Ardisiae Japonicae Herba

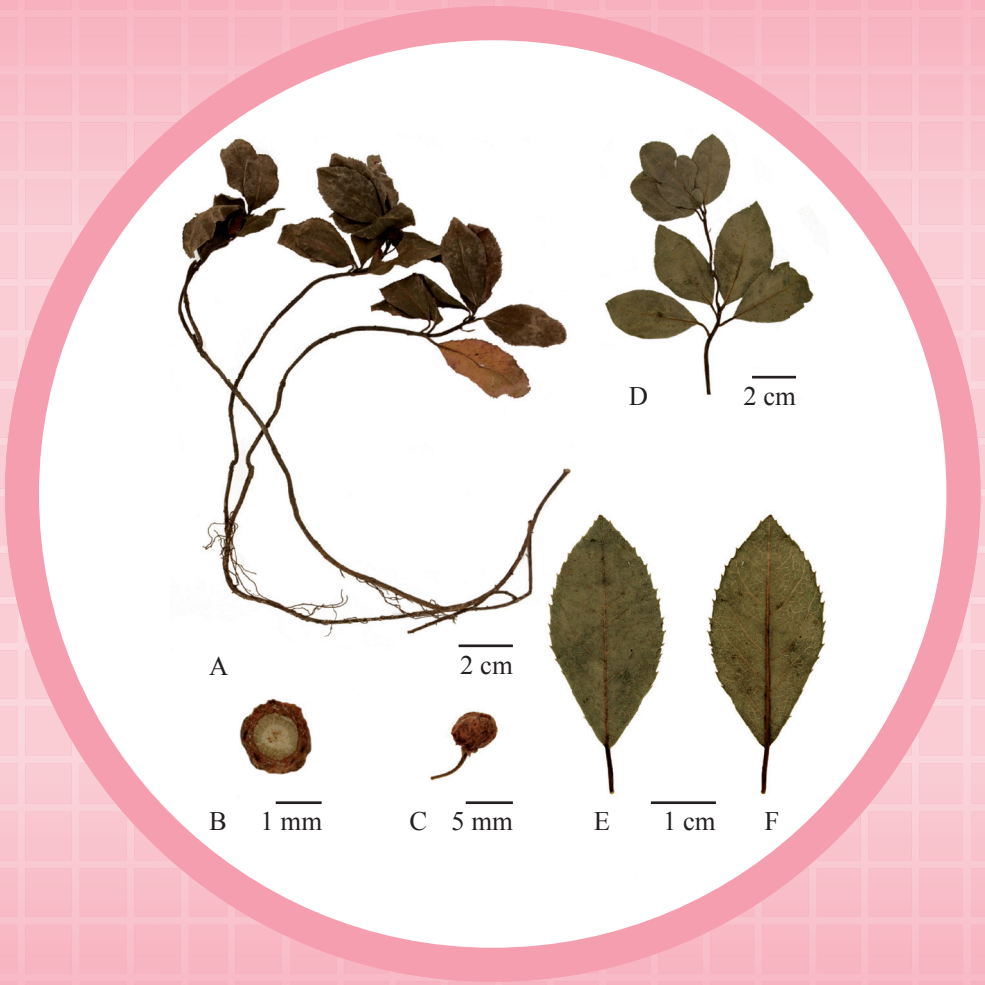


Figure 1 A photograph of Ardisiae Japonicae Herba

A. Ardisiae Japonicae Herba B. Magnified image of cut surface of stem
C. Magnified image of stone fruit D. Leaves
E. Upper surface of leaf F. Lower surface of leaf

1. NAMES

Official name: Ardisiae Japonicae Herba

Chinese name: 矮地茶

Chinese phonetic name: Aidicha

2. SOURCE

Ardisiae Japonicae Herb is the dried whole plant of *Ardisia japonica* (Thunb.) Blume (Myrsinaceae*). The whole plant is collected in summer and autumn when foliage branch growing luxuriantly, foreign matter removed, then dried under the sun to obtain Ardisiae Japonicae Herb.

