Euphorbiae Semen (unprocessed)

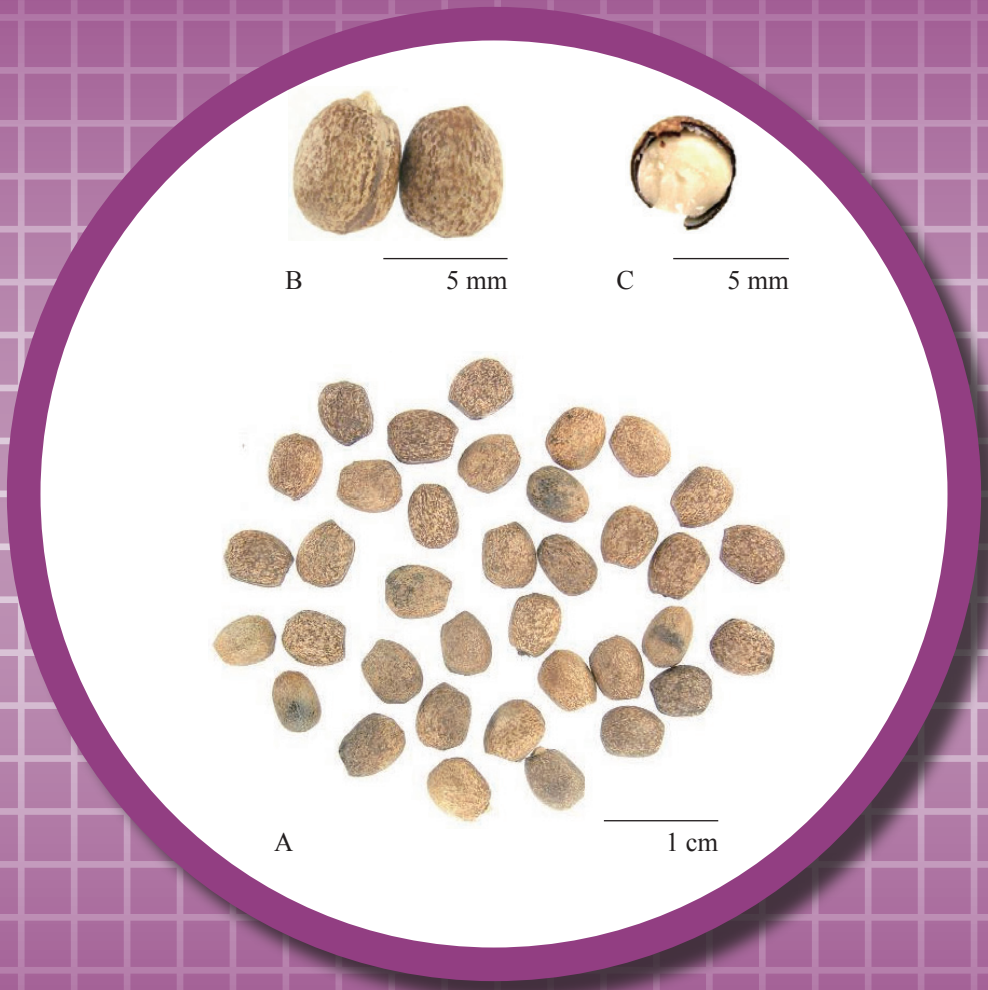

Figure 1 A photograph of Euphorbiae Semen (unprocessed)

A. Euphorbiae Semen (unprocessed) B. Magnified image of seeds
C. Magnified image of transverse section of seed

1. NAMES

Official Name: Euphorbiae Semen (unprocessed)

Chinese Name: 千金子 (生)

Chinese Phonetic Name: Qianjinzi (Sheng)

2. SOURCE

Euphorbiae Semen (unprocessed) is the unprocessed dried ripe seed of *Euphorbia lathyris* L. (Euphorbiaceae). The seed is collected in summer and autumn, foreign matter removed, then dried to obtain Euphorbiae Semen (unprocessed).

