Cyathulae Radix

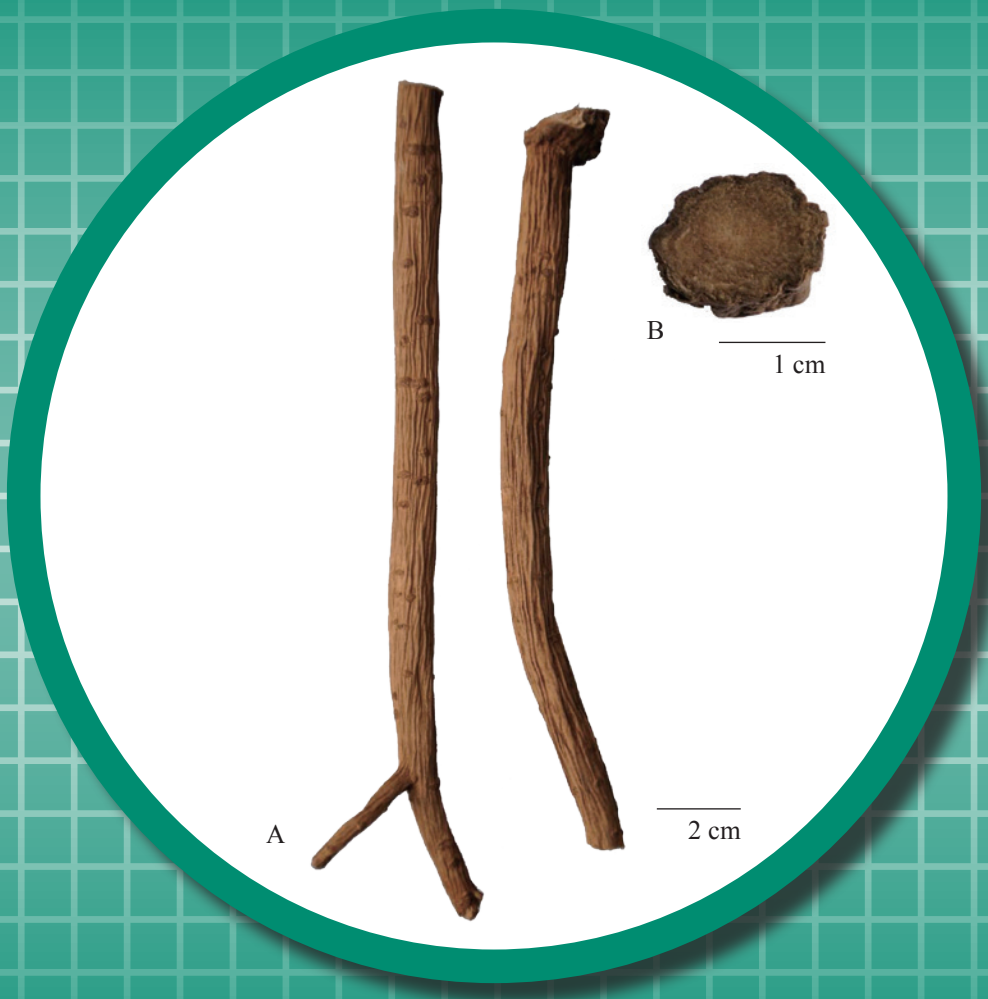

Figure 1 A photograph of Cyathulae Radix

A. Cyathulae Radix B. Magnified transverse section of root

1. NAMES

Official Name: Cyathulae Radix

Chinese Name: 川牛膝

Chinese Phonetic Name: Chuanniuxi

2. SOURCE

Cyathulae Radix is the dried root of *Cyathula officinalis* Kuan (Amaranthaceae). The root is collected in winter, root stock, rootlets and soil removed, baked or dried under the sun to half-dryness, piled up, then baked or dried under the sun thoroughly to obtain Cyathulae Radix.

