Oroxyli Semen

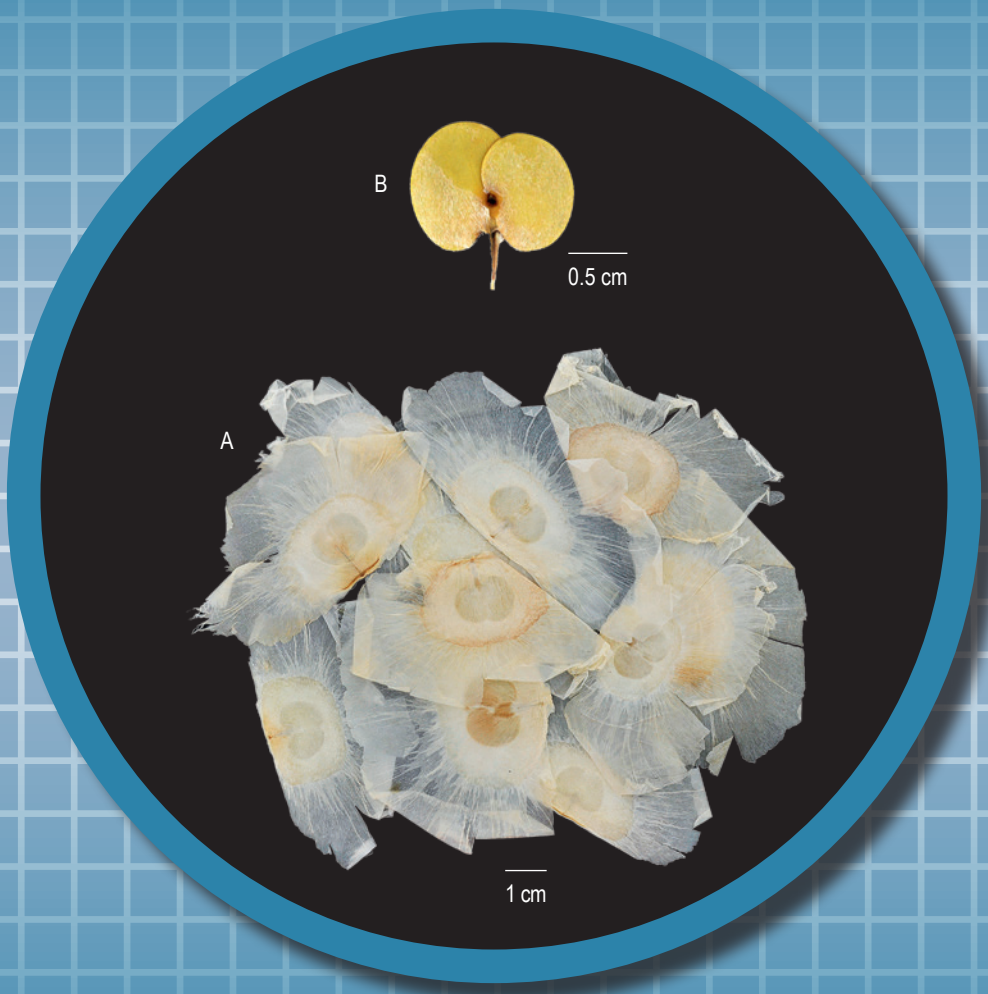


Figure 1 A photograph of Oroxyli Semen

A. Oroxyli Semen B. Cotyledons

1. NAMES

Official Name: Oroxyli Semen

Chinese Name: 木蝴蝶

Chinese Phonetic Name: Muhudie

2. SOURCE

Oroxyli Semen is the dried ripe seed of *Oroxylum indicum* (L.) Vent. (Bignoniaceae). The ripe fruit is collected in autumn and winter, dried under the sun until the fruit dehiscent, the seed gathered and dried under the sun to obtain Oroxyli Semen.

3. DESCRIPTION

Butterfly-shaped thin slices, testa extending on three sides, forming wide wings, 4.3-10 cm long, 3-5 cm wide. Externally pale yellowish-white with silky lustre; the wing membranous and translucent, with radial striations, the margin mostly broken. Texture light in weight. After removal of testa, membranous endosperms tightly cover the cotyledons. Cotyledons 2, butterfly-shaped, pale yellow to yellowish-green; 8-22 mm in diameter. Odour slight; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Endosperm consists of 2-4 layers of cells. Upper epidermal cells of cotyledon square to rectangular. Lower epidermal cells relatively small. Palisade tissue cells rectangular, containing oil droplets and chloroplasts. Spongy tissue cells oval or irregular, containing oil droplets and starch granules. Primary vascular bundles scattered in the spongy tissue (Fig. 2).

