Gynostemmatis Herba

Figure 1 (i) A photograph of Gynostemmatis Herba

Figure 1 (ii) A photograph of leaves and fruits of Gynostemmatis Herba

1. NAMES

Official Name: Gynostemmatis Herba

Chinese Name: 絞股藍

Chinese Phonetic Name: Jiaogulan

2. SOURCE

Gynostemmatis Herba is the dried aerial part of *Gynostemma pentaphyllum* (Thunb.) Makino (Cucurbitaceae). The aerial part is collected in summer and autumn, foreign matter removed, then dried under the sun to obtain Gynostemmatis Herba.

3. DESCRIPTION

Stems slender, green, greyish-green or greyish-brown, 1-7 mm in diameter, externally with longitudinal grooves and covered by fine pubescence. Compound leaves greyish-brown, greyish-green or green, usually broken; leaflets usually 5-7, membranous; petioles 1-6 cm long; lateral leaflets ovate-oblong or oblong-lanceolate; central leaflet larger, 0.6-6 cm long, 0.5-5.5 cm wide; apex acuminate, base cuneate; pilose on both surfaces; margin serrate, with arista at the apex of teeth. Fruits occasionally present, spherical and green. Odour grassy; taste slightly sweet [Fig. 1 (i) or 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

Leaf: Upper and lower epidermis consists of 1 layer of cells, with non-glandular hairs at the vein; the upper epidermal cells tangentially elongated. Mesophyll consists of 1-2 layers of palisade tissue and 3-4 layers of spongy tissue. Vascular bundle of midrib collateral. Collenchyma consists 2-3 layers of cells, arranged in the inner sides of upper and lower epidermis of midrib (Fig. 2).

Stem: Epidermis consists of 1 layer of flat cells, with thickened and cuticulate walls; sometimes with non-glandular hairs. Collenchyma consists of 4-6 layers of cells, located at the corners of the stem. Cortex relatively narrow. Pericycle fibre bundles crescent-shaped, arranged in a complete or interrupted ring. Bicollateral vascular bundles 9-10, varying in size, arranged radially. Stone cells in groups located between pericycle fibre bundles. Pith relatively large. Parenchymatous cells contain starch granules (Fig. 2).

Powder

Colour green to greenish-brown. Glandular hairs pale yellow or colourless, consisting of 4-celled elliptical head and 1- to 2-celled stalk, 19-47 µm in diameter. Non-glandular hairs consist of 1-12 cells, 29-646 µm long and 9-135 µm in diameter at base, with linear striations on the surface, sometimes middle cells narrow. Stone cells pale yellow or colourless, subsquare, subrounded or irregular in shape, 27-219 µm long and 15-94 µm in diameter, pits and pit canals distinct. Upper epidermal cells of leaf subpolygonal or irregular in surface view, with shrivelled surface, anticlinal walls almost straight. Lower epidermal cells irregular in shape, stomata anomocytic, anticlinal walls slightly wavy, with glandular hairs. Epidermal cells of stem subrectangular in surface view, stomata visible. Fibres scattered singly or in bundles, 5-47 µm in diameter, with distinct pits and pit canals; white, yellow or polychromatic under the polarized light microscope. Vessels mainly reticulate and spiral, bordered-pitted vessels occasionally visible, 5-89 µm in diameter. Simple starch granules elongated-ellipsoid, ellipsoid or subrounded, 6-49 µm long and 3-25 µm in diameter, hilum and striations indistinct; black and cruciate-shaped under the polarized light microscope; compound starch granules rare (Fig. 3).

