Herba Taxilli

Figure 1　A photograph of Herba Taxilli

1. NAMES

Official Name: Herba Taxilli

Chinese Name: 桑寄生

Chinese Phonetic Name: Sangjisheng

2. SOURCE

Herba Taxilli is the dried leaf-bearing twig of *Taxillus chinensis* (DC.) Danser (Loranthaceae). The herb is harvested in winter through the following spring. The leaf-bearing twigs are removed from the thick stems, cut into sections and dried, or steamed and dried to obtain Herba Taxilli. This plant mainly parasitizes on the host plant of *Morus alba* L.

3. DESCRIPTION

Leaf-bearing twig cylindrical, 3-4 cm long, 2-15 mm in diameter, branched. Externally reddish-brown or greyish-brown, marked with fine longitudinal striae and numerous small, protruding brown lenticels; some young stems covered with reddish-brown pubescence. Texture hard. Fracture irregular; bark thin, reddish-brown; wood pale in colour. Leaves mostly curled and with short petioles; when smoothed out, blade ovate to elliptical, 3-8 cm long, 2-5 cm wide; externally dull yellowish-brown or dull green; young leaves covered with fine pubescence. Leaf apex obtuse, base orbicular to broadly cuneate; entire; texture coriaceous. Odour slight; taste mild, and slightly astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Cork consists of 10 or more rows of cells, some containing brown contents. Cortex narrow, containing groups of stone cells in older stems, parenchyma cells with brown contents. Stone cells and fibre bundles are found in the pericycle, arranged in an interrupted ring. Phloem extremely narrow, rays scattered with stone cells. Fasicular cambium distinct. Xylem rays 1-4 rows of cells wide, stone cells also occurring near the pith; vessels scattered singly or in groups of 2-3. Pith contains groups of stone cells and parenchyma cells; the latter has brown contents. Some groups of stone cells contain prisms of calcium oxalate or brown contents (Fig. 2).

Leaf: Both the upper and lower epidermis contain stomata, cells small, subrectangular. Palisade tissue in the upper part consists of 3-4 rows of relatively long cells, that in the lower part consists of 2-3 rows of relatively short cells, arranged orderly and tightly. Spongy tissues located in the middle, containing many scattered round clusters of calcium oxalate, arranged loosely. The midrib bulge is prominent on both upper and lower leaf surfaces. Vascular bundles collateral, with fibre bundles above and below it. Many stone cells in groups are present in the parenchyma cells, containing prisms of calcium oxalate (Fig. 2).

Powder

Colour pale yellowish-brown. Stone cells relatively numerous, singly scattered or several in groups, pale yellow to nearly colourless, subsquare or suborbicular, cells wall are mostly thickened on three sides, with distinct striation, some also with distinct pit canals; bright orange colour is observed under the polarized microscope (characteristics in the stem and leaf). Prisms of calcium oxalate are relatively numerous, scattered or mostly contained in the stone cells, 4-33 μm in diameter; polychromatic observed under the polarized microscope (characteristics in the stem and leaf). Cluster of calcium oxalate subrounded; 5-37 μm in diameter, with short and obtuse angles; scattered or mostly inside pale brown parenchyma cells; polychromatic observed under the polarized microscope (characteristics in the leaf, occasionally observed in the stems). Xylem fibres are mostly in bundles, relatively long, about 6-32 μm in diameter, two ends tapering, walls relatively thin, pit canals scarce; bright yellowish-white colour is observed under the polarized microscope (characteristics in the stem). Pericyclic fibres are mostly in bundles, slender, with the top end sharp; 10-39 μm in diameter, with extremely thickened wall, lumen linear or of a ditch-shaped grain; polychromatic observed under the polarized microscope (characteristics in the stem). Bordered pitted, reticulate and spiral vessels are frequently visible (characteristics in the stem and leaf). Stellate hairs of pale yellow to yellowish-brown colour present; singly and scattered or overlapped, trifurcately or tetrafurcately branched, branches mostly curved, ends tapering, with relatively thickened wall; bright orange colour is observed under the polarized microscope (characteristics in the leaf and young stem) (Fig. 3).

