Elephantopi Herba

Figure 1 A photograph of Elephantopi Herba

A. Elephantopi Herba

B. Upper surface of leaf

C. Lower surface of leaf

D. Magnified image of upper surface of leaf

E. Magnified image of the fracture of rhizome

1. NAMES

Official Name: Elephantopi Herba

Chinese Name: 地膽草

Chinese Phonetic Name: Didancao

2. SOURCE

Elephantopi Herba is the dried whole plant of *Elephantopus scaber* L. (Asteraceae). The whole plant is collected during summer and autumn before flowering, foreign matter removed, then dried under the sun to obtain Elephantopi Herba.

3. DESCRIPTION

Whole herb 9-37 cm long. Roots fibrous, numerous, fascicled, with irregular longitudinal wrinkles. Stems erect, usually short, slightly scabrid. Rhizomes 0.5-5.5 cm long, 2-10 mm in diameter; densely pale appressed-hirsute; texture hard, uneasily broken, fracture surface pale yellow. Leaves basal, crumpled; when intact spatulate to oblanceolate, 4-24 cm long, 0.5-4.5 cm wide; externally yellowish-green to greyish-green, densely appressed with pale hirsute and glandular dots especially on the upper surface; apex blunt or shortly acute, base gradually narrow, margin crenate; veins on the lower surface more distinct. Petiole broad and short, slightly sheath-like. Odour slight; taste bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

Rhizome: Epidermis consists of 1 layer of cells. Cortex broad. Endodermis distinct, consisting of 1 layer of cells, cells sometimes contain oil droplets. Phloem narrow. Fibres occasionally scattered in the phloem, singly or several in groups. Vessels small, radially and interruptedly arranged. Pith large. Clusters of calcium oxalate numerous, scattered in the parenchymatous cells [Fig. 2 (i)].

Leaf: Upper and lower epidermis consists of 1 layer of subsquare to rectangular cells; non-glandular hairs usually fallen after staining. Several layers of collenchymatous cells arranged on the inner side of upper and lower epidermis of midrib. Palisade tissue consists of 1-2 layers of cells, irregularly arranged. Cells of spongy tissue loosely arranged. Vascular bundles 3-5, bicollateral, arranged in an arch shape; several layers of sclerenchymatous cells surround the phloem, those near the lower epidermis in crescent-shaped; vessels several, arranged in rows [Fig. 2 (ii)].

Powder

Colour greyish-green. Non-glandular hairs numerous, long, easily broken, consisting of 1-3 cells; apex cell long, base cells short, 10-52 μm in diameter; cell wall relatively thick, with longitudinal fine lines inside. Lower epidermal cells elongated, polygonal or irregular in shape; stomata anisocytic. Clusters of calcium oxalate abundant, 8-20 μm in diameter; yellowish-white under the polarized microscope. Vessels spiral or reticulate, relatively small, 7-35 μm in diameter. Fibres slender, 10-28 μm in diameter; bright bluish-white under the polarized microscope. Stone cells subsquare or irregularly rhombic, 18-100 μm in diameter (Fig. 3).

