Lonicerae Japonicae Caulis

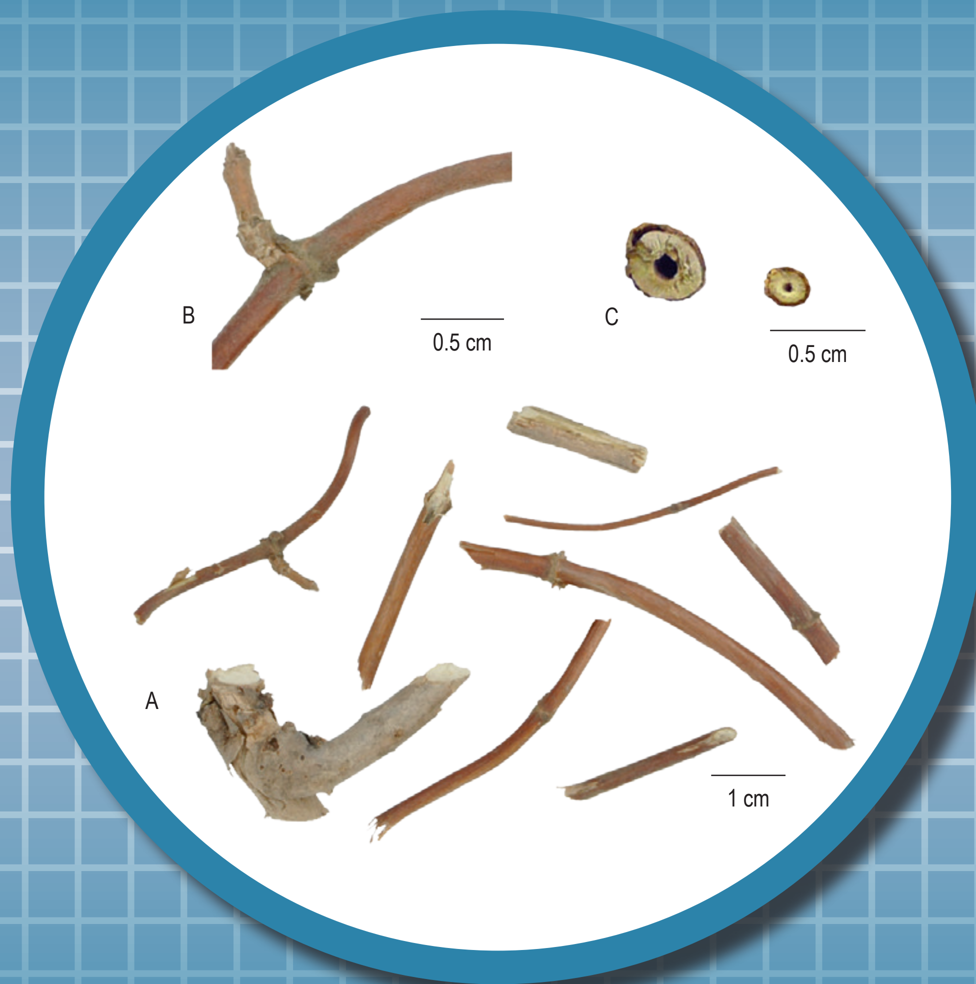

Figure 1 A photograph of Lonicerae Japonicae Caulis

A. Lonicerae Japonicae Caulis B. Young branch with node and pubescence
C. Transverse section

1. NAMES

Official Name: Lonicerae Japonicae Caulis

Chinese Name: 忍冬藤

Chinese Phonetic Name: Rendongteng

2. SOURCE

Lonicerae Japonicae Caulis is the dried stem and branch of *Lonicera japonica* Thunb. (Caprifoliaceae). The stem and branch are collected in autumn and winter, foreign matter removed, then dried under the sun to obtain Lonicerae Japonicae Caulis.

3. DESCRIPTION

Long-cylindrical, 1-7 mm in diameter. Externally brownish-red or pale grey, glabrous or pubescent; outer bark easily fallen off, appearing yellowish-white. Branches nodose, with leaf scars. Texture brittle. Fracture uneven, yellowish-white, hollow. Odour slight; taste of older branches slightly bitter, and the younger ones weak (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Young branch: Epidermis consists of 1 layer of flat cells, containing pigments. Cortex consists of several layers of compressed cells on the outer side, followed by 1-2 layers of cortex fibres on the inner side. Cork consists of several layers of subpolygonal cells. Phloem narrow, with some cells of the phloem rays contain clusters of calcium oxalate. Cambium in a ring. In xylem, the vessels usually scattered singly or in groups; xylem rays consist of 1-2 rows of cells. Pith with a hollow centre, the pith cells lignified (Fig. 2).

