Herba Desmodii Styracifolii

Figure 1(i) A photograph of Herba Desmodii Styracifolii

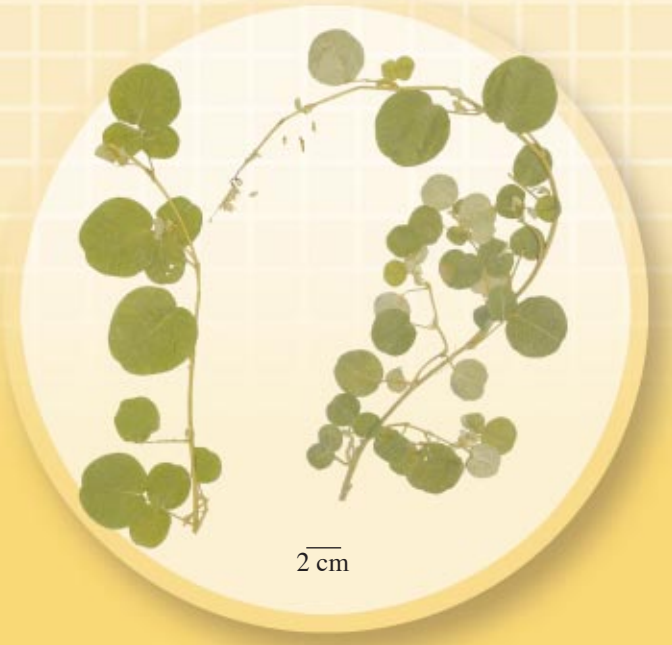

Figure 1(ii) A photograph of Herba Desmodii Styracifolii
(The dried herbs were soaked in water to obtain specimen)

1. NAMES

Official Name: Herba Desmodii Styracifolii

Chinese Name: 廣金錢草

Chinese Phonetic Name: Guangjinqiancao

2. SOURCE

Herba Desmodii Styracifolii is the dried aerial part of *Desmodium styracifolium* (Osb.) Merr. (Fabaceae/Leguminosae). The aerial part is collected in the summer and autumn, after removal of foreign matter, it is dried under the sun to obtain Herba Desmodii Styracifolii.

3. DESCRIPTION

Stem cylindrical, up to 1 m long, densely covered with yellow, spreading pubescence. Texture slightly fragile; fracture shows a pith in the center. Leaves alternate, leaflets 1-3, rounded or oblong, 15-52 mm in diameter; retuse at the apex, cordate or obtusely rounded at the base, margin entire. The upper surface yellowish-green or greyish-green, glabrous, the lower surface densely covered with white, silky tomentum, lateral veins pinnate. Petiole 1-3 cm long; stipules 2, lanceolate about 0.8 cm long. Odour slightly aromatic; taste slightly sweet [Fig. 1(i) and (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem : Epidermis consists of a layer of cells, with hooked and straight non-glandular hair. Glandular hairs, which are enlarged or swollen on the lower part. Pericycle fibre bundles distinct, connected into rings. Parenchyma cells contain pigment masses or prisms of calcium oxalate [Fig. 2(i)].

Leaf : Upper and lower epidermis consist of one layer of cells, with non-glandular and glandular hairs on the lower surface. Palisade and spongy tissues consist of palisade-shaped parenchyma

cells and irregular or subround parenchyma cells, respectively. Parenchyma cells contain pigment masses or prisms of calcium oxalate [Fig. 2(ii)].

Powder

Colour yellowish-green to pale green. Non-glandular hairs abundant; straight non-glandular hairs up to 1300 μm in length, the tip gradually sharpened; curved non-glandular hairs up to 260 μm in length, the tip bent into a hook. Glandular hairs club-like in shape, enlarged and expanded at base; the cells of the enlarged base vary in number, up to about 20 cells, the head consists of 1-2 cells. Epidermal cells of the stem subsquare, subrectangular or polygonal in shape, bearing non-glandular hairs, sometimes glandular hair and stoma observed on the surface. Fibres often in bundles, surrounded by parenchyma cells containing prisms of calcium oxalate, forming crystal fibres, showing a polychrome when examined under the polarized microscope. Vessels mainly bordered-pitted, spiral and scalariform. The stoma mostly of the paracytic type. Pigment masses orange or orange-brown [Fig. 3(i) and (ii)].

