Artemisiae Anomalae Herba

Figure 1 A photograph of Artemisiae Anomalae Herba

A. Artemisiae Anomalae Herba B. Magnified image of alternated leaves
C. Magnified image of capitula
D. Magnified image of transverse section of stem

1. NAMES

Official name: Artemisiae Anomalae Herba

Chinese name: 劉寄奴

Chinese phonetic name: Liujinu

2. SOURCE

Artemisiae Anomalae Herba is the dried aerial part of *Artemisia anomala* S. Moore (Asteraceae). The aerial part is collected in summer and autumn during flowering period, foreign matter removed, washed clean, then dried under the sun to obtain Artemisiae Anomalae Herba.

3. DESCRIPTION

Stem cylindrical, 3.1-7.5 mm in diameter, usually bent, externally brownish-yellow to brownish-green; texture fragile, easily broken, fracture yellowish-white, fiberous, with loose white pith in the centre. Leaves alternate, oblong ovate, margin dentate, usually dry shrunken or fallen off. The upper surface brownish-green; the lower surface greyish-green, densely covered with white hair. Stipes short, texture fragile, easily broken. Numerous capitula arranged densely to form sallow panicle. Odour aromatic, taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermis consists of 1 layer of subrounded or subrectangular cells, with glandular and non-glandular hairs present. Non-glandular hairs are T-shaped, many broken and the handle area easily fallen off. Sclerenchyma consists around 10 layers of cell, mainly at the corner or edges of stem. Cortex consists of 1-2 layers of rounded or oblong parenchymatous cells. Pericycle fibres arranged in an interrupted ring, located on the outer side of vascular bundles. Vascular bundles collateral, phloem relatively narrow. Xylem vessels singly scattered or sometimes 2-3 in groups. Pith broad, sometimes broken or hollow, parenchymatous cells arranged loosely and clusters of calcium oxalate present [Fig. 2(i)].

Leaf: Upper epidermis consists of 1 layer of subrounded or subrectangular cells, glandular and non-glandular hairs usually fallen off after staining process. Palisade tissue consists of 1 layer of oblong cells. Spongy tissue consists of 3-5 layers of irregular shaped cells, arranged loosely, sometimes containing clusters of calcium oxalate. Vascular bundles collateral, phloem narrow; xylem broad, with several layers of vessels. Sclerenchyma contains several layers of cells, collenchymatous cells exist between the inner side of upper and lower epidermal cells. Lower epidermis consists of 1 layer of irregular shaped cells, relatively small, glandular and non-glandular hairs usually fallen off after staining [Fig. 2(ii)].

Powder

Colour brownish-yellow to brownish-green. Glandular hairs consist of 3-4 layers cells, many shrunken, subrounded to oblong in surface view. Clusters of calcium oxalate in rosette aggregate, sometimes scattered in parenchymatous cells, extremely tiny, 1-10 μm in diameter; polychromatic under the polarized microscope. Pollen grains sub-spherical, 14-22 μm in diameter, containing 3 furrows, with fine granular sculptures visible on the surface. Non-glandular hairs are T-shaped, many broken and the handle area easily fallen off, containing light yellowish material. Lower epidermal cells of leaf subrounded or subrectangular, anticlinal walls sinuously curved; stomata anomocytic, subrounded or oblong in shape. Epidermal cells of stems subrectangular or subpolygonal shaped, some containing light yellow or red masses; stoma and the handle of the T-shaped non-glandular hairs occasionally present. Epidermal cells of bracts rectangular, containing light yellowish-brown secretion and subrounded voids. Fibres usually in bundles, 4-19 μm in diameter; polychromatic under the polarized microscope. Vessels mostly spiral, bordered-pitted and reticulate vessels, 4-45 μm, 5-38 μm and 5-42 μm in diameter respectively (Fig.3).