Powder

Colour yellow to greenish-yellow. Testa cells elongated, fibre-shaped, 15-49 μm in diameter, wall sinuously thickened; polychromatic under the polarized microscope. Endosperm cells polygonal to subsquare, wall beaded. Crystals of calcium oxalate abundant, present in testa and endosperm cells, 2-19 μm in diameter; polychromatic under the polarized microscope (Fig. 3).

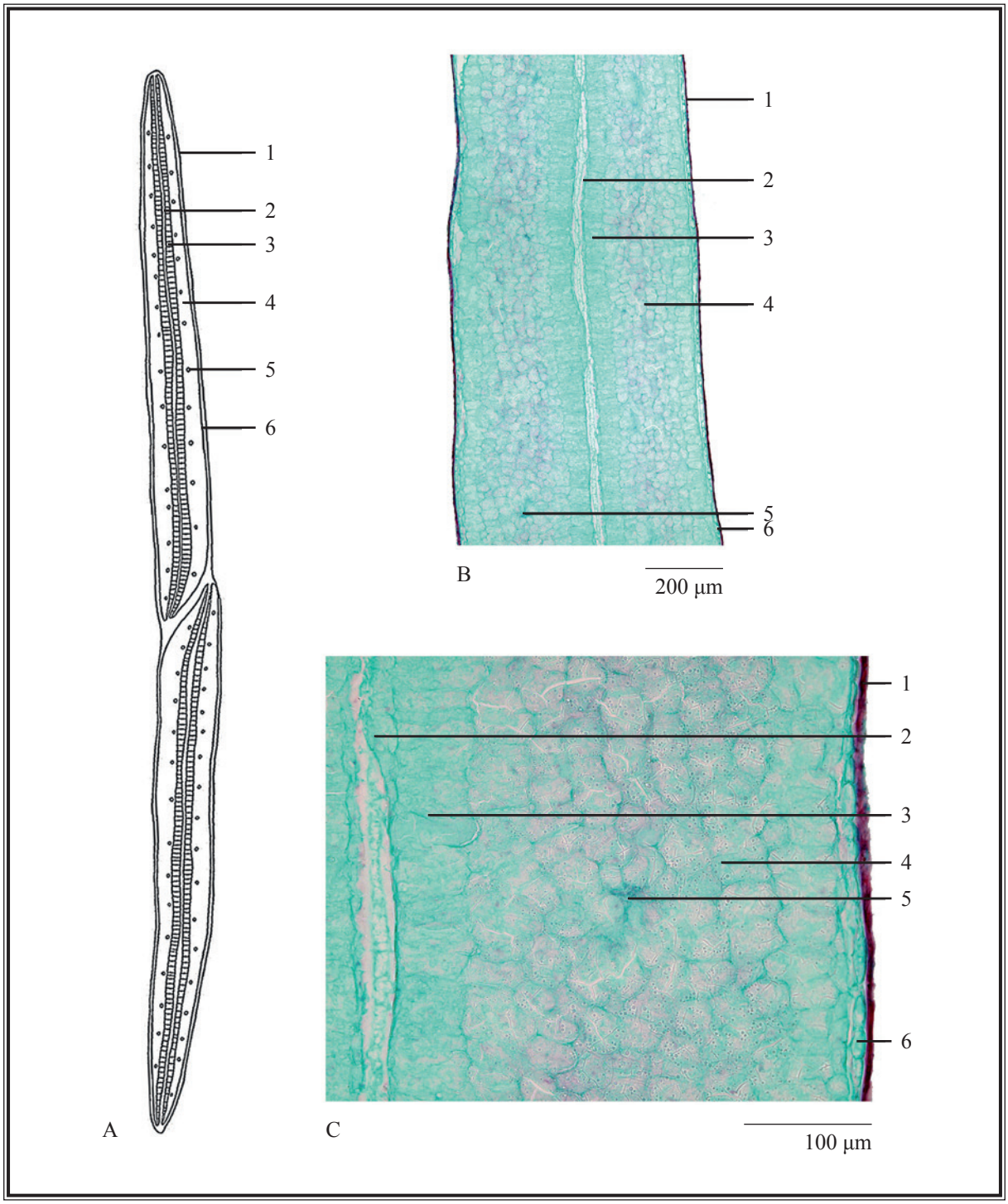


Figure 2 Microscopic features of transverse section of cotyledon of Oroxyli Semen

