Phyllanthi Urinariae Herba

Figure 1 A photograph of Phyllanthi Urinariae Herba

A. Phyllanthi Urinariae Herba
B. Magnified image of branch and capsules
C. Magnified leaf (left: upper surface, right: lower surface)
D. Magnified capsule (left: top view, right: bottom view)

1. NAMES

Official name: Phyllanthi Urinariae Herba

Chinese name: 葉下珠

Chinese phonetic name: Yexiazhu

2. SOURCE

Phyllanthi Urinariae Herba is the dried whole plant of *Phyllanthus urinaria* L. (Euphorbiaceae*). The plant is collected in summer and autumn, foreign matter removed, then dried under the sun to obtain Phyllanthi Urinariae Herba.

3. DESCRIPTION

Varying in length. Fibrous roots numerous. Stem yellowish-green to brownish-green, base brown, young one brownish-red, cylindrical, 0.4-3.3 mm in diameter, surface with fine longitudinal wrinkles, hollow, texture brittle. Leaf small, papyraceous, easily fallen, subsessile, oblong to obovate, 5-21 mm in long, 1.5-9 mm in width, apex mucronulate or obtuse, base oblique; upper surface green, lower surface greyish-green, main vein small. Capsule small, infra-axillary, triangular flat-spherical, green, brownish-yellow to reddish-brown, 1.2-2.8 mm in diameter, surface with small protuberance, with persistent sepal. Odour slightly fragrant, taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Cork consists of serval layers of cells. Cortex consists of several layers of parenchymayous cells. Phloem narrow. Xylem occupies the majority of root, consisting of vessels and fibres. Xylem ray consists of 1-2 rows of cells [Fig. 2 (i)].

Stem: Epidermis consists of 1 layer of cells. Hypodermis consists of 1 layer of relatively large cells. Cortex narrow. Pericycle fibre in bundles, arranged in an interrupted ring. Phloem narrow. Xylem consists of vessels and fibres. Xylem ray consists of 1-3 rows of cells. Pith consists of parenchymatous cells, hollow in the centre [Fig. 2 (ii)].

* Phyllanthaceae is used by APG IV (2016).

Leaf: Upper and lower epidermis each consists of 1 layer of cells, some cells outer wall papillary protruded, non-glandular hair sometimes observed at the margin of the leaf. Mesophyll consists of 1 layer of palisade cells and several layers of spongy cells; palisade cells appear across the upper part of the midrib, some containing clusters of calcium oxalate in rosette aggregates. Vascular bundle of midrib collateral, with fibre bundles on the lower side. Collenchyma consists of 1-2 layers of cells, present at the inner side of the lower epidermis of midrib [Fig. 2 (iii)].

Capsule: With six locules, each containing 1 seed. Exocarp consists of 1 layer of cells. Mesocarp consists of serval layers of subrounded cells. Endocarp consists of 1 layer of palisade cells. Testa consists of 1 layer of wall thickened cells, inner side with 1 layer of pigment cells. Endosperm consists of parenchymayous cells [Fig. 2 (iv)].

Powder

Colour brownish-green. Clusters of calcium oxalate present in mesophyll cells, parenchymatous cells, or scattered, 6-42 µm in diameter; polychromatic under the polarized microscope. Lower epidermal cells of leaf irregular in surface view, anticlinal walls deeply sinuate, stomata abundant, mainly paracytic, some cells outer wall papillary protuberance. Upper epidermal cells of leaf irregular in surface view, anticlinal walls deeply sinuate, relatively few cells with papillary protuberance on outer wall. Sclerenchymatous cells of testa linear, subpolygonal or subrectangular in surface view, with fine and dense pits and pit canals; bright yellowish-white under the polarized microscope. Epidermal cells of stem rectangular or elongated-polygonal in surface view, stomata paracytic. Non-glandular hair consists of 1-5 cells, unicellular or multicellular 24-209 µm in long, 18-67 µm in diameter, with cuticle striations on surface. Epidermal cells of persistent sepal subpolygonal or subrectangular in surface view, wall slightly thickened. Pericycle fibre 4-23 µm in diameter, wall thickened, lumen narrow or linear; bright white or orange under the polarized microscope. Mesocarp cells subrounded or subpolygonal in surface view, walls thickened at the corners; often linked with vessels. Cork cells rectangular or polygonal in surface view, brown. Vessels mainly spiral type, 3-39 µm in diameter (Fig. 3).