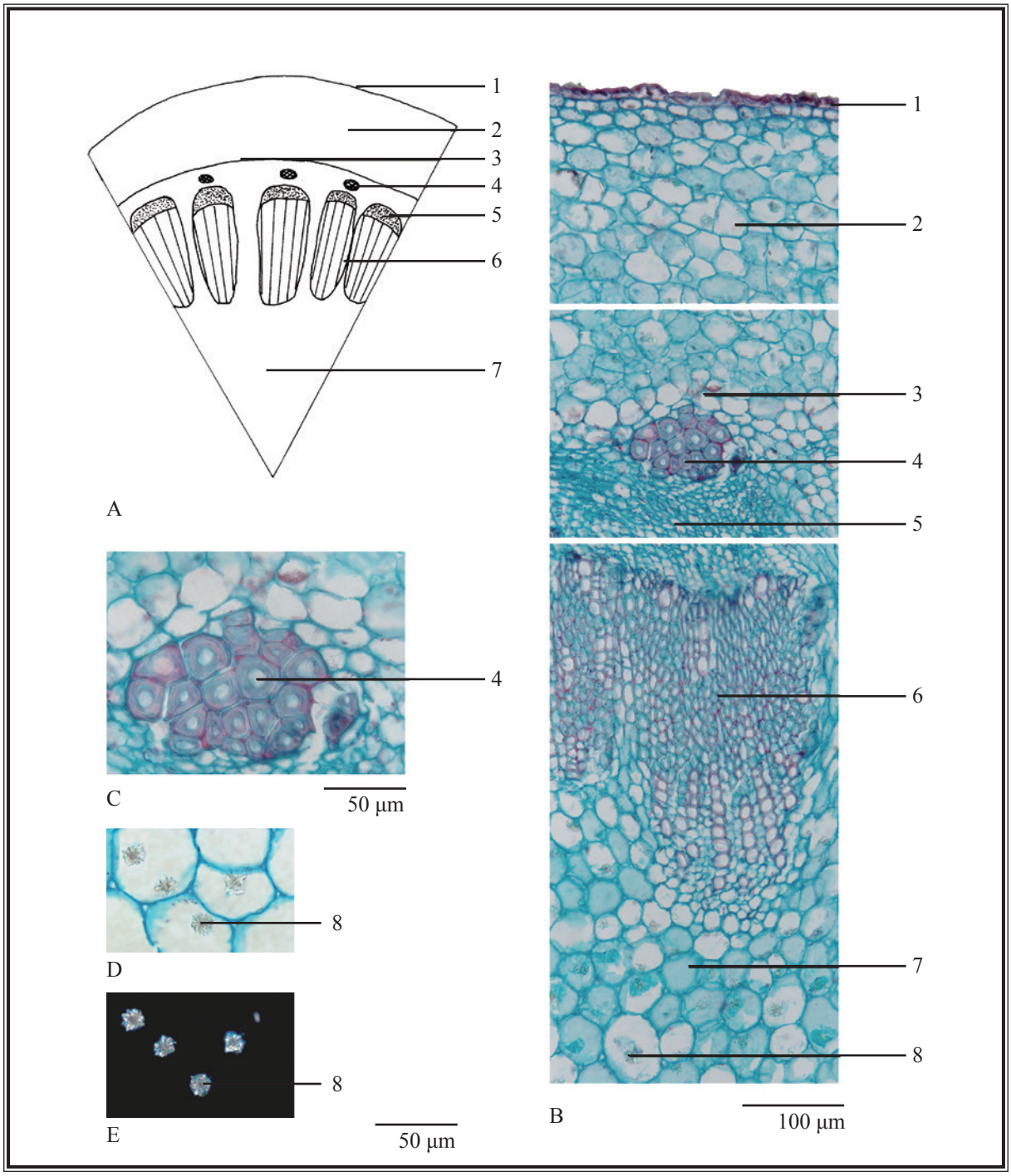

Figure 2 (i) Microscopic features of transverse section of rhizome of Elephantopi Herba

