Platycladi Cacumen

Figure 1 A photograph of Platycladi Cacumen

A. Platycladi Cacumen B. Magnified Platycladi Cacumen

1. NAMES

Official Name: Platycladi Cacumen

Chinese Name: 側柏葉

Chinese Phonetic Name: Cebaiye

2. SOURCE

Platycladi Cacumen is the dried twig and leaf of *Platycladus orientalis* (L.) Franco (Cupressaceae). The twig and leaf are collected in summer and autumn, then dried in shaded area to obtain Platycladi Cacumen.

3. DESCRIPTION

Frequently branched, twigs flattened. Leaves small, scaly, decussate, densely and closely attached to twigs, yellowish-green to dark green. Texture fragile, easily broken. Odour delicately aromatic; taste bitter, astringent and slightly pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Oblong, subrounded twigs located at the centre with 2 subtriangular leaves. Epidermis consists of 1 layer of square to rectangular epidermal cells, covered with cuticle, containing numerous microcrystals of calcium oxalate. Hypodermal fibre 1-2 layers, located underneath the epidermis, arranged discontinuously, with thickened wall, slightly lignified to lignified. Cortex consists of subrounded parenchymatous cells, arranged loosely. Large resin canals located in cortex. Stomata sunken. Leaf vascular bundles collateral, sometimes accompanied by wing-like transfusion tissue. Twig vascular tissue large, collateral; phloem relatively narrow, xylem relatively broad, medullary rays and pith present. Parenchymatous cells contain microcrystals of calcium oxalate (Fig. 2).

Powder

Colour yellowish-green. Upper epidermal cells of leaf narrowly rectangular, wall slightly thickened, containing numerous microcrystals of calcium oxalate. Lower epidermal cells subsquare, containing numerous sunken stomata, anomocytic or actinocytic type, guard cells dumbbell-shaped, 31-50 µm long and 19-36 µm wide. Tracheids bordered-pitted, sometimes present. Fibres slender, 9-24 µm in diameter (Fig. 3).

