Kansui Radix (unprocessed)

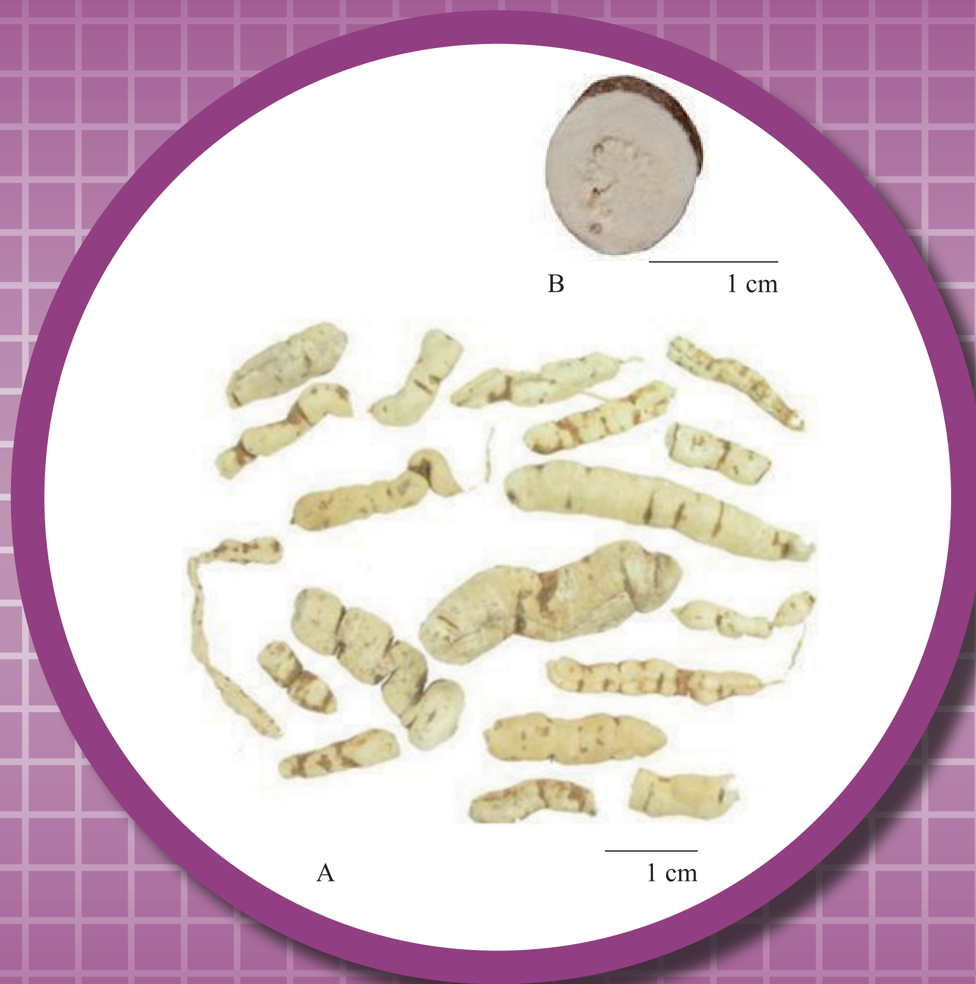

Figure 1 A photograph of Kansui Radix (unprocessed)

A. Kansui Radix (unprocessed) B. Magnified image of transverse section of root tuber

1. NAMES

Official Name: Kansui Radix (unprocessed)

Chinese Name: 甘遂 (生)

Chinese Phonetic Name: Gansui (Sheng)

2. SOURCE

Kansui Radix (unprocessed) is the unprocessed dried root tuber of *Euphorbia kansui* T. N. Liou ex T. P. Wang (Euphorbiaceae). The root tuber is collected in spring before flowering or in late autumn after the stem and leaves withered, soil removed, and the outer bark removed by dashing, then dried under the sun to obtain Kansui Radix (unprocessed).

3. DESCRIPTION

Ellipsoid, long cylindrical or moniliform, 1-6 cm long, 0.5-2.5 cm in diameter. Externally whitish or yellowish-white, with brown patches of cork remaining at pits. Texture fragile, easily broken. Fracture starchy and white, wood slightly radial-striated; long cylindrical root tubers appearing more fibrous. Odour slight (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Remaining cork consists of several layers of cork cells. Cortex narrow, scattered with oval, subtriangular, subsquare, rectangular or polygonal sclerenchymatous cells, dotted with laticiferous tubes. Phloem broad, dotted with laticiferous tubes. Cambium in a ring. Xylem vessels singly scattered, several to 10 or more in groups, arranged radially; xylem ray consists of 2-10 or more rows of cells. Parenchymatous cells filled with starch granules (Fig. 2).

