Eupatorii Chinensis Radix et Rhizoma

Figure 1 A photograph of Eupatorii Chinensis Radix et Rhizoma

A. Eupatorii Chinensis Radix et Rhizoma

B. Cut surface of rhizome

C. Magnified image of cut surface of root

1. NAMES

Official name: Eupatorii Chinensis Radix et Rhizoma

Chinese name: 廣東土牛膝

Chinese phonetic name: Guangdongtuniuxi

2. SOURCE

Eupatorii Chinensis Radix et Rhizoma is the dried root and rhizome of *Eupatorium chinense* L. (Asteraceae). The root and rhizome are collected in autumn. Soil removed, washed clean, then dried under the sun to obtain Eupatorii Chinensis Radix et Rhizoma.

3. DESCRIPTION

Rhizome stout, knotty-lumpy, 9-72 mm in diameter, upper part showing several remnants of stem bases; texture hard, not easily broken. Roots numerous; originating from stout rhizome; slender and cylindrical, some slightly curved, 1.4-54 cm long and 1-5 mm in diameter; externally greyish-brown, yellowish-brown or greyish-yellow, showing fine longitudinal wrinkles and sparse rootlet scars, occasionally with transverse crack mark; fracture fibrous-like; bark relatively thin, easily separated from wood, greyish-brown, yellowish-brown or greyish-yellow; wood broad, yellowish-white; small round pith visible at the centre. Texture hard and fragile, easily broken. Odour aromatic; taste slightly pungent and bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Epidermis consists of 1 layer of cells, cells suberized, densely arranged, subsquare, rectangular or irregular in shape, some containing brown masses. Cortex broad, parenchymatous cells subrounded, elliptic or polygonal. Stone cells scattered singly or occasionally 2 in groups, subrounded, subsquare, elongated-elliptical or polygonal, wall obviously thickened, lignified. In the inner side of cortex, near the endodermis, secretory canals scattered singly or 2-3 in groups, opposite the vascular bundle, arranged in an interrupted ring, 30-91 μm in diameter. Endodermis distinct, consisting of 1 layer of cells, forming a wavy ring. Vascular bundles collateral, fibre bundles located on the outer side of phloem; phloem narrow; xylem lignified, vessels arranged closely and irregularly. Pith relatively small, parenchymatous cells subrounded or polygonal [Fig. 2 (i)].

Rhizome: Epidermis consists of 1 layer of cells, cells suberized, densely arranged, subsquare, rectangular or irregular in shape, some containing brown masses. Root-trace vascular bundles sometimes visible. Cortex extremely narrow, parenchymatous cells subrounded, elliptic or polygonal. In the inner side of cortex, stone cells scattered singly or 2-8 in groups, subrounded, subsquare, elongated-elliptical or polygonal, wall obviously thickened, lignified. Fibres in bundles, arranged in an interrupted ring, mostly lignified. Phloem easily broken during preparation of slides, empty spaces appear between cortex and cambium. Cambium distinct. Xylem broad, consisting of vessels and xylem parenchymatous cells, lignified; xylem vessels radially arranged; xylem rays 1-7 rows of cells wide, mostly radially elongated. Pith large, parenchymatous cells subrounded or polygonal, often broken or hollow in the centre [Fig. 2 (ii)].

Powder

Colour greyish-yellow, yellowish-brown or dark brown. Stone cells abundant, singly scattered or in groups, pale yellowish-green, subrounded, subsquare, long stripe or irregular in shape, 36-525 μm long, 21-149 μm in diameter, wall heavily thickened, lignified, lumens relatively small, walls 7-28 μm thick, with striations, pit canals and pits distinct; dark brown to black masses sometimes visible around and inside; yellowish-white under the polarized microscope. Fibres elongated, fusiform, singly scattered or in bundles, wall slightly thickened, lignified, with few simple pits, pit canals distinct, 11-65 μm in diameter; white or bluish-white under the polarized microscope. Inulin occasionally found, fan-shaped or irregular, sometimes visible in parenchymatous cells, with weak radial striations; pale bluish-white under the polarized microscope. Fragments of secretory canals contain yellowish-brown secretions, with brown masses scattered around. Vessels mainly bordered-pitted, 12-150 μm in diameter (Fig. 3).

