Visci Herba

Figure 1 A photograph of Visci Herba

A. Visci Herba B. Magnified transverse section of stem

1. NAMES

Official Name: Visci Herba

Chinese Name: 槲寄生

Chinese Phonetic Name: Hujisheng

2. SOURCE

Visci Herba is the dried stem and branch, together with the leaf of *Viscum coloratum* (Komar.) Nakai (Loranthaceae). They are collected in winter to next spring. The thick stem (over 10 mm in diameter) removed, then cut into sections, then dried under the sun or in shaded area; or dried after steaming to obtain Visci Herba. This plant mainly parasitizes on the host plant of *Ulmus pumila* L. and *Pyrus* spp..

3. DESCRIPTION

Stems cylindrical, normally 2-branched in fork shape, mostly broken at the nodes into segments of varying lengths, each 2-10 mm in diameter; externally yellowish-green, golden-yellow or yellowish-brown, with longitudinal wrinkles; the nodes swollen, with branches or scars of branches. Texture light and fragile, easily broken; fracture uneven, in transverse section the bark appears yellow, wood pale yellow, with distinct annular rings and radical rays, pith often inclined to one side. Leaves opposite on the tips of branches, easily fallen off, sessile; lamina narrowly elliptic-lanceolate, asymmetrical, 2-7 cm long, 0.5-1.5 cm wide; apex obtusely rounded, base cuneate, margin entire; externally yellowish-green, with fine wrinkles, quinquenerved, the middle 3 distinct. Texture coriaceous. Odour slight; taste slightly bitter and sticky on chewing (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermal cells rectangular, covered with orange cuticle, 19-80 µm thick. Cortex consists of 8-30 layers of parenchymatous cells. Pericycle fibres arranged in bundles of several dozens of cells, strongly lignified. Phloem consists of 5-9 layers of parenchymatous cells. Cambium indistinct. Vessels and xylem rays arranged radially, scattered with fibre bundles; vessels surrounded by fibres. Pith distinct. Parenchymatous cells contains clusters of calcium oxalate and a few prisms. Stem scattered with numerous fibres, stone cells are sometimes visible [Fig. 2 (i)].

Leaf: Upper and lower epidermis consist of 1 layer of cells, covered with cuticle. Clusters of calcium oxalate scattered in parenchymatous cells. Vascular bundles collateral, each with fibre bundles above and below it [Fig. 2 (ii)].

Powder

Colour pale yellow to brown. Epidermal cells yellowish-green, subsquare, stomata paracytic. Stone cells subsquare, subpolygonal or irregular, 15-110 µm in diameter. Vessels mainly reticulate and spiral. Heterogeneous sclerenchymatous cells irregular, wall relatively thickened, slightly lignified, lumen large. Clusters of calcium oxalate 14-50 µm in diameter; polychromatic under the polarized microscope. Prisms of calcium oxalate relatively few, 4-20 µm in diameter; polychromatic under the polarized microscope. Fibres arranged in bundles, wall relatively thickened and straight, somewhat sinuous and pitted (Fig. 3).