4.2 Physicochemical Identification

Procedure

Weigh 1.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add 15 mL of water. Boil the mixture in a water bath for 10 min. Centrifuge at about 3000 × *g* for 5 min. Transfer 5 mL of the supernatant to a test tube. Evaporate the solution to about 0.5 mL on a water bath. Dissolve the residue in 2 mL of ethanol. Transfer 1.5 mL of the solution to a test tube. Add approximately 25 mg of magnesium turnings to the mixture, then add 0.5 mL of hydrochloric acid. Allow to stand for 30 min. A reddish-brown solution is observed.

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

Standard solution

Isovitexin standard solution

Weigh 1.0 mg of isovitexin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of tetrahydrofuran, methanol, water and formic acid (4:6:14:1, v/v).

Spray reagent

Dissolve 1.0 g of aluminium trichloride in 100 mL of ethanol.

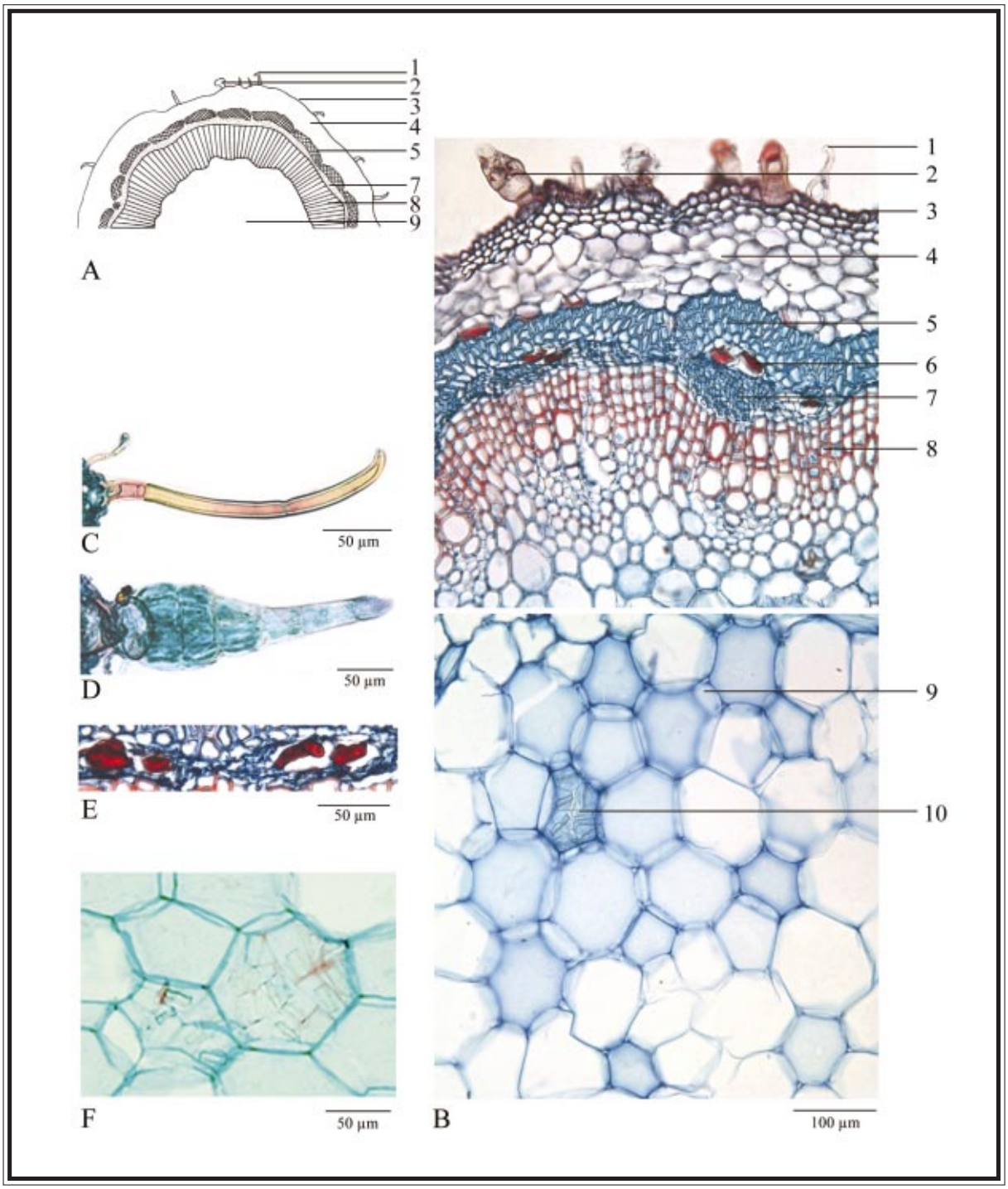

Figure 2(i) Microscopic features of transverse section of stem of Herba Desmodii Styracifolii

A. Stem sketch B. Stem C. Non-glandular hair D. Glandular hair E. Pigment masses F. Prisms of calcium oxalate

1. Non-glandular hair 2. Glandular hair 3. Epidermis 4. Cortex 5. Pericycle fibres 6. Pigment masses 7. Phloem 8. Xylem 9. Pith 10. Prisms of calcium oxalate

