depressa* Willd.:** Cork consists of several layers of cells, rectangular to elongated, tangentially extended, closely arranged. Cortex relatively narrow. Phloem broad, with clefts. Cambium distinct, undulated. Xylem broad, vessels arranged radially [Fig. 2 (ii)].

Leaf

***Plantago asiatica* L.:** Upper epidermis consists of 1 layer of subsquare to rectangular cells. Mesophyll cells rounded to subrounded. Vascular bundle collateral. Phloem cells subrounded, arranged loosely. Xylem vessels radially arranged. Collenchymatous cells subrounded, found beneath the lower epidermis and in the peripheric part of vascular bundle. Lower epidermis consists of 1 layer of square to subsquare cells, orderly arranged [Fig. 3 (i)].

***Plantago depressa* Willd.:** Upper epidermis consists of 1 layer of rectangular to elongated cells [Fig. 3 (ii)].

Powder

***Plantago asiatica* L.:** Colour greyish-green or brownish-yellow. Upper epidermal cells subrectangular, with cuticle striations. Lower epidermal cells subrectangular, walls deeply wavy and undulated. Stomata found in both surfaces, anomocytic, with 3-4 subsidiary cells. Glandular hairs colourless to yellowish-brown, with a unicellular stalk and 2-celled head, subrounded, biseriate. Non-glandular hairs from petiole and bract abundant, usually broken, intact hair composed of dozens of cells, cells sub-cylindrical, slender, usually wall shrunken or fused. Non-glandular hairs of scape rare, 2-6 cells, walls relatively thickened. Pericarp cells irregular in shape in surface view, anticlinal walls deeply undulant, lignified, pits sparse. Anticlinal walls of epidermal cells of sepal deeply undulate, usually extended. Pollen grains colourless to pale yellow, subrounded, 17-33 μm in diameter, with warty sculptures on the surface. Fragment of endothecium polygonal, with dense cylindrical striations. Fibres slender, with relatively thickened walls, slightly lignified. Vessels mainly reticulate and spiral, 8-47 μm in diameter [Fig. 4 (i)].

***Plantago depressa* Willd.:** Upper epidermal cells subrectangular, walls wavy and undulated. Non-glandular hairs from the head of root abundantly, usually broken, intact hair composed of dozens of cells, cells sub-cylindrical, slender, usually shrunken. Non-glandular hairs from scape and surface of leaf rare, 4-8 cells, apical cell relatively small, trianglular, walls relatively thickened. Pollen grains 16-30 μm in diameter. Fragment of endothecium rare. Vessels 6-55 μm in diameter [Fig. 4 (ii)].

