Apocyni Veneti Folium

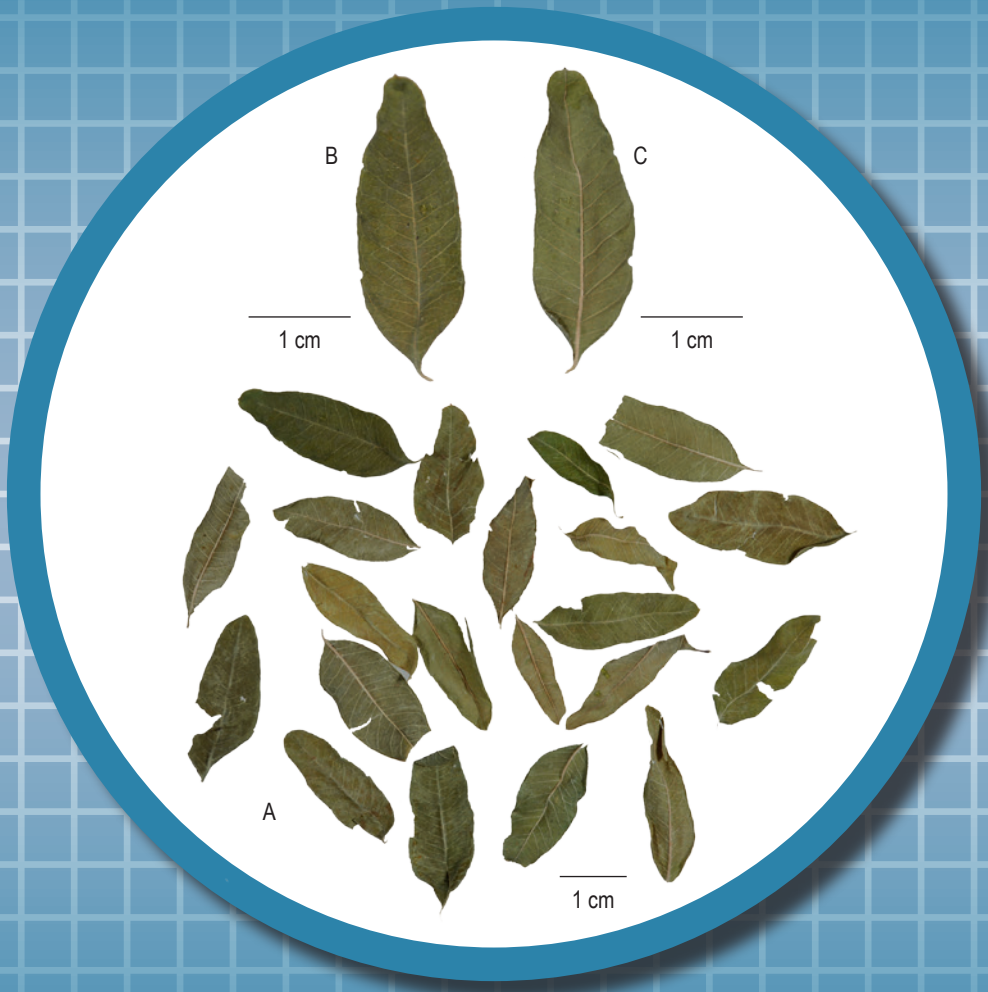


Figure 1 A photograph of Apocyni Veneti Folium

A. Apocyni Veneti Folium B. Upper part of the leaf C. Lower part of the leaf

1. NAMES

Official Name: Apocyni Veneti Folium

Chinese Name: 羅布麻葉

Chinese Phonetic Name: Luobumaye

2. SOURCE

Apocyni Veneti Folium is the dried leaf of *Apocynum venetum* L. (Apocynaceae). The leaf is collected in summer, foreign matters removed, then dried under the sun or in shaded area to obtain Apocyni Veneti Folium.