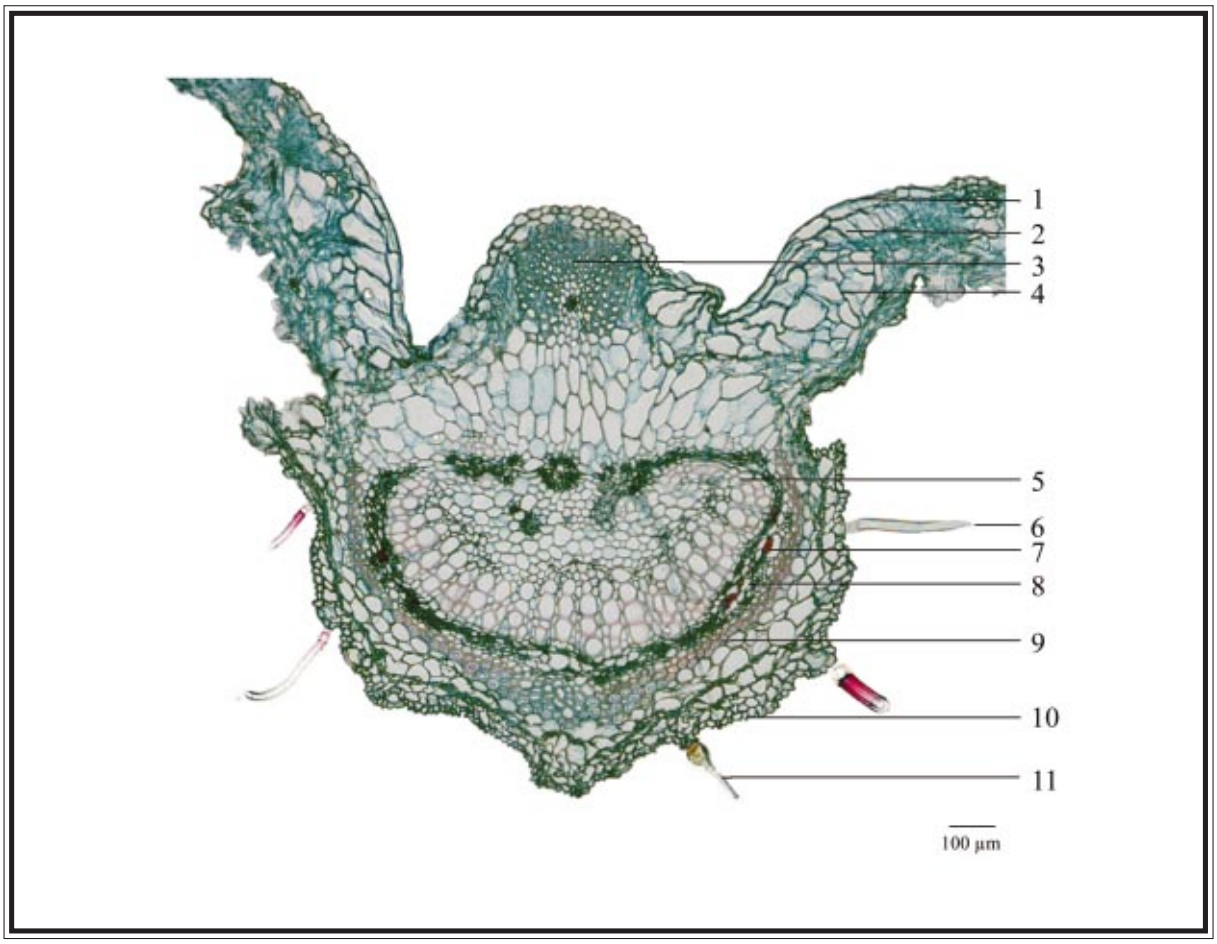

Figure 2(ii) Microscopic features of transverse section of leaf of Herba Desmodii Styracifolii

1. Upper epidermis 2. Palisade tissue 3. Fibre bundle 4. Spongy tissue 5. Xylem 6. Non-glandular hair 7. Pigment masses 8. Phloem 9. Pericycle fibres 10. Lower epidermis 11. Glandular hair

Figure 3(i) Microscopic features of powder of Herba Desmodii Styracifolii (under the light microscope)

1. Epidermal cells of the stem with non-glandular hairs and stomata 2. Glandular hair
3. Hooked non-glandular hair 4. Straight non-glandular hair