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

1. Epidemis 2. Cortex 3. Endodermis 4. Fibre 5. Phloem 6. Xylem 7. Pith
8. Cluster of calcium oxalate

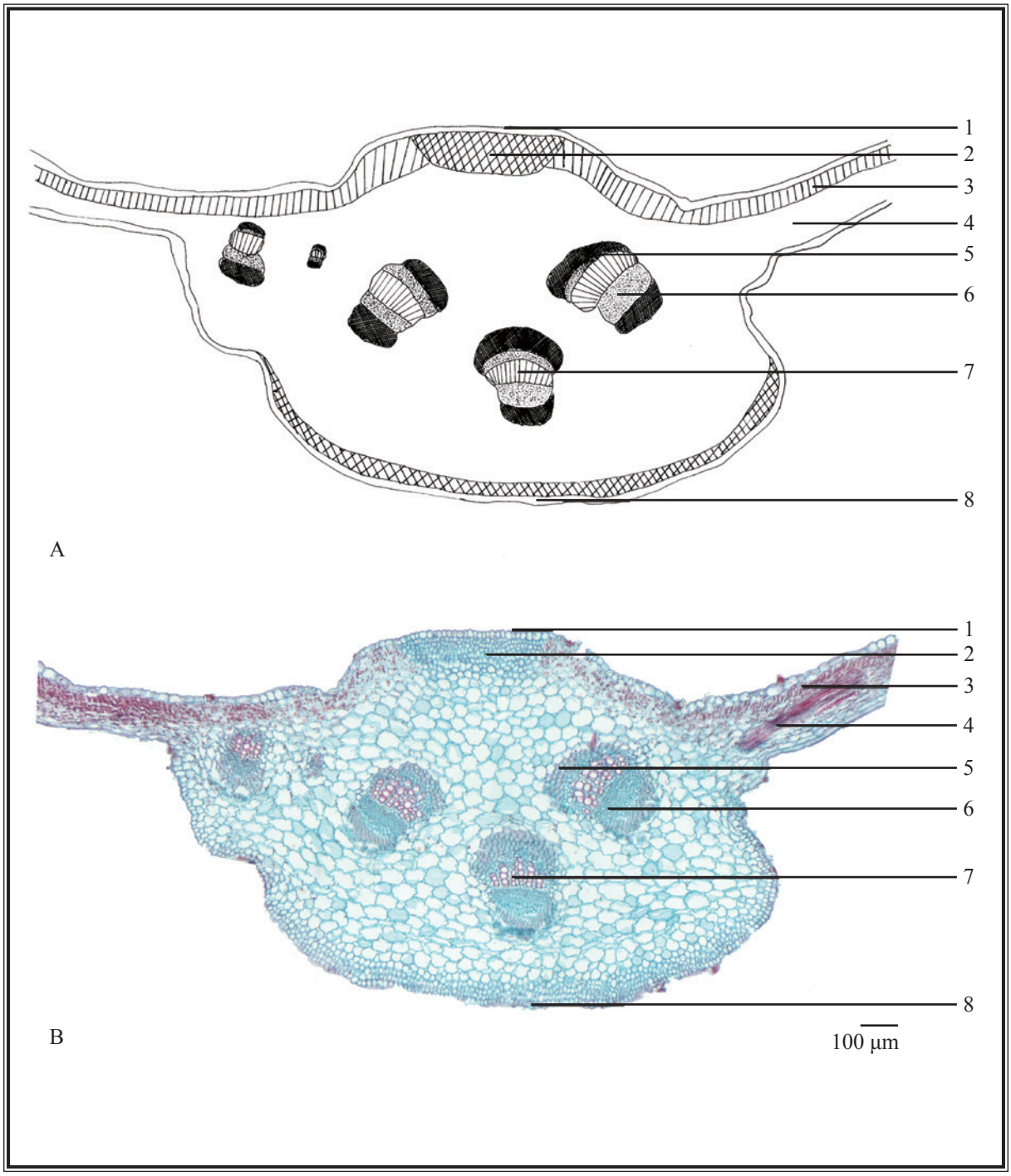

Figure 2 (ii) Microscopic features of transverse section of leaf of Elephantopi Herba

A.Sketch B. Section illustration

1. Upper epidermis 2. Collenchyma 3. Palisade tissue 4. Spongy tissue
5. Sclerenchyma 6. Phloem 7. Xylem 8. Lower epidermis