3. DESCRIPTION

Ellipsoidal or obovoid, 4.3-5.7 mm long, 3.3-4.7 mm in diameter. Externally greyish-brown, with irregular reticulate wrinkles, reticulate pits greyish-black, forming fine spots. A longitudinal furrowed raphe located on one side, a protuberant chalaza at the apex, a linear hilum at the lower end, with a whitish protuberant caruncle or its scar at the base. Testa thin and fragile, kernel white to yellowish-white, oily. Odour slight (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermal cells of testa undulated, with relatively thickened outer wall, containing brown contents. Hypodermis consists of 1-3 layers of parenchymatous cells. Inner epidermis consists of 1 layer of subsquared palisade cells, the inner part of lateral walls and inner walls prominently thickened. Endotesta consists 1 layer of brown palisade cells, the cells slender cylindrical, with thickened and lignified wall, pits occasionally visible. Perisperm consists of several layers of subsquare parenchymatous cells. Endosperm cells subrounded. Cotyledons cells squared or rectangular. Endosperm and cotyledons cells contain aleurone grains (Fig. 2).

Powder

Colour dark brown. Endotesta cells brown or dark brown, cells slender and columnar in lateral view, arranged densely, slightly curved, lower parts gradually smaller, endings truncate or obtuse, 72-281 μm long, 9-22 μm wide, walls 3-9 μm thick, pit canals slender and sparse, lumens relatively wide, containing reddish-brown or dark brown contents; cells subrounded in surface view. Inner epidermal cells rectangular or subsquare in lateral view, 9-20 μm wide, arranged in short palisade-like pattern, outer radial walls thin and relatively curved, inwards and inner walls thickened, walls 1-2 μm thick; cells polygonal in surface view, arranged densely, walls relatively thickened, without cell interspace. Epidermal cells of testa elliptic or semicircular, slightly papillae or tomentose protuberance in shape, outer walls relatively thickened, lumens usually containing yellowish-brown or reddish-brown contents, some without pigment. Endosperm cells subrounded, 36-63 μm in diameter, walls thin, lumens containing rounded or minute granular aleurone grains, also contain fatty oil droplets. Perisperm cells extremely rare, subpolygonal, walls relatively thick. Cotyledon cells pale yellowish-green, containing granular aleurone grains and fatty oil droplets (Fig. 3).