3. DESCRIPTION

Mostly crumpled, some broken; when intact and flattened, lamina elliptic-lanceolate or ovate-lanceolate, 1.3-5 cm long, 0.4-2.1 cm wide, pale green or greyish-green. Apex obtuse with small arista, base obtuse or cuneate; margin serrulate, usually recurved, glabrous on both surfaces. Veins prominent at lower surface. Petioles thin, about 1-5 mm long. Texture fragile. Odour slight; taste weak (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Upper epidermal cells flattened, with convex outer walls. Palisade tissue consists of 1-2 layers of cells. Collenchyma located in the upper and lower sides of midrib. Spongy tissue consists of 2-4 layers of cells, containing brown contents. Vascular bundle of midrib bicollateral. Lower epidermis consists 1 layer of rectangular cells (Fig. 2).

Powder

Colour pale green or greyish-green. Lower epidermal cells subpolygonal in surface view, stomata paracytic, easily observed. Upper epidermal cells with convex outer wall. Non-glandular hairs multicellular. Cells of spongy tissue subspherical, containing brown or reddish-brown content. Clusters of calcium oxalate subrounded, 8-29 μm in diameter, mainly with short and obtuse angles; polychromatic under the polarized microscope. Fibres mostly in bundles, pale, long. Laticifer contain milky juice. Vessels spiral or annular, 1-25 μm in diameter (Fig. 3).

