Rhododendri Daurici Folium

Figure 1 A photograph of Rhododendri Daurici Folium

A. Rhododendri Daurici Folium B. Magnified image of upper surface of leaf
C. Magnified image of lower surface of leaf

1. NAMES

Official name: Rhododendri Daurici Folium

Chinese name: 滿山紅

Chinese phonetic name: Manshanhong

2. SOURCE

Rhododendri Daurici Folium is the dried leaf of *Rhododendron dauricum* L. (Ericaceae). The leaf is collected in summer, foreign matter removed, then dried in shaded area to obtain Rhododendri Daurici Folium.

3. DESCRIPTION

Leaf blade rolled, torn or crumpled, elliptic or oblanceolate, 4-8 cm long, 1-3 cm wide; apex mucronate, margin slightly revolute, base obtuse; both surfaces greenish-brown to brown; papery to almost leathery. Petiole 3-7 mm, glabrescent. Odour aromatic; taste bitter, slightly acrid (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Upper epidermal cells rectangular, covered with cuticle, with unicellular non-glandular hair on the surface near the midrib. Palisade tissue consists of 2-3 layers of cells, discontinuous at the midrib. Cells of spongy tissue subrounded. Vascular bundle of midrib bicollateral, pericycle fibres arranged in an interrupted ring. Collenchymatous cells occurring inside the upper and lower epidermis at the midrib. Parenchymatous cells containing clusters of calcium oxalate in rosette aggregate. Lower epidermal cells subrounded and peltate hairs at sunken spaces (Fig. 2).

Powder

Colour yellowish-green, greenish-brown to brown. Upper epidermal cells polygonal to irregular in shape. Lower epidermal cells with sinuous walls, paracytic stomata with 2 subsidiary cells. Non-glandular hair unicellular. Reticulate and bordered-pitted vessels visible, 5-137 μm in diameter. Stone cell numerous, 25-65 μm in diameter. Cluster of calcium oxalate in rosette aggregate, scattered singly, 9-72 μm in diameter; polychromatic under the polarized microscope. Fibre occasionally found, 9-18 μm in diameter; polychromatic under the polarized microscope. Peltate hair multicellular, branched, plate-like or shield-like, occasionally found (Fig. 3).