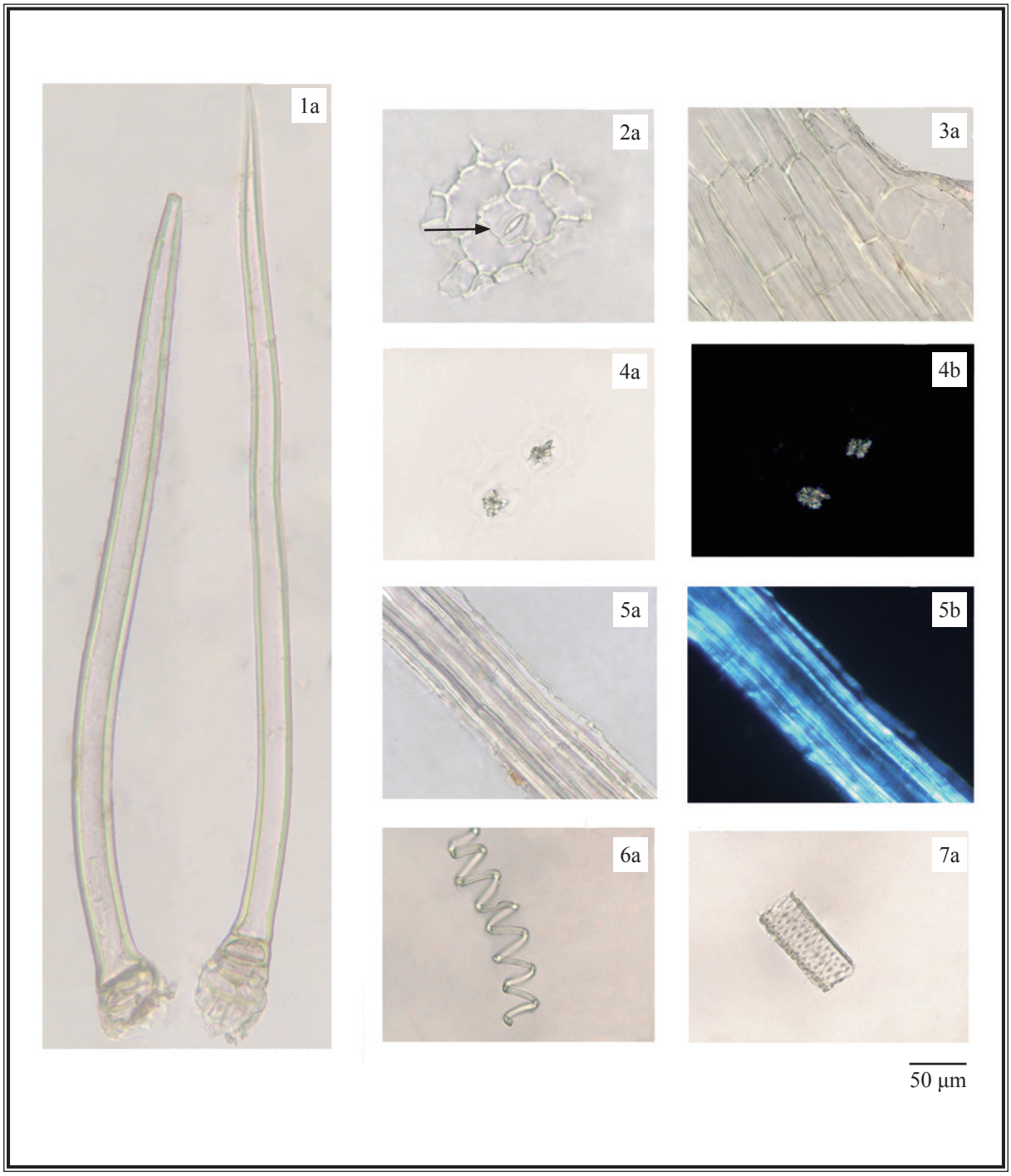

Figure 3 Microscopic features of powder of Elephantopi Herba

1. Non-glandular hairs 2. Lower epidermal cells with stoma (→) 3. Epidermal cells of rhizome
4. Clusters of calcium oxalate 5. Fibres 6. Spiral vessel 7. Reticulate vessel

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

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

Standard solution

Deoxyelephantopin standard solution

Weigh 0.8 mg of deoxyelephantopin CRS (Fig. 4) and dissolve in 2 mL of methanol (70%).

Developing solvent system

Prepare a mixture of cyclohexane, acetone and ethyl acetate (8:3:2, v/v).

Spray reagent

Sulphuric acid (95% - 98%, v/v).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 5 mL of methanol (70%). Sonicate (400 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 deoxyelephantopin standard solution (5 µL) and the test solution (15 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 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 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).