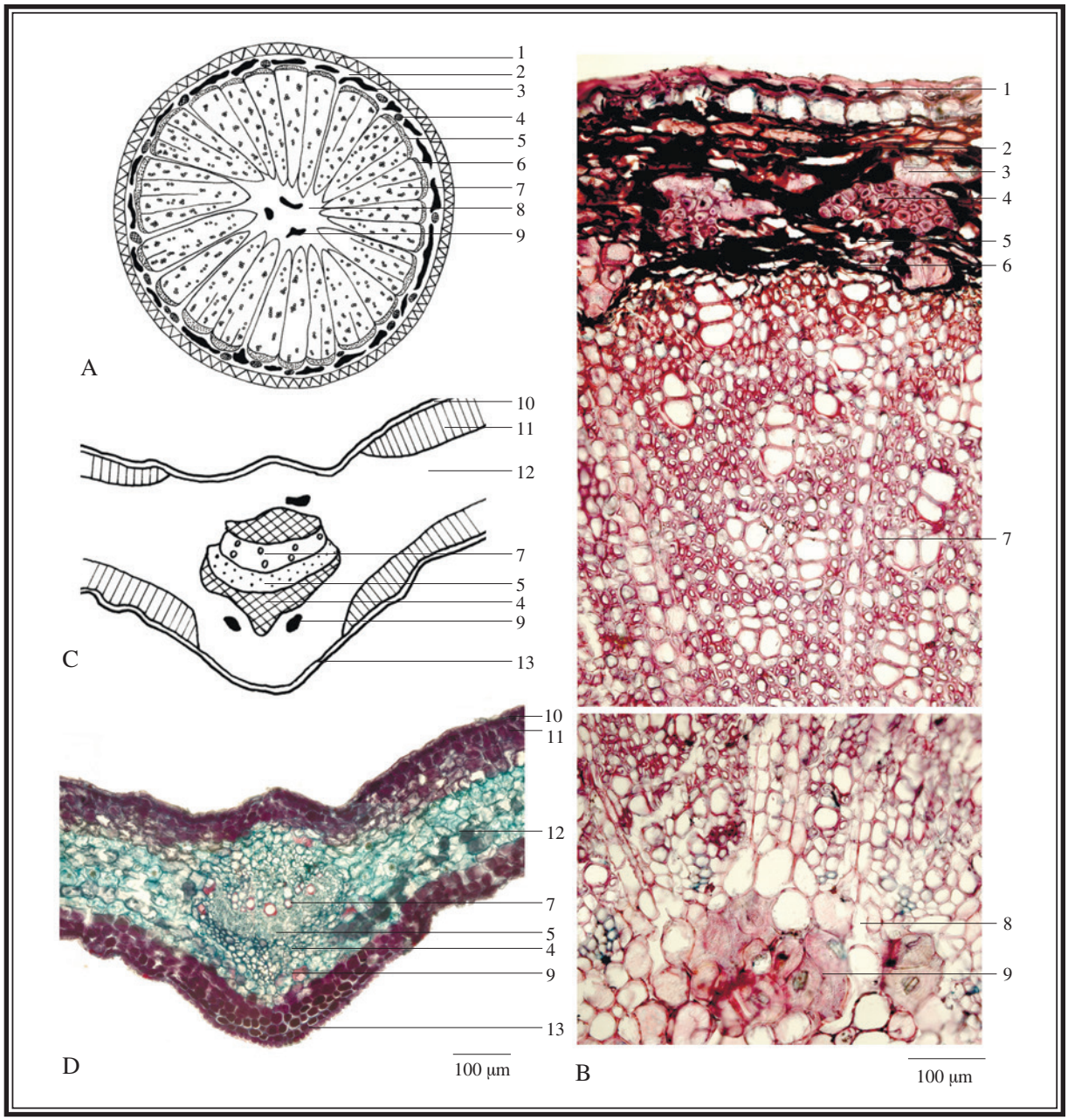

Figure 2 Microscopic features of transverse section of stem and leaf of Herba Taxilli