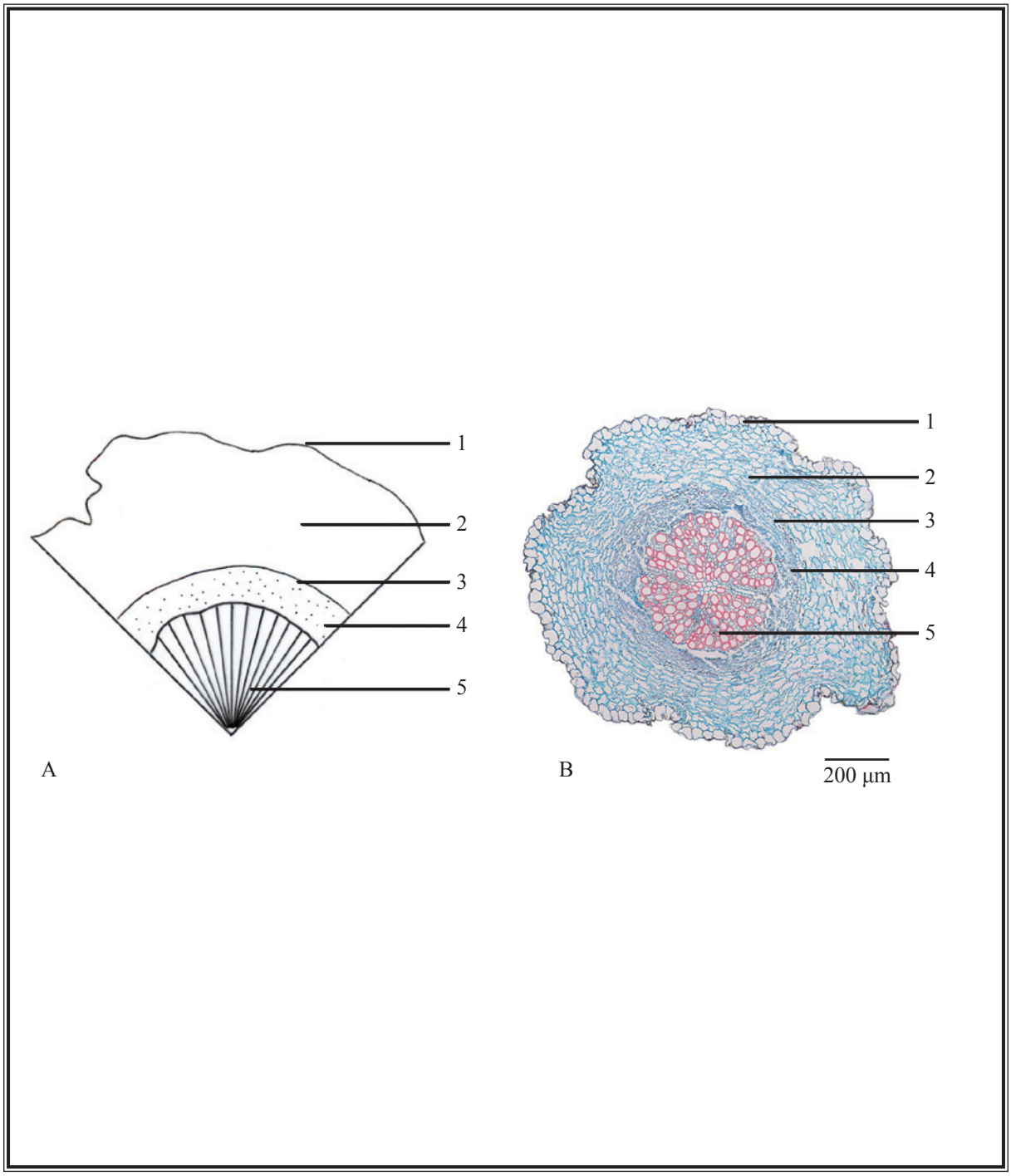

Figure 2 (i) Microscopic features of the transverse section of dried root of *Plantago asiatica* L.

A. Sketch B. Section illustration

1. Epidermis 2. Cortex 3. Endodermis 4. Phloem 5. Xylem

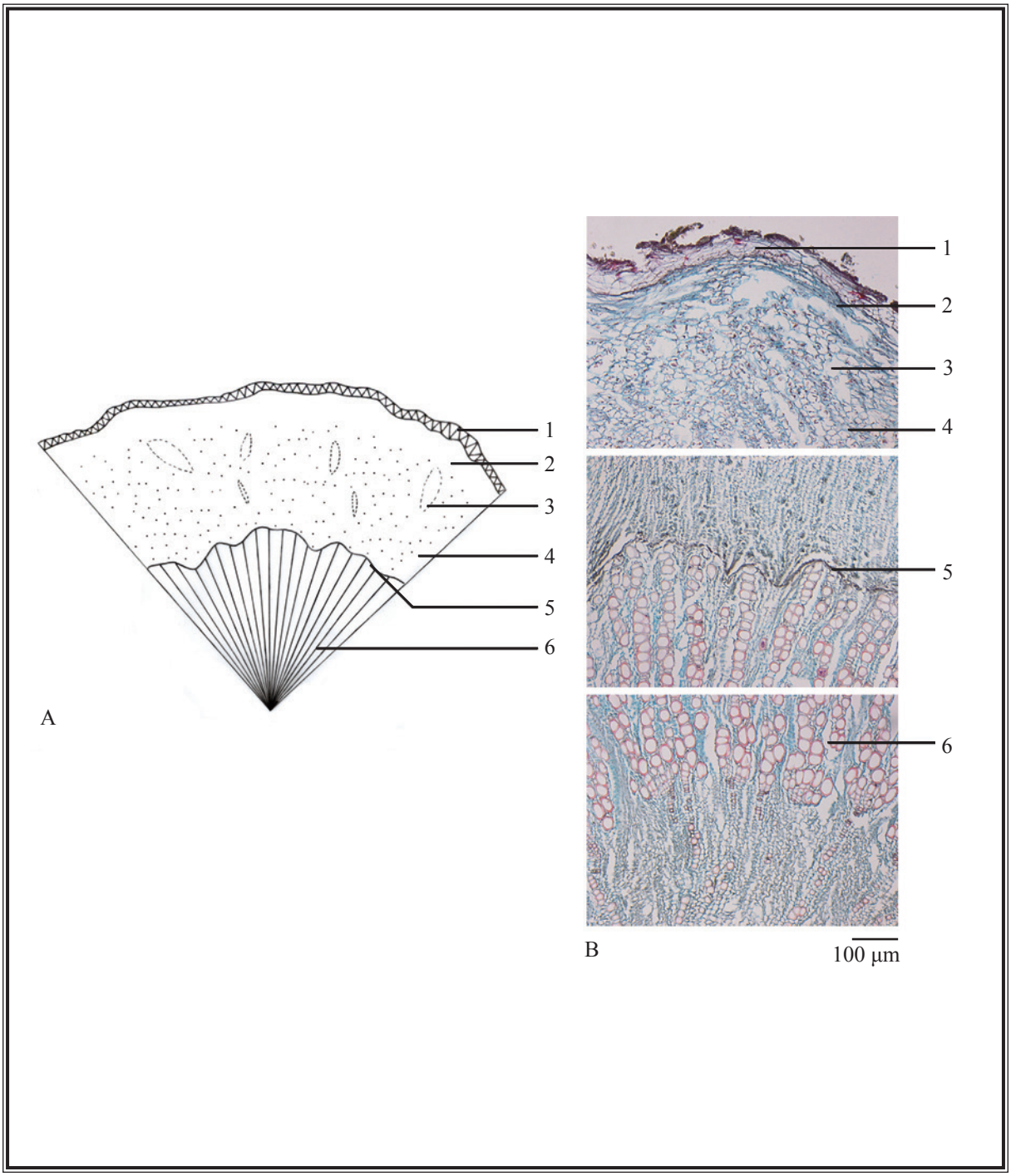

Figure 2 (ii) Microscopic features of the transverse section of dried root of *Plantago depressa* Willd.