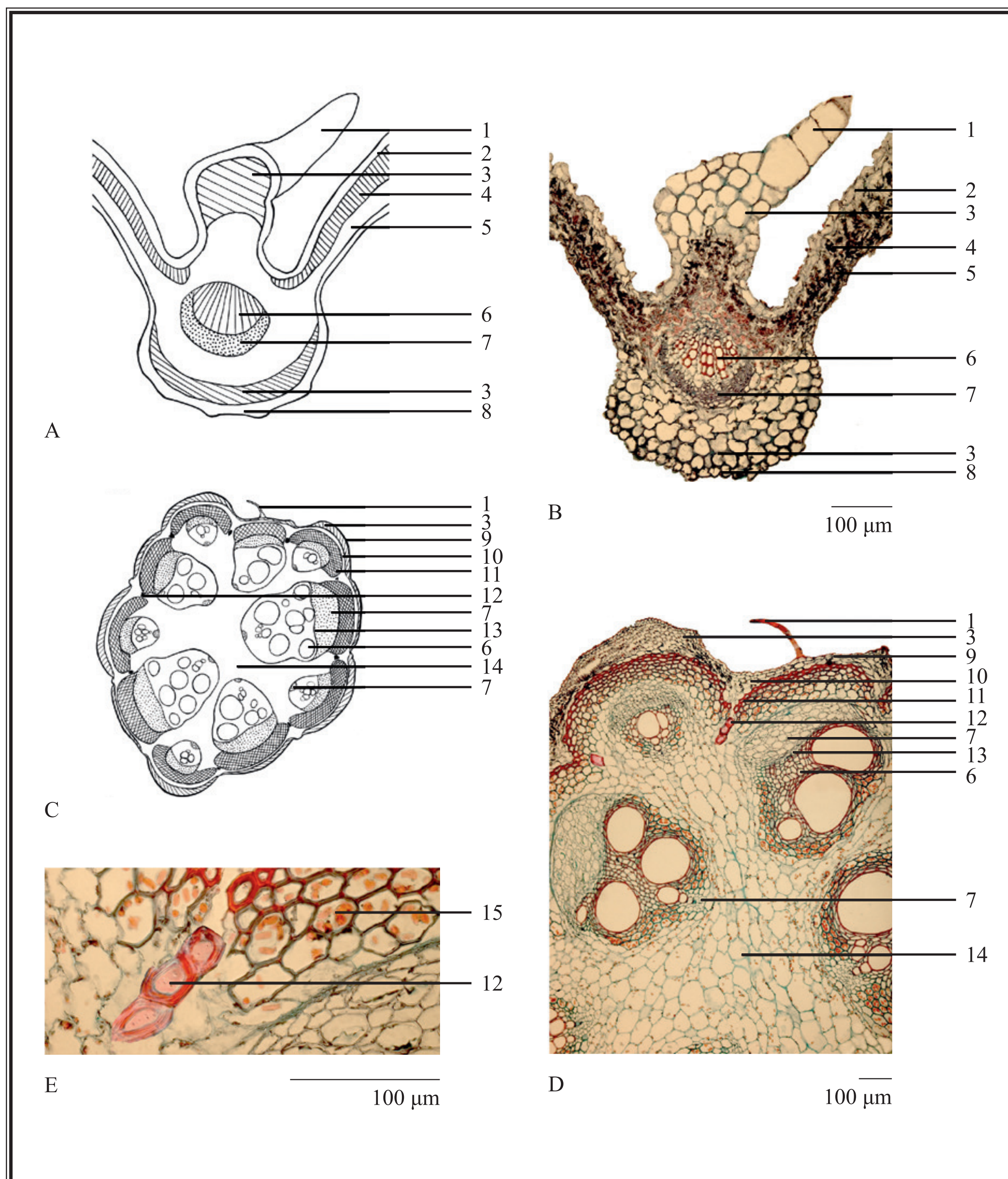

Figure 2 Microscopic features of transverse section of leaf and stem of Gynostemmatis Herba

A. Sketch of midvein B. Section illustration of midvein
C. Sketch of stem D. Section illustration of stem E. Partial magnified of Photo D

1. Non-glandular hair 2. Upper epidermis 3. Collenchyma 4. Palisade tissue
5. Spongy tissue 6. Xylem 7. Phloem 8. Lower epidermis 9. Epidermis
10. Cortex 11. Pericycle fibres 12. Stone cells 13. Cambium 14. Pith 15. Starch granules