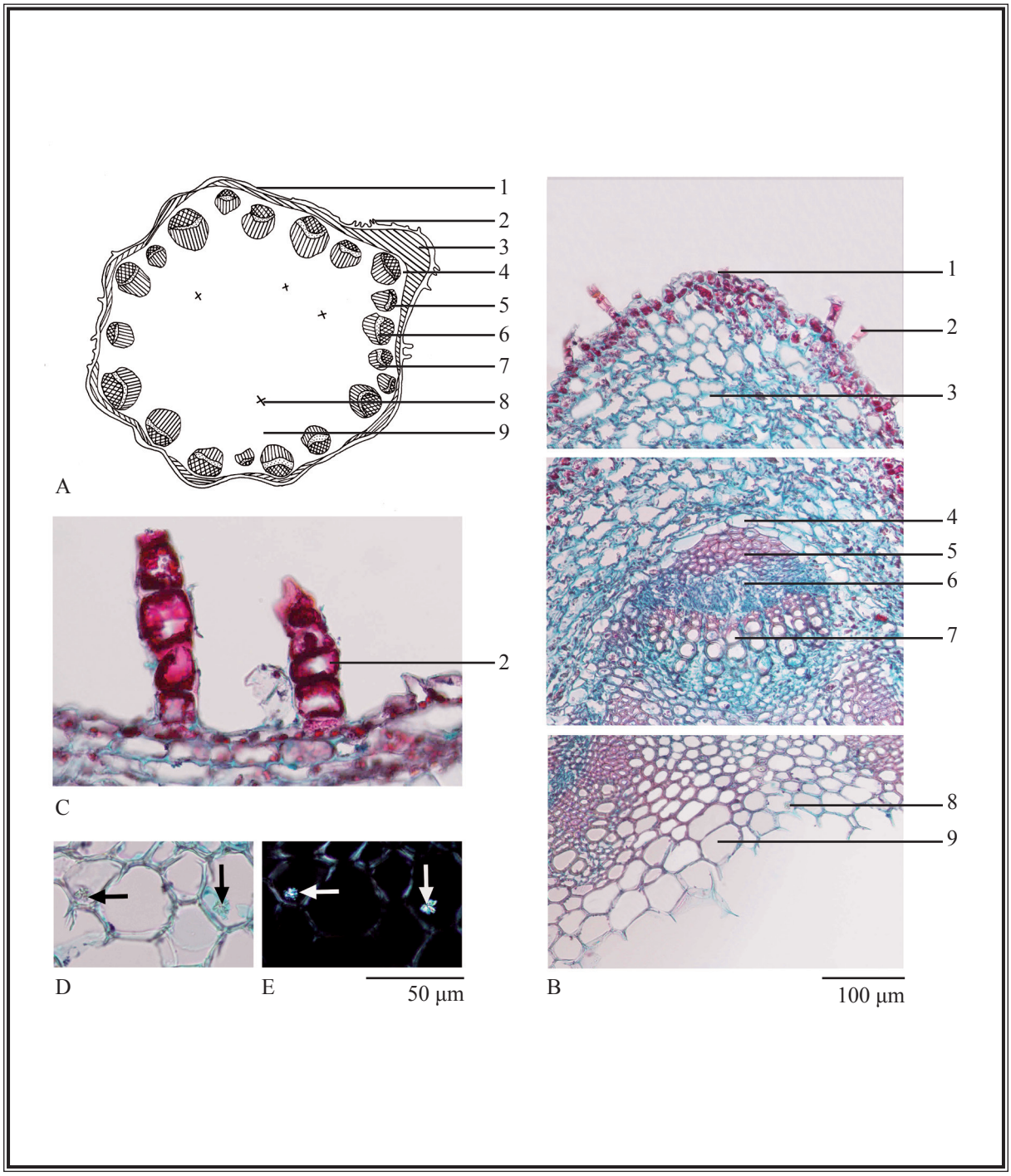


Figure 2 (i) Microscopic features of transverse section of stem of Artemisiae Anomalae Herba

A. Sketch B. Section illustration C. Non-glandular hairs
D. Clusters of calcium oxalate (under the light microscope)
E. Clusters of calcium oxalate (under the polarized microscope)

1. Epidermis 2. Non-glandular hair 3. Sclerenchyma 4. Cortex 5. Pericycle fibres
6. Phloem 7. Xylem 8. Cluster of calcium oxalate 9. Pith