A. Sketch B. Section illustration C. Section magnified

1. Endosperm 2. Upper epidermis of cotyledon 3. Palisade tissue
4. Spongy tissue 5. Primary vascular bundle 6. Lower epidermis of cotyledon

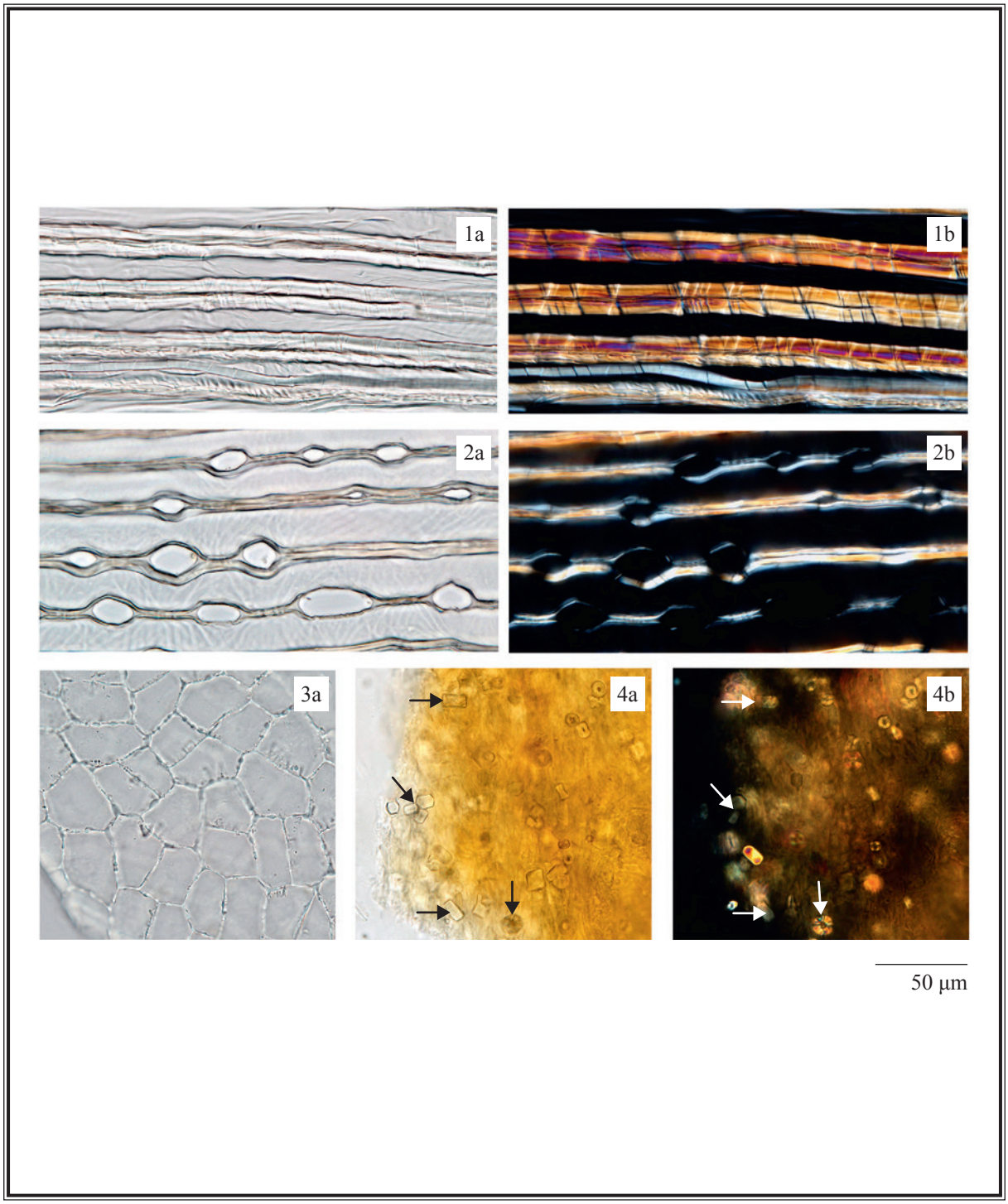


Figure 3 Microscopic features of powder of Oroxyli Semen

1, 2. Testa cells 3. Endosperm cells 4. Crystals of calcium oxalate

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

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

Standard solutions

Baicalein standard solution

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

Chrysin standard solution

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

Developing solvent system

Prepare a mixture of n-hexane, ethyl acetate and glacial acetic acid (5:5:1, v/v).