Older stem: Cork lines the outermost layer of the older stem. Phloem fibres often in bundles, with a fairly thick wall. Phloem rays distinct (Fig. 2).

Powder

Colour pale reddish-brown. Clusters of calcium oxalate usually found in a row in the parenchymatous cells, 5-28 µm in diameter; polychromatic under the polarized microscope. Non-glandular hairs unicellular, 40-374 µm long (occasionally up to 907 µm), 6-32 µm in diameter (occasionally up to 46 µm), usually broken, wall thick, basal part slightly swollen; bright yellowish-white under the polarized microscope. Epidermal cells brownish-yellow, subpolygonal, often with scars of non-glandular hairs that resemble stone cells. Cork cells subrectangular or polygonal. Cortex fibres 14-98 µm in diameter (occasionally up to 122 µm), with straight wall and large lumen; yellowish-white under the polarized microscope. Phloem fibres 8-40 µm in diameter, wall extremely thickened, pits and pit canals indistinct; bright yellowish-white under the polarized microscope. Xylem fibres 7-36 µm in diameter, pits distinct; bright white under the polarized microscope. Vessels mainly spiral and reticulate, 5-75 µm in diameter (Fig. 3).

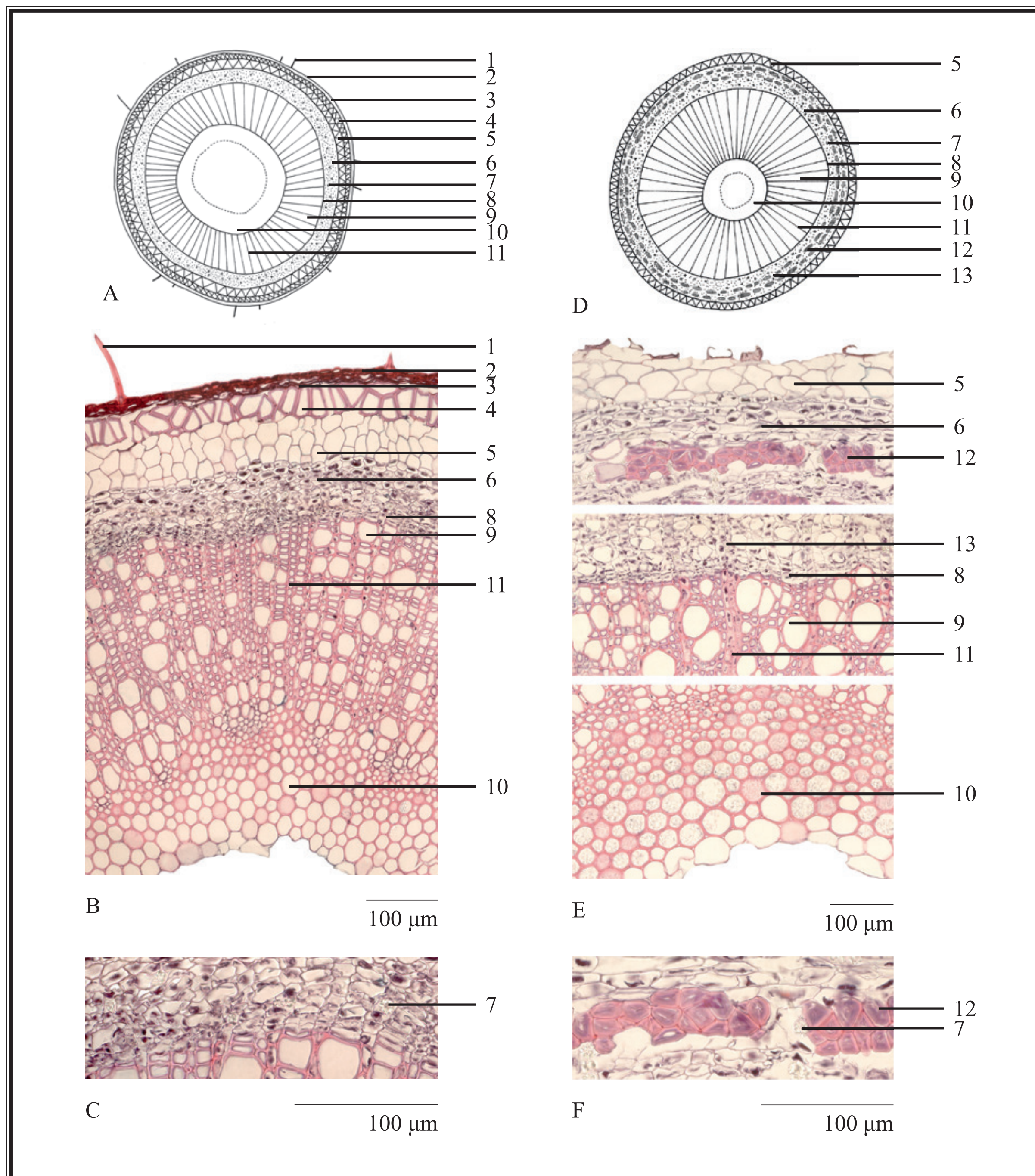

Figure 2 Microscopic features of transverse section of Lonicerae Japonicae Caulis

A. Sketch of young branch B. Section illustration of young branch
C. Clusters of calcium oxalate in phloem D. Sketch of older stem
E. Section illustration of older stem F. Clusters of calcium oxalate and phloem fibres

1. Non-glandular hair 2. Epidermis 3. Cortex 4. Cortex fibre 5. Cork
6. Phloem 7. Cluster of calcium oxalate 8. Cambium 9. Xylem 10. Pith
11. Xylem ray 12. Phloem fibre 13. Phloem ray