Powder

Colour whitish. Starch granules numerous, single starch granules spherical or hemispherical, 5-34 µm in diameter, hilum dotted, slit-shaped or stellate; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-8 units. Non-articulated

laticiferous tubes contain yellow and minute granular inclusions. Sclerenchymatous cells rectangular, fusiform, subtriangular or polygonal, 15-60 µm in diameter, walls slightly lignified or non-lignified. Bordered-pitted vessels frequently visible, usually accompanied by fibre bundles, 13-80 µm in diameter (Fig. 3).

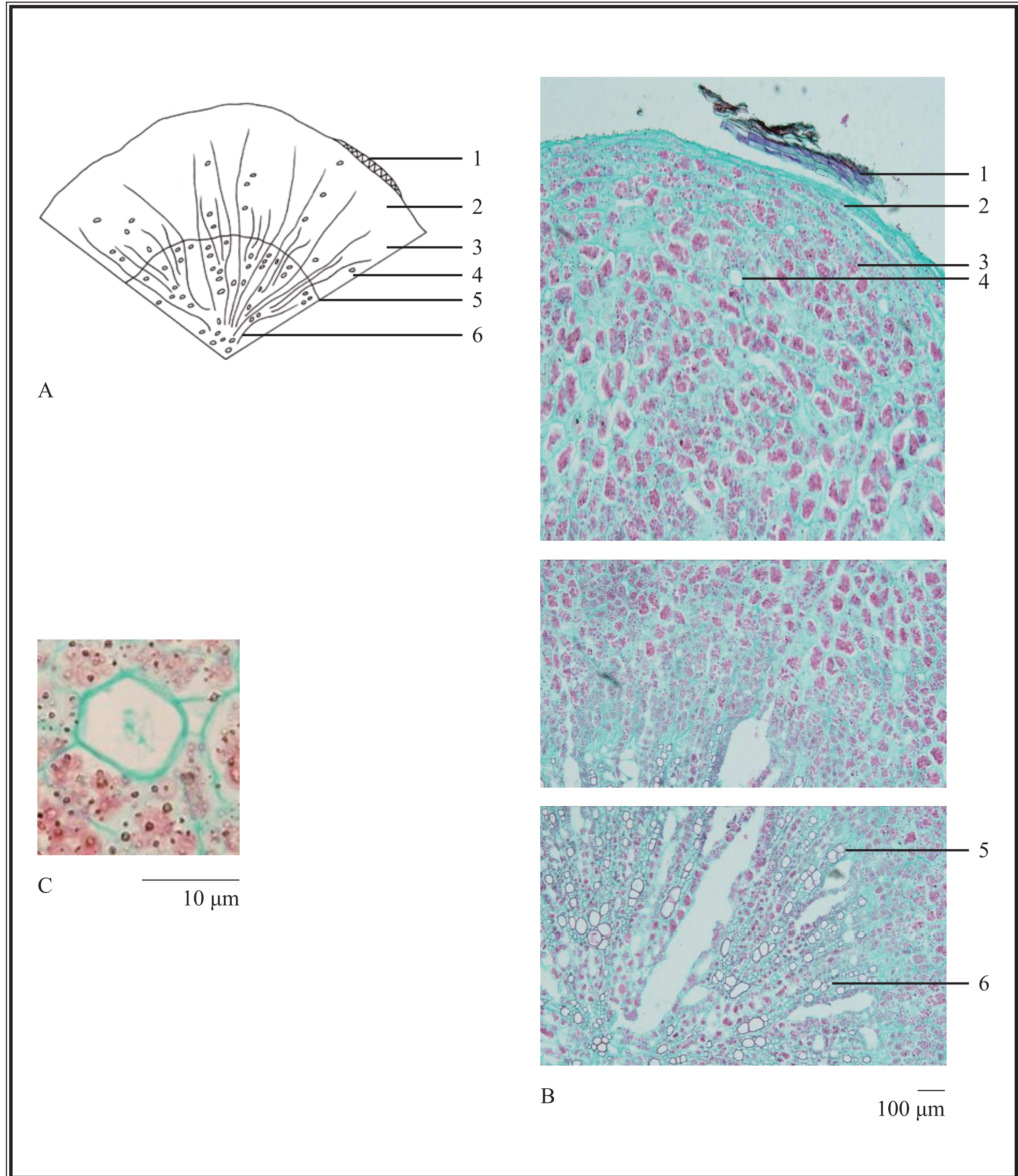

Figure 2 Microscopic features of transverse section of Kansui Radix (unprocessed)