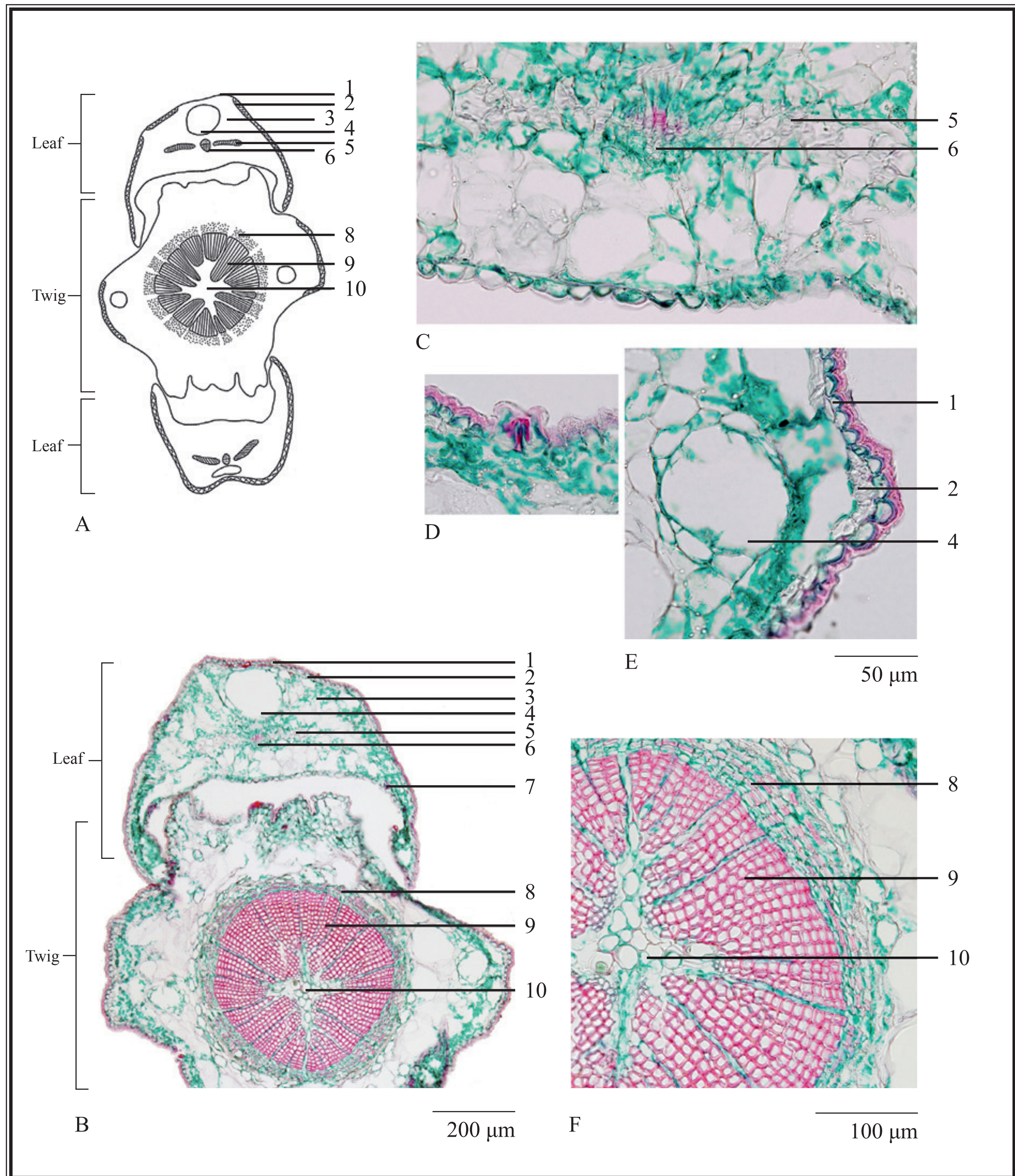

Figure 2 Microscopic features of transverse section of Platycladi Cacumen

A. Sketch B. Section illustration C. Leave vascular bundle D. Stoma
E. Epidermis F. Twig vascular bundle

1. Epidermis 2. Hypodermal fibre 3. Cortex 4. Resin canal
5. Transfusion tissue 6. Leave vascular bundle
7. Stoma 8. Phloem 9. Xylem 10. Pith