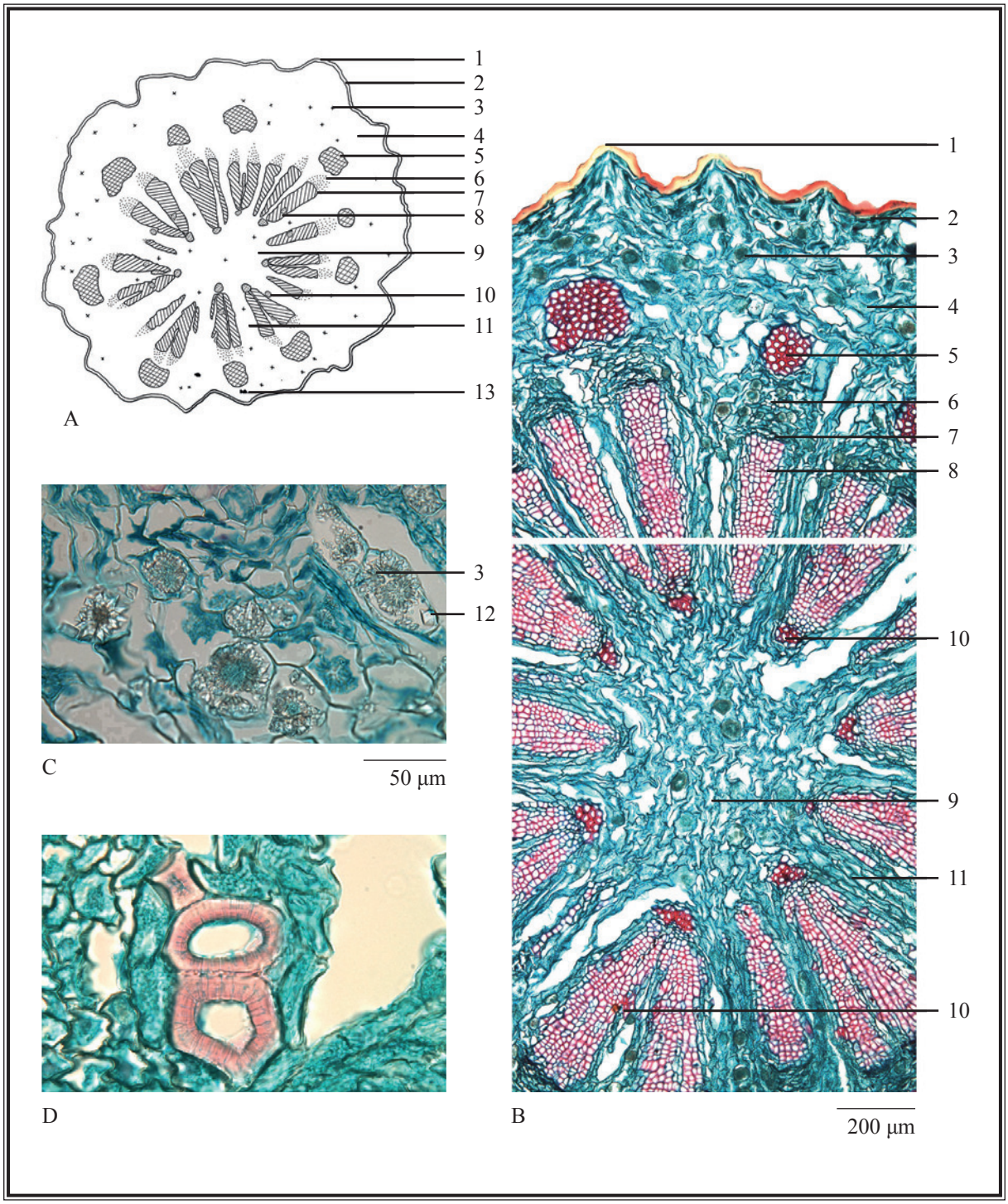


Figure 2 (i) Microscopic features of transverse section of stem of Visci Herba

A. Sketch B. Section illustration C. Crystals of calcium oxalate D. Stone cells

1. Cuticle 2. Epidermis 3. Clusters of calcium oxalate 4. Cortex
5. Pericycle fibre bundles 6. Phloem 7. Cambium 8. Xylem 9. Pith
10. Xylem fibres 11. Xylem rays 12. Prisms of calcium oxalate 13. Stone cells