Spray reagent

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

Test solution

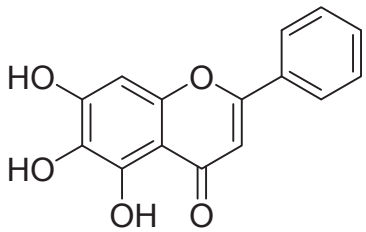
Weigh 1.0 g of the powdered sample and place it in a 25-mL conical flask, then add 10 mL of methanol (80%). Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 10-mL volumetric flask. Wash the residue with methanol (80%). Combine the solution and make up to the mark with methanol (80%). Freshly prepare the test solution.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate (2-10 µm) and a freshly prepared developing solvent system as described above. Apply separately baicalein standard solution (3 µL), chrysin standard solution (3 µL) and the test solution (4 µL) to the plate. 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 visible light. 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 baicalein and chrysin.

(i)



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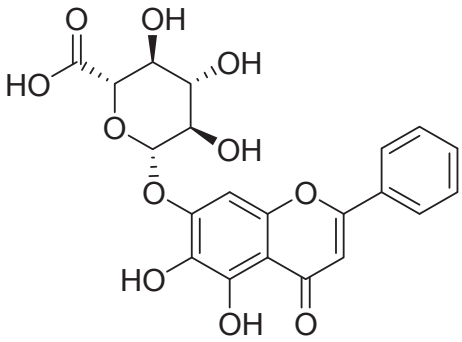

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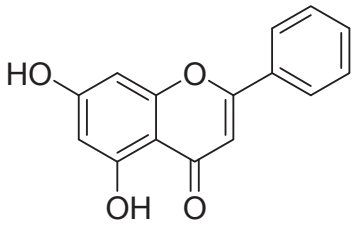

(iv)

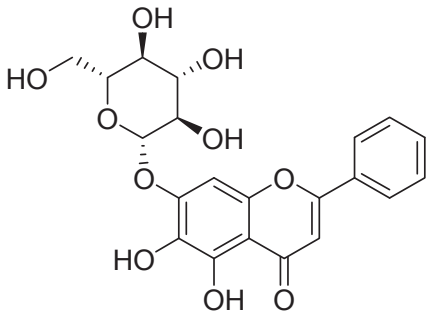

Figure 4 Chemical structures of (i) baicalein (ii) baicalin (iii) chrysin and (iv) oroxin A

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

Standard solutions

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

Weigh 0.5 mg of baicalein CRS and dissolve in 10 mL of methanol.

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

Weigh 0.5 mg of baicalin CRS (Fig. 4) and dissolve in 10 mL of methanol.

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

Weigh 0.5 mg of chrysin CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol (80%). Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Make up to the mark with methanol (80%). Filter through a 0.45-μm PTFE filter. Freshly prepare the test solution.

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)	Methanol (%, v/v)	Elution
0 – 20	55	45	isocratic
20 – 45	55 → 0	45 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μL of baicalein Std-FP, baicalin Std-FP and chrysin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of baicalein, baicalin and chrysin should not be more than 5.0%; the RSD of the retention times of baicalein, baicalin and chrysin peaks should not be more than 2.0%; the column efficiencies determined from baicalein, baicalin and chrysin peaks should not be less than 110000, 10000 and 200000 theoretical plates respectively.

The *R* value between peak 3 and the closest peak; the *R* value between peak 5 and the closest peak; and the *R* value between peak 6 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject baicalein Std-FP, baicalin Std-FP, chrysin Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention times of baicalein, baicalin and chrysin peaks in the chromatograms of baicalein Std-FP, baicalin Std-FP, chrysin Std-FP and the retention times of the six characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify baicalein, baicalin and chrysin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of baicalein Std-FP, baicalin Std-FP and chrysin Std-FP. The retention times of baicalein, baicalin and chrysin 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 six characteristic peaks of Oroxyli Semen extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Oroxyli Semen extract