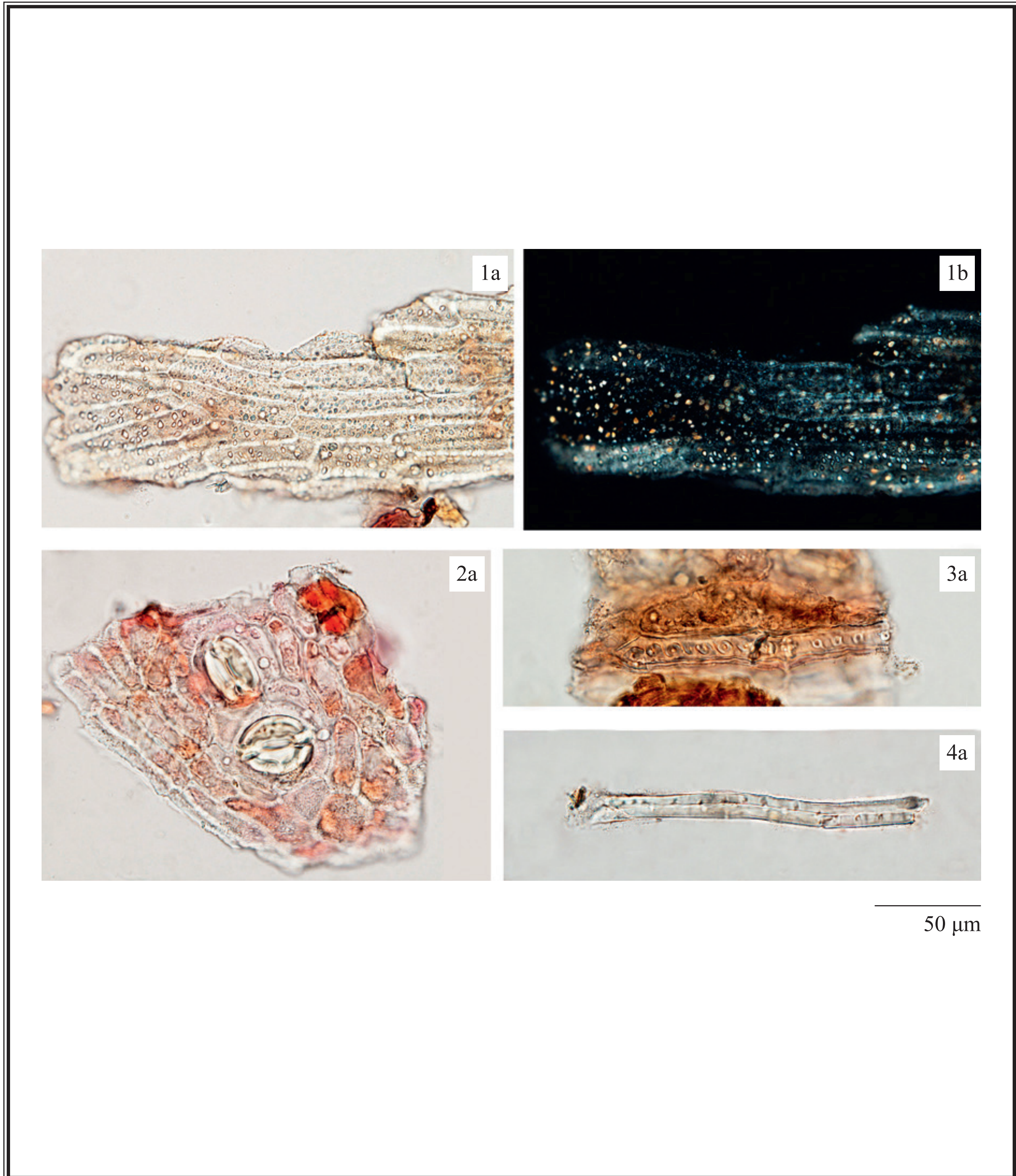

Figure 3 Microscopic features of powder of Platycladi Cacumen

1. Upper epidermal cells with microcrystals of calcium oxalate 2. Lower epidermal cells with sunken stomata 3. Bordered-pitted tracheid 4. Fibre

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 5 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, acetone, formic acid and water (20:2:1:1, v/v).

Spray reagent

Weigh 1 g of aluminium trichloride and dissolve in 100 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 10-mL volumetric flask. Make up to the mark with methanol.

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 and the test solution (6 μL each) 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 it in air until the spots or bands become visible. 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.

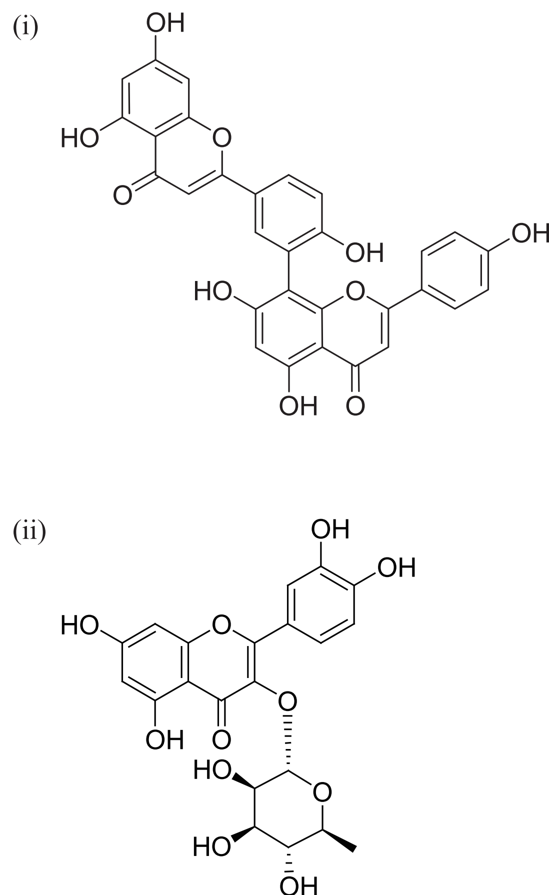

Figure 4 Chemical structures of (i) amentoflavone and (ii) quercitrin

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

Standard solutions

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

Weigh 0.5 mg of amentoflavone CRS (Fig. 4) and dissolve in 10 mL of methanol.

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

Weigh 0.5 mg of quercitrin 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 10 mL of methanol. Sonicate (220 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Filter and transfer the filtrate to a 10-mL volumetric flask. Make up to the mark with methanol. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (350 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)	0.1% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 50	60 → 10	40 → 90	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of amentoflavone Std-FP and quercitrin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of amentoflavone and quercitrin should not be more than 5.0%; the RSD of the retention times of amentoflavone and quercitrin peaks should not be more than 2.0%; the column efficiencies determined from amentoflavone and quercitrin peaks should not be less than 80000 and 13000 theoretical plates respectively.