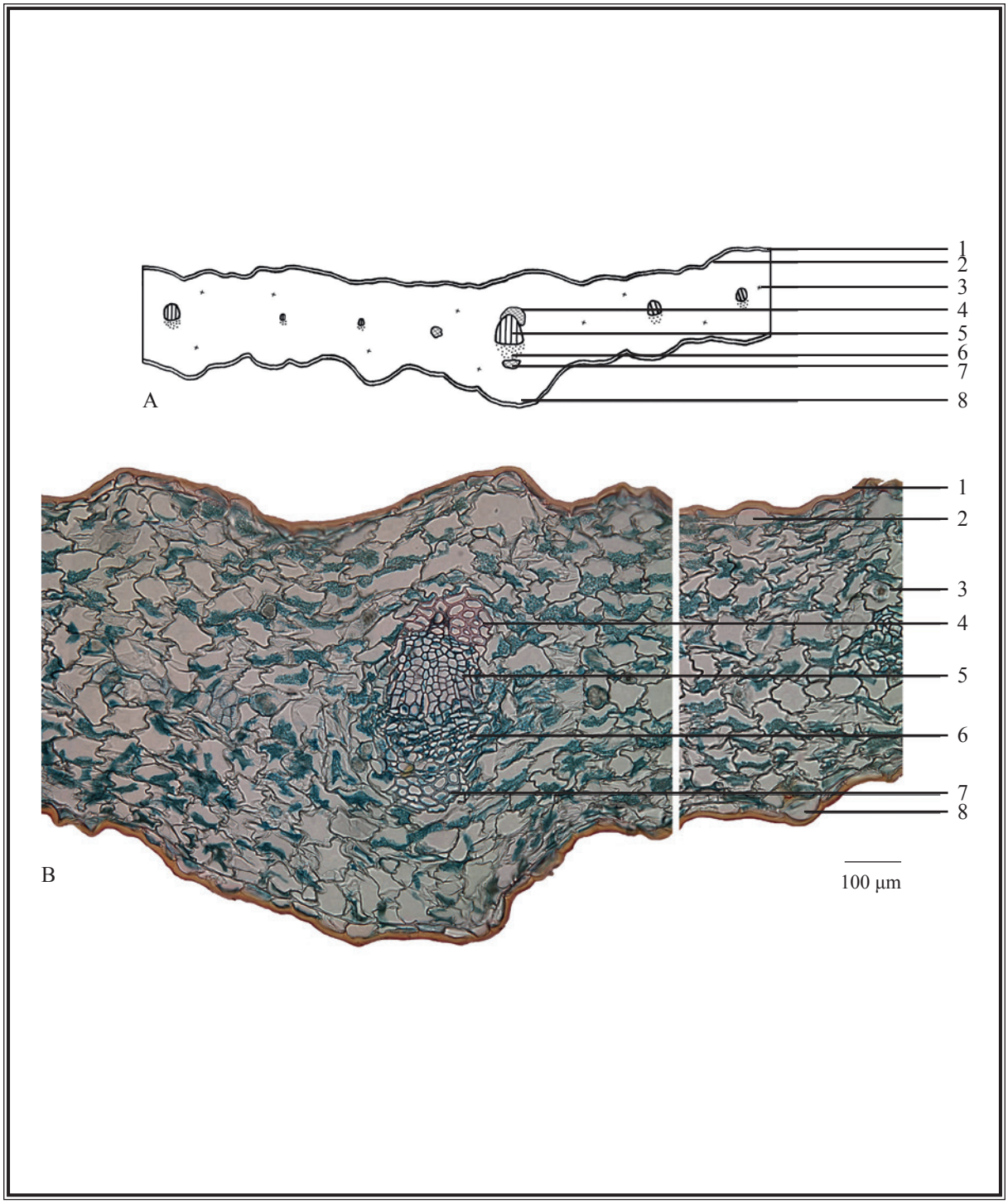


Figure 2 (ii) Microscopic features of transverse section of leaf of Visci Herba

A. Sketch B. Section illustration

1. Cuticle 2. Upper epidermis 3. Clusters of calcium oxalate
4. Xylem fibres 5. Xylem 6. Phloem 7. Pericycle fibre bundles
8. Lower epidermis