A. Sketch of stem B. Section illustration of stem

C. Sketch of leaf D. Section illustration of leaf

1. Cork 2. Cortex 3. Stone cells 4. Pericyclic fibre bundles 5. Phloem 6. Cambium

7. Xylem 8. Pith 9. Group of stone cells 10. Upper epidermal cells

11. Palisade tissue 12. Spongy tissue 13. Lower epidermal cells

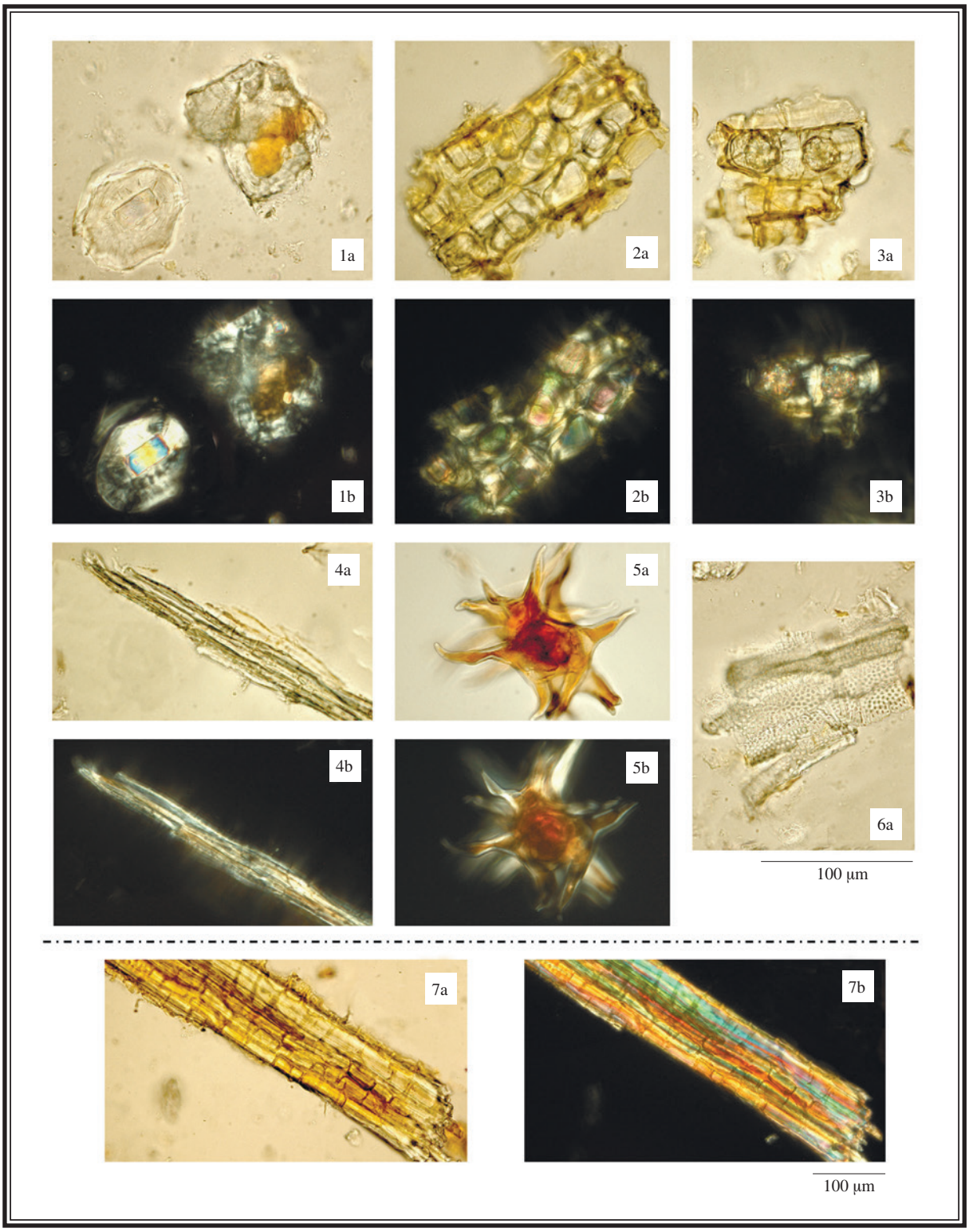

Figure 3 Microscopic features of powder of Herba Taxilli

1. Stone cells 2. Prisms of calcium oxalate 3. Cluster crystals of calcium oxalate
4. Xylem fibres 5. Stellate hairs 6. Vessels 7. Pericyclic fibres

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

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

Standard solution

Quercitrin standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, butan-2-one, formic acid and water (24:3.6:1.5:0.9, v/v).

Spray reagent

Dissolve 2.5 g of aluminum chloride in 100 mL of ethanol.

Test solution

Weigh 5.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of methanol (50%). Sonicate (140 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min and then filter.

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 quercitrin standard solution (0.5 μL) and the test solution (2 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 (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 quercitrin.