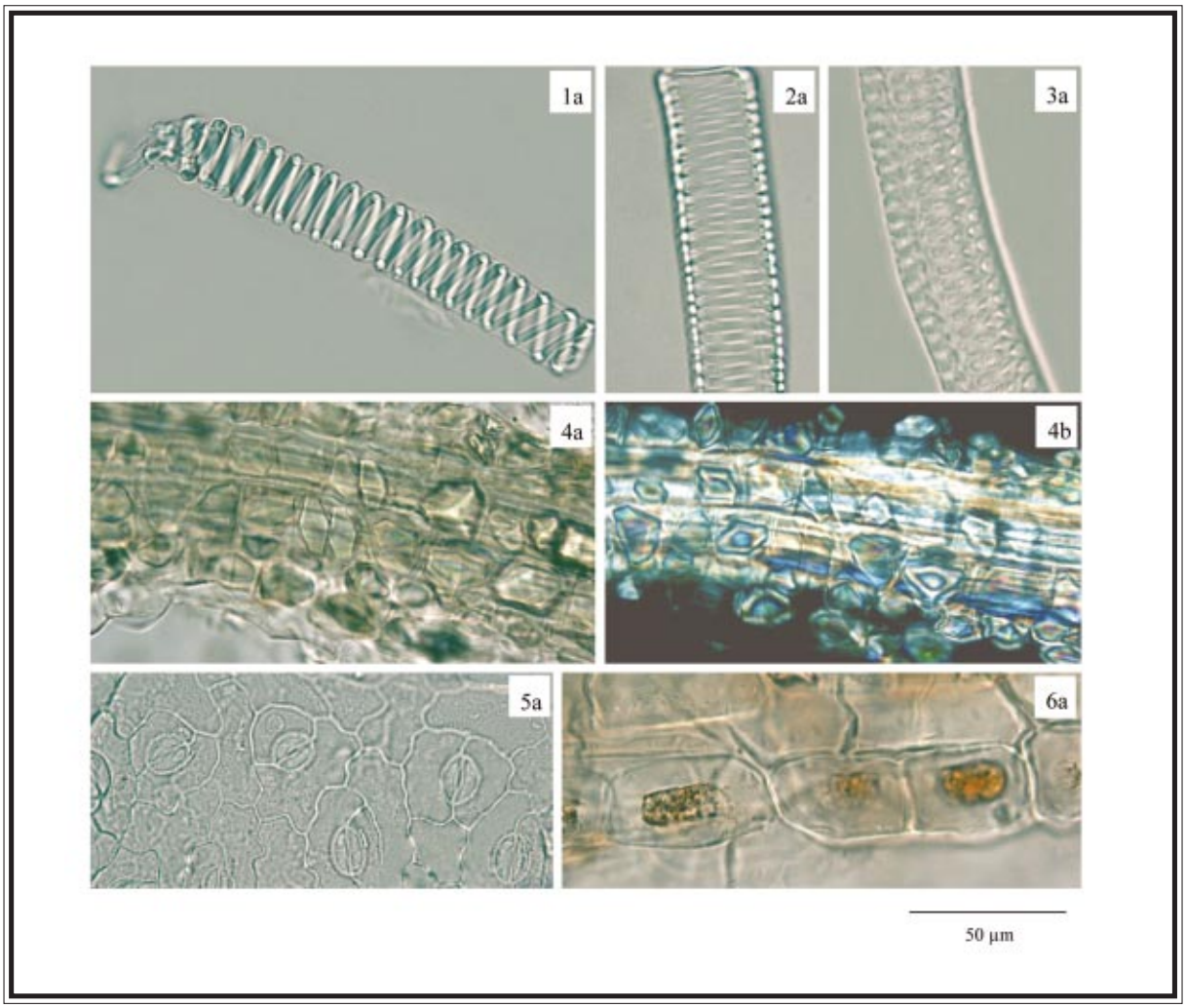

Figure 3(ii) Microscopic features of powder of Herba Desmodii Styracifolii

1. Spiral vessel 2. Scalariform vessel 3. Bordered-pitted vessel 4. Crystal fibres 5. Stomata
6. Pigment masses

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

Test solution

Weigh 3.0 g of the powdered sample and put into a 100-mL conical flask, then add 50 mL of methanol. Sonicate (560 W) the mixture for 30 min. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 30 mL of water. Transfer the aqueous solution to a separatory funnel. Shake the solution with 100 mL of dichloromethane and discard the lower layer. Extract twice each with 100 mL of 1-butanol. Combine the 1-butanol extracts, wash with 100 mL of water and discard the lower layer. Evaporate the 1-butanol extracts to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of methanol.

Procedure

Carry out the method by using a TLC RP-18 F_{254} (40-63 μm) plate and a freshly prepared developing solvent system as described above. Apply separately isovitexin standard solution (1 μL) and the test solution (1.5 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 dry in air. Examine the plate under UV light (365 nm). Calculate the R_f value by using the equation as indicated in Appendix IV(A).