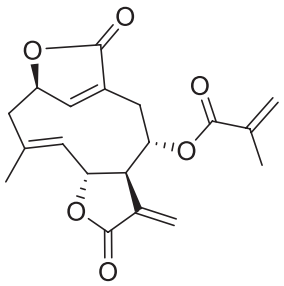

Figure 4 Chemical structure of deoxyelephantopin

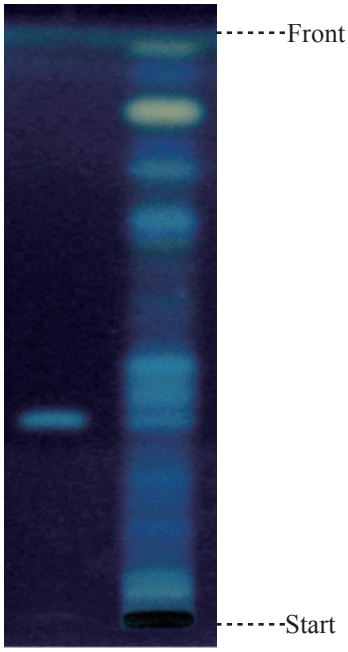

Figure 5 A reference HPTLC chromatogram of Elephantopi Herba extract observed under UV light (366 nm) after staining

1. Deoxyelephantopin 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 deoxyelephantopin (Fig. 5).

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

Standard solution

Deoxyelephantopin standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.1 mg of deoxyelephantopin CRS and dissolve in 10 mL of methanol (70%).

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 (70%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (208 nm) and a column (4.6 × 250 mm) packed with ODS 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)	0.03% Phosphoric acid (%, v/v)	Phosphoric acid: Acetonitrile (0.03:99.97, v/v) (%, v/v)	Elution
0 – 10	75 → 70	25 → 30	linear gradient
10 – 30	70 → 65	30 → 35	linear gradient
30 – 40	65	35	isocratic
40 – 60	65 → 60	35 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of deoxyelephantopin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of deoxyelephantopin should not be more than 5.0%; the RSD of the retention time of deoxyelephantopin peak should not be more than 2.0%; the column efficiency determined from deoxyelephantopin peak should not be less than 25000 theoretical plates.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject deoxyelephantopin Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention time of deoxyelephantopin peak in the chromatogram of deoxyelephantopin Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify deoxyelephantopin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of deoxyelephantopin Std-FP. The retention times of deoxyelephantopin 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 Elephantopi Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Elephantopi Herba extract

Peak No.	RRT	Acceptable Range
1 (marker, deoxyelephantopin)	1.00	-
2	1.09	± 0.03
3	1.41	± 0.03
4	1.54	± 0.04