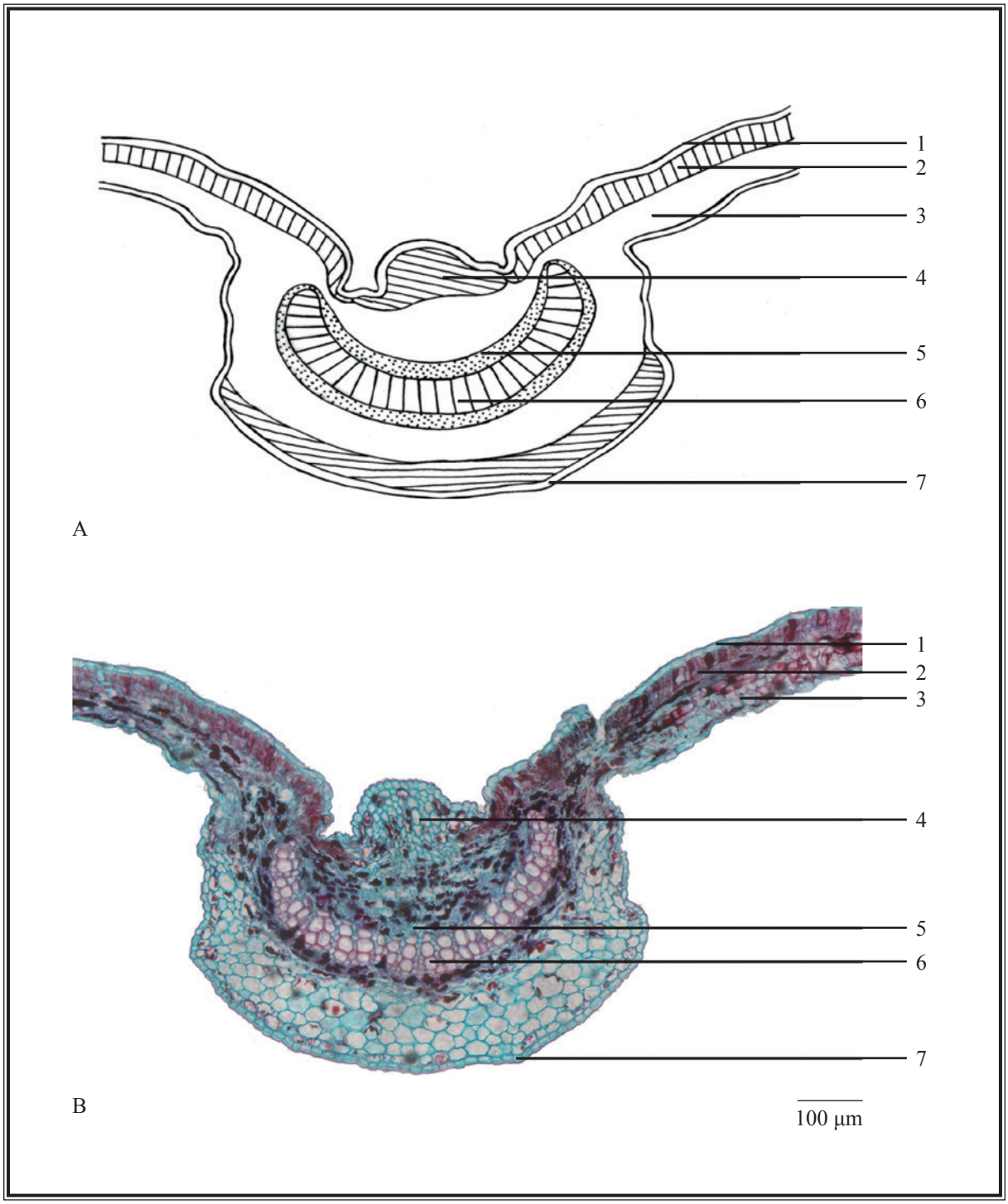


Figure 2 Microscopic features of transverse section of Apocyni Veneti Folium

A. Sketch B. Section illustration

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Collenchyma 5. Phloem
6. Xylem 7. Lower epidermis