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 isovitexin.

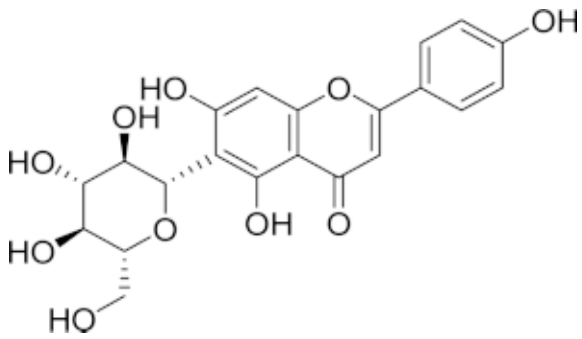

Figure 4 Chemical structure of isovitexin

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

Standard solution

Isovitexin standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of isovitexin CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and put into a 50-mL test tube, then add 10 mL of methanol. Sonicate (560 W) the mixture for 30 min. Filter through a 0.45-μm RC filter. Repeat the extraction twice. Combine the filtrate to a 50-mL volumetric flask. Make up to the mark with methanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (340 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.7 mL/min. Programme the chromatographic system as follows –

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 15	85	15	isocratic
15 – 60	85 → 78	15 → 22	linear gradient

System suitability requirements

Perform at least five replicate injections each with 10 μL of isovitexin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of isovitexin should not be more than 5.0%; the RSD of the retention time of isovitexin peak should not be more than 2.0%; the column efficiency determined from isovitexin peak should not be less than 26000 theoretical plates.