3. DESCRIPTION

Rhizome cylindrical, sparsely with rootlets. Stem somewhat flattened-cylindrical, slightly twisted, 8-56 cm long, 1-2.5 mm in diameter; externally reddish-brown, with fine longitudinal striations, leaf scars and nodes; texture hard, easily broken. Leaves alternate, gathered at the tip of stems, lamina slightly crumped or broken, when whole, ellipsoid, 2.3-7.8 cm long, 1.0-3.7 cm wide, greyish-green, dark brown to pale reddish-brown; apex acute, base cuneate, margin serrulate; texture nearly leathery. Stone fruit red and spheroidal, at the apex of stem, rarely found. Odour slight; taste slightly astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: The walls of epidermal cells thickened, with glandular hairs. Glandular hair head unicellular or bicellular, with brown or dark brown content. Exodermis distinct, consisting of 1 layer of cells, subrectangular or subsquare. Cortex relatively broad, several layers of collenchymatous cells occurring in the outer part of cortex, with prisms of calcium oxalate and secretory cavities. Endodermis with distinct casparian dots. Phloem fairly narrow, a few of fibres outside of phloem. Cambium indistinct. Xylem broad, vessels lignified. Pith relatively large, with prisms of calcium oxalate and secretory cavities. Parenchymatous cells containing prisms of calcium oxalate, starch granules (could be observed before stained and occasionally found after stained) and occasionally brown masses [Fig. 2(i)].

* Primulaceae is used by APG IV (2016).

Leaf: Glandular hair found at both upper and lower epidermis, head unicellular or bicellular, with brown or dark brown content. Collenchyma located at the upper and lower part of vascular bundle: upper part consists of several layers of collenchymatous cells, subrounded or oblate; lower part consists of 1-2 layers of collenchymatous cells, subrounded or oblate. Upper epidermis consists of 1 layer of cells, subrectangular or subsqaure. Mesophyll consists of 5-6 layers of cells, palisade tissue and spongy tissue indistinct. Vascular bundle heart-shaped, occupying about 2/5 of midrib of leaf. Xylem distinct, radially arranged in a heart shape. Phloem distinct and orderly arranged, located in between the xylem, forming a heart-shaped phloem zone. Prisms of calcium oxalate abundant, found in mesophyll and collenchyma, occasionally in parenchymatous cells. Lower epidermis consists of 1 layer of cells, irregular in shape [Fig. 2(ii)].

Powder

Colour dark brown. Glandular hairs two types: head unicellular or bicellular, stalk unicellular; some contain brownish-yellow contents in the cell cavity. Glandular scales round or oblate, stalk very short, most contain brownish-yellow contents. Single starch granules ovate-rounded or rounded, 4-22 μm in diameter, hilum dotted or cleft; compound starch granules composed of 2-3 units; black and cruciate-shaped under the polarized microscope. Prisms of calcium oxalate abundant, 5-32 μm in diameter; polychromatic under the polarized microscope. Secretory cavities mostly broken, some cavities contain yellowish-brown secretory contents, and some secretory cells contain brown contents. Upper epidermal cells of leaf polygonal, the anticlinal cell walls sinuous, stomata rarely observed. Lower epidermal cells of leaf polygonal, the anticlinal cell walls sinuous, stomata frequently observed, stomata oblong to subrounded in shape, anisocytic. Vessels frequently found, mainly spiral and scalariform vessels, 5-28 μm in diameter. Fibres with thick-walled, relative long, scattered singly. Exodermis brown in colour, subsquare, orderly arranged. Epidermal cells suberized, yellowish-brown to brown in colour, subsquare, cell walls thickened (Fig. 3).