3. DESCRIPTION

Cylindrical, straight or slightly curved, the upper part broad, tapering downward towards the distal end, occasionally branched, 14-54 cm long, 5-15 mm in diameter. Externally yellowish-brown, with slightly twisted and fine longitudinal wrinkles, transverse lenticel-like protuberances and sparsely distributed rootlet scars. Texture tenacious, uneasily broken; fracture pale yellow to brownish-yellow; vascular bundles many scattered and spotted, arranged in several whorls, yellowish-white; those on the outer part small, the one in the centre relatively large. Odour slight; taste sweet (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of cells. Cortex relatively narrow, parenchymatous cells elongated and arranged in rows tangentially. Vascular bundles arranged in several interrupted whorls, relatively small in the outermost whorl, sometimes having only 1 to several vessels. Cambium nearly in a ring in the outermost whorl. Xylem mainly consists of vessels and small xylem fibres; central xylem aggregated into 2 or more groups. Parenchymatous cells contain microcrystals of calcium oxalate (Fig. 2).

Powder

Colour greyish-brown to brown. Microcrystals of calcium oxalate scattered in parenchymatous cells, triangular, arrow-pointed, subsquare or irregular, 1-12 μm in diameter; polychromatic under the polarized microscope. Fibres mostly in bundles, strip-shaped, with curved and tapered end, 6-49 μm in diameter, walls slightly thickened, pits oblique or V-shaped, bordered pits observed, pit canals distinct with different width; pale blue or polychromatic under the polarized microscope. Vessels mainly bordered-pitted, 12-73 μm in diameter. Cork cells subsquare or subrectangular (Fig. 3).