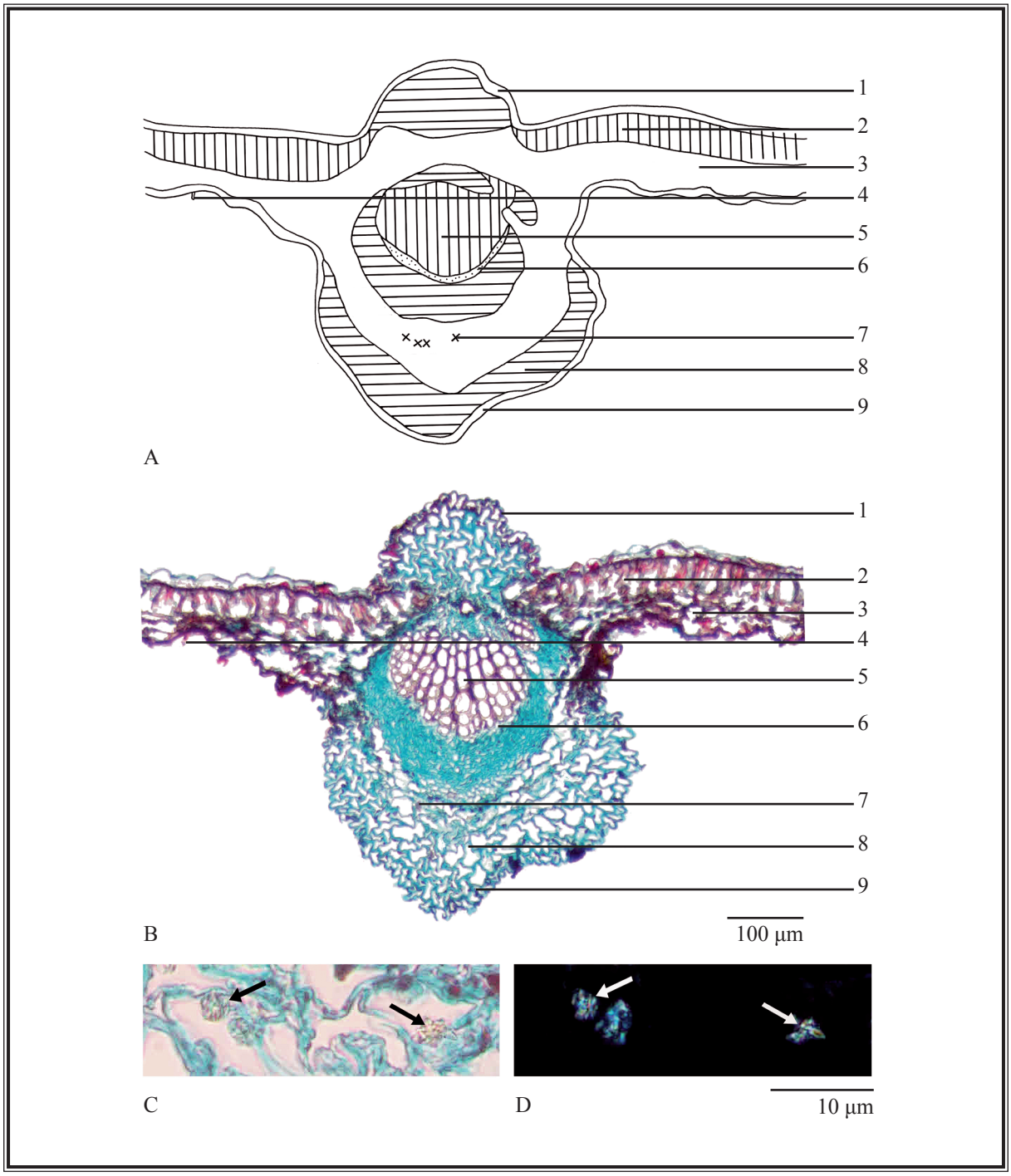


Figure 2 (ii) Microscopic features of transverse section of leaf of Artemisiae Anomalae Herba

A. Sketch B. Section illustration
C. Clusters of calcium oxalate (under the light microscope)
D. Clusters of calcium oxalate (under the polarized microscope)

1. Upper Epidermis 2. Palisade tissue 3. Spongy tissue 4. Non-glandular hair
5. Xylem 6. Phloem 7. Cluster of calcium oxalate 8. Sclerenchyma
9. Lower epidermis