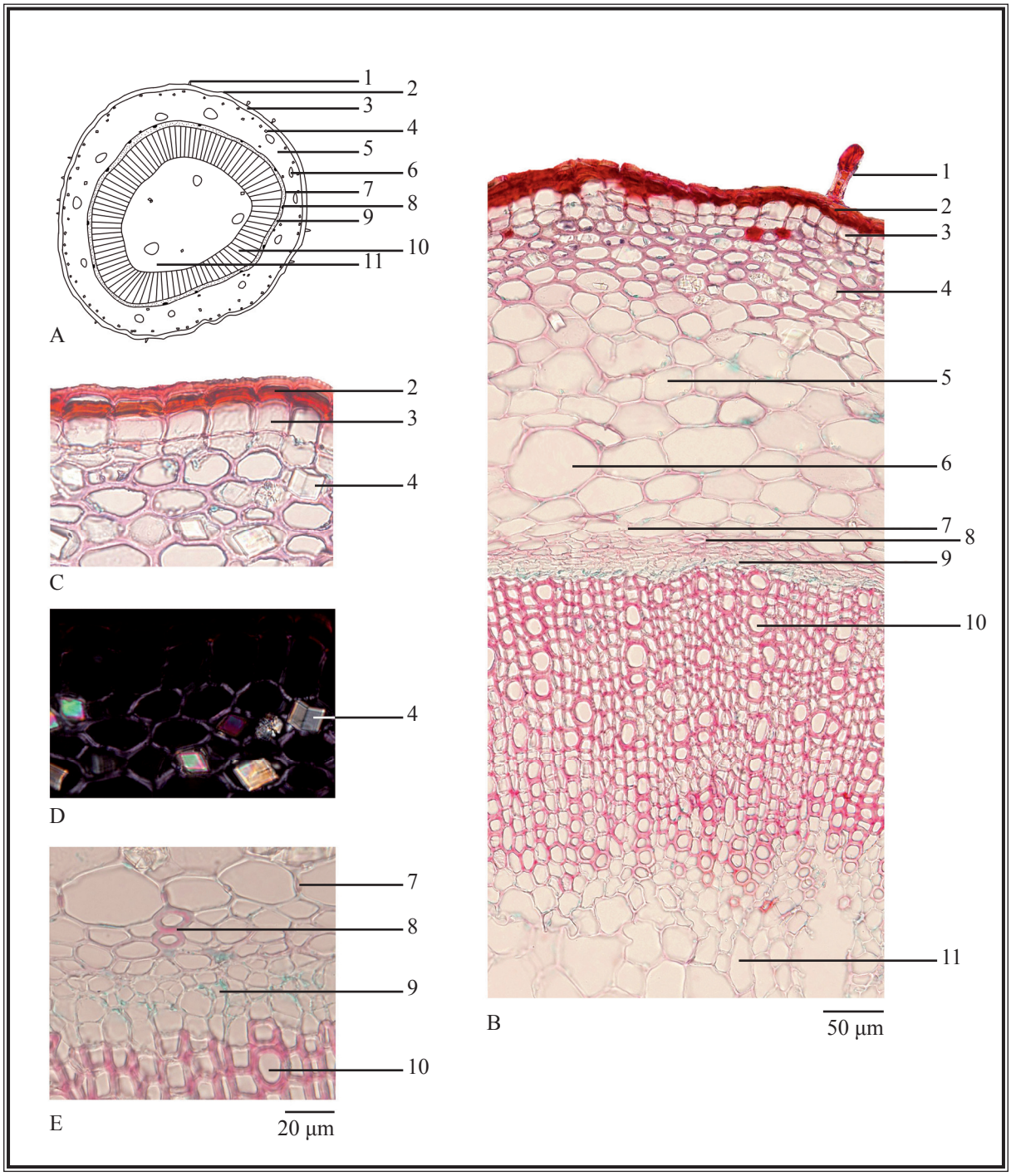


Figure 2 (i) Microscopic features of transverse section of stem of Ardisiae Japonicae Herba