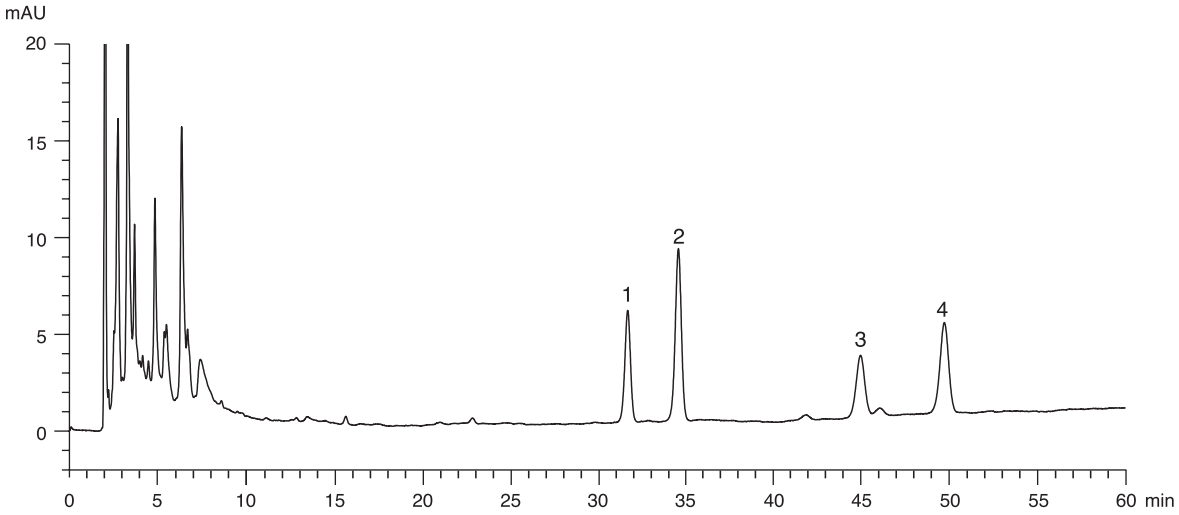

Figure 6 A reference fingerprint chromatogram of Elephantopi Herba extract

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 14.5%.
Acid-insoluble ash: not more than 7.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 10.0%.
Ethanol-soluble extractives (cold extraction method): not less than 4.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 1.0 mg of deoxyelephantopin CRS and dissolve in 10 mL of methanol (70%).

Deoxyelephantopin standard solution for assay, Std-AS

Measure accurately the volume of the deoxyelephantopin Std-Stock, dilute with methanol (70%) to produce a series of solutions of 1, 3, 6, 12, 15 mg/L for deoxyelephantopin.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (70%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Wash the residue with methanol (70%). Combine the solutions and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (208 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.03% phosphoric acid and acetonitrile with 0.03% phosphoric acid (66:34, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 5 µL of deoxyelephantopin Std-AS (6 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of deoxyelephantopin should not be more than 5.0%; the RSD of the retention time of deoxyelephantopin peak should not be more than 2.0%; the column efficiency determined from deoxyelephantopin peak should not be less than 10000 theoretical plates.

The *R* value between deoxyelephantopin 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 deoxyelephantopin Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of deoxyelephantopin against the corresponding concentrations of deoxyelephantopin 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 deoxyelephantopin peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of deoxyelephantopin Std-AS. The retention times of deoxyelephantopin 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 deoxyelephantopin in the test solution, and calculate the percentage content of deoxyelephantopin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.053% of deoxyelephantopin ($C_{19}H_{20}O_6$), calculated with reference to the dried substance.

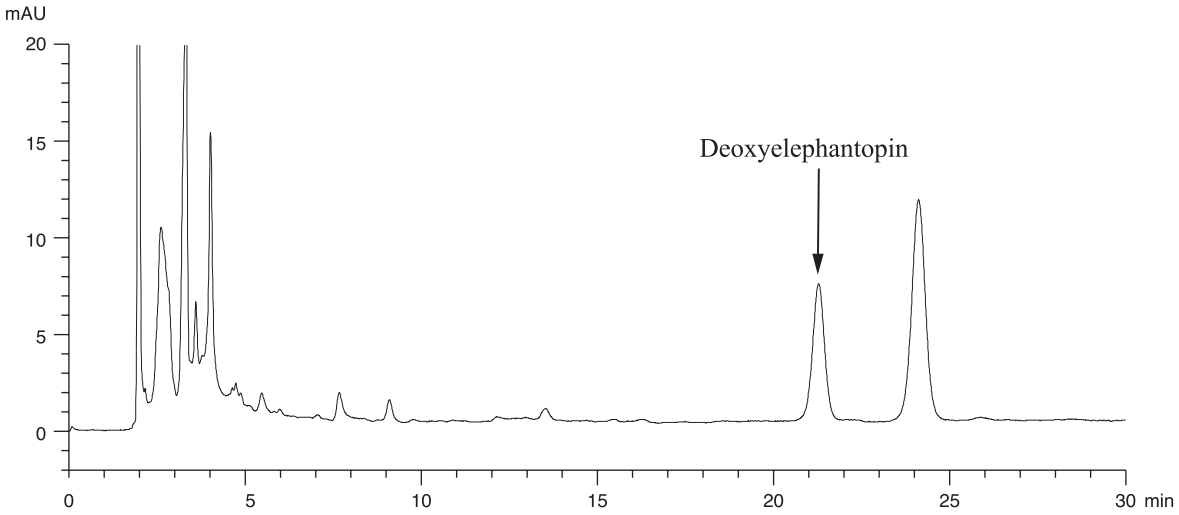

Figure 7 A reference assay chromatogram of Elephantopi Herba extract