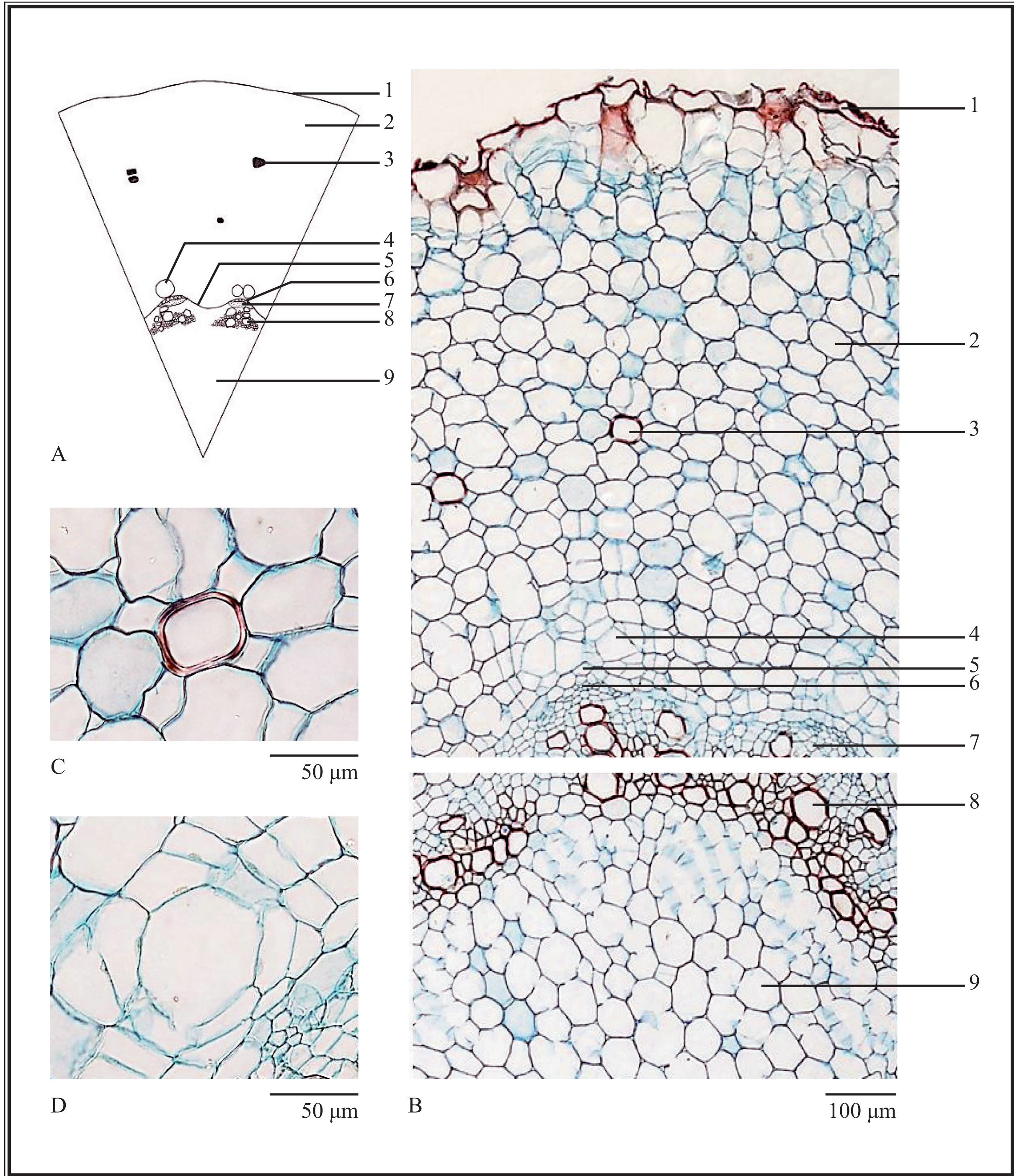

Figure 2 (i) Microscopic features of transverse section of root of Eupatorii Chinensis Radix et Rhizoma