A. Sketch B. Section illustration

C. Prisms of calcium oxalate (under the light microscope)

D. Prisms of calcium oxalate (under the polarized microscope) E. Section magnified

1. Glandular hair 2. Epidermis 3. Exodermis 4. Prism of calcium oxalate

5. Cortex 6. Secretory cavity 7. Endodermis 8. Fibre 9. Phloem

10. Xylem 11. Pith

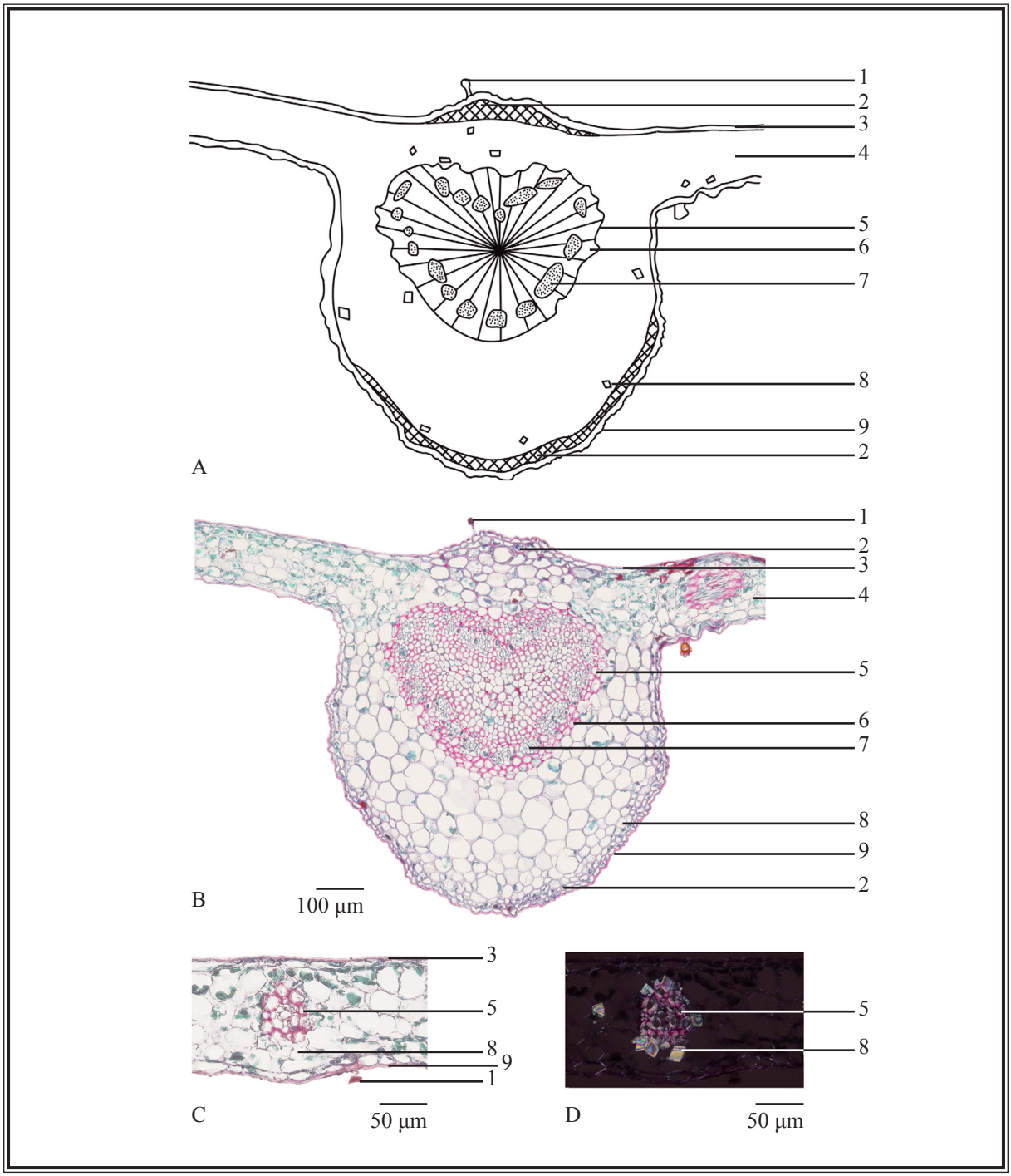


Figure 2 (ii) Microscopic features of transverse section of leaf of Ardisiae Japonicae Herba

A. Sketch B. Section illustration
C. Section magnified (under the light microscope)
D. Section magnified (under the polarized microscope)