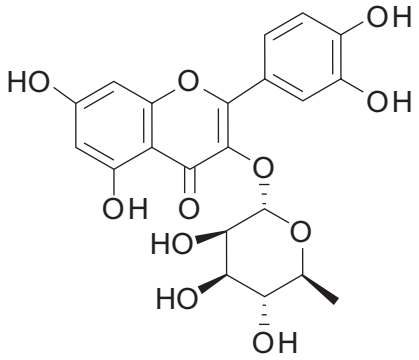

Figure 4 Chemical structure of quercitrin

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

Standard solution

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

Weigh 5.0 mg of quercitrin CRS and dissolve in 100 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the extracts 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 (256 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30°C 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)	0.001M Sodium dihydrogen phosphate (%, v/v)	Acetonitrile (%, v/v)	Elution
0–25	95→85	5→15	linear gradient
25–35	85→81	15→19	linear gradient
35–60	81	19	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of quercitrin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of quercitrin should not be more than 3.0%; the RSD of the retention time of quercitrin peak should not be more than 2.0%; the column efficiency determined from quercitrin peak should not be less than 50000 theoretical plates.

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

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

Peak No.	RRT	Acceptable Range
1	0.34	±0.03
2	0.35	±0.03
3	0.41	±0.03
4	0.77	±0.03
5 (marker, quercitrin)	1.00	-