A. Sketch B. Section illustration C. Stone cell D. Secretory canal

1. Epidermis 2. Cortex 3. Stone cell 4. Secretory canal 5. Endodermis 6. Fibre bundle 7. Phloem 8. Xylem 9. Pith

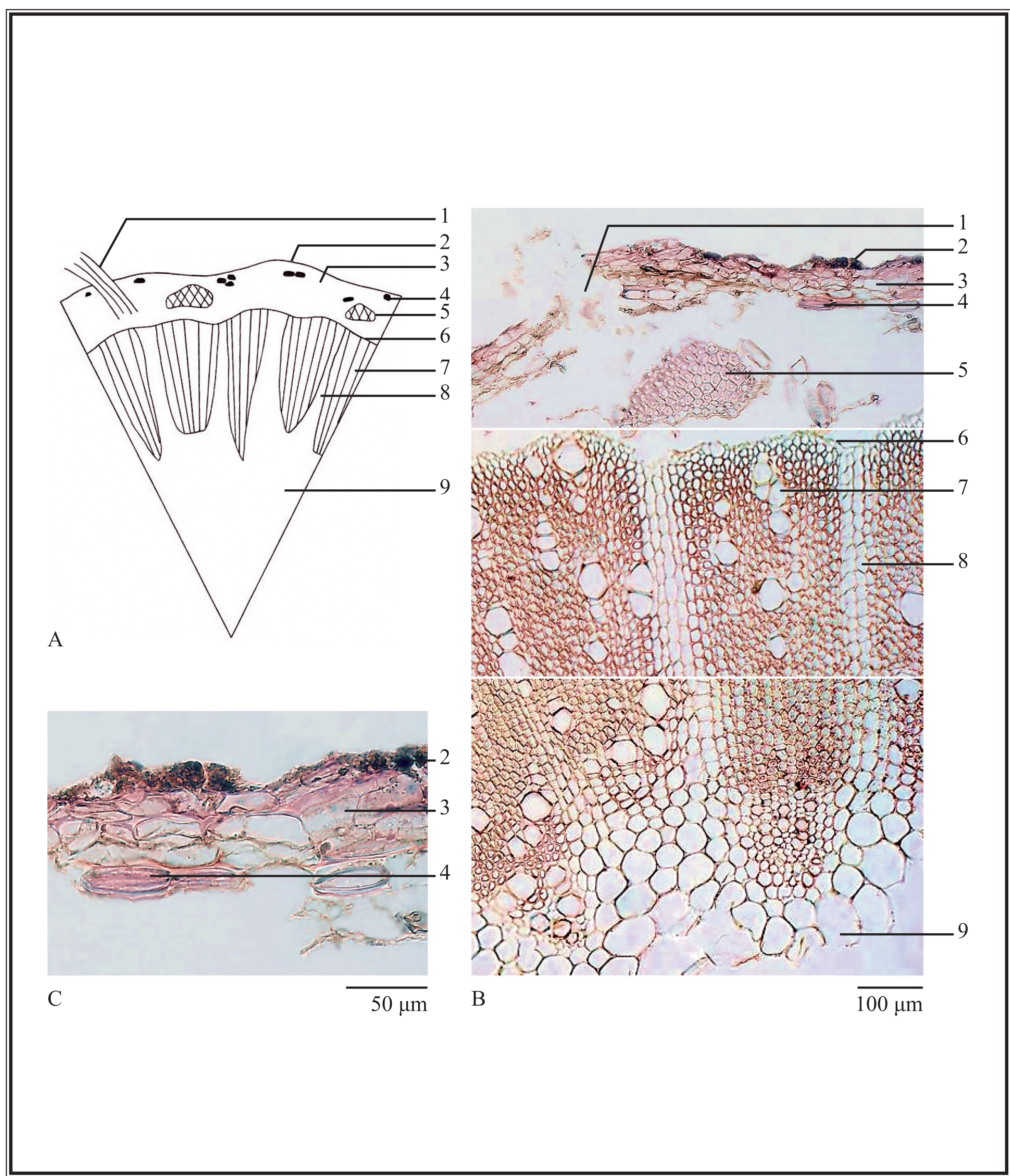

Figure 2 (ii) Microscopic features of transverse section of rhizome of Eupatorii Chinensis Radix et Rhizoma

