Trachelospermi Caulis et Folium

Figure 1 A photograph of Trachelospermi Caulis et Folium

A. Trachelospermi Caulis et Folium B. Leaf
C. Transverse section of stem

1. NAMES

Official Name: Trachelospermi Caulis et Folium

Chinese Name: 絡石藤

Chinese Phonetic Name: Luoshiteng

2. SOURCE

Trachelospermi Caulis et Folium is the dried stem with leaf of *Trachelospermum jasminoides* (Lindl.) Lem. (Apocynaceae). The stem with leaf is collected in winter to next spring, foreign matter removed, then dried under the sun to obtain Trachelospermi Caulis et Folium.

3. DESCRIPTION

Stem cylindrical, branched, varying in length, 1-5 mm in diameter. Externally reddish-brown, with dotted lenticels and adventitious roots; texture hard; fracture pale yellow and frequently hollow. Leaves opposite, slightly curled and with short petiole; lamina elliptical to ovate-lanceolate, 2.1-6.9 cm long, 0.7-3.4 cm wide; upper surface dark green or brownish-green, glossy; lower surface greyish-green, margin entire; texture coriaceous. Odour slight; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Cork consists of several layers of brownish-red cells. Stone cells located inside the cork layers, arranged in an interrupted ring, prisms of calcium oxalate scattered between the cork and the stone cells band. Cortex narrow. Phloem narrow, with non-lignified fibre bundles on the outer side, arranged in an interrupted ring. Cambium in a ring. Xylem consists of lignified cells; the vessels subrounded, arranged radially. On the inner part of the xylem, internal cambium and internal phloem situated. Pith relatively small, containing large clefts, fibres and prisms of calcium oxalate [Fig. 2 (i)].

Leaf: The upper epidermis consists of 1 layer of cells, subsquare or rectangular. Palisade tissue consists of 1 layer of palisade cells, arranged in compact order, going through the upper part of the midrib. Spongy cells subrounded, arranged loosely, clusters of calcium oxalate occasionally found. Vascular bundles collateral, xylem vessels radially arranged, with non-lignified fibres lining up the outer side of the phloem. Lower epidermis consists of 1 layer of cells, the cells subrounded to subrectangular. Non-lignified fibres and prisms of calcium oxalate present in the inner side of the epidermis. Non-glandular hairs occasionally visible [Fig. 2 (ii)].

Powder

Colour greyish-yellow to brown. Epidermal cells with curved anticlinal wall, stomata anomocytic, subsidiary cells 4-8. Non-glandular hairs unicellular or multicellular, scattered, wall thick, with warty protuberance. Stone cells scattered singly or in groups, subrectangular, oblong ovate or triangular, 14-98 μm in diameter, wall thick, striations distinct. Prisms of calcium oxalate relatively numerous, scattered or occasionally present in stone cells, 6-51 μm in diameter. Clusters of calcium oxalate occasionally visible, subrounded, 13-52 μm in diameter, with short and obtuse angles; polychromatic under the polarized microscope. Vessels mainly bordered-pitted, 7-134 μm in diameter. Fibres scattered singly or in bundles, relatively long, 6-56 μm in diameter, wall relatively thick, with distinct pit canals, polychromatic under the polarized microscope. Cork cells yellowish-brown, subpolygonal, rectangular or triangular (Fig. 3).

