Euphorbiae Pekinensis Radix

Figure 1 A photograph of Euphorbiae Pekinensis Radix

A. Euphorbiae Pekinensis Radix

B. Magnified image of fracture surface of root

1. NAMES

Official name: Euphorbiae Pekinensis Radix

Chinese name: 京大戟

Chinese phonetic name: Jingdaji

2. SOURCE

Euphorbiae Pekinensis Radix is the dried root of *Euphorbia pekinensis* Rupr. (Euphorbiaceae). The root is collected during autumn and winter, washed clean, foreign matter removed, then dried under the sun to obtain Euphorbiae Pekinensis Radix.

3. DESCRIPTION

Irregularly long-conical, slightly curved, some with branches, 4.5-25.6 cm long, 2-25 mm in diameter; the apex usually enlarged, with stem scars and remnants of stem base. Externally greyish-yellow to greyish-brown, rough, loose with longitudinal wrinkles and lateral lenticels; branch root few and twisted. Texture hard, uneasily broken, fracture greyish-white to yellowish-brown, fibrous. Odour slight; taste slightly bitter and astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of rectangular flat cells, outer layers usually broken. Cortex narrow. Laticiferous tubes abundant in phloem. Cambium distinct, arranged in a ring. Xylem relatively broad; vessels singly scattered or several in bundles, radially arranged, surrounded by fibres. Rays broad. Clusters of calcium oxalates in rosette aggregates and scattered in parenchyma (Fig. 2).

Powder

Colour yellowish-brown. Single starch granules subspheroid or ovoid, 3-22 µm in diameter, hilum pointed or slit-shaped; compound starch granules composed of 2-4 units; distinct black cruciate shape under the polarized microscope. Prisms of calcium oxalate 4-28 µm in diameter; polychromatic under the polarized light microscope. Clusters of calcium oxalate abundant, 5-45 µm in diameter, in rosette aggregates; polychromatic under the polarized light microscope. Fibres scattered singly or in bundles, walls thick but non-lignified, 6-50 µm in diameter. Bordered-pitted and reticulate vessels, usually broken, 6-92 µm in diameter. Non-articulate laticiferous tubes usually broken, containing yellowish and minute-granular inclusions. Cork cells polygonal (Fig. 3).