1. Glandular hair 2. Collenchyma 3. Upper epidermis 4. Mesophyll
5. Vascular bundle 6. Xylem 7. Phloem 8. Prism of calcium oxalate
9. Lower epidermis

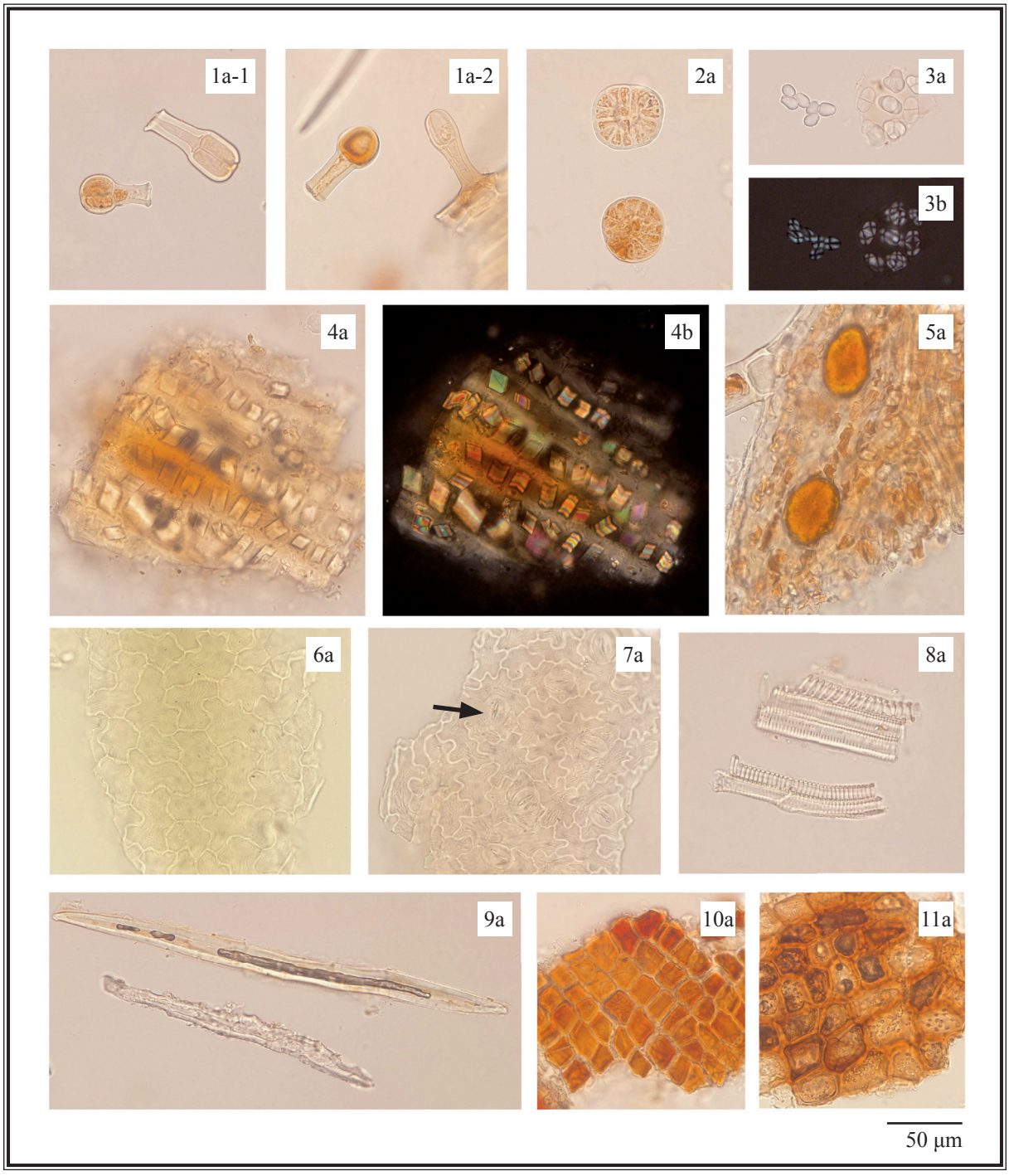


Figure 3 Microscopic features of powder of Ardisiae Japonicae Herba

1. Glandular hairs (1-1 glandular hairs with bicellular head, 1-2 glandular hairs with unicellular head)
2. Glandular scales 3.Starch granules 4. Prisms of calcium oxalate 5. Secretory cavities
6. Upper epidermal cells of leaf 7. Lower epidermal cells of leaf with stomata (⟶) 8. Vessels
9. Fibres 10. Exodermis 11. Suberized epidermal cells