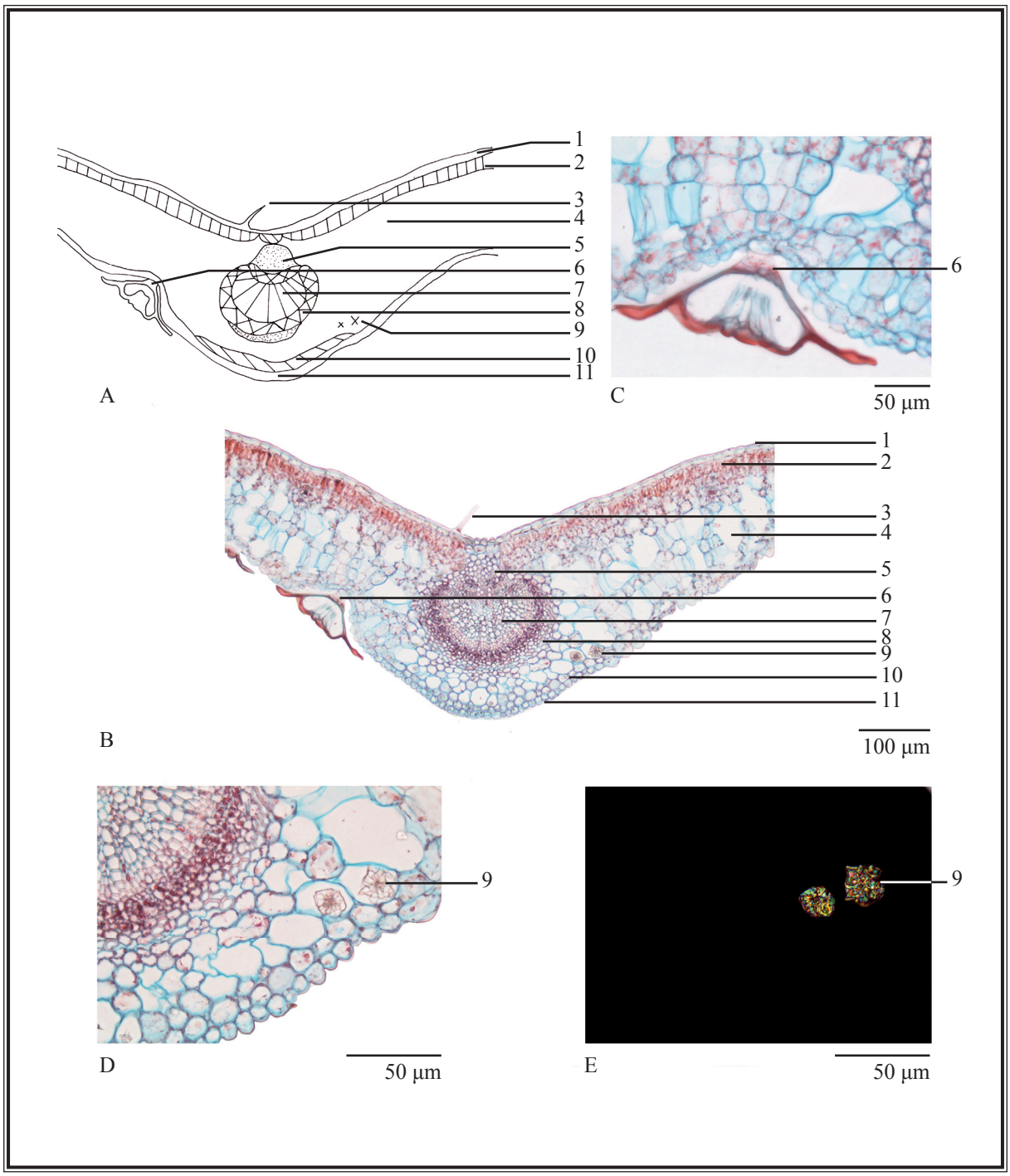


Figure 2 Microscopic features of the transverse section of Rhododendri Daurici Folium

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

1. Upper epidermis 2. Palisade tissue 3. Non-glandular hair 4. Spongy tissue
5. Phloem 6. Peltate hair 7. Xylem 8. Pericycle fibre 9. Cluster of calcium oxalate
10. Collenchyma 11. Lower epidermis