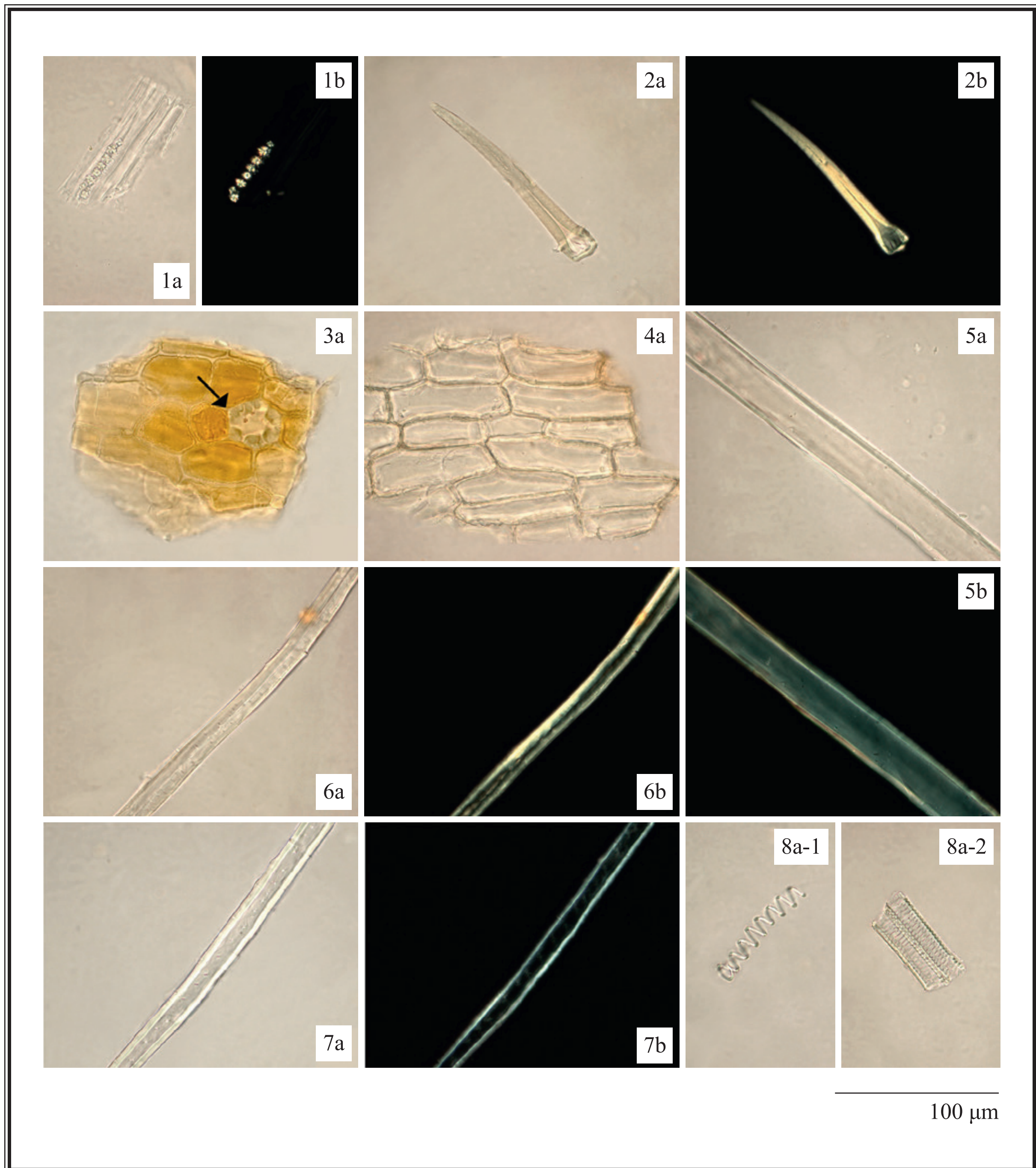

Figure 3 Microscopic features of powder of Lonicerae Japonicae Caulis

1. Clusters of calcium oxalate 2. Non-glandular hair
3. Epidermal cells with scar of non-glandular hair 4. Cork cells
5. Cortex fibre 6. Phloem fibre 7. Xylem fibre
8. Vessels (8-1 spiral vessel, 8-2 reticulate vessels)

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

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

Standard solution

Loganin standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, methanol and water (10:1.5:0.5, 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 15-mL centrifuge tube, then add 10 mL of methanol. Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Filter through a 0.45-µm nylon filter.