A. Sketch B. Section illustration C. Section magnified

1. Root-trace vascular bundle 2. Epidermis 3. Cortex 4. Stone cell
5. Fibre bundle 6. Cambium 7. Xylem 8. Xylem ray 9. Pith

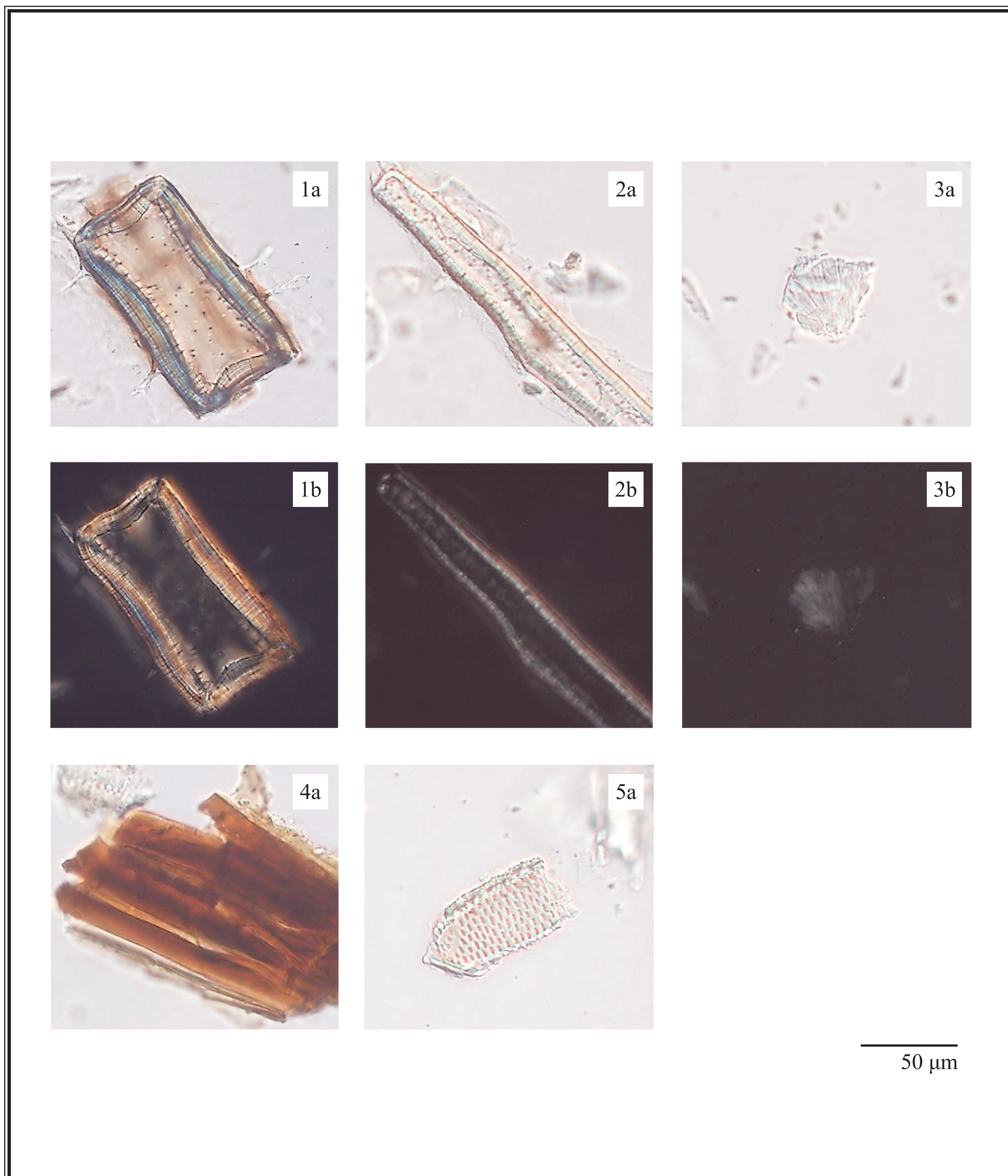

Figure 3 Microscopic features of powder of Eupatorii Chinensis Radix et Rhizoma

1. Stone cell 2. Fibre 3. Inulin 4. Fragments of secretory canals
5. Bordered-pitted vessel

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

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

Standard solution

Euparin standard solution

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

Developing solvent system

Prepare a mixture of petroleum ether (60 – 80ºC) and ethyl acetate (9.4:0.6, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 1200 × *g* for 5 min. Collect the supernatant.