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

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

Standard solutions

Bergenin standard solution

Weigh 1.0 mg of bergenin CRS (Fig. 4) and dissolve in 1 mL of methanol (50%).

Quercitrin standard solution

Weigh 0.5 mg of quercitrin CRS (Fig. 4) and dissolve in 1 mL of methanol (50%).

Developing solvent system

Prepare a mixture of ethyl acetate, *n*-butanol, formic acid and water (10:1:1:1, v/v).

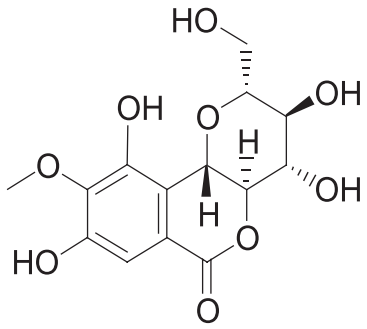
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol (50%). Sonicate (220 W) the mixture for 30 min. Filter the solution.

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 bergenin standard solution (3 μL), quercitrin standard solution (3 μL) and the test solution (3 − 9 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 8 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 values by using the equation as indicated in Appendix IV (A).

(i)



(ii)

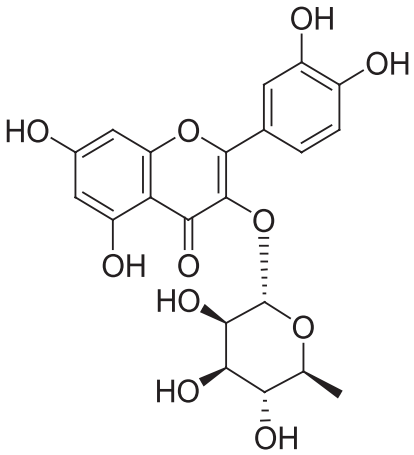


Figure 4 Chemical structures of (i) bergenin and (ii) quercitrin

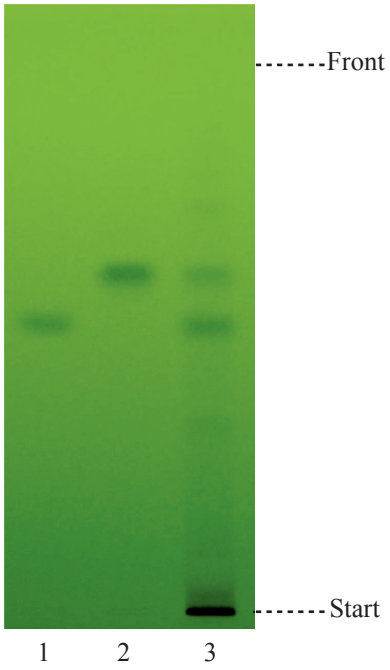


Figure 5 A reference HPTLC chromatogram of Ardisiae Japonicae Herba extract observed under UV light (254 nm)

1. Bergenin standard solution 2. Quercitrin 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 bergenin and quercitrin (Fig. 5).

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

Standard solutions

Bergenin standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 0.5 mg of bergenin CRS and dissolve in 10 mL of methanol (50%).

Quercitrin standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.1 mg of quercitrin CRS and dissolve in 10 mL of methanol (50%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 80 mL of methanol (50%). Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL volumetric flask. Wash the residue with methanol (50%). Combine the extracts and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (275 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 40	95 → 65	5 → 35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of bergenin Std-FP and quercitrin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of bergenin and quercitrin should not be more than 5.0%; the RSD of the retention times of bergenin and quercitrin peaks should not be more than 2.0%; the column efficiencies determined from bergenin and quercitrin peaks should not be less than 40000 and 120000 theoretical plates respectively.