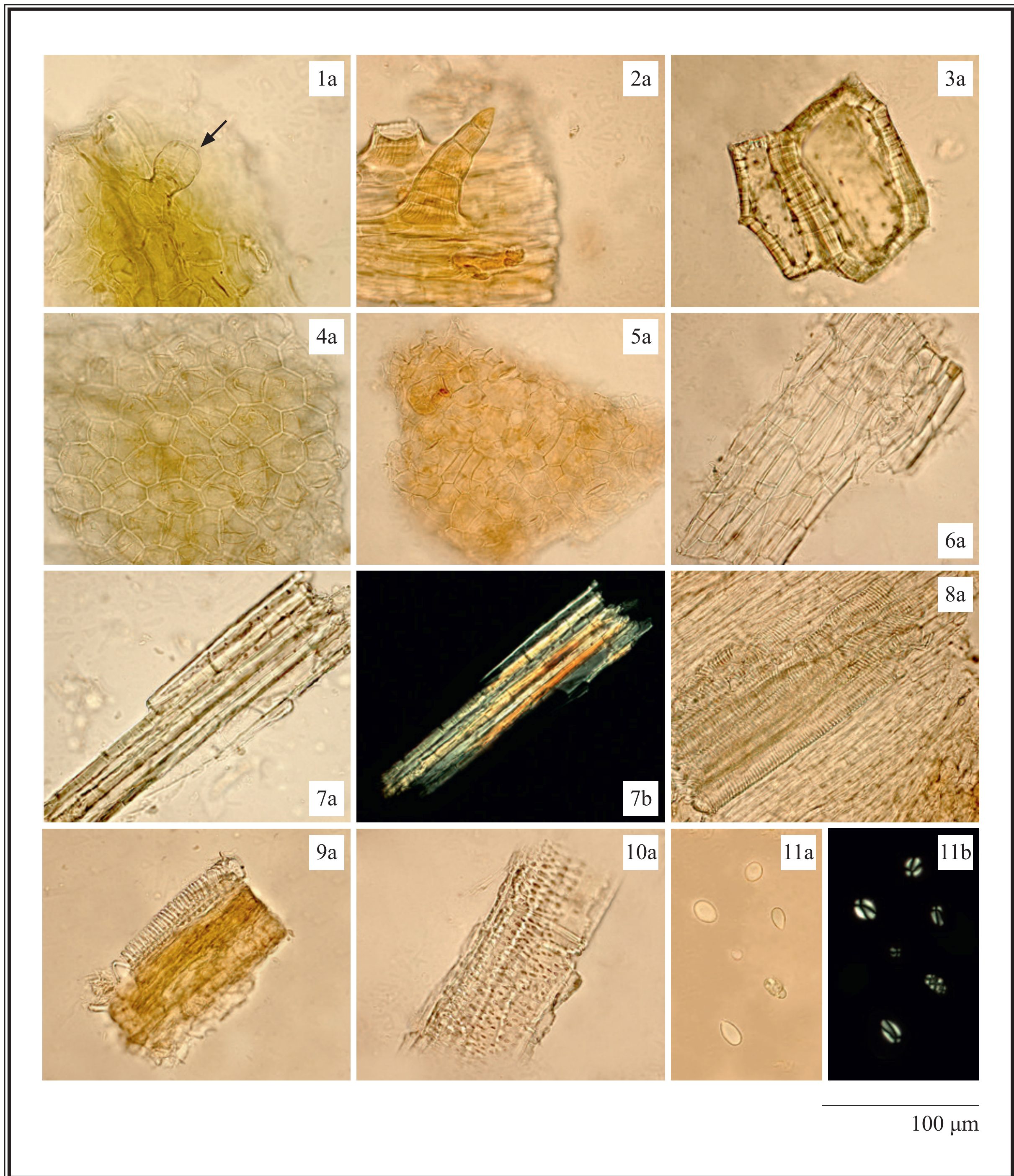

Figure 3 Microscopic features of powder of Gynostemmatis Herba

1. Glandular hair 2. Non-glandular hair 3. Stone cells 4. Upper epidermal cells of leaf
5. Lower epidermal cells of leaf 6. Epidermal cells of stem 7. Fibres 8. Reticulate vessels
9. Spiral vessels 10. Bordered-pitted vessels 11. Starch granules

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

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

Standard solutions

Ombuoside standard solution

Weigh 0.1 mg of ombuoside CRS (Fig. 4) and dissolve in 1 mL of methanol. Place it in a water bath at about 60ºC to dissolve the standard.