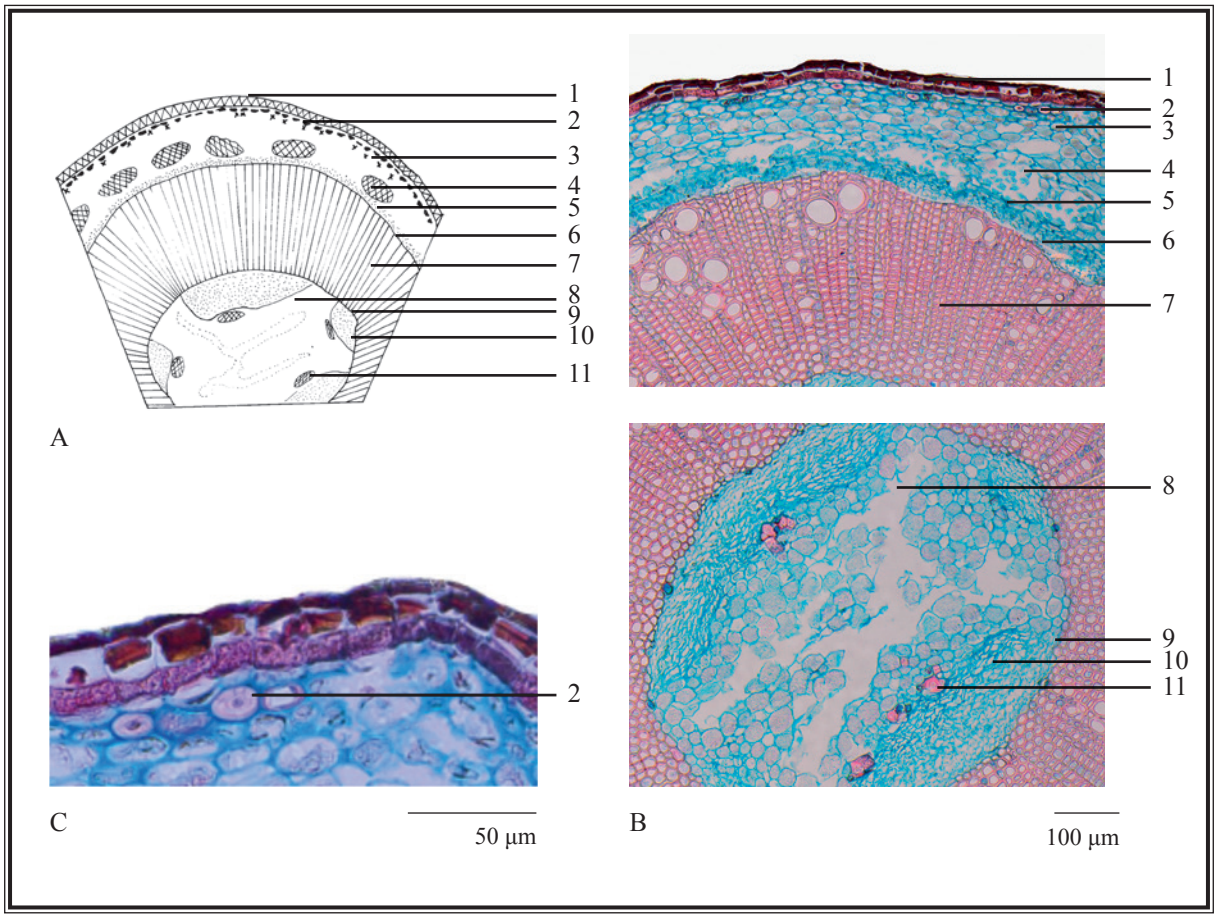

Figure 2 (i) Microscopic features of transverse section of stem of Trachelospermi Caulis et Folium

A. Sketch B. Section illustration C. Stone cells

1. Cork 2. Stone cells 3. Cortex 4. Phloem fibres 5. Phloem 6. Cambium 7. Xylem
8. Pith 9. Internal cambium 10. Internal phloem 11. Fibres of pith

