Rosae Laevigatae Fructus

Figure 1 A photograph of Rosae Laevigatae Fructus

A. Rosae Laevigatae Fructus
B. Magnified longitudinal section of receptacle and achenes
C. Magnified inner surface of receptacle with tomentum

1. NAMES

Official Name: Rosae Laevigatae Fructus

Chinese Name: 金櫻子

Chinese Phonetic Name: Jinyingzi

2. SOURCE

Rosae Laevigatae Fructus is the dried ripe fruit of *Rosa laevigata* Michx. (Rosaceae). The fruit is collected in October and November when it turns red, dried under the sun, then bristles removed to obtain Rosae Laevigatae Fructus.

3. DESCRIPTION

Pseudocarp developed from the receptacle, obovoid, 1.7-4.4 cm long, 8-25 mm in diameter. Externally reddish-yellow to reddish-brown, with protuberant brown small scars of the fallen bristles. A dish-like remnants of calyx at the apex, with a yellow style base in the middle and the lower part tapered. Texture hard. When cutting in longitudinal section, the wall of the receptacle 2-3 mm thick, with numerous small and hard achenes inside; the inner surface of the receptacle and the achenes covered with pale yellow tomentum. Odour slight; taste sweet and slightly astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Receptacle wall: Outer epidermal cells subsquare or slightly radially elongated, with thickened and cutinized outer and lateral walls. Longitudinal section of epidermal bristle shows radially elongated cells. Parenchymatous cells with slightly thickened and distinctly pitted walls, containing oil droplets and yellowish-orange contents, some containing prisms and clusters of calcium oxalate. Fibre bundles scattered in the outer part of cortex; collateral vascular bundles mostly situated in the central part of cortex, with fibre bundles locate outside the phloem, vessels scattered or radially arranged. Inner epidermal cells rectangular, with thickened and cutinized inner walls. Non-glandular hairs or their remains lignified (Fig. 2).

Powder

Colour pale flesh. Non-glandular hairs unicellular or multicellular, 489-1208 μm long, 15-39 μm in diameter, walls lignified or slightly lignified, with slightly curved and oblique striations on the surface, lumens contain yellowish-brown contents. Epidermal cells polygonal, walls thick, containing yellowish-brown contents. Parenchymatous cells polygonal, lignified, with pits and contain yellowish-brown contents. Hypodermal cells subsquare or polygonal, walls relatively thick. Vessels spiral, reticulate, annular and bordered-pitted, 6-24 μm in diameter. Fibres colourless to yellow, fusiform or strip-shaped, 13-23 μm in diameter, walls lignified; polychromatic under the polarized microscope. Masses of resin translucent and yellowish-brown, irregular in shape. Prisms of calcium oxalate abundant, rectangular or irregular, 14-39 μm in diameter; polychromatic under the polarized microscope. Clusters of calcium oxalate sometimes visible, 24-53 μm in diameter; polychromatic under the polarized microscope (Fig. 3).