The *R* value between peak 3 and the closest peak; and 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 bergenin Std-FP, quercitrin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of bergenin and quercitrin peaks in the chromatograms of bergenin Std-FP, quercitrin Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify bergenin and quercitrin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of bergenin Std-FP and quercitrin Std-FP. The retention times of bergenin and quercitrin 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 five characteristic peaks of Ardisiae Japonicae Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.55	± 0.03
2	0.70	± 0.03
3 (marker, bergenin)	1.00	-
4	1.09	± 0.03
5 (quercitrin)	2.07	± 0.03

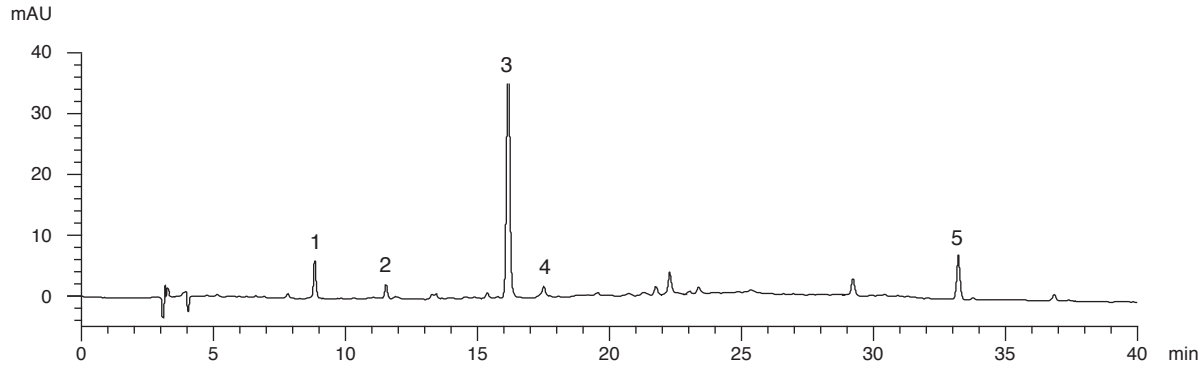


Figure 6 A reference fingerprint chromatogram of Ardisiae Japonicae Herba extract

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 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 8.0%.
Acid-insoluble ash: not more than 2.0%.

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 8.0%.
Ethanol-soluble extractives (cold extraction method): not less than 8.0%.

7. ASSAY

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

Standard solution

Mixed bergenin and quercitrin standard stock solution, Std-Stock (100 mg/L for bergenin and 20 mg/L for quercitrin)

Weigh accurately 1.0 mg of bergenin CRS and 0.2 mg of quercitrin CRS, and dissolve in 10 mL of methanol (50%).

Mixed bergenin and quercitrin standard solution for assay, Std-AS

Measure accurately the volume of the mixed bergenin and quercitrin Std-Stock, dilute with methanol (50%) to produce a series of solutions of 5, 10, 25, 50, 75 mg/L for bergenin and 0.1, 0.4, 1, 10, 20 mg/L for quercitrin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 80 mL of methanol (50%). Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL volumetric flask. Wash the residue with methanol (50%). Combine the extracts and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (275 nm for bergenin and 256 nm for quercitrin) 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 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 40	95 → 65	5 → 35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed bergenin and quercitrin Std-AS (25 mg/L for bergenin and 1 mg/L for quercitrin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of bergenin and quercitrin should not be more than 5.0%; the RSD of the retention times of bergenin and quercitrin peaks should not be more than 2.0%; the column efficiencies determined from bergenin and quercitrin peaks should not be less than 40000 and 120000 theoretical plates respectively.

The *R* value between bergenin peak and the closest peak; and the *R* value between quercitrin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 [Fig. 7 (i) and (ii)].