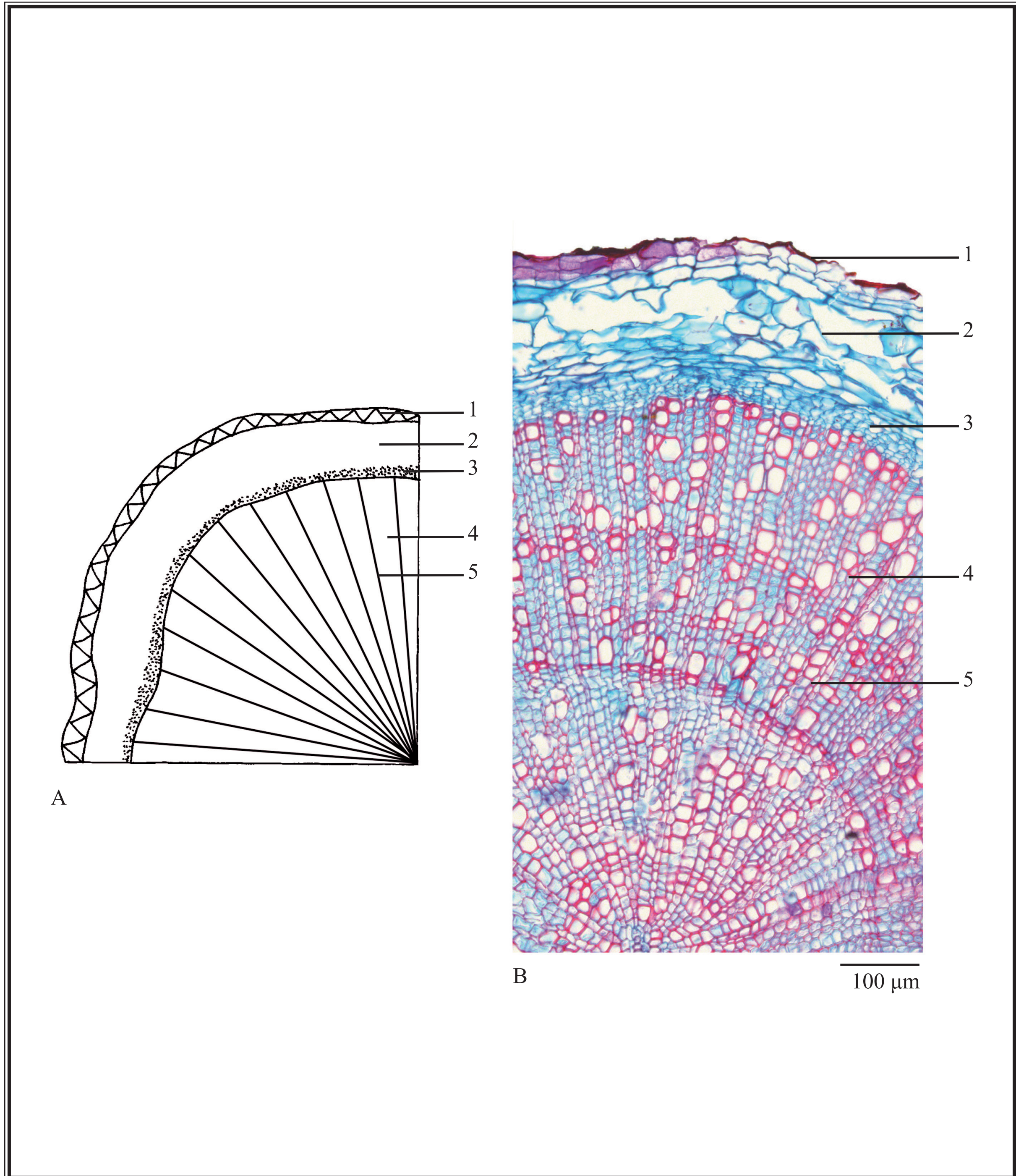

Figure 2 (i) Microscopic features of transverse section of root of Phyllanthi Urinariae Herba