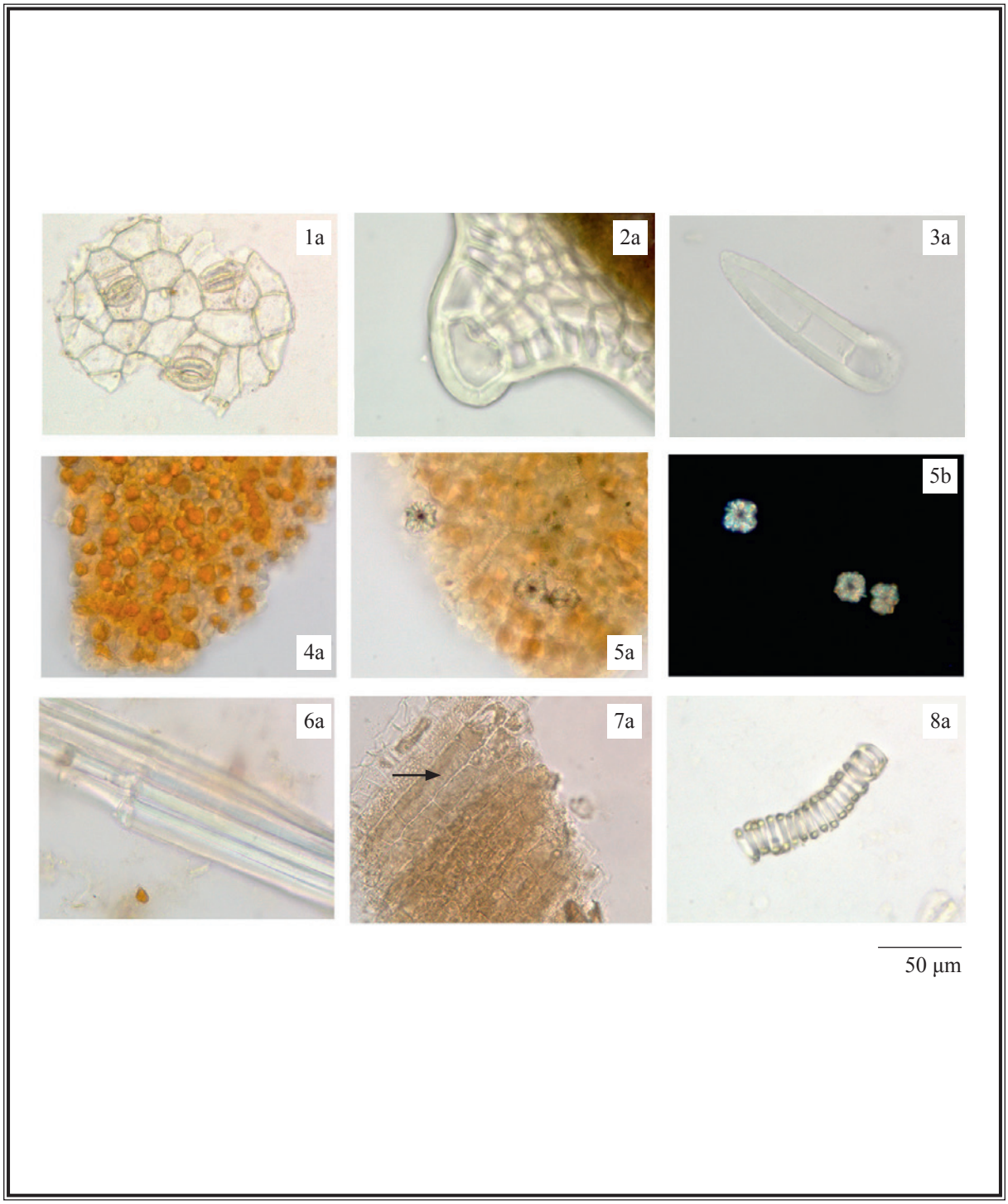


Figure 3 Microscopic features of powder of Apocyni Veneti Folium

1. Lower epidermal cells with stomata 2. Upper epidermal cells with palisade tissue
3. Non-glandular hair 4. Spongy tissue 5. Clusters of calcium oxalate 6. Fibres
7. Laticifer 8. Spiral vessel

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

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

Standard solutions

Hyperoside standard solution

Weigh 4.5 mg of hyperoside CRS (Fig. 4) and dissolve in 5 mL of ethanol (50%).

Isoquercitrin standard solution

Weigh 5.0 mg of isoquercitrin CRS (Fig. 4) and dissolve in 5 mL of ethanol (50%).

Developing solvent system

Prepare a mixture of ethyl acetate, butanone, formic acid and water (10:2:2:0.9, v/v).

Spray reagent

Weigh 1 g of aluminium trichloride and dissolve in 100 mL of ethanol.

Test solution

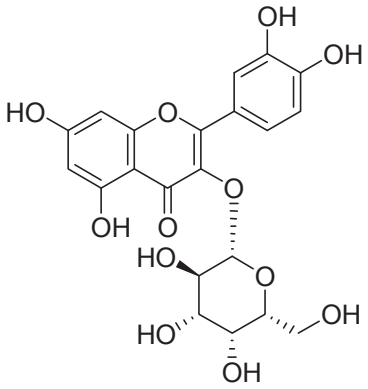
Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 30 mL of ethanol (50%). Sonicate (150 W) the mixture for 10 min. Filter the mixture.

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 hyperoside standard solution (0.3 µL), isoquercitrin standard solution (0.5 µL) and the test solution (3 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. Spray the plate evenly with the spray reagent and dry it in air until the spots or bands become visible. Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

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 hyperoside and isoquercitrin.

(i)



(ii)

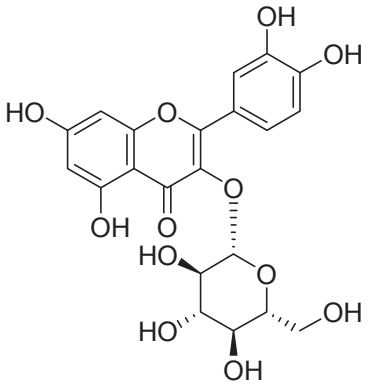


Figure 4 Chemical structures of (i) hyperoside and (ii) isoquercitrin

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

Standard solutions

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

Weigh 2.5 mg of hyperoside CRS and dissolve in 50 mL of ethanol (70%).

Isoquercitrin standard solution for fingerprinting, Std-FP (60 mg/L)

Weigh 3.0 mg of isoquercitrin CRS and dissolve in 50 mL of ethanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (70%). Sonicate (150 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (360 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)	Acetonitrile (%, v/v)	0.1% Formic acid (%, v/v)	Elution
0 – 10	12 → 17	88 → 83	linear gradient
10 – 23	17	83	isocratic
23 – 35	17 → 20	83 → 80	linear gradient
35 – 45	20 → 30	80 → 70	linear gradient
45 – 50	30 → 70	70 → 30	linear gradient

System suitability requirements

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

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 1.5 (Fig. 5).