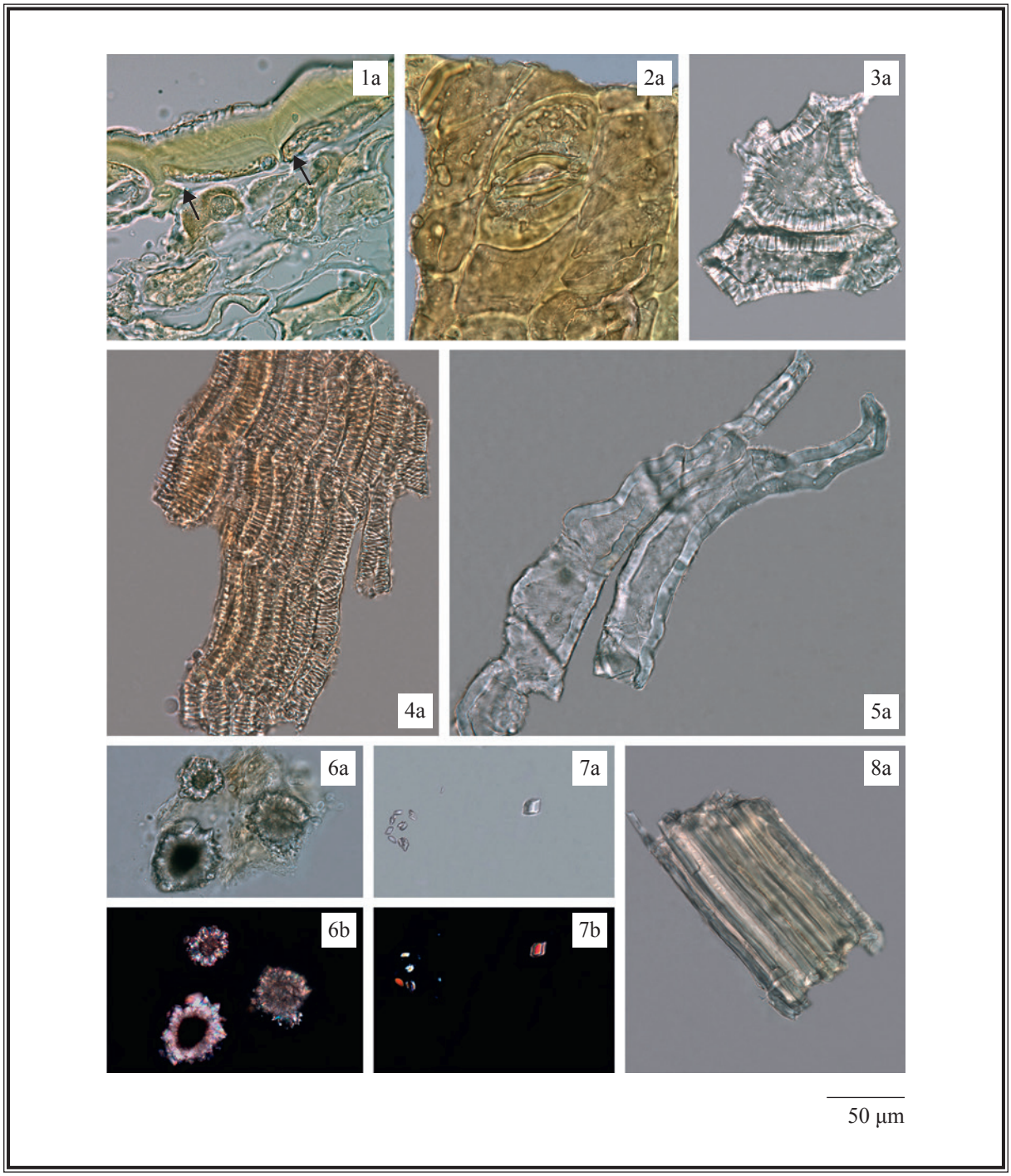


Figure 3 Microscopic features of powder of Visci Herba

1. Epidermal cells (in lateral view)
2. Epidermal cells with stomata (in surface view) 3. Stone cells
4. Vessels 5. Heterogeneous sclerenchymatous cells 6. Clusters of calcium oxalate
7. Prisms of calcium oxalate 8. Fibres

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

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

Standard solution

Oleanolic acid standard solution

Weigh 1.0 mg of oleanolic acid CRS (Fig. 4) and dissolve in 0.5 mL of ethanol.

Developing solvent system

Prepare a mixture of n-hexane, ethyl acetate and acetic acid (20:6:1, v/v).

Spray reagent

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

Test solution

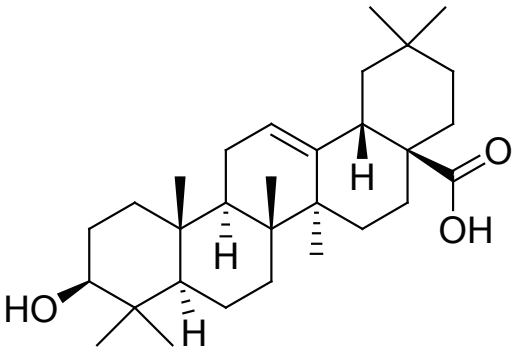
Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of ethanol. Sonicate (160 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately oleanolic acid standard solution and the test solution (2 µL each) to the plate. Develop over a path of about 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 80˚C until the spots or bands become visible (about 15 min). Examine the plate under visible light. 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 oleanolic acid.

(i)



(ii)

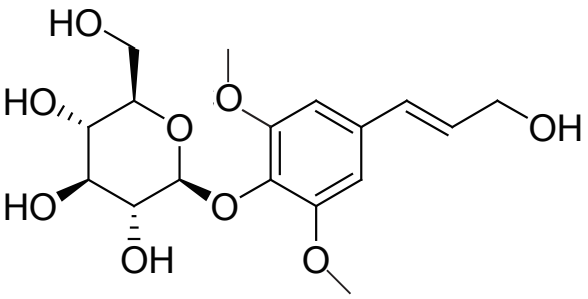


Figure 4 Chemical structures of (i) oleanolic acid and (ii) syringin

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

Standard solution

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

Weigh 0.2 mg of syringin CRS (Fig. 4) and dissolve in 20 mL of ethanol (75%).