Rutin standard solution

Weigh 0.5 mg of rutin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, formic acid, water and methanol (8:1:1:0.5, v/v).

Spray reagent

Weigh 2.5 g of aluminium trichloride and dissolve in 100 mL of ethanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (80%). Sonicate (140 W) the mixture for 1 h. Centrifuge at about 1800 × *g* for 15 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 ombuoside standard solution (3 μL), rutin standard solution (2 μL) and the test solution (15 μL) 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. Spray the plate evenly with the spray reagent and heat at about 85ºC until the spots or bands become visible (about 5-10 min). Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of ombuoside and rutin.

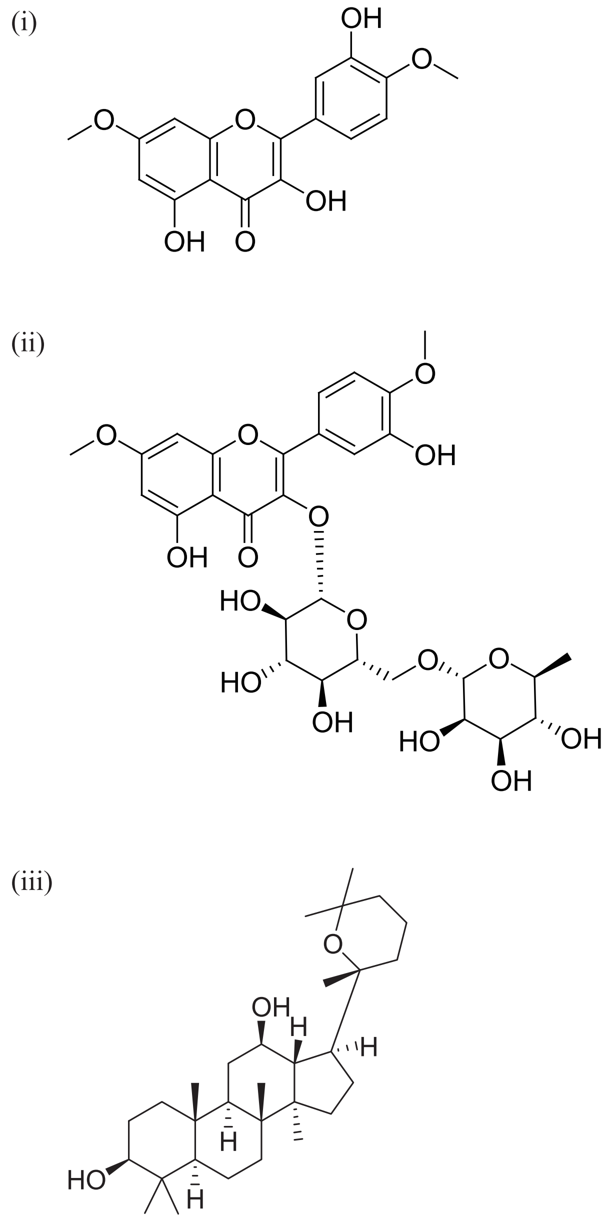

Figure 4 Chemical structures of (i) ombuin (ii) ombuoside and (iii) panaxadiol

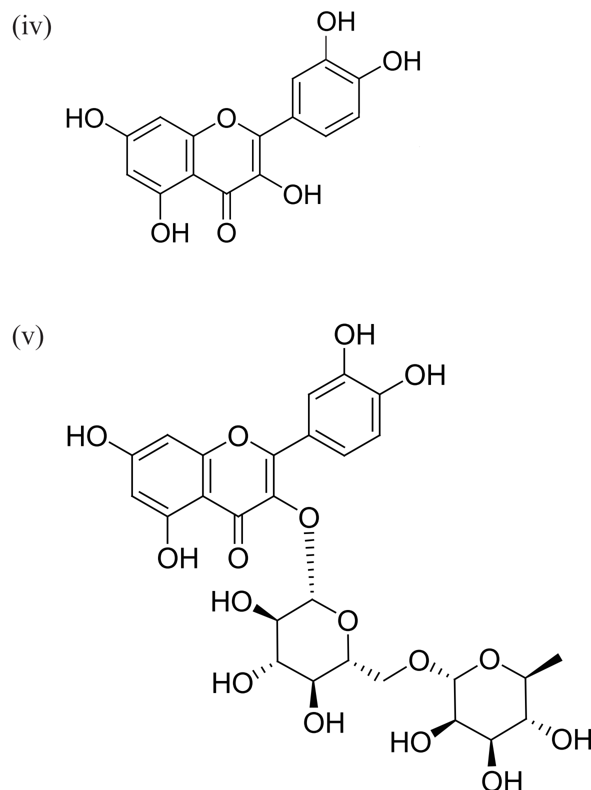

Figure 4 Chemical structures of (iv) quercetin and (v) rutin

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

Standard solutions

Ombuin standard solution for fingerprinting, Std-FP (30 mg/L)

Weigh 0.3 mg of ombuin CRS (Fig. 4) and dissolve in 10 mL of methanol.

Ombuoside standard solution for fingerprinting, Std-FP (35 mg/L)

Weigh 0.35 mg of ombuoside CRS and dissolve in 10 mL of methanol. Place it in a water bath at about 60ºC to dissolve the standard.

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

Weigh 0.5 mg of quercetin CRS (Fig. 4) and dissolve in 10 mL of methanol.

Rutin standard solution for fingerprinting, Std-FP (120 mg/L)

Weigh 1.2 mg of rutin CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (270 W) the mixture for 1 h. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. 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 (370 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 40ºC 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 15	90 → 80	10 → 20	linear gradient