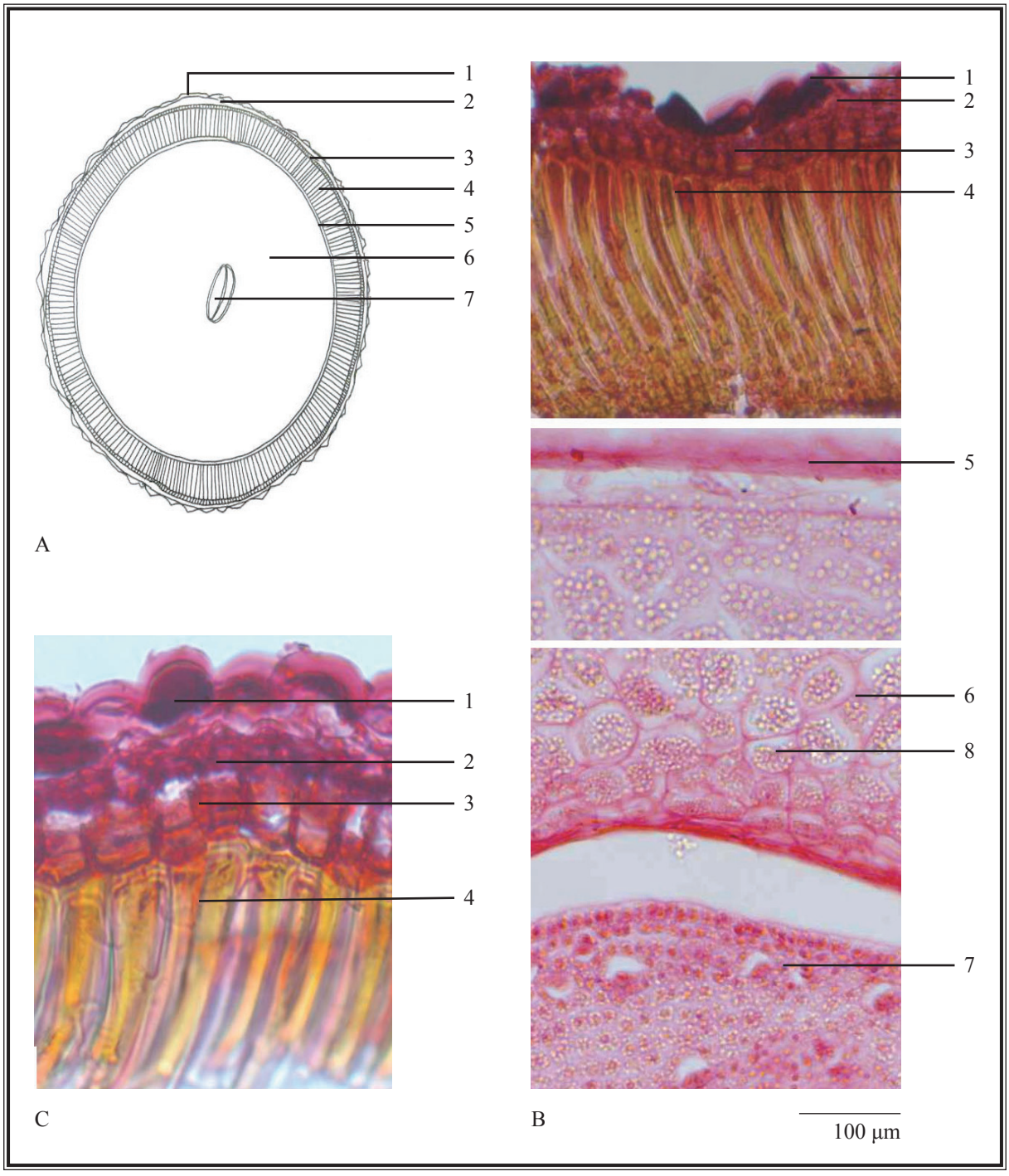

Figure 2 Microscopic features of transverse section of Euphorbiae Semen (unprocessed)

A. Sketch B. Section illustration C. Section magnified

1. Epidermis of testa 2. Hypodermis 3. Inner epidermis 4. Endotesta 5. Perisperm 6. Endosperm 7. Cotyledons 8. Aleurone grains