A. Sketch B. Section illustration

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

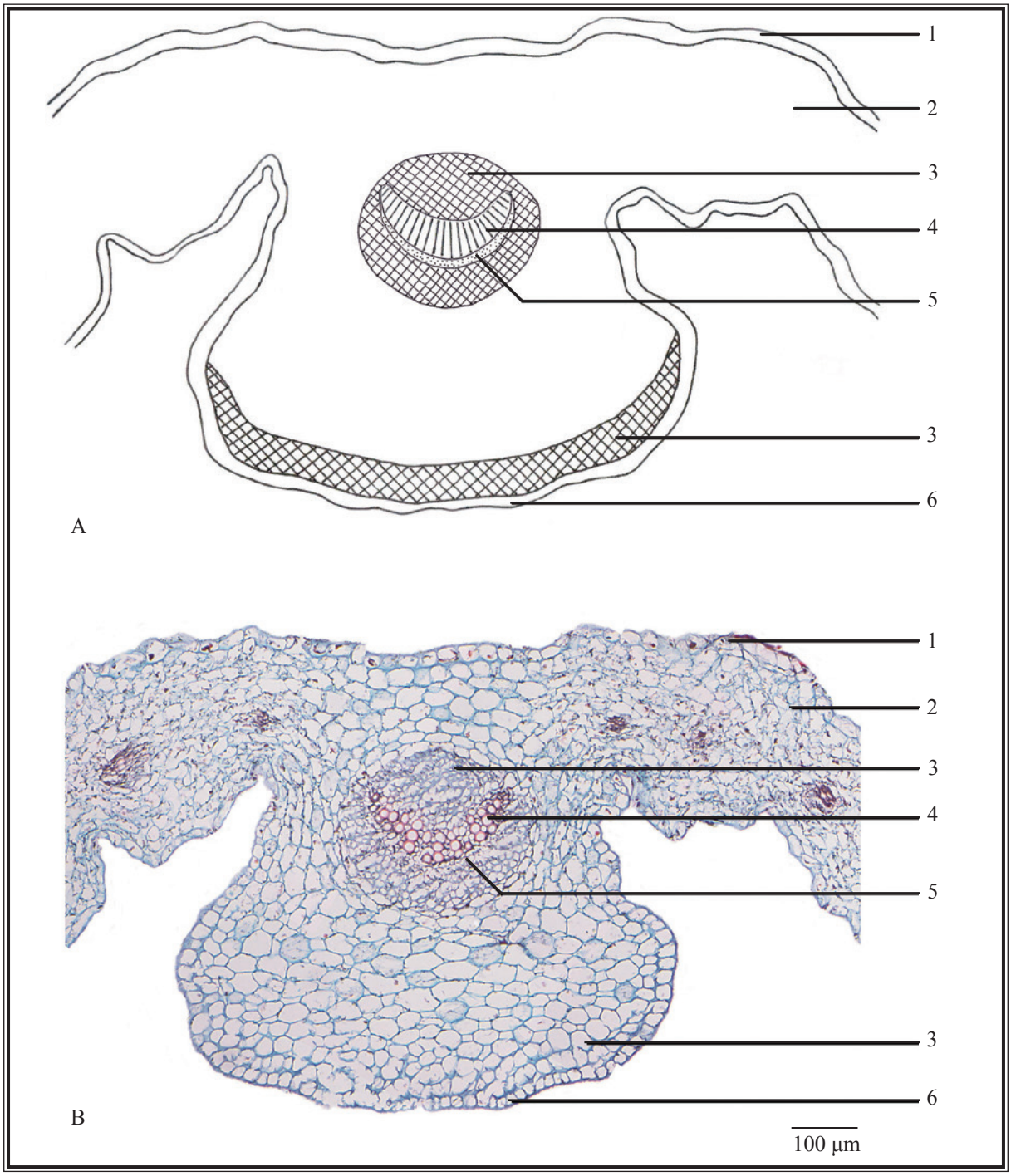

Figure 3 (i) Microscopic features of the transverse section of dried leaf of *Plantago asiatica* L.