A. Sketch B. Section illustration C. Laticiferous tube

1. Cork 2. Cortex 3. Phloem 4. Laticiferous tube 5. Cambium 6. Xylem

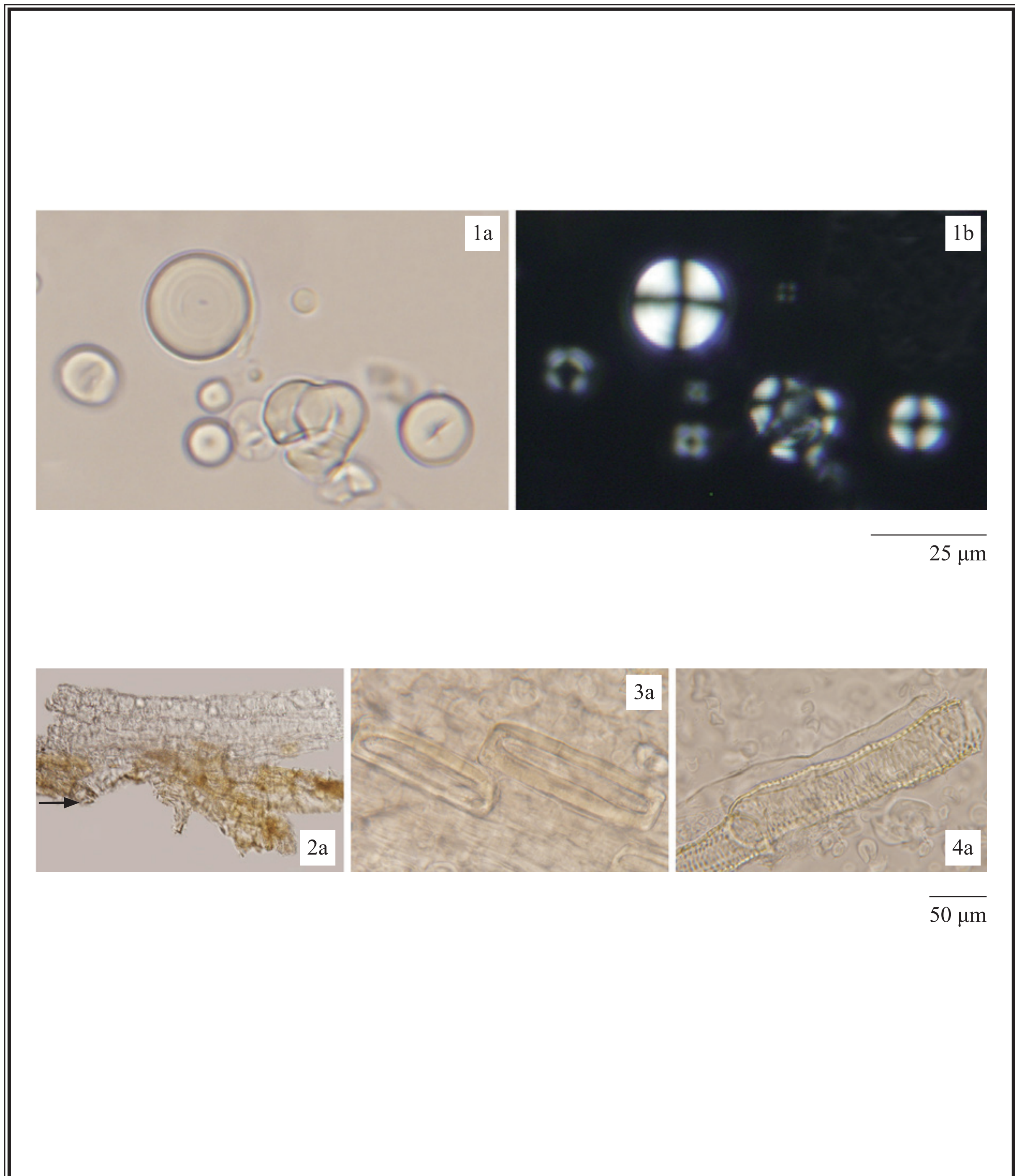

Figure 3 Microscopic features of powder of Kansui Radix (unprocessed)

1. Starch granules 2. Non-articulated laticiferous tube 3. Sclerenchymatous cells
4. Bordered-pitted vessels

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 1 mL of ethanol.

Developing solvent system

Prepare a mixture of petroleum ether (30-60ºC) and acetone (5:1, v/v).

Spray reagent

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

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol. Sonicate (160 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask and evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol.