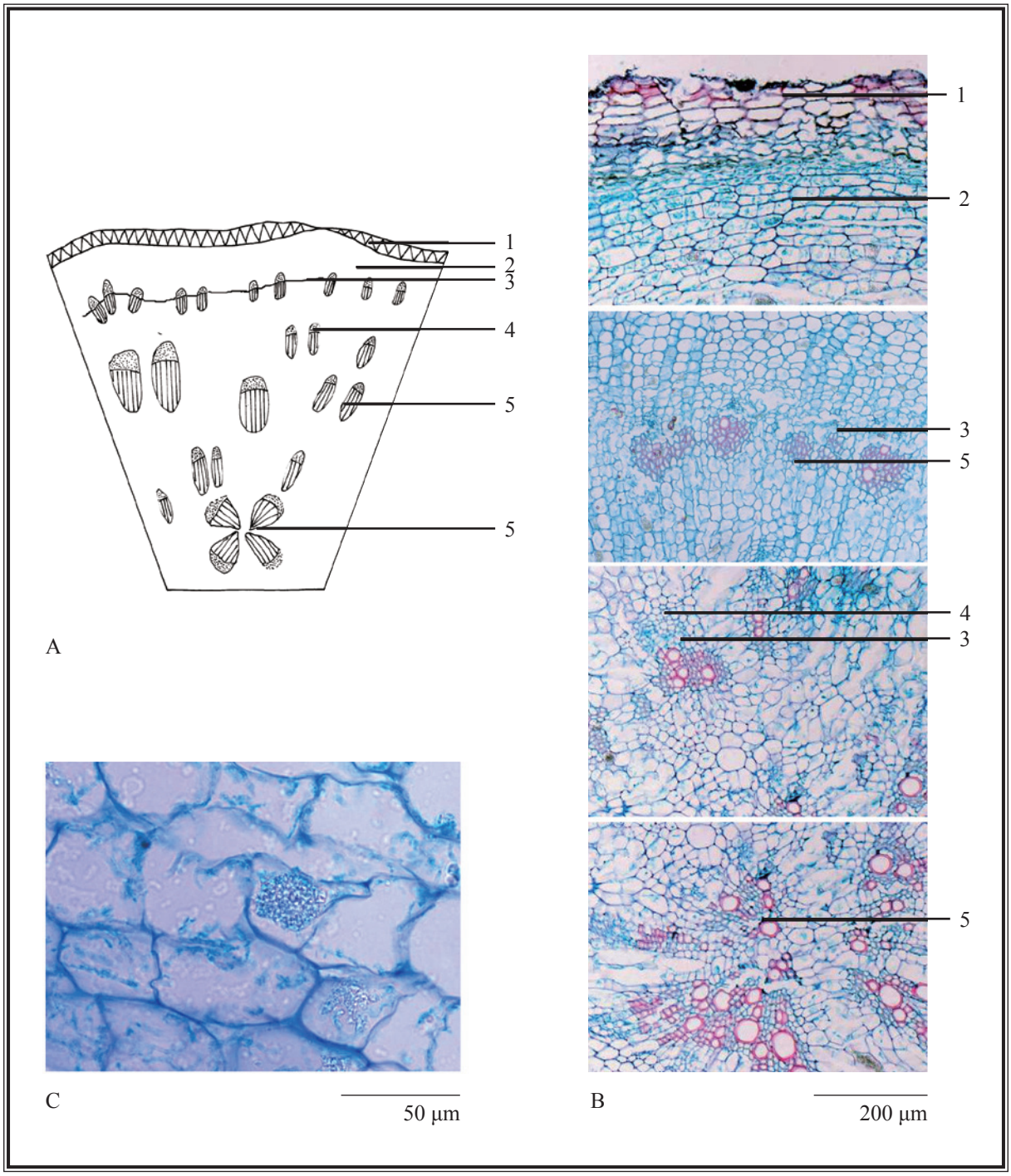

Figure 2 Microscopic features of transverse section of Cyathulae Radix

A. Sketch B. Section illustration C. Microcrystals of calcium oxalate

1. Cork 2. Cortex 3. Cambium 4. Phloem 5. Xylem

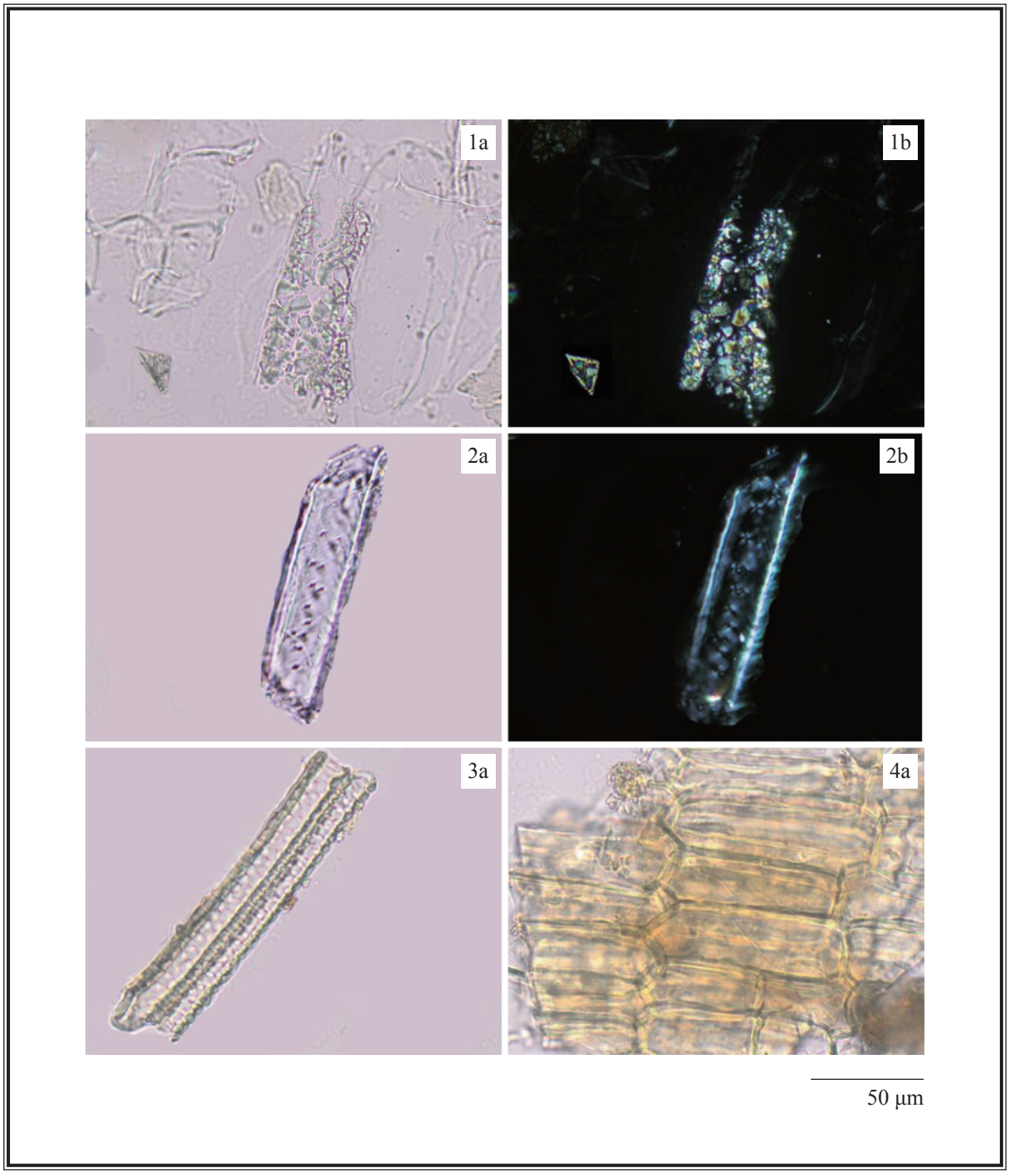

Figure 3 Microscopic features of powder of Cyathulae Radix

1. Microcrystals of calcium oxalate 2. Fibres 3. Bordered-pitted vessels 4. 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

Cyasterone standard solution

Weigh 0.05 mg of cyasterone CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, cyclohexane, methanol and glacial acetic acid (4:3:1.5:0.2, v/v).

Spray reagent

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

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. Sonicate (350 W) the mixture for 30 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 cyasterone standard solution (20 μL) and the test solution (10 μL) 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 8.5 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 until the spots or bands become visible (about 4 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

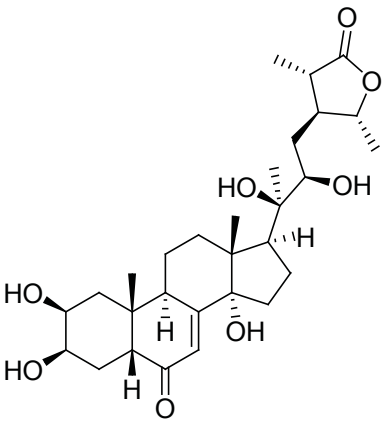

Figure 4 Chemical structure of cyasterone

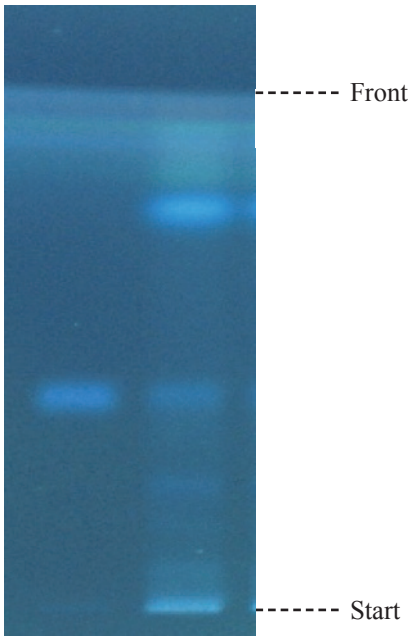

Figure 5 A reference HPTLC chromatogram of Cyathulae Radix extract observed under UV light (366 nm) after staining

1. Cyasterone 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 cyasterone (Fig. 5).

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

Standard solution

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

Weigh 0.5 mg of cyasterone CRS and dissolve in 10 mL of methanol (80%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (80%). Sonicate (350 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (246 nm) and a column (4.6 × 150 mm) packed with ODS bonded silica gel (2.6 μm particle size). The column temperature is maintained at 27ºC during the separation. The flow rate is about 0.5 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Methanol (%, v/v)	Elution
0 – 5	90	10	isocratic
5 – 15	90 → 66	10 → 34	linear gradient
15 – 48	66	34	isocratic
48 – 60	66 → 55	34 → 45	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of cyasterone Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of cyasterone should not be more than 5.0%; the RSD of the retention time of cyasterone peak should not be more than 2.0%; the column efficiency determined from cyasterone peak should not be less than 40000 theoretical plates.