A. Sketch B. Section illustration

1. Upper epidermis 2. Mesophyll 3. Collenchyma 4. Xylem 5. Phloem 6. Lower epidermis

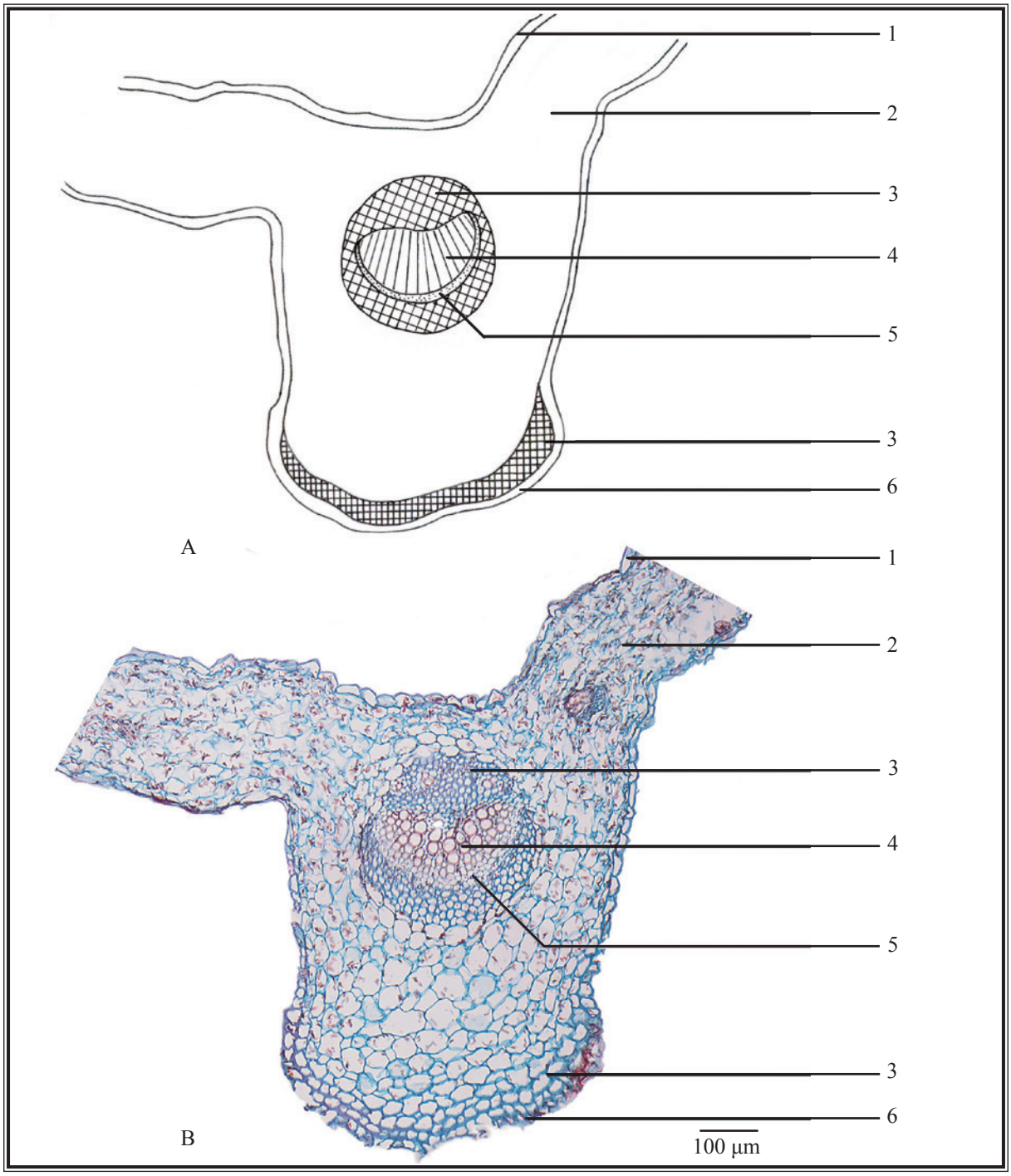

Figure 3 (ii) Microscopic features of the transverse section of dried leaf of *Plantago depressa* Willd.