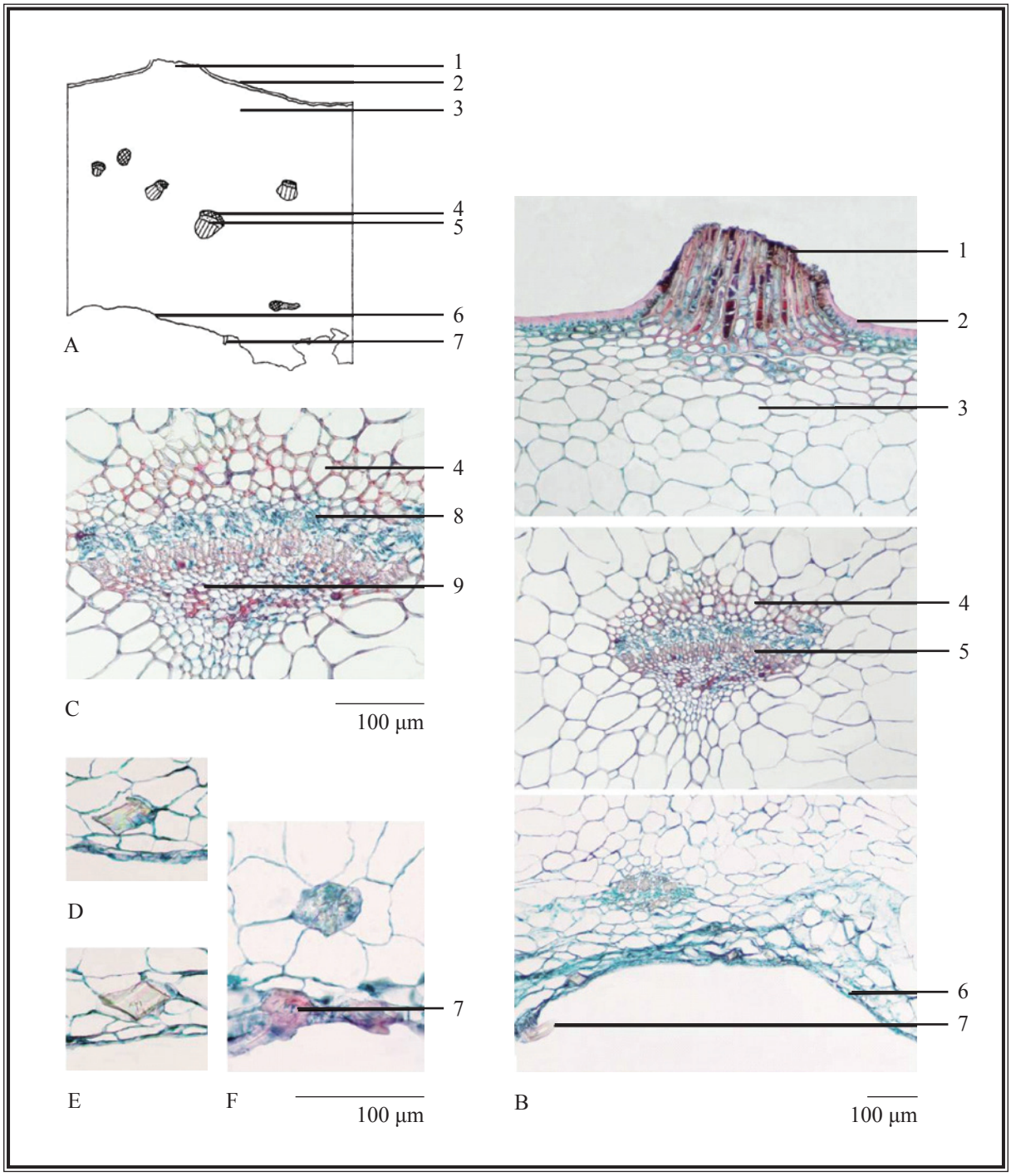

Figure 2 Microscopic features of transverse section of receptacle wall of Rosae Laevigatae Fructus

A. Sketch B. Section illustration C. Vascular bundle
D, E. Prisms of calcium oxalate F. Clusters of calcium oxalate and non-glandular hair

1. Scars of the fallen bristle 2. Outer epidermis 3. Cortex 4. Fibre bundles
5. Vascular bundle 6. Inner epidermis 7. Non-glandular hair 8. Phloem 9. Xylem