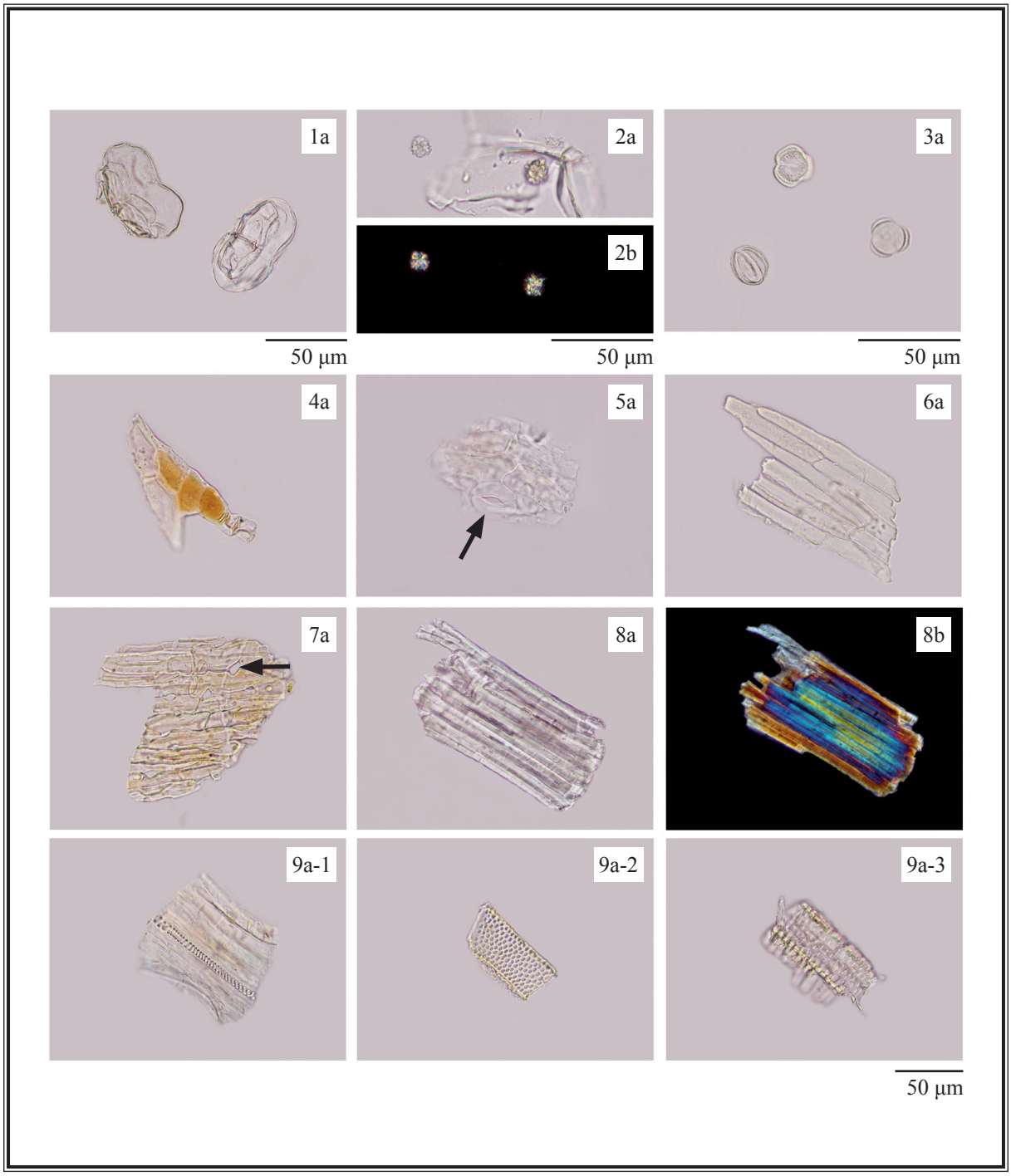


Figure 3 Microscopic features of the powder of Artemisiae Anomalae Herba

1. Glandular hairs 2. Clusters of calcium oxalate 3. Pollen grains
4. Non-glandular hair 5. Lower epidermal cells of leaf with stoma (⟶)
6. Epidermal cells of stem 7. Epidermal cells of bract (subrounded void ⟶)
8. Fibres 9. Vessels (9-1 spiral vessel, 9-2 bordered-pitted vessel, 9-3 reticulate vessel)

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

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

Standard solutions

3,5-Dicaffeoylquinic acid standard solution

Weigh 0.5 mg of 3,5-dicaffeoylquinic acid CRS (Fig. 4) and dissolve in 1 mL of methanol (70%).