A. Sketch B. Section illustration

1. Upper epidermis 2. Mesophyll 3. Collenchyma 4. Xylem 5. Phloem 6. Lower epidermis

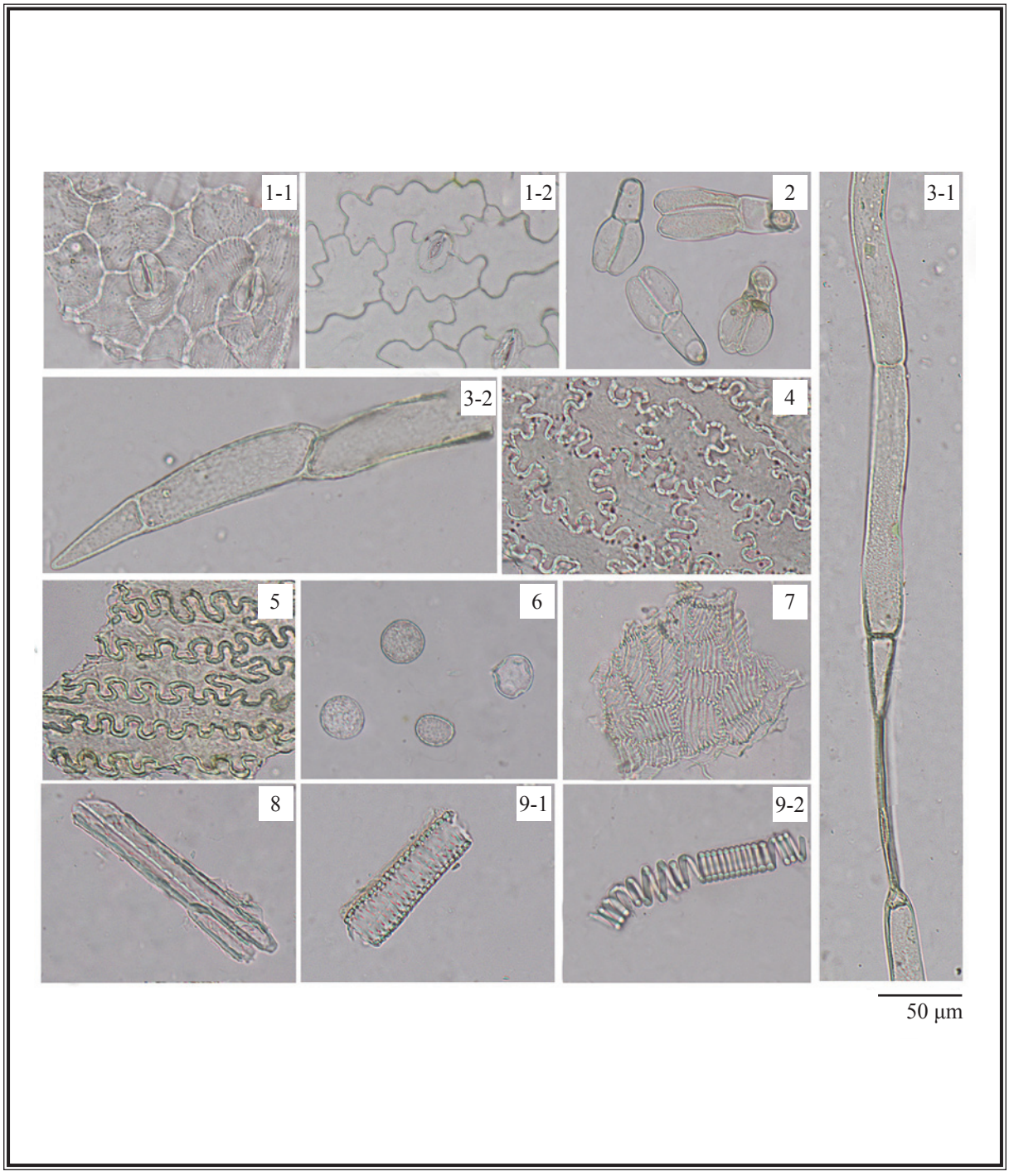

Figure 4 (i) Microscopic features of powder of dried herb of *Plantago asiatica* L. (under the light microscope)

1. Epidermal cells and stomata of leaf (1-1 upper epidermal cells, 1-2 lower epidermal cells)
2. Glandular hairs 3. Non-glandular hairs (3-1 petiole and bract, 3-2 scape) 4. Pericarp cells
5. Epidermal cells of sepal 6. Pollen grains 7. Fragment of endothecium 8. Fibres
9. Vessels (9-1 reticulate vessel, 9-2 spiral vessel)

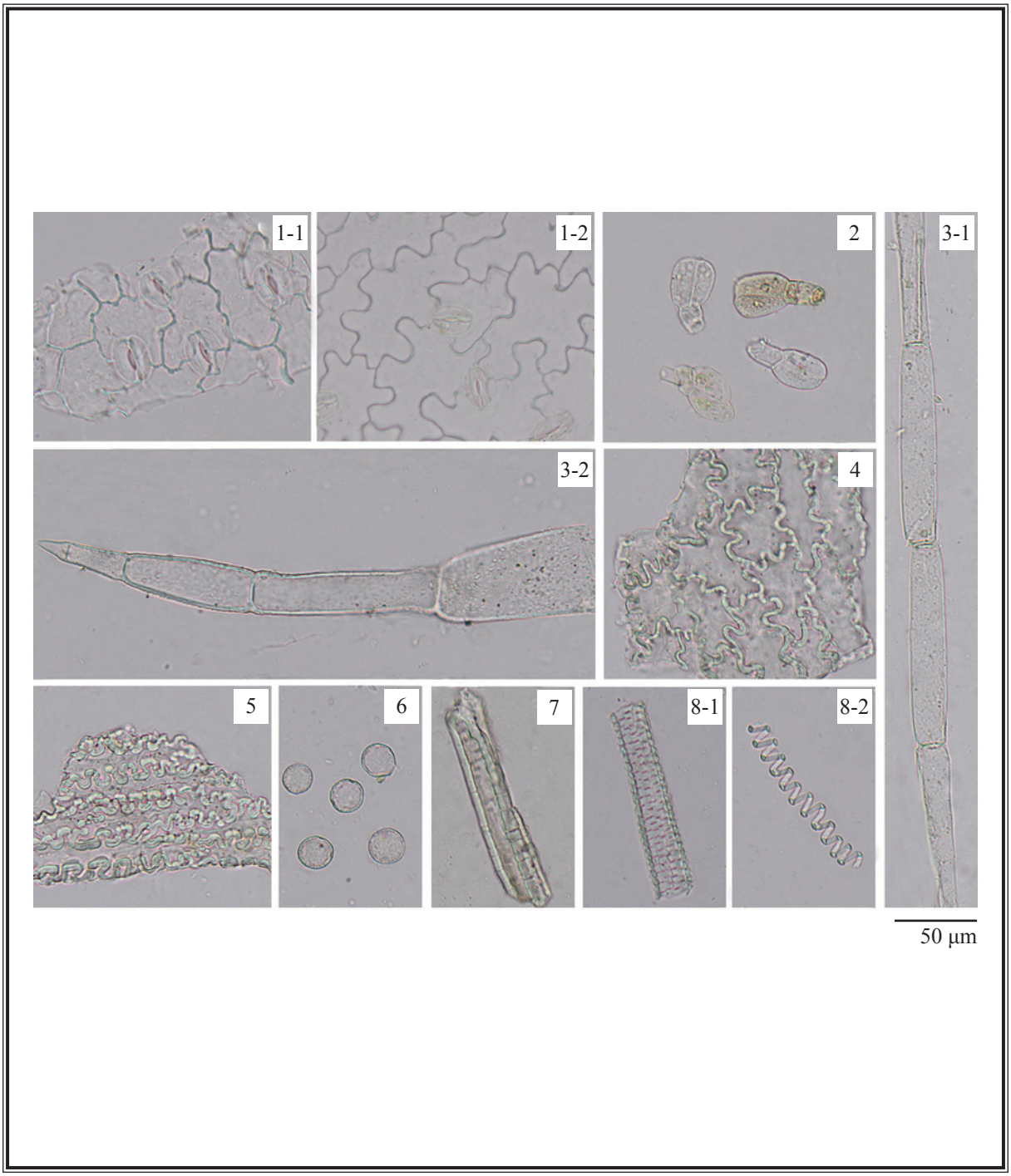

Figure 4 (ii) Microscopic features of powder of dried herb of *Plantago depressa* Willd. (under the light microscope)

1. Epidermal cells and stomata of leaf (1-1 upper epidermal cells, 1-2 lower epidermal cells)
2. Glandular hairs 3. Non-glandular hair (3-1 head of root, 3-2 scape and surface of leaf)
4. Pericarp cells 5. Epidermal cells of sepal 6. Pollen grains 7. Fibre
8. Vessels (8-1 reticulate vessel, 8-2 spiral vessel)

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

Standard solutions

Acteoside standard solution

Weigh 1.0 mg of acteoside CRS (Fig. 5) and dissolve in 1 mL of methanol (60%).