Calibration curves

Inject a series of the mixed bergenin and quercitrin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of bergenin and quercitrin against the corresponding concentrations of the mixed bergenin and quercitrin 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 bergenin and quercitrin peaks [Fig. 7 (i) and (ii)] in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed bergenin and quercitrin Std-AS. The retention times of bergenin and quercitrin 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 bergenin and quercitrin in the test solution, and calculate the percentage contents of bergenin and quercitrin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.50% of bergenin ($C_{14}H_{16}O_9$) and not less than 0.012% of quercitrin ($C_{21}H_{20}O_{11}$), calculated with reference to the dried substance.

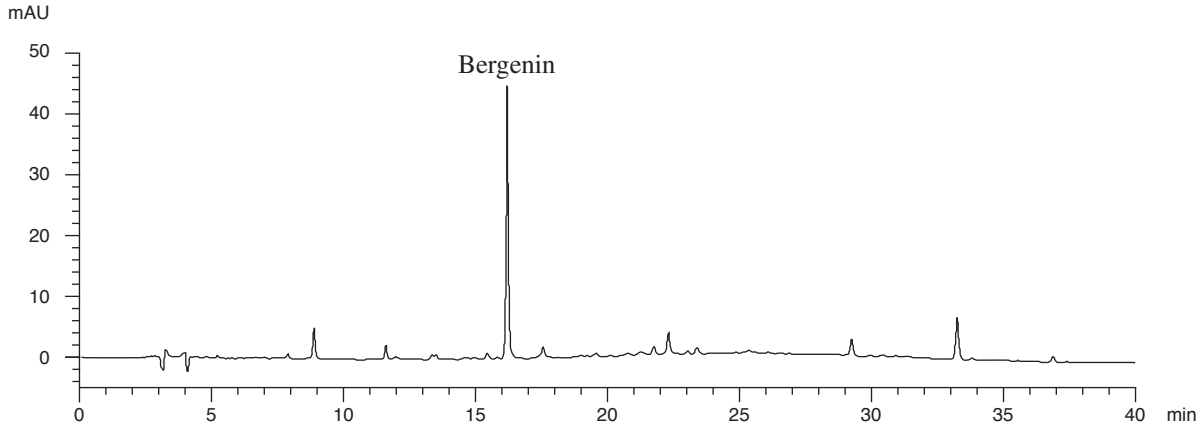


Figure 7 (i) A reference assay chromatogram of bergenin of Ardisiae Japonicae Herba extract at 275 nm

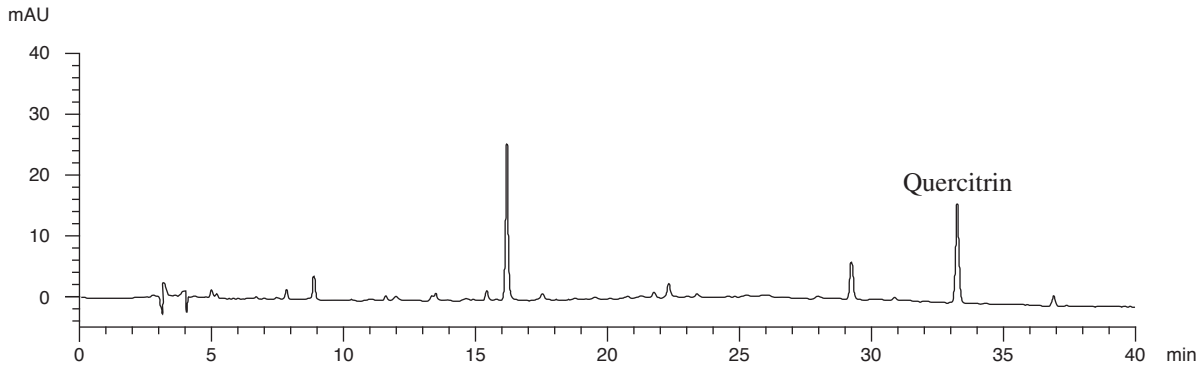


Figure 7 (ii) A reference assay chromatogram of quercitrin of Ardisiae Japonicae Herba extract at 256 nm