4,5-Dicaffeoylquinic acid standard solution

Weigh 0.5 mg of 4,5-dicaffeoylquinic acid CRS (Fig. 4) and dissolve in 1 mL of methanol (70%).

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC), *n*-butyl acetate, formic acid and water (2:7:5:3, v/v). Use the upper layer.

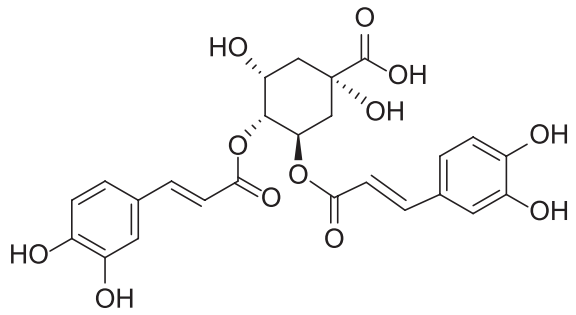
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol (70%). Sonicate (400 W) the mixture for 30 min. Filter through a 0.45-μm PTFE filter.

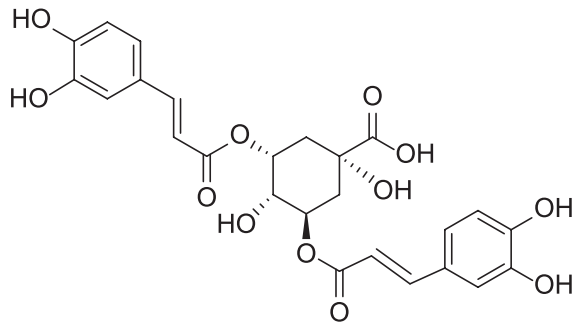
Procedure

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 3,5-dicaffeoylquinic acid standard solution (1 μL), 4,5-dicaffeoylquinic acid standard solution (1 μL) and the test solution (2 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 (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



(ii)



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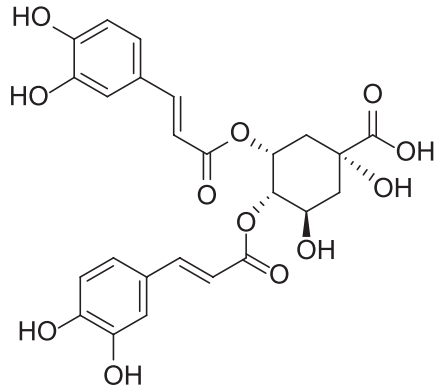

Figure 4 Chemical structures of (i) 3,4-dicaffeoylquinic acid, (ii) 3,5-dicaffeoylquinic acid and (iii) 4,5-dicaffeoylquinic acid

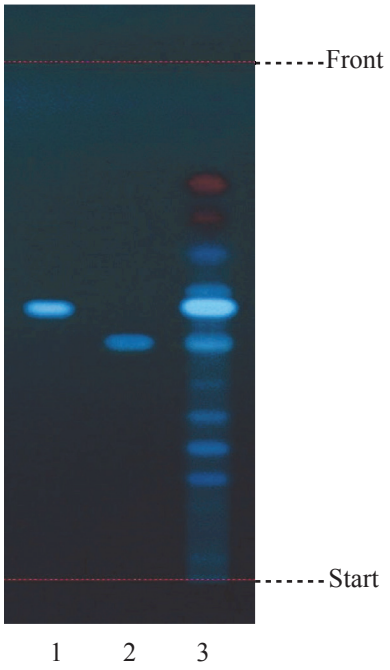

Figure 5 A reference HPTLC chromatogram of Artemisiae Anomalae Herba extract observed under UV light (366 nm)

1. 3,5-Dicaffeoylquinic acid standard solution
2. 4,5-Dicaffeoylquinic 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 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid (Fig. 5).