Plantamajoside standard solution

Weigh 1.0 mg of plantamajoside CRS (Fig. 5) and dissolve in 1 mL of methanol (60%) containing 0.05% phosphoric acid.

Developing solvent system

Prepare a mixture of ethyl acetate, ethanol, formic acid and water (18:3:1.5:1, v/v).

Test solution

Weigh 2.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (60%). Sonicate (350 W) the mixture for 15 min. Centrifuge at about 4000 × *g* for 5 min. 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 acteoside standard solution (2 μL), plantamajoside standard solution (5 μL) and the test solution (3-5 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 6 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

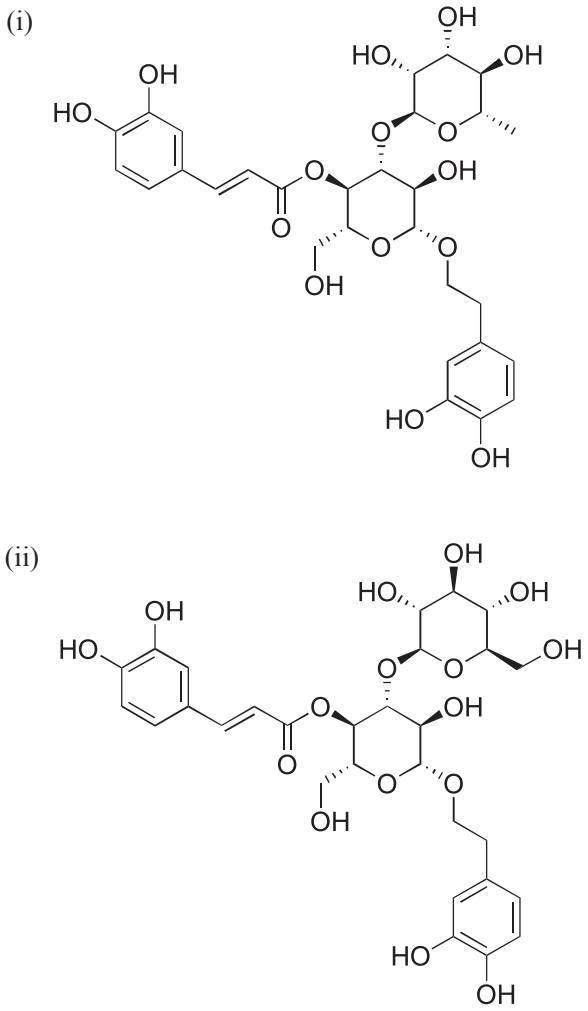

Figure 5 Chemical structures of (i) acteoside and (ii) plantamajoside

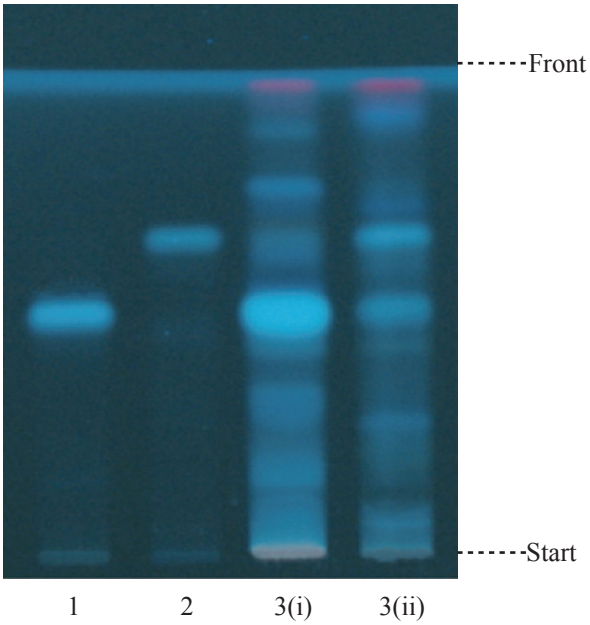

Figure 6 A reference HPTLC chromatogram of Plantaginis Herba extract observed under UV light (366 nm)

1. Plantamajoside standard solution 2. Acteoside standard solution
3. Test solution of
(i) dried herb of *Plantago asiatica* L.
(ii) dried herb of *Plantago depressa* Willd.

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 acteoside and plantamajoside (Fig. 6).

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

(I) Dried herb of *Plantago asiatica* L.

Standard solutions

Acteoside standard solution for fingerprinting, Std-FP (160 mg/L)

Weigh 1.6 mg of acteoside CRS and dissolve in 10 mL of methanol (60%).

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

Weigh 1.0 mg of plantamajoside CRS and dissolve in 10 mL of methanol (60%) containing 0.05% phosphoric acid.