15 – 50	80 → 30	20 → 70	linear gradient
50 – 60	30 → 0	70 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of ombuin Std-FP, ombuoside Std-FP, quercetin Std-FP and rutin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of ombuin, ombuoside, quercetin and rutin should not be more than 5.0%; the RSD of the retention times of ombuin, ombuoside, quercetin and rutin peaks should not be more than 2.0%; the column efficiencies determined from ombuin, ombuoside, quercetin and rutin peaks should not be less than 20000 theoretical plates.

The *R* value between peak 1 and the closest peak; the *R* value between peak 2 and the closest peak; the *R* value between peak 3 and the closest peak; and the *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

Separately inject ombuin Std-FP, ombuoside Std-FP, quercetin Std-FP, rutin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of ombuin, ombuoside, quercetin and rutin peaks in the chromatograms of ombuin Std-FP, ombuoside Std-FP, quercetin Std-FP, rutin Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify ombuin, ombuoside, quercetin and rutin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of ombuin Std-FP, ombuoside Std-FP, quercetin Std-FP and rutin Std-FP. The retention times of ombuin, ombuoside, quercetin and rutin peaks in the chromatograms of the test solution and the corresponding Std-FP 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 Gynostemmatis Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Gynostemmatis Herba extract

Peak No.	RRT	Acceptable Range
1 (rutin)	0.48	± 0.03
2 (ombuoside)	0.66	± 0.03
3 (quercetin)	0.70	± 0.03
4	0.87	± 0.03
5 (marker, ombuin)	1.00	-