The *R* value between peak 3 and peak 4 in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

Separately inject isovitexin Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of isovitexin peak in the chromatogram of isovitexin Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Under the same HPLC conditions, identify isovitexin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of isovitexin Std-FP. The retention times of isovitexin 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 Herba Desmodii Styracifolii extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the five characteristic peaks of Herba Desmodii Styracifolii extract

Peak No.	RRT	Acceptable Range
1	0.54	±0.03
2	0.65	±0.03
3	0.79	±0.03
4	0.82	±0.03
5 (marker, isovitexin)	1.00	-

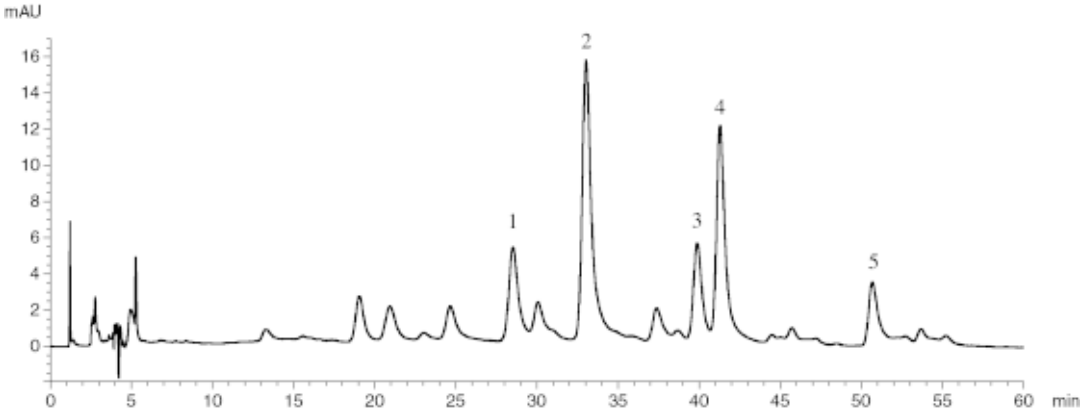

Figure 5 A reference fingerprint chromatogram of Herba Desmodii Styracifolii extract

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 4.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 18.0%.
Acid-insoluble ash: not more than 12.0%.

5.7 Water Content *(Appendix X)*: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 9.0%.
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
Isovitexin standard stock solution, Std-Stock (100 mg/L)
Weigh accurately 5.0 mg of isovitexin CRS and dissolve in 50 mL of methanol.
Isovitexin standard solution for assay, Std-AS
Measure accurately the volume of the isovitexin Std-Stock, dilute with methanol to produce a series of solutions of 1, 5, 10, 15, 20 mg/L for isovitexin.

Test solution
Weigh accurately 0.5 g of the powdered sample and put into a 50-mL test tube, then add 10 mL of methanol. Sonicate (560 W) the mixture for 30 min. Filter through a 0.45-μm RC filter. Repeat the extraction twice. Combine the filtrate to a 50-mL volumetric flask. Make up to the mark with methanol. Filter through a 0.45-μm RC filter.

Chromatographic system
The liquid chromatograph is equipped with a detector (340 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.7 mL/min. The mobile phase is a mixture of acetonitrile and water (21:79, v/v). The elution time is about 22 min.

System suitability requirements

Perform at least five replicate injections each with 10 µL of isovitexin Std-AS (15 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of isovitexin should not be more than 3.0%; the RSD of the retention time of isovitexin peak should not be more than 1.0%; the column efficiency determined from isovitexin peak should not be less than 6000 theoretical plates.

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

Limits

The sample contains not less than 0.048% of isovitexin ($C_{21}H_{20}O_{10}$), calculated with reference to the dried substance.