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 (60%). Sonicate (100 W) the mixture for 15 min. Centrifuge at about 4000 × *g* for 10 min. Filter through a 0.45-µm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (330 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 40ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.05% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	88 → 87	12 → 13	linear gradient
35 – 45	87 → 85	13 → 15	linear gradient
45 – 60	85 → 80	15 → 20	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of acteoside Std-FP and plantamajoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of acteoside and plantamajoside should not be more than 5.0%; the RSD of the retention times of acteoside and plantamajoside peaks should not be more than 2.0%; the column efficiencies determined from acteoside and plantamajoside peaks should not be less than 30000 and 13000 theoretical plates respectively.

The *R* value between peak 2 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 1.0 (Fig. 7).

Procedure

Separately inject acteoside Std-FP, plantamajoside Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of acteoside and plantamajoside peaks in the chromatograms of acteoside Std-FP, plantamajoside Std-FP and the retention times of the six characteristic peaks (Fig. 7) in the chromatogram of the test solution. Identify acteoside and plantamajoside peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of acteoside Std-FP and plantamajoside Std-FP. The retention times of acteoside and plantamajoside 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 dried herb of *Plantago asiatica* L. extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of dried herb of *Plantago asiatica* L. extract

Peak No.	RRT	Acceptable Range
1	0.96	± 0.03
2 (marker, plantamajoside)	1.00	-
3	1.36	± 0.03
4 (acteoside)	1.46	± 0.03
5	1.51	± 0.03
6	1.83	± 0.07

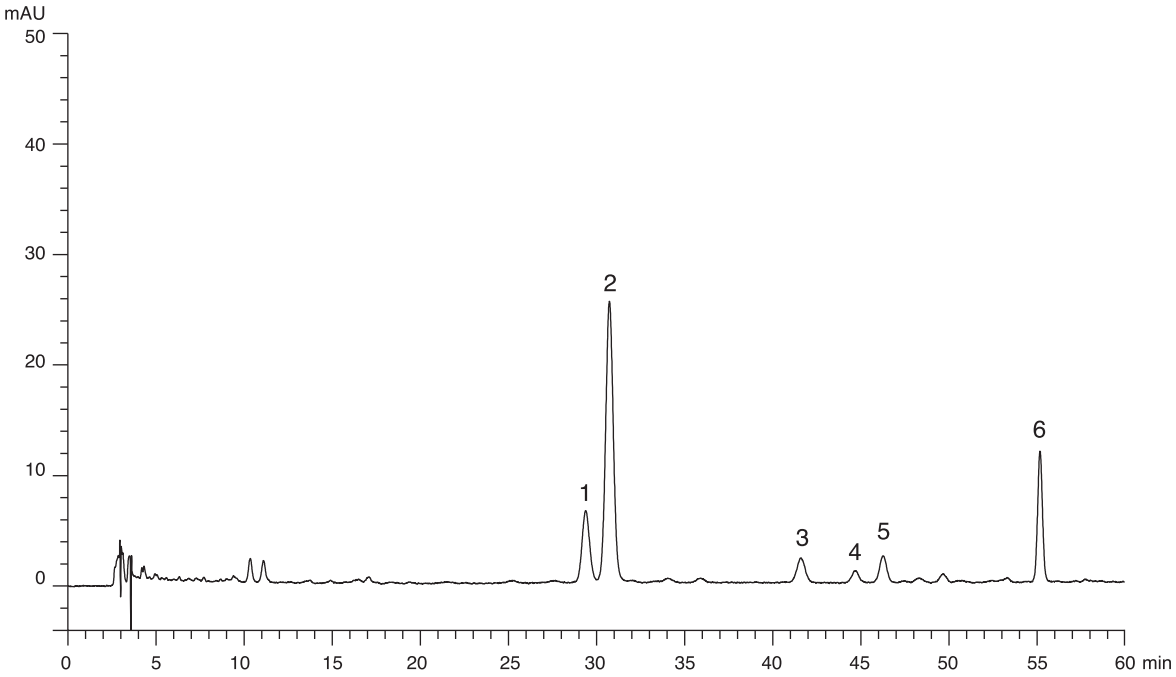

Figure 7 A reference fingerprint chromatogram of dried herb of *Plantago asiatica* L. extract

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. 7).

(II) Dried herb of *Plantago depressa* Willd.

Standard solutions

Acteoside standard solution for fingerprinting, Std-FP (160 mg/L)

Weigh 1.6 mg of acteoside CRS and dissolve in 10 mL of methanol (60%).

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

Weigh 1.0 mg of plantamajoside CRS and dissolve in 10 mL of methanol (60%) containing 0.05% phosphoric acid.

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 (60%). Sonicate (100 W) the mixture for 15 min. Centrifuge at about 4000 × *g* for 10 min. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (330 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 40ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.05% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	88 → 87	12 → 13	linear gradient
35 – 45	87 → 85	13 → 15	linear gradient
45 – 60	85 → 80	15 → 20	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of acteoside Std-FP and plantamajoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of acteoside and plantamajoside should not be more than 5.0%; the RSD of the retention times of acteoside and plantamajoside peaks should not be more than 2.0%; the column efficiencies determined from acteoside and plantamajoside peaks should not be less than 30000 and 13000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and the *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 8).

Procedure

Separately inject acteoside Std-FP, plantamajoside Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of acteoside and plantamajoside peaks in the chromatograms of acteoside Std-FP, plantamajoside Std-FP and the retention times of the three characteristic peaks (Fig. 8) in the chromatogram of the test solution. Identify acteoside and plantamajoside peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of acteoside Std-FP and plantamajoside Std-FP. The retention times of acteoside and plantamajoside 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 three characteristic peaks of dried herb of *Plantago depressa* Willd. extract are listed in Table 4.