Procedure

Separately inject hyperoside Std-FP, isoquercitrin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of hyperoside and isoquercitrin peaks in the chromatograms of hyperoside Std-FP, isoquercitrin Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify hyperoside and isoquercitrin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of hyperoside Std-FP and isoquercitrin Std-FP. The retention times of hyperoside and isoquercitrin 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 Apocyni Veneti Folium extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Apocyni Veneti Folium extract

Peak No.	RRT	Acceptable Range
1 (hyperoside)	0.95	± 0.03
2 (marker, isoquercitrin)	1.00	-
3	1.20	± 0.03
4	1.58	± 0.03
5	1.82	± 0.05

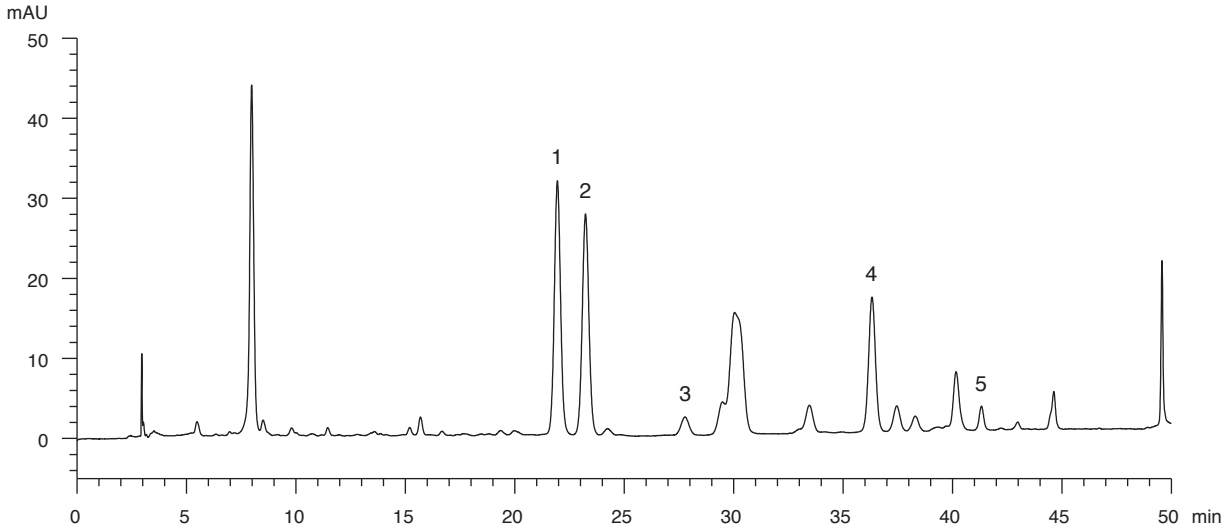


Figure 5 A reference fingerprint chromatogram of Apocyni Veneti Folium extract

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. 5).

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 XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 12.0%.

Acid-insoluble ash: not more than 3.0%.

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 21.0%.
Ethanol-soluble extractives (cold extraction method): not less than 20.0%.

7. ASSAY

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

Standard solution

Mixed hyperoside and isoquercitrin standard stock solution, Std-Stock (100 mg/L each)

Weigh accurately 1.0 mg of hyperoside CRS and 1.0 mg of isoquercitrin CRS, and dissolve in 10 mL of methanol (70%).

Mixed hyperoside and isoquercitrin standard solution for assay, Std-AS

Measure accurately the volume of the mixed hyperoside and isoquercitrin Std-Stock, dilute with methanol (70%) to produce a series of solutions of 2, 10, 20, 30, 60 mg/L for both hyperoside and isoquercitrin.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 40 mL of methanol (70%). Reflux the mixture for 3 h. Cool down to room temperature. Transfer the mixture to a 50-mL centrifuge tube. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Wash the residue with methanol (70%). Centrifuge at about 3000 × *g* for 5 min. Combine the supernatants and make up to the mark with methanol (70%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (256 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 3) –

Table 3 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.05% Trifluoroacetic acid (%, v/v)	Elution
0 – 22	17 → 18	83 → 82	linear gradient
22 – 30	18 → 35	82 → 65	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed hyperoside and isoquercitrin Std-AS (20 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of hyperoside and isoquercitrin should not be more than 5.0%; the RSD of the retention times of hyperoside and isoquercitrin peaks should not be more than 2.0%; the column efficiencies determined from hyperoside and isoquercitrin peaks should not be less than 8000 theoretical plates.

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

Limits

The sample contains not less than 0.45% of hyperoside ($C_{21}H_{20}O_{12}$) and not less than 0.36% of isoquercitrin ($C_{21}H_{20}O_{12}$), calculated with reference to the dried substance.