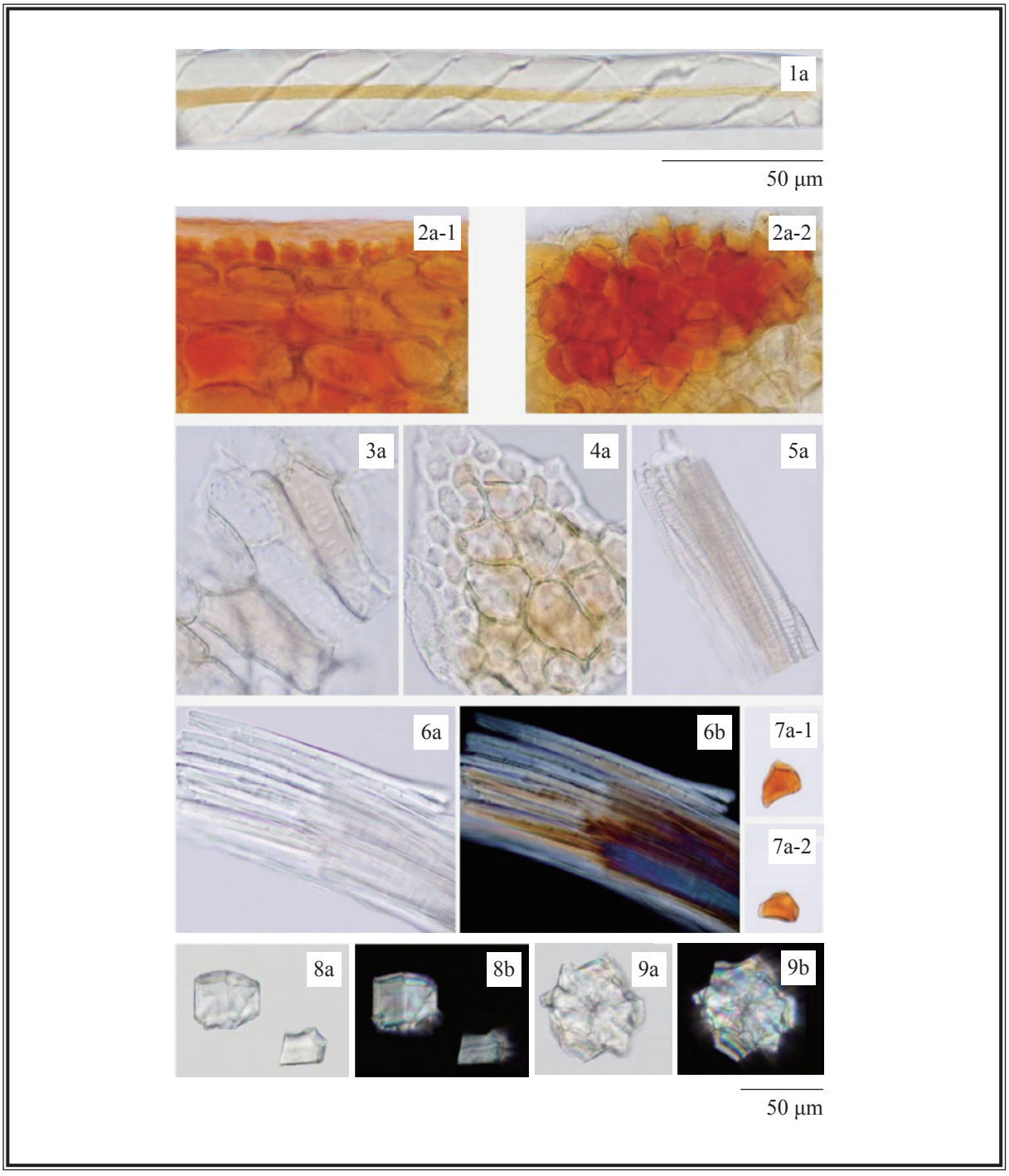

Figure 3 Microscopic features of powder of Rosae Laevigatae Fructus

1. Non-glandular hair 2. Epidermal cells (2-1 in lateral view, 2-2 in surface view)
3. Parenchymatous cells 4. Hypodermal cells 5. Vessels 6. Fibres
7. Resin masses 8. Prisms of calcium oxalate 9. Cluster of calcium oxalate

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

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

Standard solution

(+)-Catechin standard solution

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

Developing solvent system

Prepare a mixture of dichloromethane, ethyl acetate, methanol and formic acid (5:5:1:0.1, v/v).

Spray reagent

Add slowly 80 mL of sulphuric acid to 20 mL of ethanol and dissolve 0.5 g of vanillin.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 100-mL conical flask, then add 30 mL of ethanol (95%). Sonicate (400 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 20 mL of water. Extract for two times each with 30 mL of ethyl acetate. Combine the ethyl acetate extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of methanol and then 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 (+)-catechin standard solution (1 μL) and the test solution (10 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 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 1 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

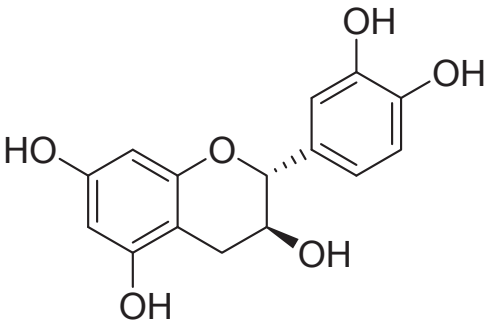

Figure 4 Chemical structure of (+)-catechin

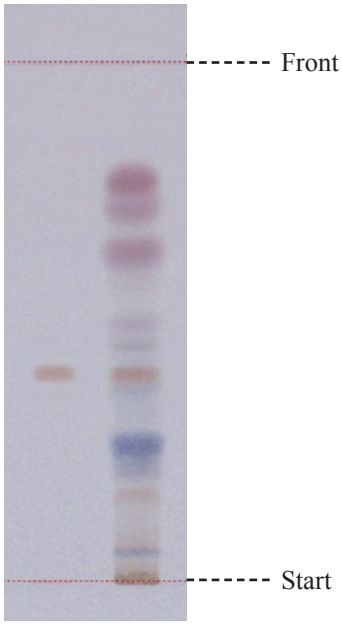

Figure 5 A reference HPTLC chromatogram of Rosae Laevigatae Fructus extract observed under visible light after staining

1. (+)-Catechin standard solution 2. Test solution

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 (+)-catechin (Fig. 5).