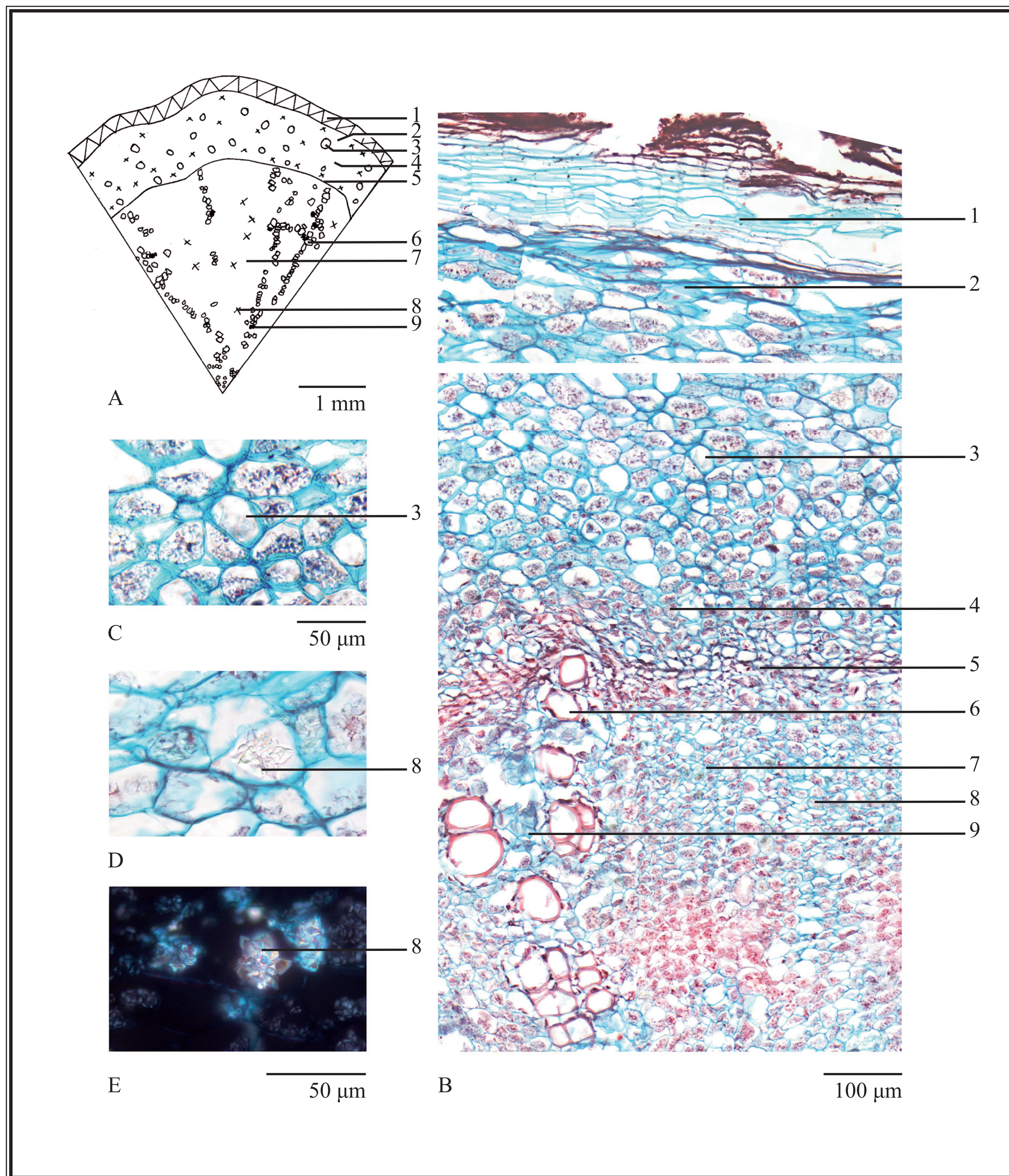

Figure 2 Microscopic features of transverse section of Euphorbiae Pekinensis Radix