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

Standard solutions

3,4-Dicaffeoylquinic acid standard solution for fingerprinting, Std-FP (2 mg/L)

Weigh 0.1 mg of 3,4-dicaffeoylquinic acid CRS (Fig. 4) and dissolve in 50 mL of methanol (70%).

3,5-Dicaffeoylquinic acid standard solution for fingerprinting, Std-FP (9 mg/L)

Weigh 0.45 mg of 3,5-dicaffeoylquinic acid CRS and dissolve in 50 mL of methanol (70%).

4,5-Dicaffeoylquinic acid standard solution for fingerprinting, Std-FP (5 mg/L)

Weigh 0.25 mg of 4,5-dicaffeoylquinic acid CRS and dissolve in 50 mL of methanol (70%).

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (70%). Sonicate (400 W) the extract for 30 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction one more time. Wash the residue with methanol (70%). Combine the extracts and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (325 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.2% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	85	15	isocratic
5 – 25	85 → 70	15 → 30	linear gradient
25 – 40	70 → 60	30 → 40	linear gradient
40 – 50	60 → 10	40 → 90	linear gradient
50 – 55	10	90	isocratic
55 – 60	10 → 85	90 → 15	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of 3,4-dicaffeoylquinic acid Std-FP, 3,5-dicaffeoylquinic acid Std-FP and 4,5-dicaffeoylquinic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid should not be more than 5.0%; the RSD of the retention times of 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid peaks should not be more than 2.0%; the column efficiencies determined from 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid peaks should not be less than 75000 theoretical plates.

The *R* value between peak 2 and the closest peak; the *R* value between peak 3 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 1.5 (Fig. 6).

Procedure

Separately inject 3,4-dicaffeoylquinic acid Std-FP, 3,5-dicaffeoylquinic acid Std-FP, 4,5-dicaffeoylquinic acid Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid peaks in the chromatograms of 3,4-dicaffeoylquinic acid Std-FP, 3,5-dicaffeoylquinic acid Std-FP, 4,5-dicaffeoylquinic acid Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of 3,4-dicaffeoylquinic acid Std-FP, 3,5-dicaffeoylquinic acid Std-FP and 4,5-dicaffeoylquinic acid Std-FP. The retention times of 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid 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 Artemisiae Anomalae Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.40	± 0.03
2 (3,4-dicaffeoylquinic acid)	0.91	± 0.03
3 (marker, 3,5-dicaffeoylquinic acid)	1.00	-
4 (4,5-dicaffeoylquinic acid)	1.05	± 0.03
5	1.41	± 0.03

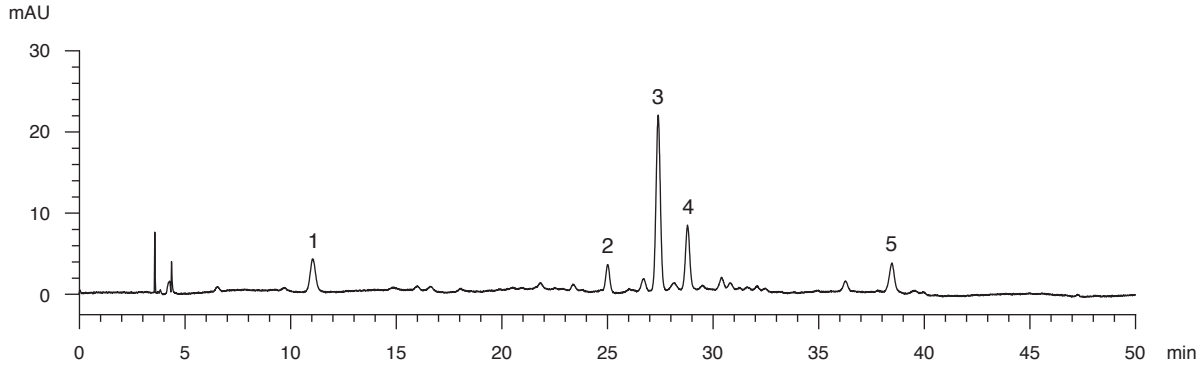

Figure 6 A reference fingerprint chromatogram of Artemisiae Anomalae Herba extract

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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 6.0%.
Acid-insoluble ash: not more than 1.0%.