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 loganin standard solution (1 µL) and the test solution (2 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 6 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 3-5 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

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 loganin.

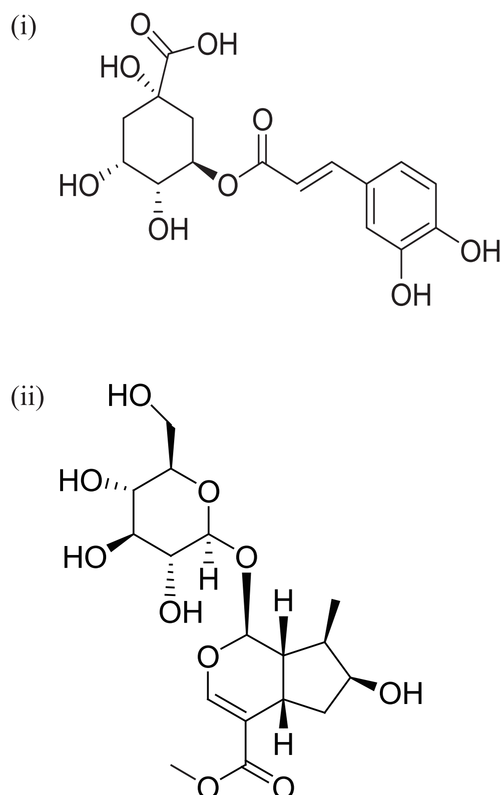

Figure 4 Chemical structures of (i) chlorogenic acid and (ii) loganin

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

Standard solutions

Chlorogenic acid standard solution for fingerprinting, Std-FP (60 mg/L)

Weigh 1.5 mg of chlorogenic acid CRS (Fig. 4) and dissolve in 25 mL of methanol (50%).