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

1. Cork 2. Cortex 3. Laticiferous tube 4. Phloem 5. Cambium 6. Xylem
7. Ray 8. Clusters of calcium oxalate 9. Fibre

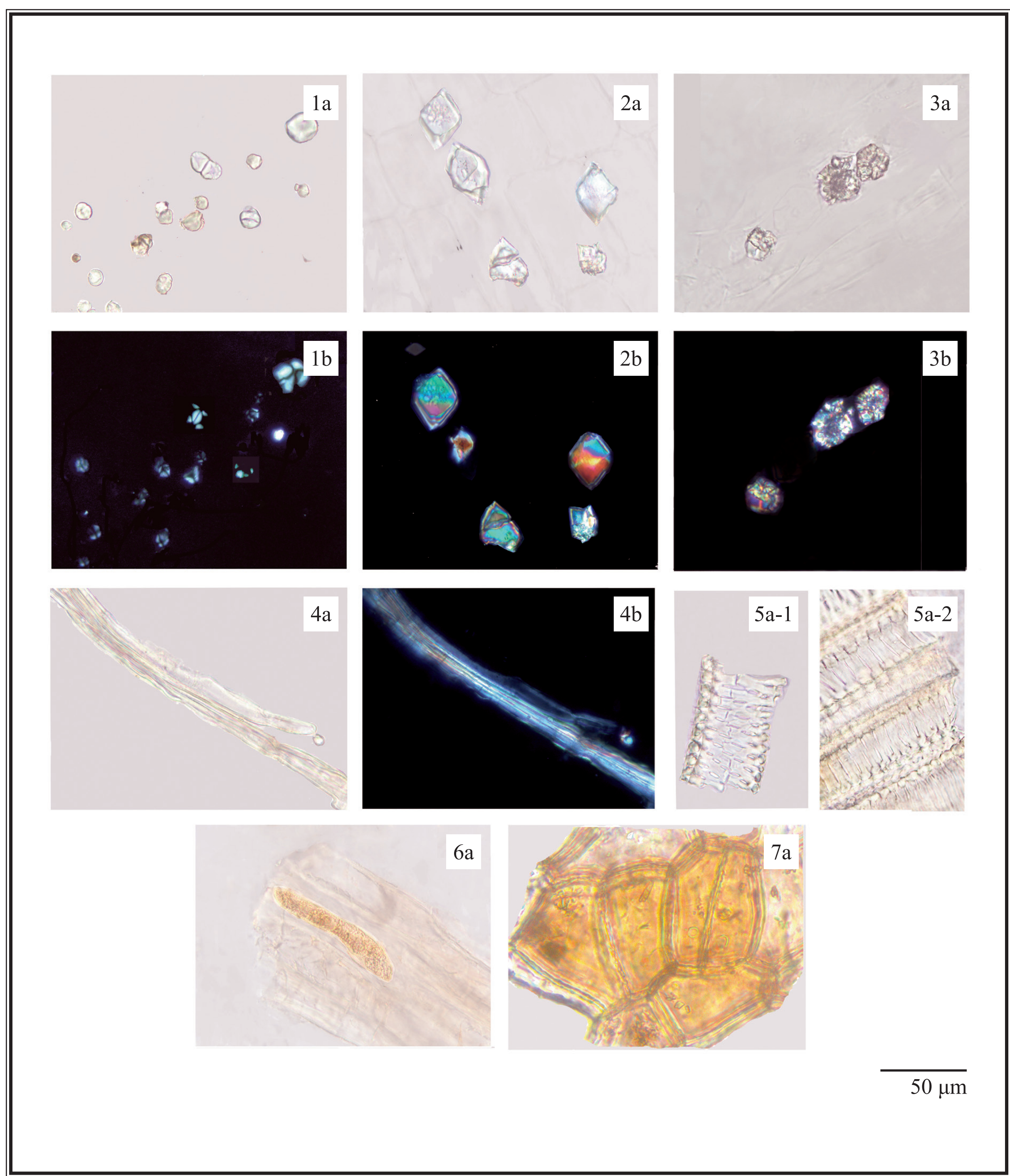

Figure 3 Microscopic features of powder of Euphorbiae Pekinensis Radix

1. Starch granules 2. Prisms of calcium oxalate 3. Clusters of calcium oxalate
4. Fibres 5. Vessels (5-1 bordered-pitted vessels, 5-2 reticulate vessels)
6. Laticiferous tube 7. Cork cells

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

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

Standard solution

Euphol standard solution

Weigh 1.0 mg of euphol CRS (Fig. 4) and dissolve in 10 mL of methanol.

Developing solvent system

Prepare a mixture of petroleum ether (60-80°C) and ethyl acetate (10:1.5, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 0.3 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (400 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 euphol standard solution and the test solution (2 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 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 heat at about 110ºC (about 5 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