Peak No.	RRT	Acceptable Range
1 (oroxin B)	0.41	± 0.06
2 (oroxin A)	0.70	± 0.05
3 (baicalin)	0.77	± 0.06
4	0.89	± 0.04
5 (marker, baicalein)	1.00	-
6 (chrysin)	1.12	± 0.03

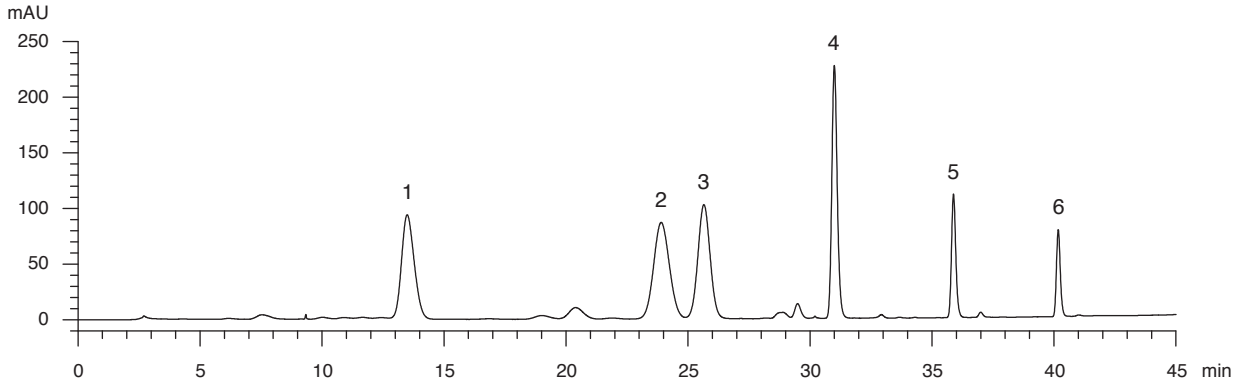

Figure 5 A reference fingerprint chromatogram of Oroxyli Semen extract

For positive identification, the sample must give the above six 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 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 5.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 14.0%.
Ethanol-soluble extractives (hot extraction method): not less than 20.0%.

7. ASSAY

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

Standard solution

Mixed baicalin and oroxin A standard stock solution, Std-Stock (200 mg/L each)

Weigh accurately 1.0 mg of baicalin CRS and 1.0 mg of oroxin A CRS (Fig. 4), and dissolve in 5 mL of methanol. Keep at about 4°C.

Mixed baicalin and oroxin A standard solution for assay, Std-AS

Measure accurately the volume of the mixed baicalin and oroxin A Std-Stock, dilute with methanol to produce a series of solutions of 0.5, 1, 10, 20, 30 mg/L for both baicalin and oroxin A. Keep at about 4°C.

Test solution

Weigh accurately 0.05 g of the powdered sample and place it in a 50-mL conical flask, then add 30 mL of methanol. Sonicate (180 W) the mixture for 15 min in an ice water bath at about 4°C. Filter and transfer the filtrate to a 100-mL volumetric flask. Keep the solution at about 4°C. Repeat the extraction for two more times. Combine the filtrates and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter. Freshly prepare the test solution and keep at about 4°C.

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 test sample solution is maintained at a temperature of 4°C. The internal diameter of inlet column tubing is about 0.5 mm. 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)	Methanol (%, v/v)	Elution
0 – 30	60	40	isocratic
30 – 60	60 → 25	40 → 75	linear gradient
60 – 70	25	75	isocratic

System suitability requirements

Perform at least five replicate injections, each using 20 μL of the mixed baicalin and oroxin A Std-AS (10 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of baicalin and oroxin A should not be more than 5.0%; the RSD of the retention times of baicalin and oroxin A peaks should not be more than 2.0%; the column efficiencies determined from baicalin and oroxin A peaks should not be less than 20000 theoretical plates.

The *R* value between baicalin and the closest peak; and the *R* value between oroxin A 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 baicalin and oroxin A Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of baicalin and oroxin A against the corresponding concentrations of the mixed baicalin and oroxin A Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 20 μL of the test solution into the HPLC system and record the chromatogram. Identify baicalin and oroxin A peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed baicalin and oroxin A Std-AS. The retention times of baicalin and oroxin A 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 baicalin and oroxin A in the test solution, and calculate the percentage contents of baicalin and oroxin A in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 2.0% baicalin ($C_{21}H_{18}O_{11}$) and not less than 1.5% of oroxin A ($C_{21}H_{20}O_{10}$), calculated with reference to the dried substance.