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 euparin standard solution and the test solution (3 μL each) 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 7 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 value by using the equation as indicated in Appendix IV (A).

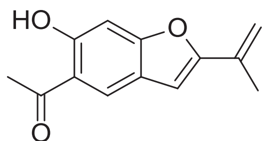

Figure 4 Chemical structure of euparin

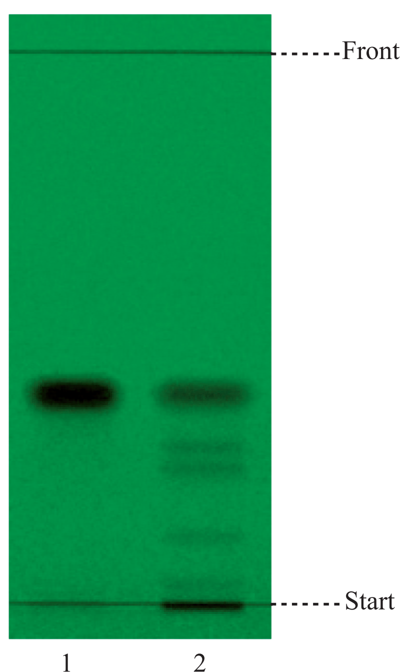

Figure 5 A reference HPTLC chromatogram of Eupatorii Chinensis Radix et Rhizoma extract observed under UV light (254 nm)

1. Euparin standard solution 2. Test solution

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the R_f value, corresponding to that of euparin (Fig. 5).

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

Standard solution

Euparin standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of euparin CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 6000 × *g* for 10 min. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (240 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size, 90 Å pore size and 14.8% carbon loading). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	90 → 80	10 → 20	linear gradient
10 – 20	80 → 60	20 → 40	linear gradient
20 – 25	60 → 45	40 → 55	linear gradient
25 – 60	45 → 20	55 → 80	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of euparin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of euparin should not be more than 5.0%; the RSD of the retention time of euparin peak should not be more than 2.0%; the column efficiency determined from euparin peak should not be less than 150000 theoretical plates.

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

Procedure

Separately inject euparin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of euparin peak in the chromatogram of euparin Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify euparin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of euparin Std-FP. The retention times of euparin peaks from the two chromatograms 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 three characteristic peaks of Eupatorii Chinensis Radix et Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Eupatorii Chinensis Radix et Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.50	± 0.03
2	0.82	± 0.03
3 (marker, euparin)	1.00	-

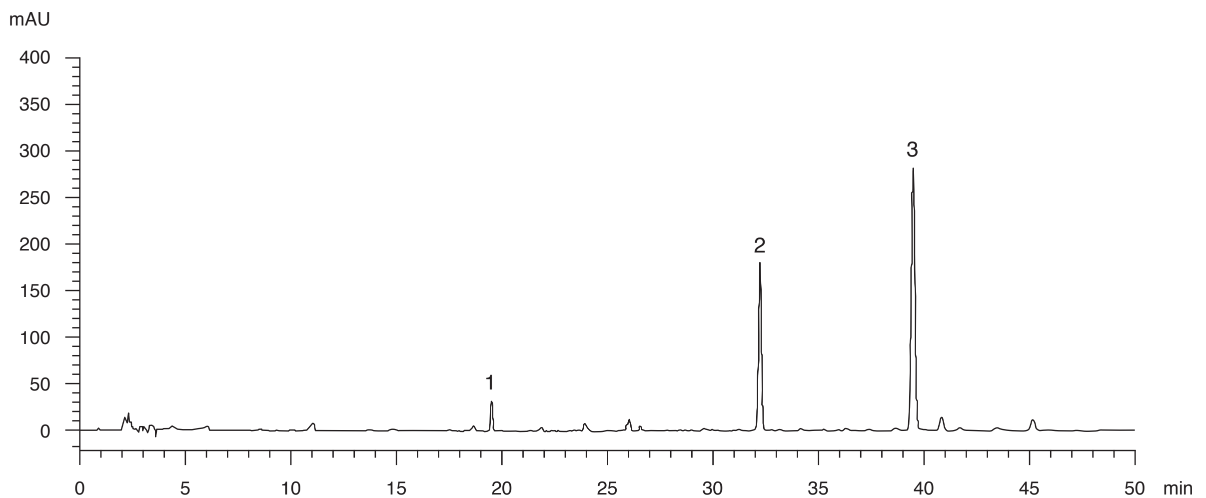

Figure 6 A reference fingerprint chromatogram of Eupatorii Chinensis Radix et Rhizoma extract