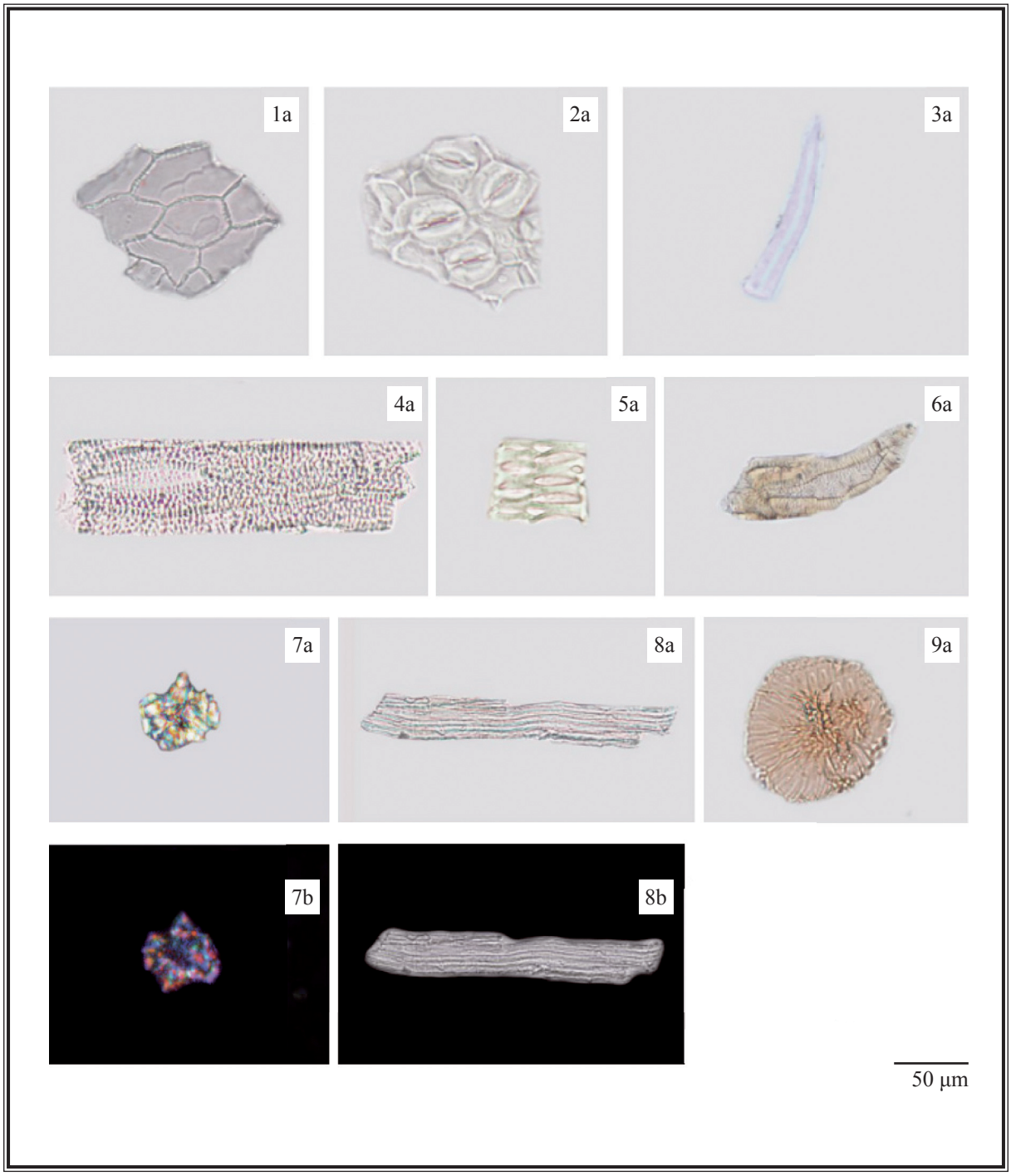


Figure 3 Microscopic features of the powder of Rhododendri Daurici Folium

1. Upper epidermal cells 2. Lower epidermal cells with paracytic stomata
3. Non-glandular hair 4. Bordered-pitted vessel 5. Reticulate vessel 6. Stone cell
7. Cluster of calcium oxalate 8. Fibres 9. Peltate hair

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

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

Standard solutions

Farrerol standard solution

Weigh 0.3 mg of farrerol CRS (Fig. 4) and dissolve in 1 mL of ethanol (70%).

Hyperoside standard solution

Weigh 0.6 mg of hyperoside CRS (Fig. 4) and dissolve in 1 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of methanol, water, tetrahydrofuran and formic acid (3.5: 2.5: 1.5: 0.3, v/v).

Spray reagent

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

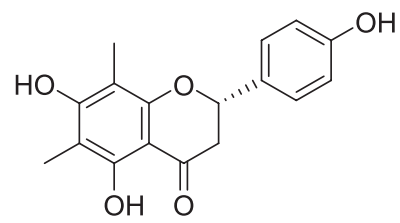
Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (70%). Sonicate (200 W) the mixture for 15 min. Filter through a 0.45-μm PTFE filter.

Procedure

Carry out the method by using a HPTLC RP-18 F_{254S} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately farrerol standard solution (2 μL), hyperoside standard solution (1 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 8.5 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 heat at about 105ºC (about 10 min). 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)