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 12.0%.
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 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid standard stock solution, Std-Stock (120 mg/L for 3,4-dicaffeoylquinic acid, 280 mg/L for 3,5-dicaffeoylquinic acid and 140 mg/L for 4,5-dicaffeoylquinic acid)

Weigh accurately 3.0 mg of 3,4-dicaffeoylquinic acid CRS, 7.0 mg of 3,5-dicaffeoylquinic acid CRS and 3.5 mg of 4,5-dicaffeoylquinic acid CRS, and dissolve in 25 mL of methanol (70%).

Mixed 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid standard solution for assay, Std-AS

Measure accurately the volume of the mixed 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid Std-Stock, dilute with methanol (70%) to produce a series of solutions of 0.25, 0.5, 1, 2, 4 mg/L for 3,4-dicaffeoylquinic acid, 0.75, 1.5, 3, 6, 12 mg/L for 3,5-dicaffeoylquinic acid and 0.5, 1, 2, 4, 8 mg/L for 4,5-dicaffeoylquinic acid.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (70%). Sonicate (400 W) the extract for 30 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction one more time. Wash the residue with methanol (70%). Combine the extracts and make up to the mark with methanol (70%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (325 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.2% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	85	15	isocratic
5 – 25	85 → 70	15 → 30	linear gradient
25 – 40	70 → 60	30 → 40	linear gradient
40 – 50	60 → 10	40 → 90	linear gradient
50 – 55	10	90	isocratic
55 – 60	10 → 85	90 → 15	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid Std-AS (1 mg/L for 3,4-dicaffeoylquinic acid, 3 mg/L for 3,5-dicaffeoylquinic acid and 2 mg/L for 4,5-dicaffeoylquinic acid). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid should not be more than 5.0%; the RSD of the retention times of 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid peaks should not be more than 2.0%; the column efficiencies determined from 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid peaks should not be less than 75000 theoretical plates.

The *R* value between 3,4-dicaffeoylquinic acid peak and the closest peak; the *R* value between 3,5-dicaffeoylquinic acid peak and the closest peak; and the *R* value between 4,5-dicaffeoylquinic acid peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curves

Inject a series of the mixed 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid against the corresponding concentrations of the mixed 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid 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 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid Std-AS. The retention times of 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid 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 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid in the test solution, and calculate the percentage contents of 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.25% of the total content of 3,4-dicaffeoylquinic acid ($C_{25}H_{24}O_{12}$), 3,5-dicaffeoylquinic acid ($C_{25}H_{24}O_{12}$) and 4,5-dicaffeoylquinic acid ($C_{25}H_{24}O_{12}$), calculated with reference to the dried substance.

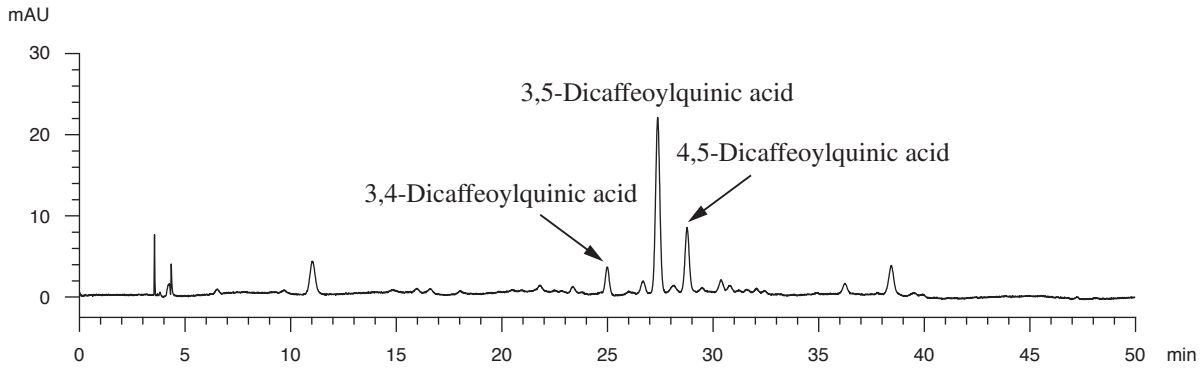

Figure 7 A reference assay chromatogram of Artemisiae Anomalae Herba extract