For positive identification, the sample must give the above three 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 9.5%.
Acid-insoluble ash: not more than 4.5%.

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 17.0%.
Ethanol-soluble extractives (cold extraction method): not less than 6.0%.

7. ASSAY

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

Standard solution

Euparin standard stock solution, Std-Stock (100 mg/L)

Weigh accurately 1.0 mg of euparin CRS and dissolve in 10 mL of methanol.

Euparin standard solution for assay, Std-AS

Measure accurately the volume of the euparin Std-Stock, dilute with methanol to produce a series of solutions of 1, 5, 10, 20, 50 mg/L for euparin.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 25 mL of methanol. Reflux the mixture in a water bath at 80ºC for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 25-mL volumetric flask. Make up to the mark with methanol. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size, 90 Å pore size and 14.8% carbon loading). 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)	0.2% Phosphoric acid (%, v/v)	Elution
0 – 14	60 → 72	40 → 28	linear gradient
14 – 24	72	28	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of euparin Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of euparin should not be more than 5.0%; the RSD of the retention time of euparin peak should not be more than 2.0%; the column efficiency determined from euparin peak should not be less than 25000 theoretical plates.

The *R* value between euparin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curve

Inject a series of euparin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of euparin against the corresponding concentrations of euparin Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify euparin peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of euparin Std-AS. The retention times of euparin peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of euparin in the test solution, and calculate the percentage content of euparin in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.21% of euparin ($C_{13}H_{12}O_3$), calculated with reference to the dried substance.

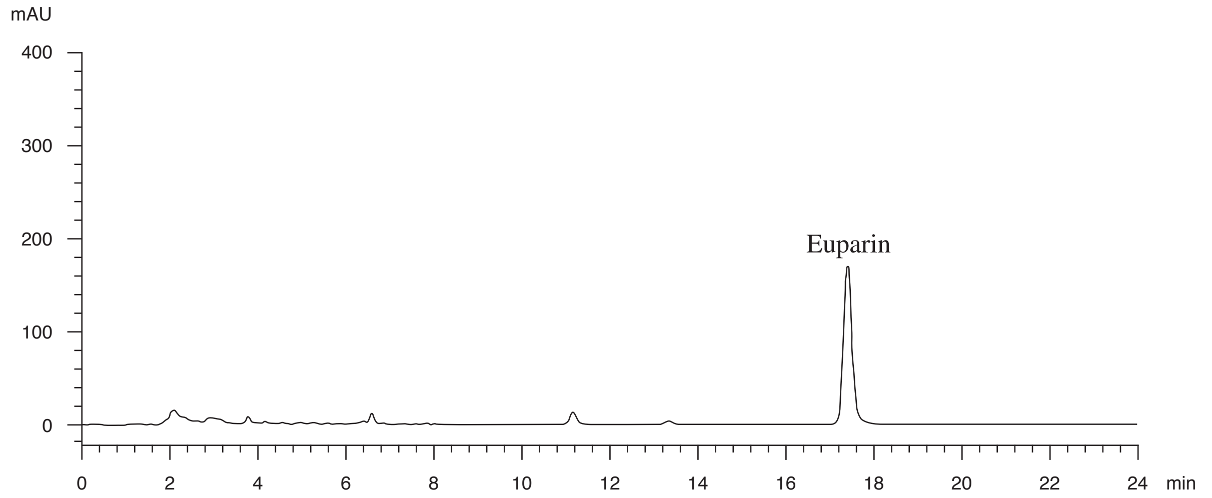

Figure 7 A reference assay chromatogram of Eupatorii Chinensis Radix et Rhizoma extract