Procedure

Carry out the method by using a HPTLC silica gel G60 plate 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. 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. Spray the plate evenly with the spray reagent and heat at about 105ºC (about 2 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

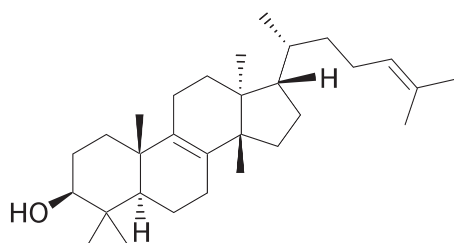

Figure 4 Chemical structure of euphol

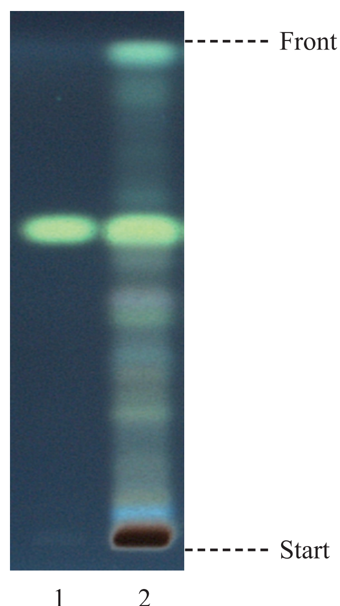

Figure 5 A reference HPTLC chromatogram of Kansui Radix (unprocessed) extract observed under UV light (366 nm) after staining

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 (150 mg/L)

Weigh 1.5 mg of euphol CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of ethyl acetate. Sonicate (160 W) the mixture for 40 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue for three times each with 5 mL of ethyl acetate. Repeat the extraction for one more time. Combine the solutions and evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol and transfer the solution

to a 25-mL volumetric flask. Wash the residue for four times each with 5 mL of methanol. Combine the solutions 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 (210 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)	Water (%, v/v)	Elution
0 – 20	45 → 20	55 → 80	linear gradient
20 – 50	20 → 15	80 → 85	linear gradient
50 – 60	15 → 5	85 → 95	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 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 25000 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 euphol Std-FP and the test solution (10 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 Kansui Radix (unprocessed) extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Kansui Radix (unprocessed) extract

Peak No.	RRT	Acceptable Range
1	0.24	± 0.04
2	0.34	± 0.03
3	0.97	± 0.03
4 (marker, euphol)	1.00	-

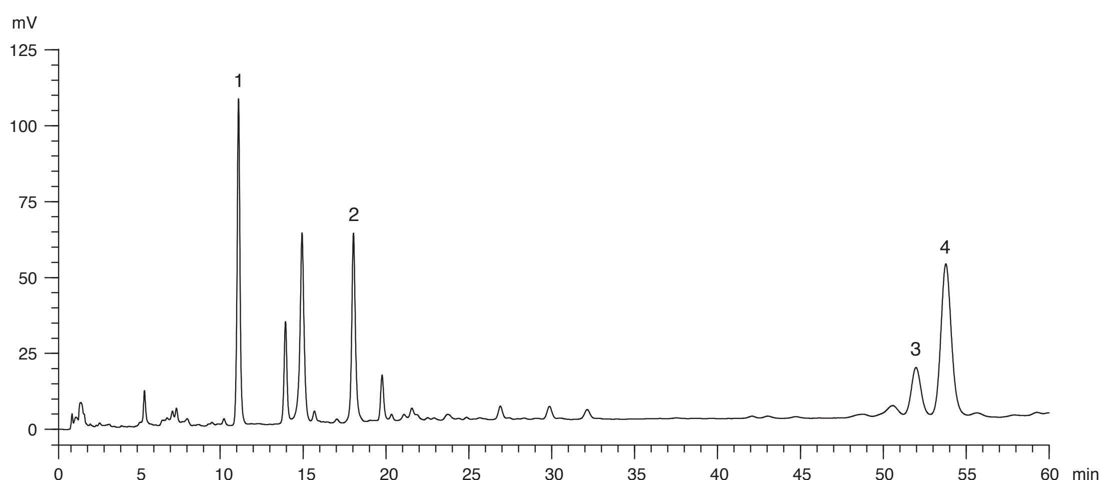

Figure 6 A reference fingerprint chromatogram of Kansui Radix (unprocessed) extract

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 XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 **Ash** *(Appendix IX)*

Total ash: not more than 2.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 **Water Content** *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 58.0%.
Ethanol-soluble extractives (hot extraction method): not less than 11.0%.

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 25, 50, 100, 200, 500 mg/L for euphol.

Test solution

Weigh accurately 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of ethyl acetate. Sonicate (160 W) the mixture for 40 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue for three times each with 5 mL of ethyl acetate. Repeat the extraction for one more time. Combine the solutions and evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol and transfer the solution to a 25-mL volumetric flask. Wash the residue for four times each with 5 mL of methanol. Combine the solutions 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 (210 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)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	45 → 20	55 → 80	linear gradient
20 – 50	20 → 15	80 → 85	linear gradient
50 – 60	15 → 5	85 → 95	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of euphol Std-AS (100 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 25000 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.

Calibration curve

Inject a series of euphol Std-AS (10 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 10 μL of the test solution into the HPLC system and record the chromatogram. Identify euphol peak 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 0.12% of euphol ($C_{30}H_{50}O$), calculated with reference to the dried substance.

8. CAUTION

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