The *R* value between peak 2 and the closest peak; and the *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

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

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Platycladi Cacumen extract

Peak No.	RRT	Acceptable Range
1	0.36	± 0.03
2 (quercitrin)	0.48	± 0.03
3	0.59	± 0.03
4 (marker, amentoflavone)	1.00	-
5	1.17	± 0.03

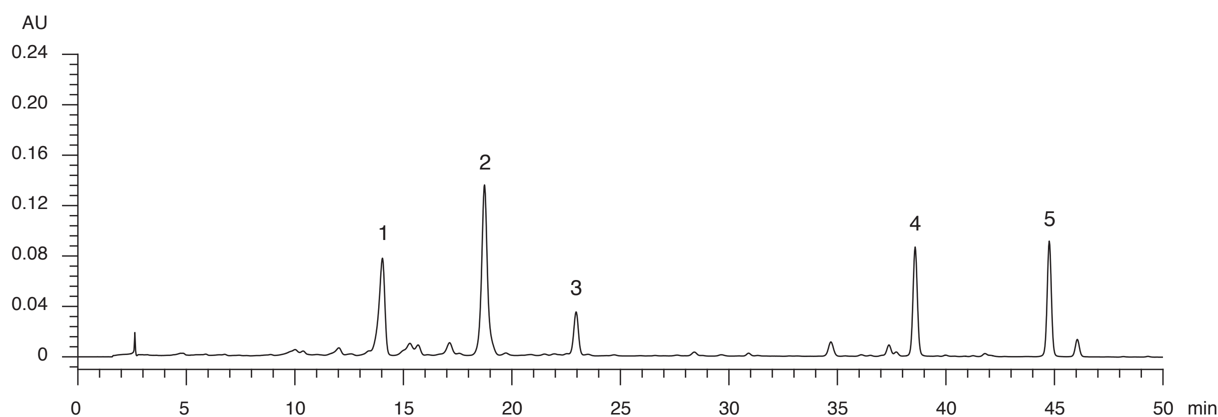

Figure 5 A reference fingerprint chromatogram of Platycladi Cacumen extract

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 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 10.0%.
Acid-insoluble ash: not more than 2.0%.

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 16.0%.
Ethanol-soluble extractives (hot extraction method): not less than 21.0%.

7. ASSAY

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

Standard solution

Mixed amentoflavone and quercitrin standard stock solution, Std-Stock (200 mg/L for amentoflavone and 400 mg/L for quercitrin)

Weigh accurately 2.0 mg of amentoflavone CRS and 4.0 mg of quercitrin CRS, and dissolve in 10 mL of methanol.

Mixed amentoflavone and quercitrin standard solution for assay, Std-AS

Measure accurately the volume of the mixed amentoflavone and quercitrin Std-Stock, dilute with methanol to produce a series of solutions of 1, 2.5, 5, 8, 10 mg/L for amentoflavone and 5, 10, 16, 20, 40 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 25 mL of methanol. Sonicate (220 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Repeat the extraction for three more times. Combine the filtrates and make up to the mark with methanol. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (350 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)	0.1% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 50	60 → 10	40 → 90	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed amentoflavone and quercitrin Std-AS (5 mg/L for amentoflavone and 16 mg/L for quercitrin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of amentoflavone and quercitrin should not be more than 5.0%; the RSD of the retention times of amentoflavone and quercitrin peaks should not be more than 2.0%; the column efficiencies determined from amentoflavone and quercitrin peaks should not be less than 100000 and 20000 theoretical plates respectively.

The *R* value between amentoflavone peak and the closest peak; and 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 curves

Inject a series of the mixed amentoflavone and quercitrin Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of amentoflavone and quercitrin against the corresponding concentrations of the mixed amentoflavone and quercitrin 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 amentoflavone and quercitrin peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed amentoflavone and quercitrin Std-AS. The retention times of amentoflavone and quercitrin peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of amentoflavone and quercitrin in the test solution, and calculate the percentage contents of amentoflavone and quercitrin in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.079% of amentoflavone ($C_{30}H_{18}O_{10}$) and not less than 0.33% of quercitrin ($C_{21}H_{20}O_{11}$), calculated with reference to the dried substance.