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (75%). Sonicate (160 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol (75%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (264 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The internal diameter of the inlet column tubing is about 0.5 mm. 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% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	95 → 85	5 → 15	linear gradient
30 – 60	85 → 65	15 → 35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of syringin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of syringin should not be more than 5.0%; the RSD of the retention time of syringin peak should not be more than 2.0%; the column efficiency determined from syringin peak should not be less than 20000 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. 5).

Procedure

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

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

Peak No.	RRT	Acceptable Range
1 (marker, syringin)	1.00	-
2	1.08	± 0.03
3	1.56	± 0.05
4	2.22	± 0.03
5	2.33	± 0.03

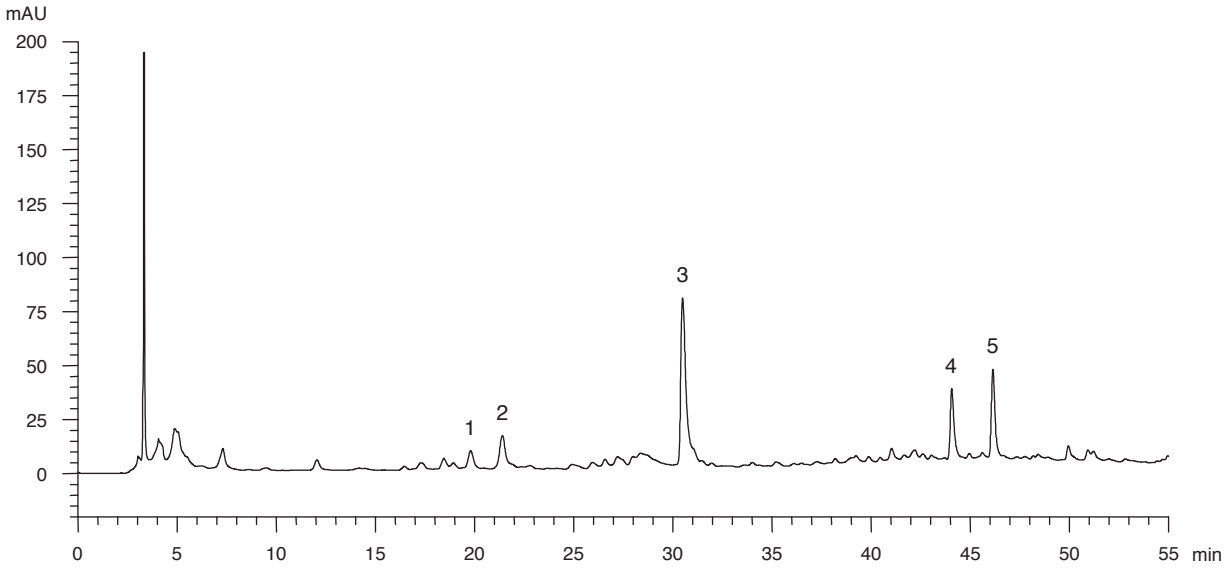


Figure 5 A reference fingerprint chromatogram of Visci Herba extract

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 8.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 9.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 22.0%.
Ethanol-soluble extractives (hot extraction method): not less than 20.0%.

7. ASSAY

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

Standard solution

Syringin standard stock solution, Std-Stock (200 mg/L)
Weigh accurately 2.0 mg of syringin CRS and dissolve in 10 mL of ethanol (75%).
Syringin standard solution for assay, Std-AS
Measure accurately the volume of the syringin Std-Stock, dilute with ethanol (75%) to produce a series of solutions of 0.5, 2, 10, 20, 30 mg/L for syringin.

Test solution

Weigh accurately 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (75%). Sonicate (160 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol (75%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (264 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The internal diameter of the inlet column tubing is about 0.5 mm. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	95 → 85	5 → 15	linear gradient
30 – 60	85 → 65	15 → 35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of syringin Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of syringin should not be more than 5.0%; the RSD of the retention time of syringin peak should not be more than 2.0%; the column efficiency determined from syringin peak should not be less than 20000 theoretical plates.

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

Limits

The sample contains not less than 0.010% of syringin ($C_{17}H_{24}O_9$), calculated with reference to the dried substance.