A. Sketch B. Section illustration

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

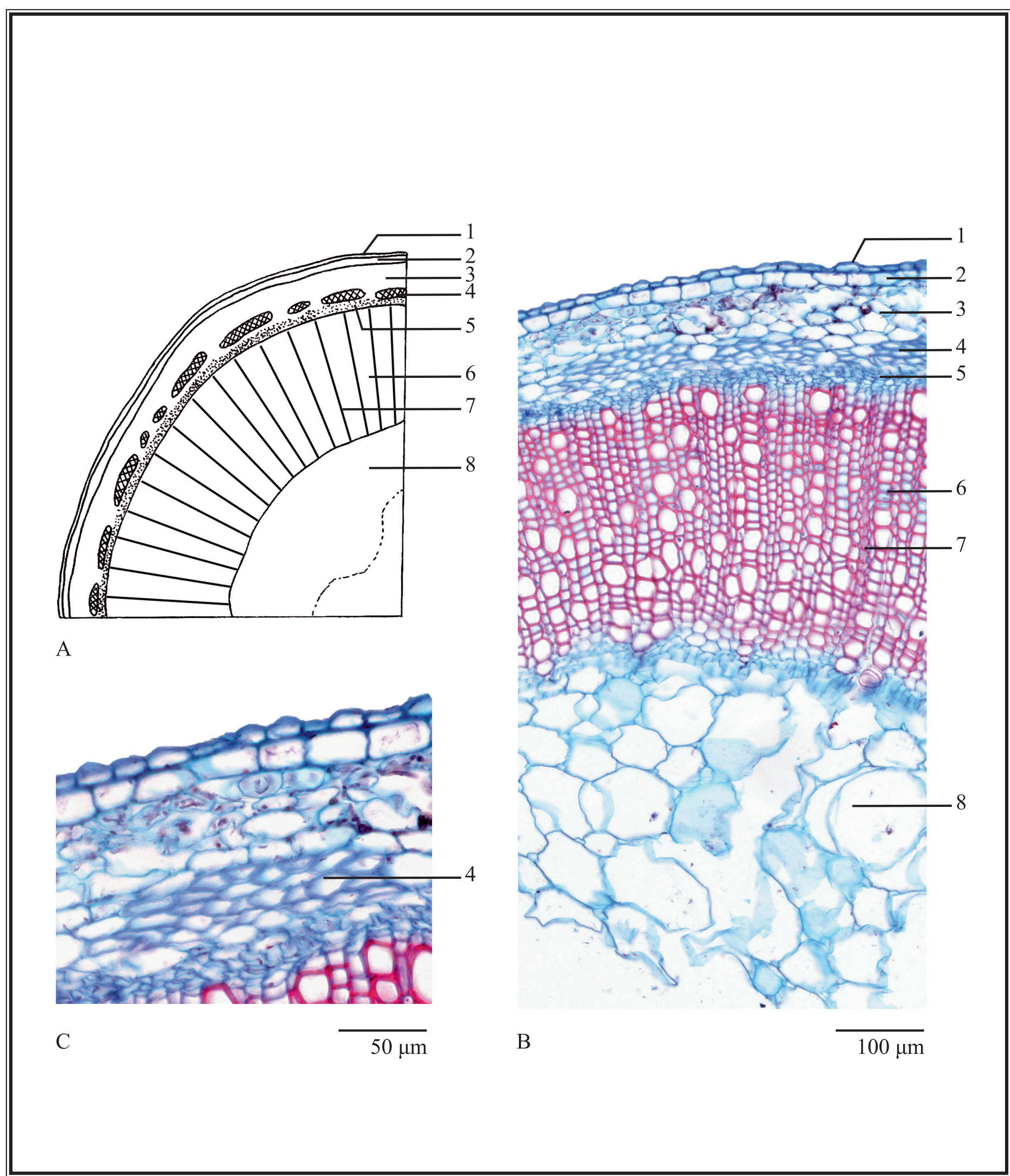

Figure 2 (ii) Microscopic features of transverse section of stem of Phyllanthi Urinariae Herba