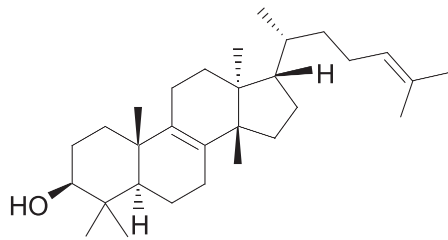

Figure 4 Chemical structure of euphol

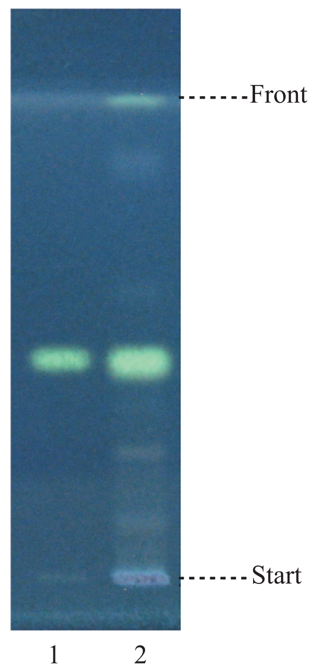

Figure 5 A reference HPTLC chromatogram of Euphorbiae Pekinensis Radix extract observed under UV light (366 nm) after staining

1. Euphol 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 euphol (Fig. 5).

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

Standard solution

Euphol standard solution for fingerprinting, Std-FP (90 mg/L)

Weigh 4.5 mg of euphol CRS and dissolve in 50 mL of methanol.

Test solution

Weigh 0.3 g of the powdered sample and place it in a 50-mL round-bottomed flask, then add 10 mL of methanol. Reflux the mixture for 1 h. Cool down to room temperature. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (203 nm) and a column (4.6 × 150 mm) packed with OS 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% Phosphoric acid (%, v/v)	Elution
0 – 10	60 → 70	40 → 30	linear gradient
10 – 30	70 → 85	30 → 15	linear gradient
30 – 40	85 → 95	15 → 5	linear gradient
40 – 60	95 → 99	5 → 1	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 µL of euphol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of euphol should not be more than 5.0%; the RSD of the retention time of euphol peak should not be more than 2.0%; the column efficiency determined from euphol peak should not be less than 100000 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 euphol Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention time of euphol peak in the chromatogram of euphol Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify euphol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of euphol Std-FP. The retention times of euphol 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 four characteristic peaks of Euphorbiae Pekinensis Radix extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Euphorbiae Pekinensis Radix extract

Peak No.	RRT	Acceptable Range
1	0.31	± 0.03
2	0.98	± 0.03
3 (marker, euphol)	1.00	-
4	1.04	± 0.03

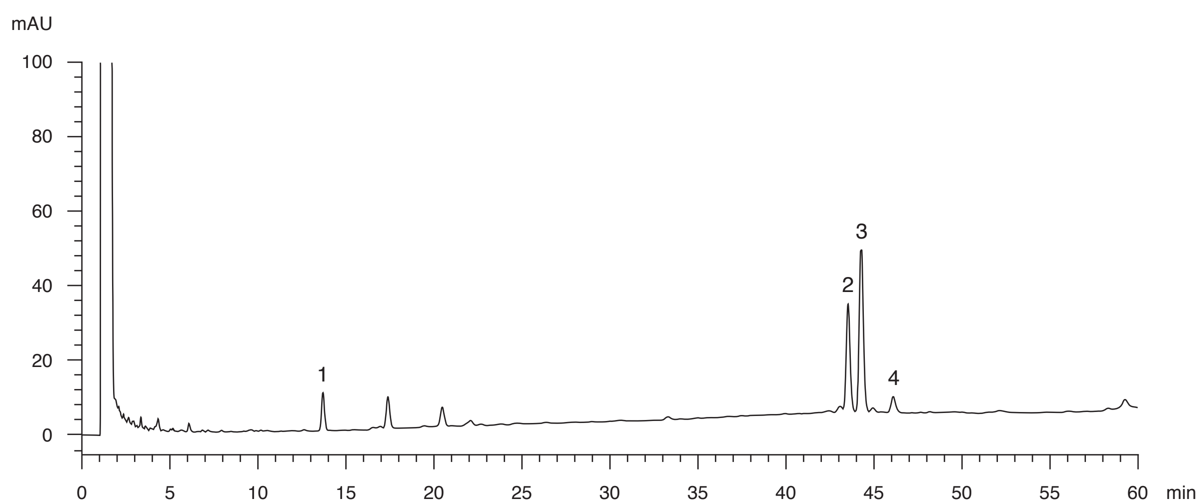

Figure 6 A reference fingerprint chromatogram of Euphorbiae Pekinensis Radix extract

For positive identification, the sample must give the above four 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 8.5%.
Acid-insoluble ash: not more than 2.0%.