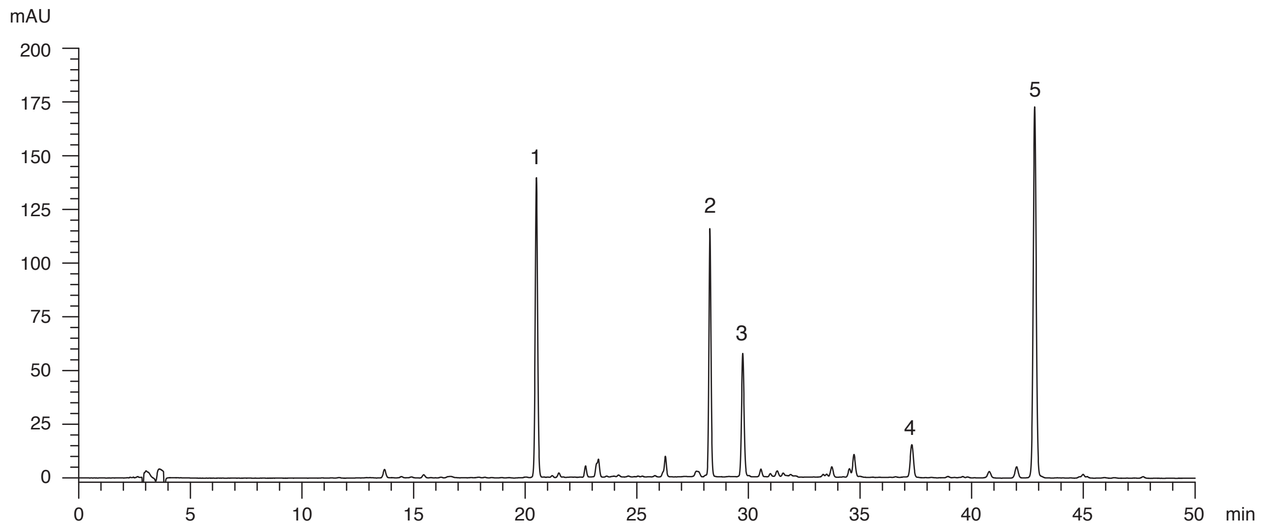

Figure 5 A reference fingerprint chromatogram of Gynostemmatis Herba extract

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. 5).

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 10.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

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

Ethanol-soluble extractives (cold extraction method): not less than 11.0%.

7. ASSAY

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

Standard solution

Panaxadiol standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 10.0 mg of panaxadiol CRS (Fig. 4) and dissolve in 10 mL of methanol.

Panaxadiol standard solution for assay, Std-AS

Measure accurately the volume of the panaxadiol Std-Stock, dilute with methanol to produce a series of solutions of 16, 40, 80, 200, 500 mg/L for panaxadiol.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of methanol. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 100-mL round-bottomed flask. Repeat the extraction for two more times. Wash the residue with 5 mL of methanol. Combine the solutions. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 20 mL of hydrochloric acid (29.5%, w/v). Reflux the mixture for 4 h. Cool down to room temperature. Transfer the solution to a separating funnel. Extract for three times each with 30 mL of dichloromethane. Combine the dichloromethane extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 75ºC; nebulizer gas (N_2) flow: 2.0 L/min] and a column (4.6 × 150 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of methanol and water (88:12, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 20 µL of panaxadiol Std-AS (80 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of panaxadiol should not be more than 5.0%; the RSD of the retention time of panaxadiol peak should not be more than 2.0%; the column efficiency determined from panaxadiol peak should not be less than 5000 theoretical plates.

The *R* value between panaxadiol 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 panaxadiol Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of panaxadiol against the natural logarithm of the corresponding concentrations of panaxadiol Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify panaxadiol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of panaxadiol Std-AS. The retention times of panaxadiol peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of panaxadiol in the test solution by using the following equation –

$$\text{Concentration of panaxadiol in the test solution} = e^{[\mathrm{Ln}\,(A) - I]/m}$$

Where A = the peak area of panaxadiol in the test solution,
I = the y-intercept of the 5-point calibration curve of panaxadiol,
m = the slope of the 5-point calibration curve of panaxadiol.

Calculate the percentage content of panaxadiol in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.094% of panaxadiol ($C_{30}H_{52}O_3$), calculated with reference to the dried substance.