A. Sketch B. Section illustration C. Pericycle fibres

1. Epidermis 2. Hypodermis 3. Cortex 4. Pericycle fibre 5. Phloem
6. Xylem 7. Xylem ray 8. Pith

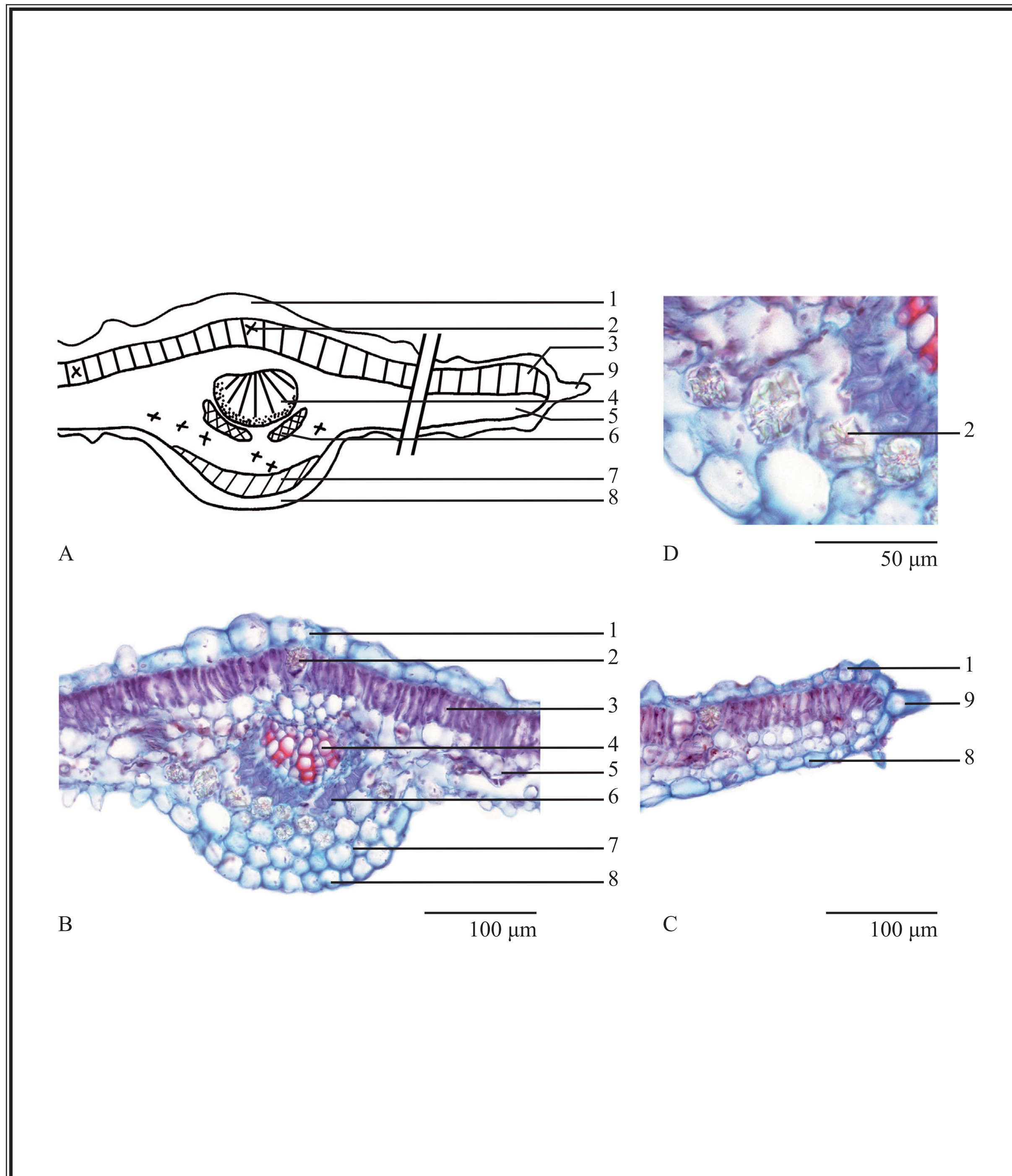

Figure 2 (iii) Microscopic features of transverse section of leaf of Phyllanthi Urinariae Herba

A. Sketch B. Section illustration C. Section illustration of margin
D. Clusters of calcium oxalate

1. Upper epidermis 2. Cluster of calcium oxalate 3. Palisade tissue 4. Vascular bundle of midrib
5. Spongy tissue 6. Fibre bundle 7. Collenchyma 8. Lower epidermis 9. Non-glandular hair