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 16.0%.
Ethanol-soluble extractives (cold extraction method): not less than 14.0%.

7. ASSAY

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

Standard solution
Euphol standard stock solution, Std-Stock (1000 mg/L)
Weigh accurately 5.0 mg of euphol CRS and dissolve in 5 mL of methanol.
Euphol standard solution for assay, Std-AS
Measure accurately the volume of the euphol Std-Stock, dilute with methanol to produce a series of solutions of 5, 20, 40, 80, 120 mg/L for euphol.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL round-bottomed flask, then add 10 mL of methanol. Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction one more time. Combine the filtrates and 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 (203 nm) and a column (4.6 × 150 mm) packed with OS 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)	0.1% Phosphoric acid (%, v/v)	Elution
0 – 30	90 → 95	10 → 5	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 µL of euphol Std-AS (40 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of euphol should not be more than 5.0%; the RSD of the retention time of euphol peak should not be more than 2.0%; the column efficiency determined from euphol peak should not be less than 12000 theoretical plates.

The *R* value between euphol 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 euphol Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of euphol against the corresponding concentrations of euphol Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify euphol peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of euphol Std-AS. The retention times of euphol 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 euphol in the test solution, and calculate the percentage content of euphol in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.36% of euphol ($C_{30}H_{50}O$), calculated with reference to the dried substance.

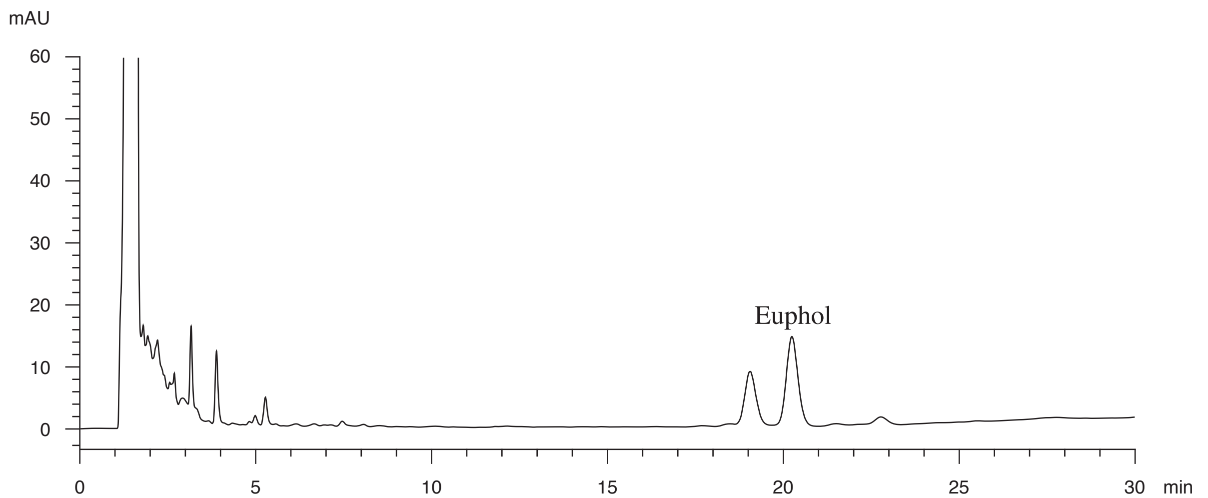

Figure 7 A reference assay chromatogram of Euphorbiae Pekinensis Radix extract