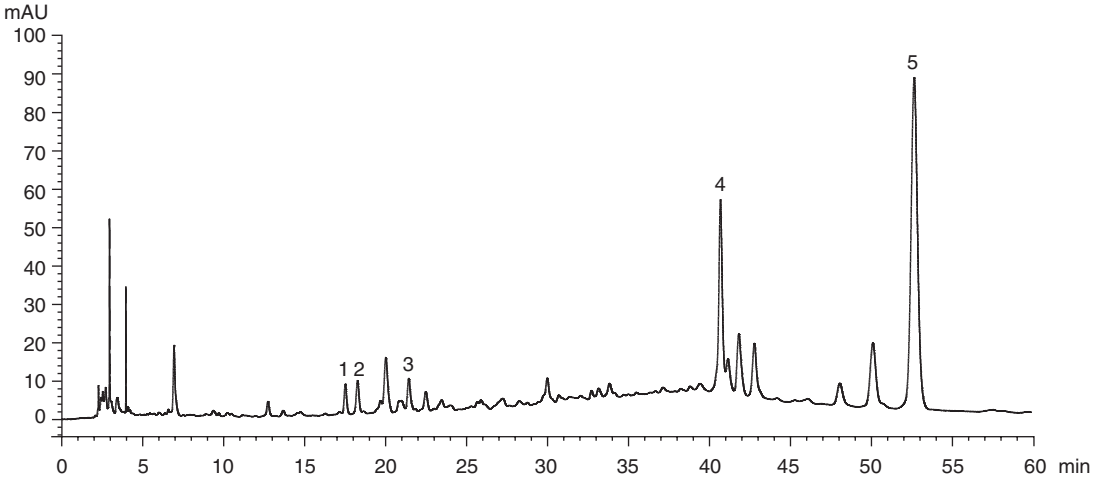

Figure 5 A reference fingerprint chromatogram of Herba Taxilli extract

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 XV)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 7.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*: not more than 13.0%.

5.8 Cardiac glycoside

Reagent
Alkaline 3,5-dinitrobenzoic acid solution
Dissolve 1.0 g of dinitrobenzoic acid in 100 mL of ethanol.
Dissolve 4.3 g of sodium hydroxide in 100 mL of water.
Mix 1 mL of each of the above solution.

Procedure
Weigh 10.0 g of powdered sample and place it in a 100-mL round-bottomed flask, then add 50 mL of ethanol (80%). Reflux the mixture for 30 min. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 10 mL of hot water and then filter. Transfer the aqueous solution to a separatory funnel. Extract for four times each with 15 mL of diethyl ether and discard the diethyl ether layer. Add appropriate amount of saturated lead acetate solution to the aqueous solution until the precipitation is completed and then filter. Transfer the filtrate to a 50-mL conical flask, add 10 mL of ethanol and appropriate amount of saturated sodium sulphate solution to remove the lead ion and then filter. Extract for three times each with 15 mL of dichloromethane. Combine the dichloromethane extracts. Evaporate the extracts to 1 mL at reduced pressure in a rotary evaporator. Spot the concentrated solution onto a filter paper. Dry in air. Apply the alkaline 3,5-dinitrobenzoic acid solution to the dried spot. No purplish-red colour should be observed.

Note: Herba Taxilli contains toxic cardiac glycoside when parasitizes on the oleander trees.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 10.0%.
Ethanol-soluble extractives (cold extraction method): not less than 14.0%.

7. ASSAY

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

Standard solution

Quercitrin standard stock solution, Std-Stock (500 mg/L)

Weigh accurately 5.0 mg of quercitrin CRS and dissolve in 10 mL of methanol.

Quercitrin standard solution for assay, Std-AS

Measure accurately the volume of the quercitrin Std-Stock, dilute with methanol to produce a series of solutions of 0.5, 5, 10, 15, 20 mg/L for quercitrin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of methanol (75%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with 3 mL of methanol (75%). Centrifuge at about 1800 × *g* for 5 min. Combine the extracts and make up to the mark with methanol (75%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (256 nm) and a column (4.6 × 150 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30°C during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.001M Sodium dihydrogen phosphate (%, v/v)	Acetonitrile (%, v/v)	Elution
0–40	85→81	15→19	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μL of quercitrin Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of quercitrin should not be more than 3.0%; the RSD of the retention time of quercitrin peak should not be more than 2.0%; the column efficiency determined from quercitrin peak should not be less than 7000 theoretical plates.

The *R* value between quercitrin 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 quercitrin Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of quercitrin against the corresponding concentrations of quercitrin 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 quercitrin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of quercitrin Std-AS. The retention times of quercitrin 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 quercitrin in the test solution, and calculate the percentage content of quercitrin in the sample by using the equations indicated in Appendix IV (B).

Limits

The sample contains not less than 0.015% of quercitrin ($C_{21}H_{20}O_{11}$), calculated with reference to the dried substance.