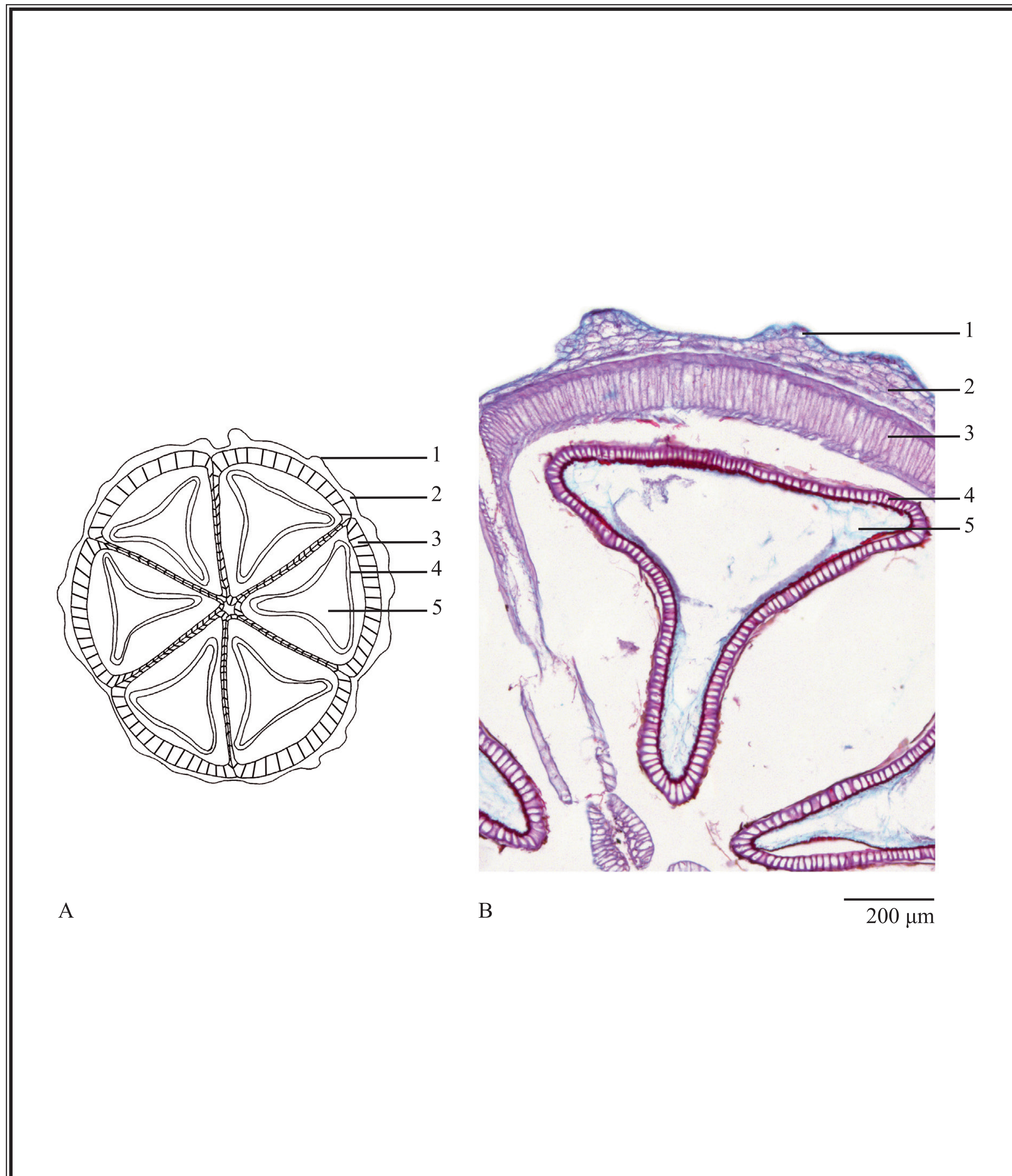

Figure 2 (iv) Microscopic features of transverse section of capsule of Phyllanthi Urinariae Herba