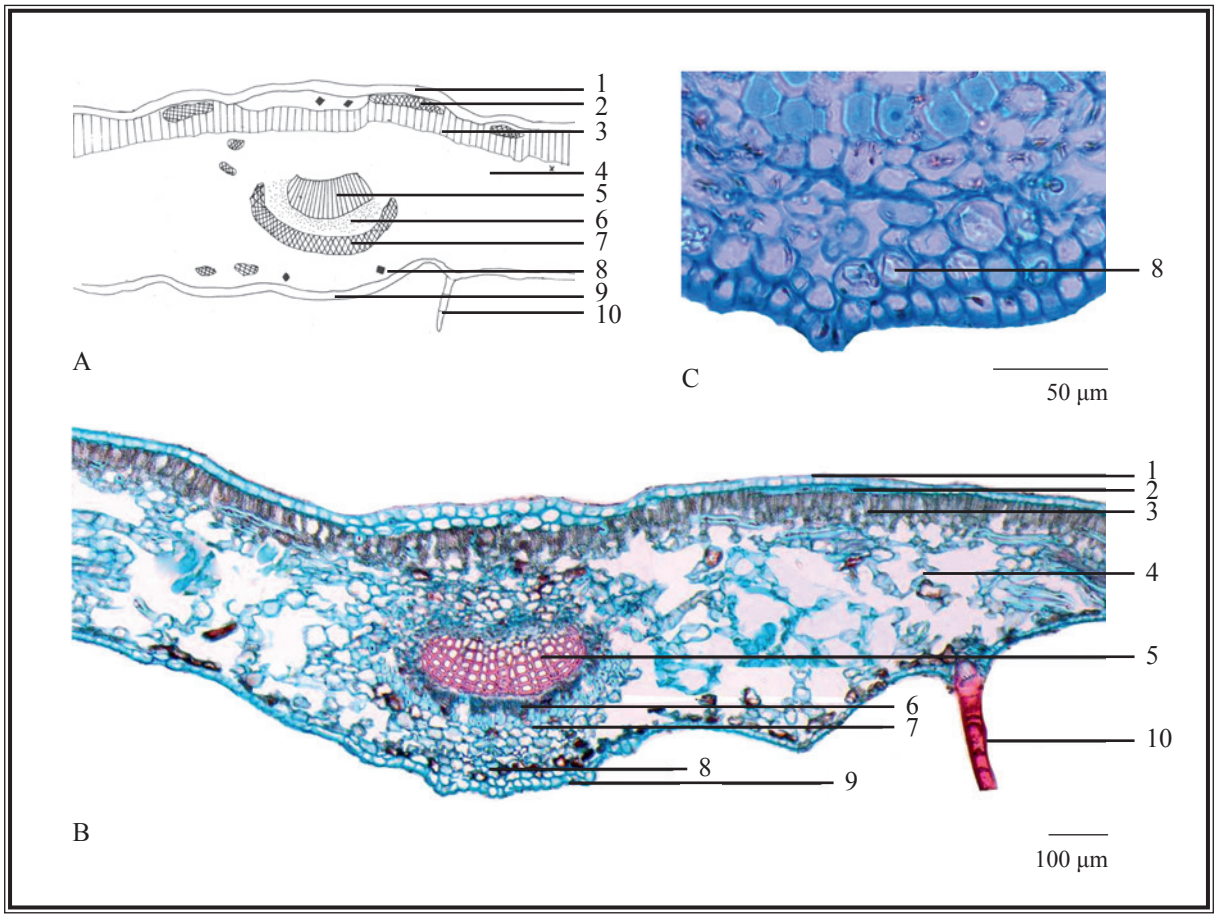

Figure 2 (ii) Microscopic features of transverse section of leaf of Trachelospermi Caulis et Folium

A. Sketch B. Section illustration C. Prisms of calcium oxalate

1. Upper epidermis 2. Fibres 3. Palisade tissue 4. Sponge tissue 5. Xylem 6. Phloem
7. Fibres 8. Prisms of calcium oxalate 9. Lower epidermis 10.Non-glandular hair