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

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

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Cyathulae Radix extract

Peak No.	RRT	Acceptable Range
1	0.97	± 0.03
2 (marker, cyasterone)	1.00	-
3	1.03	± 0.03
4	1.24	± 0.03
5	1.28	± 0.03

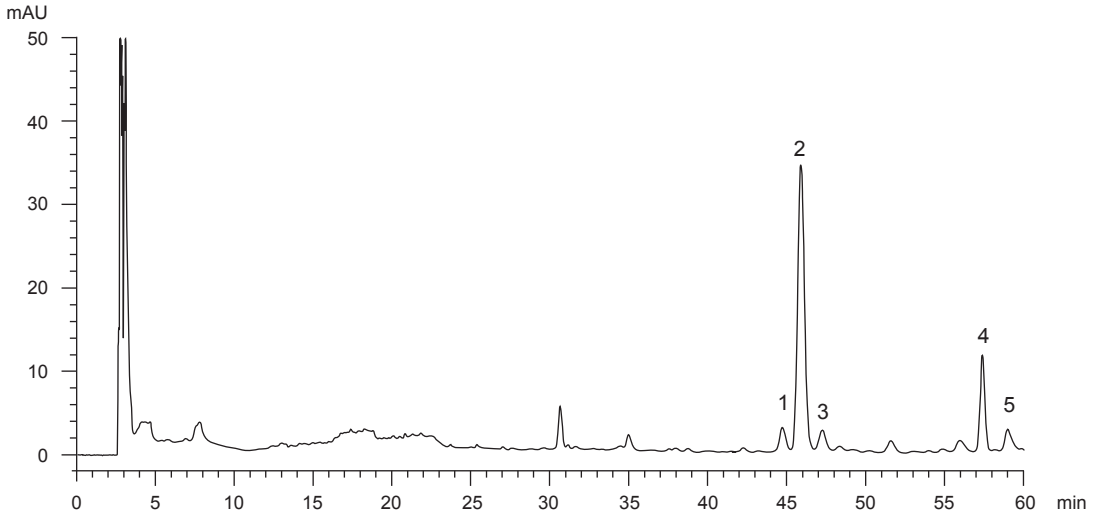

Figure 6 A reference fingerprint chromatogram of Cyathulae Radix extract

For positive identification, the sample must give the above five 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 – Aflatoxins *(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 7.0%.
Acid-insoluble ash: not more than 1.0%.

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 65.0%.
Ethanol-soluble extractives (hot extraction method): not less than 54.0%.

7. ASSAY

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

Standard solution

Cyasterone standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 1.0 mg of cyasterone CRS and dissolve in 5 mL of methanol (80%).

Cyasterone standard solution for assay, Std-AS

Measure accurately the volume of the cyasterone Std-Stock, dilute with methanol (80%) to produce a series of solutions of 4, 8, 16, 32, 48 mg/L for cyasterone.

Test solution

Weigh accurately 0.8 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (80%). Sonicate (350 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (80%). Combine the solutions and make up to the mark with methanol (80%). Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (246 nm) and a column (4.6 × 150 mm) packed with ODS bonded silica gel (2.6 μm particle size). The column temperature is maintained at 27ºC during the separation. The flow rate is about 0.5 mL/min. The mobile phase is a mixture of water and methanol (68:32, v/v). The elution time is about 45 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of cyasterone Std-AS (16 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of cyasterone should not be more than 5.0%; the RSD of the retention time of cyasterone peak should not be more than 2.0%; the column efficiency determined from cyasterone peak should not be less than 10000 theoretical plates.

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

Limits

The sample contains not less than 0.046% of cyasterone ($C_{29}H_{44}O_8$), calculated with reference to the dried substance.