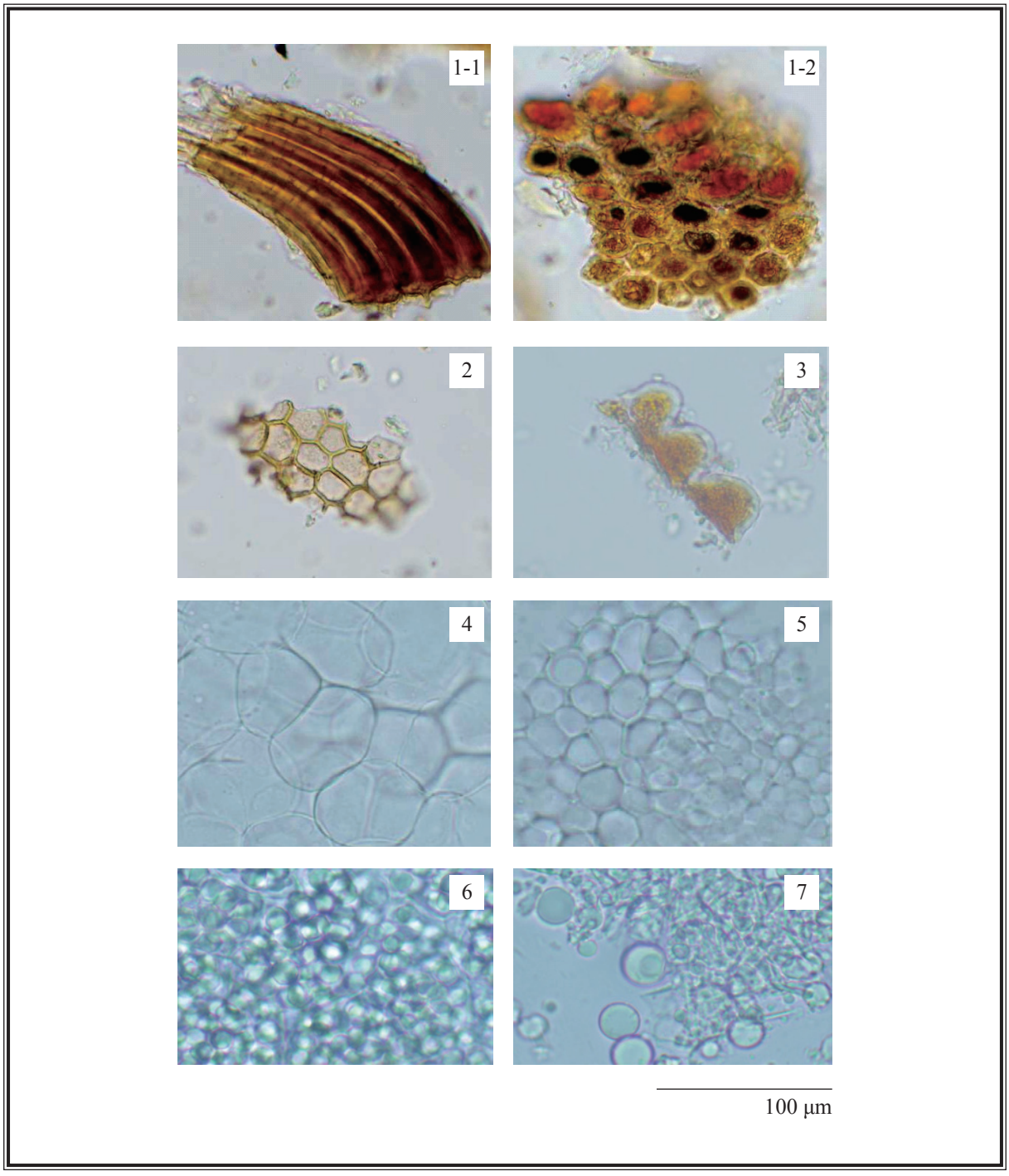

Figure 3 Microscopic features of powder of Euphorbiae Semen (unprocessed) (under the light microscope)

1. Endotesta cells (1-1 in lateral view, 1-2 in surface view) 2. Inner epidermal cells (in surface view)
3. Epidermal cells of testa 4. Endosperm cells 5. Cotyledon cells 6. Aleurone grains 7. Fatty oil droplets

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

Standard solution

Euphorbia factor L_3 standard solution

Weigh 1.0 mg of euphorbia factor L_3 CRS (Fig. 4) and dissolve in 2 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of petroleum ether (60-80°C) and ethyl acetate (4:1, v/v).

Test solution

Equal amount of kieselguhr are added to the sample before powdering. Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethyl acetate. Sonicate (250 W) the mixture for 30 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately euphorbia factor L_3 standard solution and the test solution (5 µL each) 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. Examine the plate under UV light (254 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

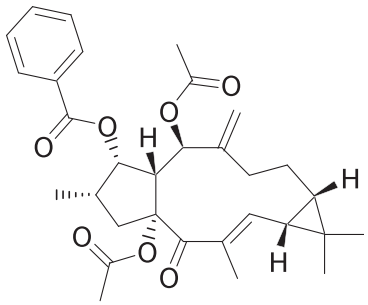

Figure 4 Chemical structure of euphorbia factor L_3

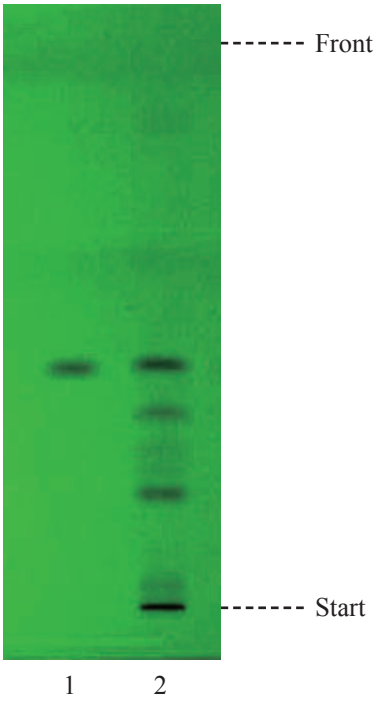

Figure 5 A reference HPTLC chromatogram of Euphorbiae Semen (unprocessed) extract observed under UV light (254 nm)

1. Euphorbia factor L_3 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 euphorbia factor L_3 (Fig. 5).

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

Standard solution

Euphorbia factor L_3 standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 1.0 mg of euphorbia factor L_3 CRS and dissolve in 20 mL of methanol. Keep at about 4°C.

Test solution

Equal amount of kieselguhr are added to the sample before powdering. Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. Sonicate (500 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Wash the residue with 5 mL of methanol. Filter and combine the filtrates. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30°C 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)	Methanol (%, v/v)	Water (%, v/v)	Elution
0 – 10	70	30	isocratic
10 – 25	70 → 74	30 → 26	linear gradient
25 – 31	74 → 90	26 → 10	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of euphorbia factor L_3 Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of euphorbia factor L_3 should not be more than 5.0%; the RSD of the retention time of euphorbia factor L_3 peak should not be more than 2.0%; the column efficiency determined from euphorbia factor L_3 peak should not be less than 9000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject euphorbia factor L_3 Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of euphorbia factor L_3 peak in the chromatogram of euphorbia factor L_3 Std-FP and the retention times of the seven characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify euphorbia factor L_3 peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of euphorbia factor L_3 Std-FP. The retention times of euphorbia factor L_3 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 seven characteristic peaks of Euphorbiae Semen (unprocessed) extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the seven characteristic peaks of Euphorbiae Semen (unprocessed) extract