A. Sketch B. Section illustration

1. Exocarp 2. Mesocarp 3. Endocarp 4. Testa 5. Endosperm

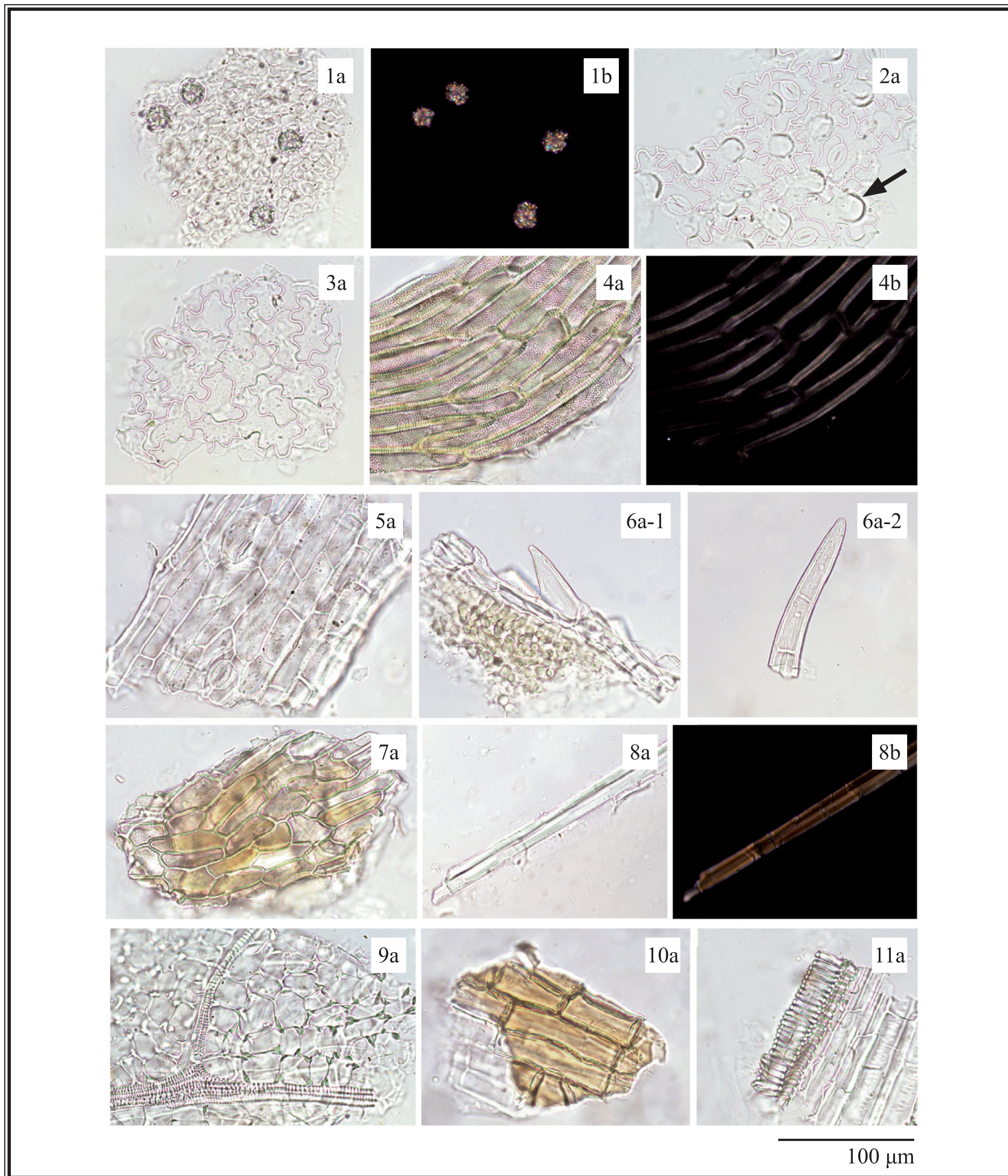

Figure 3 Microscopic features of powder of Phyllanthi Urinariae Herba

1. Clusters of calcium oxalate
2. Lower epidermal cells of leaf with paracytic stomata (papillary protuberance ⟶)
3. Upper epidermal cells of leaf 4. Sclerenchymatous cells of testa 5. Epidermal cells of stem
6. Non-glandular hairs (6-1 unicellular, 6-2 multicellular) 7. Epidermal cells of persistent sepal
8. Pericycle fibre 9. Mesocarp cells 10. Cork cells 11. 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

Corilagin standard solution

Weigh 1.0 mg of corilagin CRS (Fig. 4) and dissolve in 4 mL of water.

Developing solvent system

Prepare a mixture of dichloromethane, formic acid and methanol (5:1.5:1, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of water. Reflux the mixture for 30 min. Cool down to room temperature. Filter the mixture.