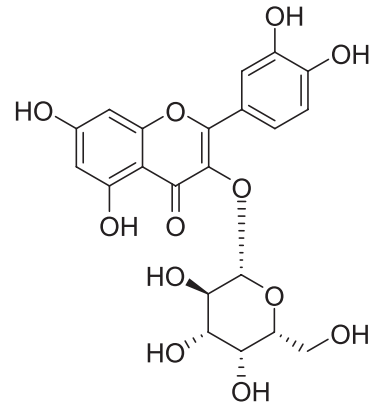


Figure 4 Chemical structures of (i) farrerol and (ii) hyperoside

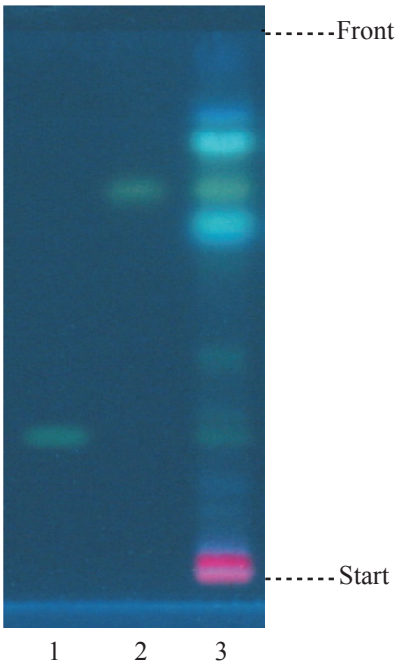


Figure 5 A reference HPTLC chromatogram of Rhododendri Daurici Folium extract observed under UV light (366 nm) after staining

1. Farrerol standard solution 2. Hyperoside 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 farrerol and hyperoside (Fig. 5).

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

Standard solutions

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

Weigh 1.0 mg of farrerol CRS and dissolve in 100 mL ethanol (70%).

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

Weigh 5.0 mg of hyperoside CRS and dissolve in 100 mL of ethanol (70%).

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (70%). Sonicate (120 W) the mixture for 40 min. Centrifuge at about 4000 × *g* for 10 min. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (310 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)	0.2% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 25	80 → 62	20 → 38	linear gradient
25 – 45	62 → 55	38 → 45	linear gradient
45 – 60	55 → 25	45 → 75	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 µL of farrerol Std-FP and hyperoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of farrerol and hyperoside should not be more than 5.0%; the RSD of the retention times of farrerol and hyperoside peaks should not be more than 2.0%; the column efficiencies determined from farrerol and hyperoside peaks should not be less than 800000 and 50000 theoretical plates respectively.

The *R* value between peak 1 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 1.0 (Fig. 6).

Procedure

Separately inject farrerol Std-FP, hyperoside Std-FP and the test solution (5 µL each) into the HPLC system and record the chromatograms. Measure the retention times of farrerol and hyperoside peaks in the chromatograms of farrerol Std-FP, hyperoside Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify farrerol and hyperoside peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of farrerol Std-FP and hyperoside Std-FP. The retention times of farrerol and hyperoside 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 Rhododendri Daurici Folium extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Rhododendri Daurici Folium extract

Peak No.	RRT	Acceptable Range
1 (marker, hyperoside)	1.00	-
2	1.18	± 0.03
3	1.47	± 0.03
4	2.16	± 0.03
5 (farrerol)	2.23	± 0.03

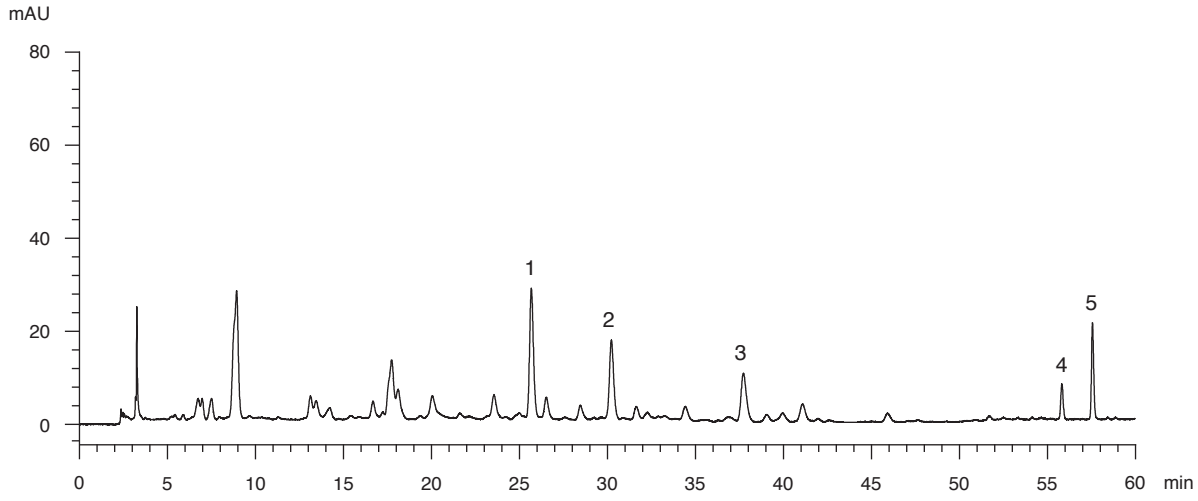


Figure 6 A reference fingerprint chromatogram of Rhododendri Daurici Folium extract

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. 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 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 6.5%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 9.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 15.0%.
Ethanol-soluble extractives (cold extraction method): not less than 23.0%.