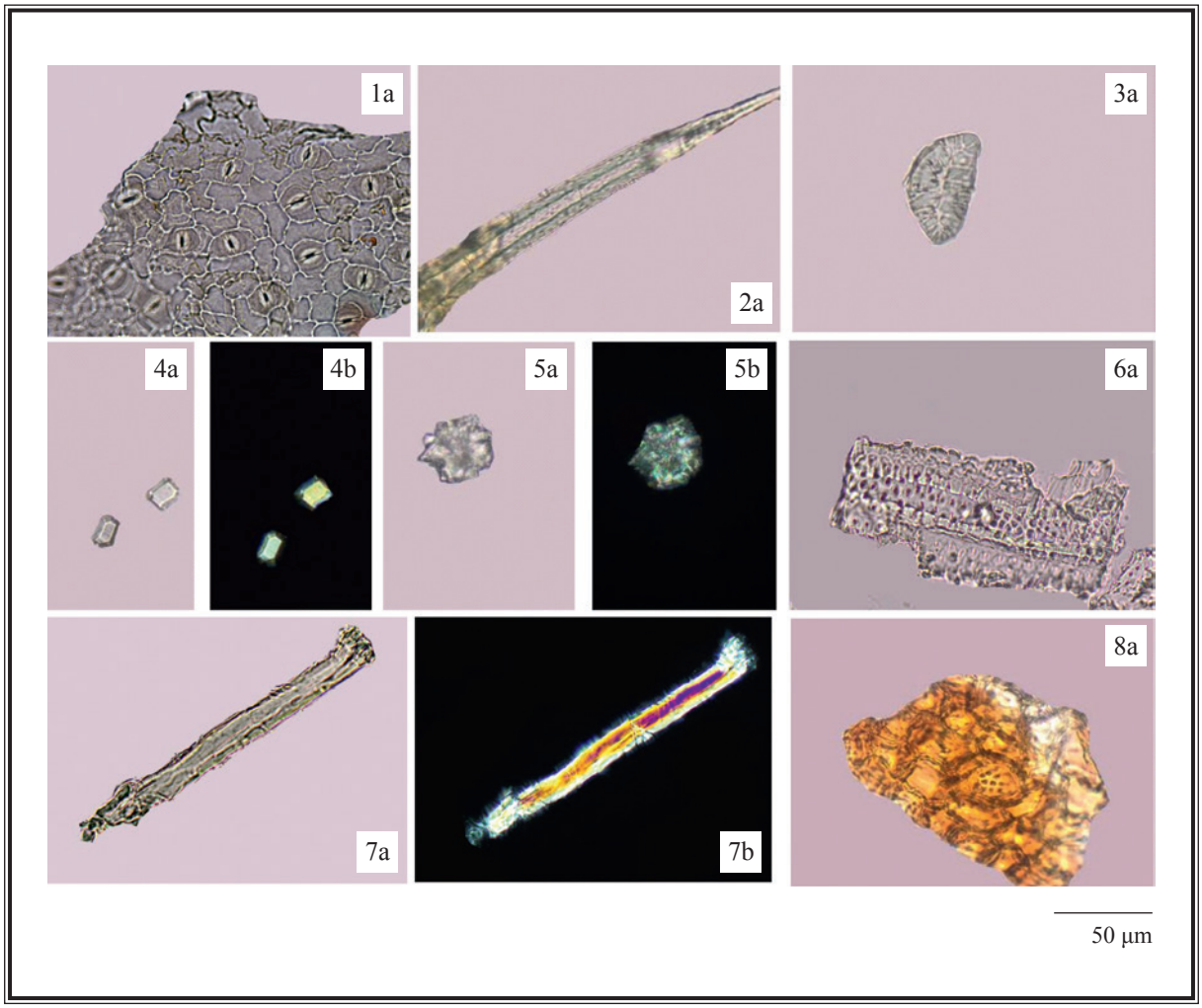

Figure 3 Microscopic features of powder of Trachelospermi Caulis et Folium

1. Epidermal cells of leaf 2. Non-glandular hair 3. Stone cell
4. Prisms of calcium oxalate 5. Cluster of calcium oxalate 6. Bordered-pitted vessel
7. Fibres 8. Cork cells

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

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

Standard solution

Tracheloside standard solution

Weigh 0.2 mg of tracheloside CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, water, formic acid and glacial acetic acid (8:1.5:0.8:0.8, 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 100-mL round-bottomed flask, then add 10 mL of methanol. Reflux the mixture for 15 min. Cool down to room temperature. 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 tracheloside standard solution (15 µL) and the test solution (6 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 8.5 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 105˚C until the spots or bands become visible (about 4 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 tracheloside.

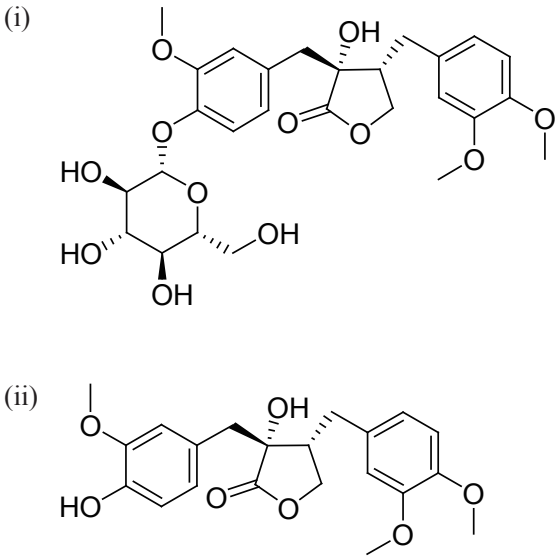

Figure 4 Chemical structures of (i) tracheloside and (ii) trachelogenin

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

Standard solution

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

Weigh 1.0 mg of tracheloside 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 25 mL of ethanol (70%). Sonicate (200 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (228 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)	Water (%, v/v)	Methanol (%, v/v)	Elution
0 – 10	75 → 65	25 → 35	linear gradient
10 – 20	65 → 60	35 → 40	linear gradient
20 – 40	60 → 40	40 → 60	linear gradient
40 – 50	40	60	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of tracheloside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of tracheloside should not be more than 5.0%; the RSD of the retention time of tracheloside peak should not be more than 2.0%; the column efficiency determined from tracheloside peak should not be less than 40000 theoretical plates.