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 corilagin standard solution (2 μL) and the test solution (1 μL) to the plate. Develop over a path of about 4 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

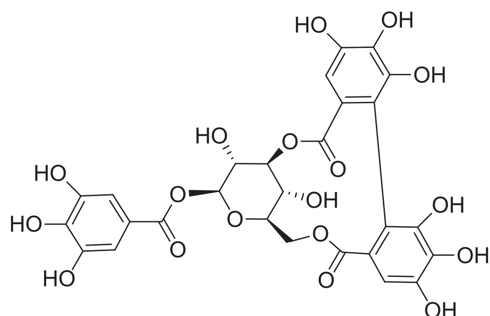

Figure 4 Chemical structure of corilagin

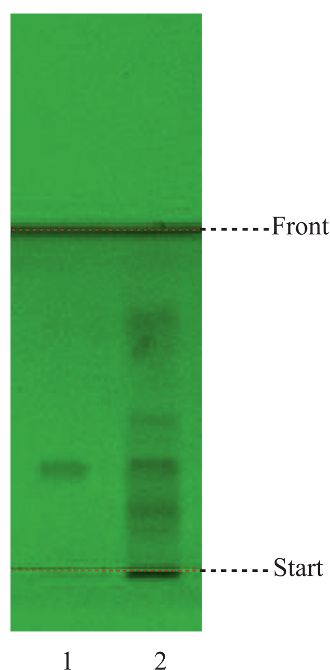

Figure 5 A reference HPTLC chromatogram of Phyllanthi Urinariae Herba extract observed under UV light (254 nm)

1. Corilagin 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 corilagin (Fig. 5).

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

Standard solution

Corilagin standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 1.0 mg of corilagin CRS and dissolve in 50 mL of methanol (50%).

Test solution

Weigh 0.25 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of methanol (50%). Reflux the mixture at about 100ºC for 1 h. Cool down to room temperature. Transfer the extract to a 50-mL centrifuge tube. Centrifuge at about 3200 × *g* for 10 min. Transfer the supernatant to a 100-mL volumetric flask. Repeat the extraction one more time. Wash the residue for two times each with 10 mL of methanol (50%). Combine the extracts and make up to the mark with methanol (50%). Filter through a 0.22-µm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (266 nm) and a column (2.1 × 100 mm) packed with ODS bonded silica gel (1.7 µm particle size). The column temperature is maintained at 40ºC during the separation. The flow rate is about 0.2 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.5% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 4	95 → 91	5 → 9	linear gradient
4 – 9	91	9	isocratic
9 – 14	91 → 86	9 → 14	linear gradient
14 – 20	86	14	isocratic

System suitability requirements

Perform at least five replicate injections, each using 1 µL of corilagin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of corilagin should not be more than 5.0%; the RSD of the retention time of corilagin peak should not be more than 2.0%; the column efficiency determined from corilagin peak should not be less than 19000 theoretical plates.

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 corilagin Std-FP and the test solution (1 µL each) into the UHPLC system and record the chromatograms. Measure the retention time of corilagin peak in the chromatogram of corilagin Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify corilagin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of corilagin Std-FP. The retention times of corilagin 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 Phyllanthi Urinariae Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.21	± 0.03
2	0.44	± 0.03
3	0.94	± 0.03
4 (marker, corilagin)	1.00	-

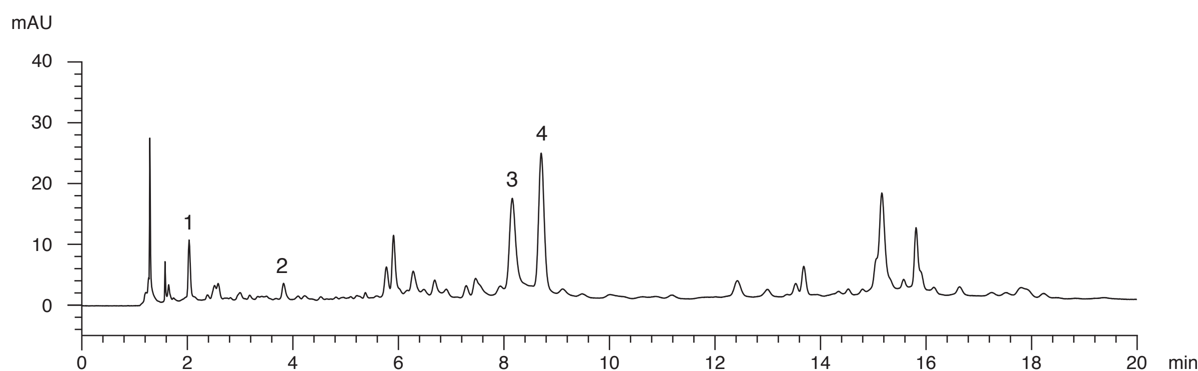

Figure 6 A reference fingerprint chromatogram of Phyllanthi Urinariae Herba extract

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)*: The CMM shall meet the requirements for arsenic, lead and mercury as specified in Appendix V. For cadmium, Phyllanthi Urinariae Herba should meet the specified limit of not more than 3.3 mg/kg, when the CMM will be processed as a decoction in the final consumption form; otherwise, the limit for cadmium specified in Appendix V shall be applied.