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

Standard solution

(+)-Catechin standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 1.0 mg of (+)-catechin CRS and dissolve in 50 mL of methanol (50%).

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 (50%). Sonicate (200 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Combine the solutions 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 (202 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 0.9 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.1% Phosphoric acid (%, v/v)	Elution
0 – 48	4 → 8	96 → 92	linear gradient
48 – 75	8 → 11	92 → 89	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of (+)-catechin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of (+)-catechin should not be more than 5.0%; the RSD of the retention time of (+)-catechin peak should not be more than 2.0%; the column efficiency determined from (+)-catechin peak should not be less than 30000 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. 6).

Procedure

Separately inject (+)-catechin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of (+)-catechin peak in the chromatogram of (+)-catechin Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify (+)-catechin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of (+)-catechin Std-FP. The retention times of (+)-catechin 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 Rosae Laevigatae Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Rosae Laevigatae Fructus extract

Peak No.	RRT	Acceptable Range
1 [marker, (+)-catechin]	1.00	-
2	1.17	± 0.03
3	1.27	± 0.03

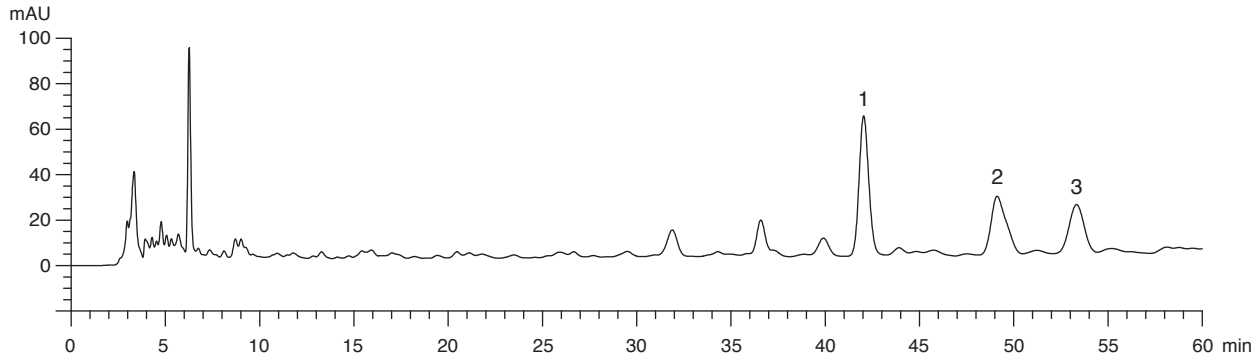

Figure 6 A reference fingerprint chromatogram of Rosae Laevigatae Fructus extract

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. 6).

5. TESTS

5.1 Heavy Metals *(Appendix V)*: meet the requirements.

5.2 Pesticide Residues *(Appendix VI)*: meet the requirements.

5.3 Mycotoxins – Aflatoxins *(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 5.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 22.0%.
Ethanol-soluble extractives (cold extraction method): not less than 17.0%.

7. ASSAY

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

Standard solution

(+)-Catechin standard stock solution, Std-Stock (400 mg/L)
Weigh accurately 4.0 mg of (+)-catechin CRS and dissolve in 10 mL of methanol (50%).
(+)-Catechin standard solution for assay, Std-AS
Measure accurately the volume of the (+)-catechin Std-Stock, dilute with methanol (50%) to produce a series of solutions of 2, 4, 8, 16, 32 mg/L for (+)-catechin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (50%). Sonicate (200 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Combine the solutions 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 (202 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 0.9 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.1% Phosphoric acid (%, v/v)	Elution
0 – 48	4 → 8	96 → 92	linear gradient
48 – 75	8 → 11	92 → 89	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of (+)-catechin Std-AS (8 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of (+)-catechin should not be more than 5.0%; the RSD of the retention time of (+)-catechin peak should not be more than 2.0%; the column efficiency determined from (+)-catechin peak should not be less than 30000 theoretical plates.

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

Limits

The sample contains not less than 0.039% of (+)-catechin ($C_{15}H_{14}O_6$), calculated with reference to the dried substance.