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

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

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Trachelospermi Caulis et Folium extract

Peak No.	RRT	Acceptable Range
1	0.70	± 0.03
2 (marker, tracheloside)	1.00	-
3 (trachelogenin)	1.41	± 0.03

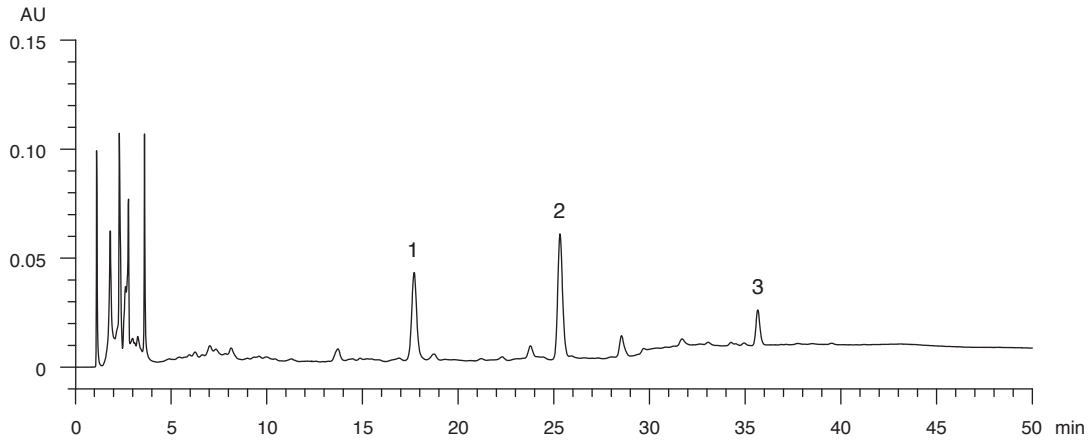

Figure 5 A reference fingerprint chromatogram of Trachelospermi Caulis et Folium extract

For positive identification, the sample must give the above three 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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 8.0 %.
Acid-insoluble ash: not more than 3.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 8.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 11.0%.
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

Mixed tracheloside and trachelogenin standard stock solution, Std-Stock (320 mg/L for tracheloside and 80 mg/L for trachelogenin)

Weigh accurately 1.6 mg of tracheloside CRS and 0.4 mg of trachelogenin CRS (Fig. 4) and dissolve in 5 mL of ethanol (70%).

Mixed tracheloside and trachelogenin standard solution for assay, Std-AS

Measure accurately the volume of the mixed tracheloside and trachelogenin Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 8, 16, 32, 48, 80 mg/L for tracheloside and 1, 2, 4, 8, 12 mg/L for trachelogenin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of ethanol (70%). Sonicate (200 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time with 20 mL of ethanol (70%). Combine the supernatants and make up to the mark with ethanol (70%). Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 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)	Water (%, v/v)	Methanol (%, v/v)	Elution
0 – 10	75 → 65	25 → 35	linear gradient
10 – 20	65 → 60	35 → 40	linear gradient
20 – 40	60 → 40	40 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed tracheloside and trachelogenin Std-AS (32 mg/L for tracheloside and 4 mg/L for trachelogenin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of tracheloside and trachelogenin should not be more than 5.0%; the RSD of the retention times of tracheloside and trachelogenin peaks should not be more than 2.0%; the column efficiencies determined from tracheloside and trachelogenin peaks should not be less than 35000 and 100000 theoretical plates respectively.

The *R* value between tracheloside peak and the closest peak; and the *R* value between trachelogenin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed tracheloside and trachelogenin Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of tracheloside and trachelogenin against the corresponding concentrations of the mixed tracheloside and trachelogenin Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify tracheloside and trachelogenin peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed tracheloside and trachelogenin Std-AS. The retention times of tracheloside and trachelogenin peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of tracheloside and trachelogenin in the test solution, and calculate the percentage contents of tracheloside and trachelogenin in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.32% of the total content of tracheloside ($C_{27}H_{34}O_{12}$) and trachelogenin ($C_{21}H_{24}O_7$), calculated with reference to the dried substance.