7. ASSAY

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

Standard solution

Mixed farrerol and hyperoside standard stock solution, Std-Stock (100 mg/L for farrerol and 500 mg/L for hyperoside)

Weigh accurately 1.0 mg of farrerol CRS and 5.0 mg of hyperoside CRS, and dissolve in 10 mL of ethanol (70%).

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

Measure accurately the volume of the mixed farrerol and hyperoside Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 1, 2.5, 5, 10, 20 mg/L for farrerol and 5, 12.5, 25, 50, 100 mg/L for hyperoside.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (70%). Sonicate (120 W) the mixture for 40 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction one more time. Combine the supernatants and make up to the mark with ethanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (310 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)	0.2% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 5	75 → 69	25 → 31	linear gradient
5 – 25	69	31	isocratic
25 – 40	69 → 29	31→ 71	linear gradient
40 – 45	29	71	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 μL of the mixed farrerol and hyperoside Std-AS (5 mg/L for farrerol and 25 mg/L for hyperoside). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of farrerol and hyperoside should not be more than 5.0%; the RSD of the retention times of farrerol and hyperoside peaks should not be more than 2.0%; the column efficiencies determined from farrerol and hyperoside peaks should not be less than 1000000 and 10000 theoretical plates respectively.

The *R* value between farrerol peak and the closest peak; and the *R* value between hyperoside 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 farrerol and hyperoside Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of farrerol and hyperoside against the corresponding concentrations of the mixed farrerol and hyperoside Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify farrerol and hyperoside peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed farrerol and hyperoside Std-AS. The retention times of farrerol and hyperoside 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 farrerol and hyperoside in the test solution, and calculate the percentage contents of farrerol and hyperoside in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.080% of farrerol ($C_{17}H_{16}O_5$) and 0.16% of hyperoside ($C_{21}H_{20}O_{12}$), calculated with reference to the dried substance.

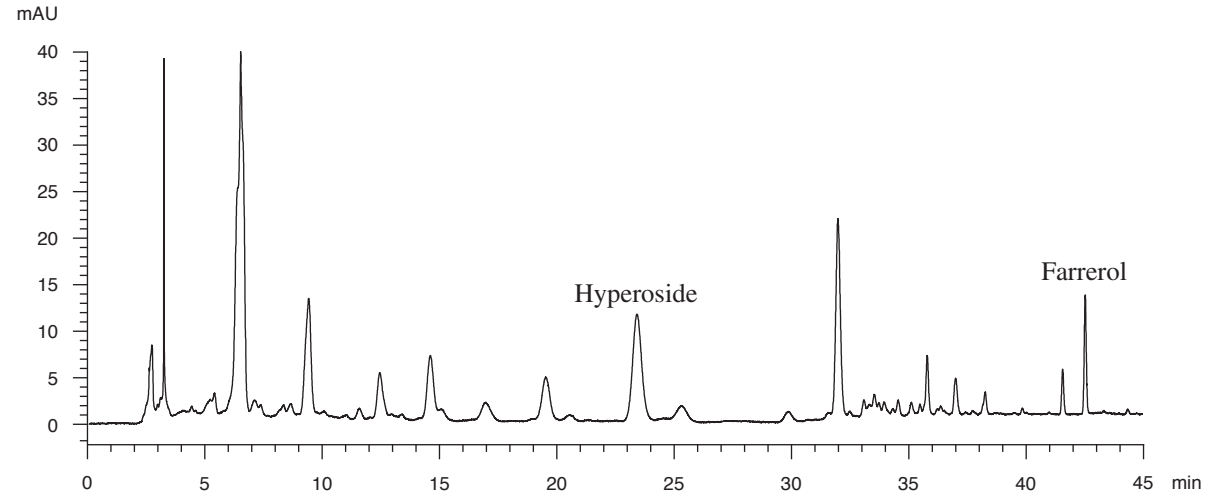


Figure 7 A reference assay chromatogram of Rhododendri Daurici Folium extract