Loganin standard solution for fingerprinting, Std-FP (40 mg/L)

Weigh 1.0 mg of loganin CRS and dissolve in 25 mL of methanol (50%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 45 mL of methanol (50%). Reflux the mixture for 40 min. Cool down to room temperature. Centrifuge at about 3800 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask and make up to the mark with methanol (50%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (236 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)	0.4% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 6	88	12	isocratic
6 – 7	88 → 87	12 → 13	linear gradient
7 – 9	87 → 86	13 → 14	linear gradient
9 – 35	86	14	isocratic

System suitability requirements

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

The R value between peak 4 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 chlorogenic acid Std-FP, loganin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of chlorogenic acid and loganin peaks in the chromatograms of chlorogenic acid Std-FP, loganin Std-FP and the retention times of the six characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify chlorogenic acid and loganin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of chlorogenic acid Std-FP and loganin Std-FP. The retention times of chlorogenic acid and loganin 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 six characteristic peaks of Lonicerae Japonicae Caulis extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Lonicerae Japonicae Caulis extract

Peak No.	RRT	Acceptable Range
1	0.37	± 0.03
2	0.46	± 0.03
3	0.71	± 0.03
4 (chlorogenic acid)	0.89	± 0.03
5 (marker, loganin)	1.00	-
6	1.07	± 0.03

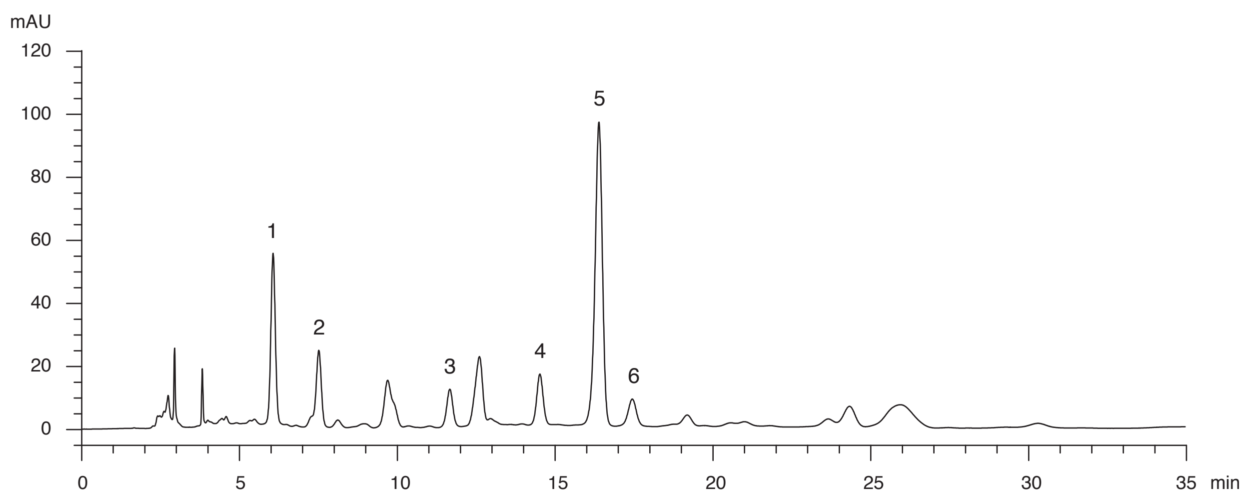

Figure 5 A reference fingerprint chromatogram of Lonicerae Japonicae Caulis extract

For positive identification, the sample must give the above six 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 4.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 6.0%.
Ethanol-soluble extractives (hot extraction method): not less than 9.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 1.0 mg of loganin CRS and dissolve in 10 mL of methanol (50%).

Loganin standard solution for assay, Std-AS

Measure accurately the volume of the loganin Std-Stock, dilute with methanol (50%) to produce a series of solutions of 10, 20, 40, 80, 120 mg/L for loganin.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 45 mL of methanol (50%). Reflux the mixture for 40 min. Cool down to room temperature. Centrifuge at about 3800 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask and make up to the mark with methanol (50%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (236 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 3) –

Table 3 Chromatographic system conditions

Time (min)	0.4% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 6	88	12	isocratic
6 – 7	88 → 87	12 → 13	linear gradient
7 – 9	87 → 86	13 → 14	linear gradient
9 – 35	86	14	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of loganin Std-AS (40 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of loganin should not be more than 5.0%; the RSD of the retention time of loganin peak should not be more than 2.0%; the column efficiency determined from loganin peak should not be less than 20000 theoretical plates.

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

Limits

The sample contains not less than 0.15% of loganin ($C_{17}H_{26}O_{10}$), calculated with reference to the dried substance.