Peak No.	RRT	Acceptable Range
1	0.58	± 0.03
2	0.71	± 0.03
3	0.75	± 0.03
4 (marker, euphorbia factor L_3)	1.00	-
5	1.15	± 0.03
6	1.35	± 0.03
7	1.55	± 0.03

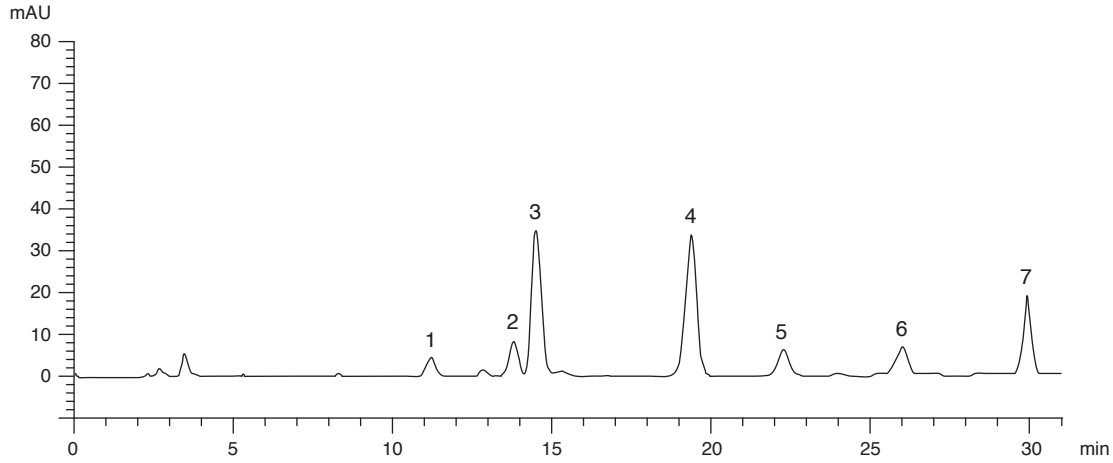

Figure 6 A reference fingerprint chromatogram of Euphorbiae Semen (unprocessed) extract

For positive identification, the sample must give the above seven 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 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 4.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 6.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 6.0%.
Ethanol-soluble extractives (hot extraction method): not less than 6.0%.

7. ASSAY

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

Standard solution

Euphorbia factor L_3 standard stock solution, Std-Stock (150 mg/L)

Weigh accurately 3.0 mg of euphorbia factor L_3 CRS and dissolve in 20 mL of methanol. Keep at about 4°C.

Euphorbia factor L_3 standard solution for assay, Std-AS

Measure accurately the volume of the euphorbia factor L_3 Std-Stock, dilute with methanol to produce a series of solutions of 12, 30, 60, 90, 120 mg/L for euphorbia factor L_3. Keep at about 4°C.

Test solution

Equal amount of kieselguhr are added to the sample before powdering. Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. Sonicate (500 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Wash the residue with 5 mL of methanol. Filter and combine the filtrates. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30°C 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)	Methanol (%, v/v)	Water (%, v/v)	Elution
0 – 10	70	30	isocratic
10 – 25	70 → 74	30 → 26	linear gradient
25 – 31	74 → 90	26 → 10	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of euphorbia factor L_3 Std-AS (60 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of euphorbia factor L_3 should not be more than 5.0%; the RSD of the retention time of euphorbia factor L_3 peak should not be more than 2.0%; the column efficiency determined from euphorbia factor L_3 peak should not be less than 9000 theoretical plates.

The *R* value between euphorbia factor L_3 peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curve

Inject a series of euphorbia factor L_3 Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of euphorbia factor L_3 against the corresponding concentrations of euphorbia factor L_3 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 euphorbia factor L_3 peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of euphorbia factor L_3 Std-AS. The retention times of euphorbia factor L_3 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 euphorbia factor L_3 in the test solution, and calculate the percentage content of euphorbia factor L_3 in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.41% of euphorbia factor L_3 ($C_{31}H_{38}O_7$), calculated with reference to the dried substance.

8. CAUTION

This CMM is potent/toxic and should be prescribed by registered Chinese medicine practitioner.