Table 4 The RRTs and acceptable ranges of the three characteristic peaks of dried herb of *Plantago depressa* Willd. extract

Peak No.	RRT	Acceptable Range
1 (marker, plantamajoside)	1.00	-
2 (acteoside)	1.46	± 0.03
3	1.69	± 0.04

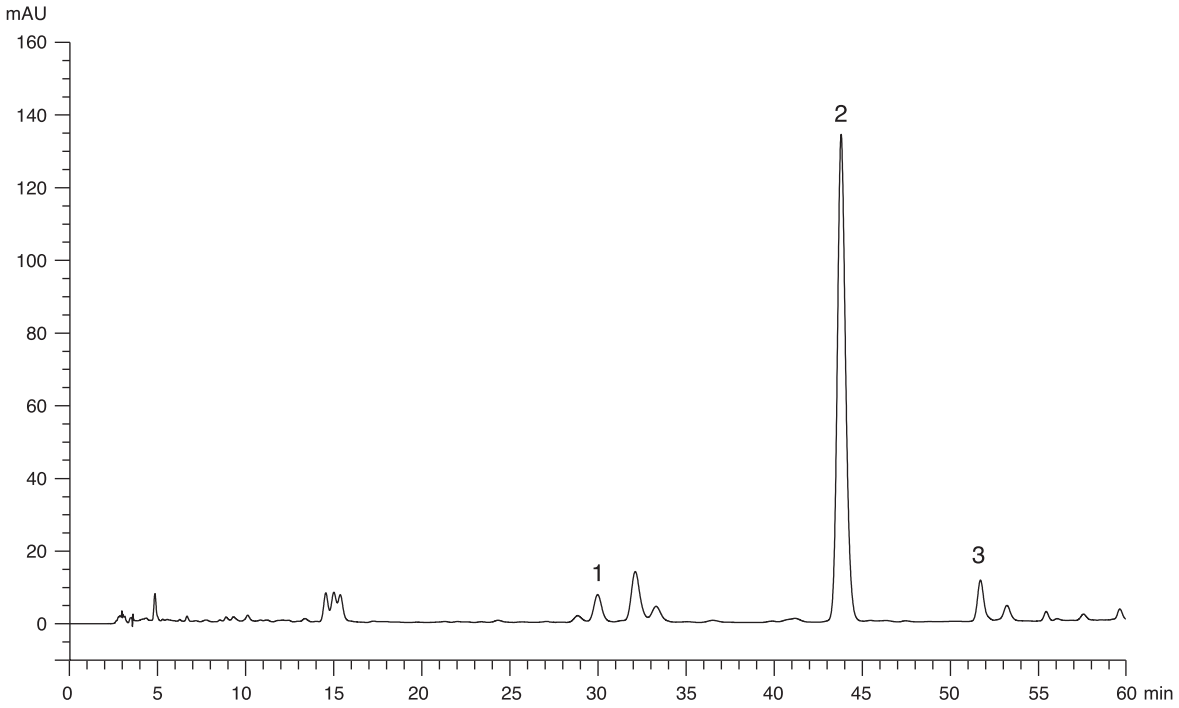

Figure 8 A reference fingerprint chromatogram of dried herb of *Plantago depressa* Willd. extract

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. 8).

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 6.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 15.0%.
Acid-insoluble ash: not more than 5.0%.

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 9.0%.
Ethanol-soluble extractives (cold extraction method): not less than 9.0%.

7. ASSAY

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

Standard solution

Mixed acteoside and plantamajoside standard stock solution, Std-Stock (500 mg/L each)

Weigh accurately 5.0 mg of acteoside CRS and 5.0 mg of plantamajoside CRS, and dissolve in 10 mL of methanol (60%) containing 0.05% phosphoric acid.

Mixed acteoside and plantamajoside standard solution for assay, Std-AS

Measure accurately the volume of the mixed acteoside and plantamajoside Std-Stock, dilute with methanol (60%) containing 0.05% phosphoric acid to produce a series of solutions of 0.5, 4, 62.5, 125, 250 mg/L for both acteoside and plantamajoside.

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 methanol (60%). Sonicate (100 W) the mixture for 15 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times with 20 mL of methanol (60%) and 10 mL of methanol (60%) respectively. Wash the residue with methanol (60%). Combine the solutions and make up to the mark with methanol (60%). Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (330 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 5) –

Table 5 Chromatographic system conditions

Time (min)	0.05% Trifluoroacetic acid containing 2% isopropanol (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	90 → 89.5	10 → 10.5	linear gradient
35 – 45	89.5 → 85	10.5 → 15	linear gradient
45 – 50	85 → 83	15 → 17	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed acteoside and plantamajoside Std-AS (62.5 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of acteoside and plantamajoside should not be more than 5.0%; the RSD of the retention times of acteoside and plantamajoside peaks should not be more than 2.0%; the column efficiencies determined from acteoside and plantamajoside peaks should not be less than 60000 and 10000 theoretical plates respectively.

The *R* value between acteoside peak and the closest peak; and the *R* value between plantamajoside 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 acteoside and plantamajoside Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of acteoside and plantamajoside against the corresponding concentrations of the mixed acteoside and plantamajoside 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 acteoside and plantamajoside peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed acteoside and plantamajoside Std-AS. The retention times of acteoside and plantamajoside 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 acteoside and plantamajoside in the test solution, and calculate the percentage contents of acteoside and plantamajoside in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.11% of the total content of acteoside ($C_{29}H_{36}O_{15}$) and plantamajoside ($C_{29}H_{36}O_{16}$), calculated with reference to the dried substance.