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 4.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 12.5%.
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 15.0%.
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
Corilagin standard stock solution, Std-Stock (1000 mg/L)
Weigh accurately 5.0 mg of corilagin CRS and dissolve in 5 mL of methanol (50%).
Corilagin standard solution for assay, Std-AS
Measure accurately the volume of the corilagin Std-Stock, dilute with methanol (50%) to produce a series of solutions of 8, 10, 20, 40, 80 mg/L for corilagin.

Test solution
Weigh accurately 0.25 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of methanol (50%). Reflux the mixture at about 100ºC for 1 h. Cool down to room temperature. Transfer the extract to a 50-mL centrifuge tube. Centrifuge at about 3200 × *g* for 10 min. Transfer the supernatant to a 100-mL volumetric flask. Repeat the extraction one more time. Wash the residue for two times each with 10 mL of methanol (50%). Combine the extracts and make up to the mark with methanol (50%). Filter through a 0.22-µm nylon filter.

Chromatographic system
The liquid chromatograph is equipped with a DAD (266 nm) and a column (2.1 × 100 mm) packed with ODS bonded silica gel (1.7 µm particle size). The column temperature is maintained at 40ºC during the separation. The flow rate is about 0.2 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.5% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 4	95 → 91	5 → 9	linear gradient
4 – 9	91	9	isocratic
9 – 14	91 → 86	9 → 14	linear gradient
14 – 20	86	14	isocratic

System suitability requirements

Perform at least five replicate injections, each using 1 μL of corilagin Std-AS (20 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of corilagin should not be more than 5.0%; the RSD of the retention time of corilagin peak should not be more than 2.0%; the column efficiency determined from corilagin peak should not be less than 19000 theoretical plates.

The *R* value between corilagin 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 corilagin Std-AS (1 μL each) into the UHPLC system and record the chromatograms. Plot the peak areas of corilagin against the corresponding concentrations of corilagin Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 1 μL of the test solution into the UHPLC system and record the chromatogram. Identify corilagin peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of corilagin Std-AS. The retention times of corilagin 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 corilagin in the test solution, and calculate the percentage content of corilagin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.74% of corilagin ($C_{27}H_{22}O_{18}$), calculated with reference to the dried substance.

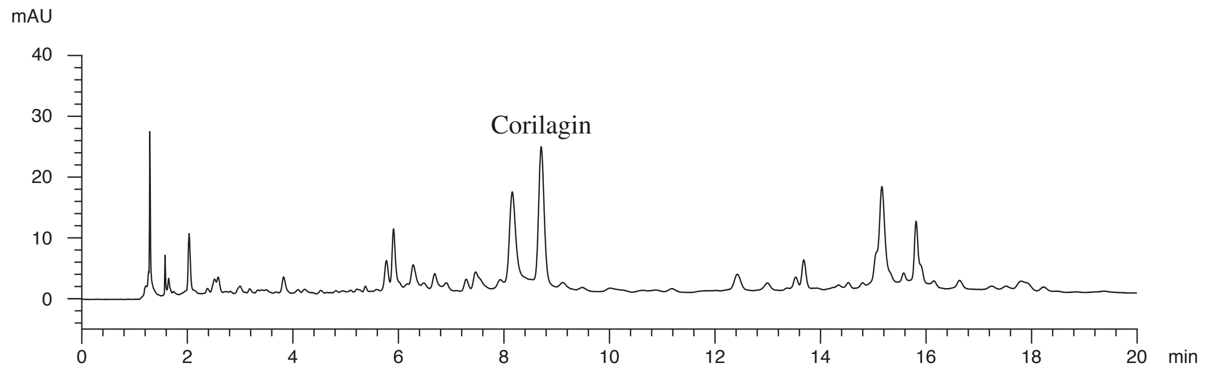

Figure 7 A reference assay chromatogram of Phyllanthi Urinariae Herba extract

8. CAUTION

This CMM should be used after proper processing (such as decoction).