Ampelopsis Radix

Figure 1 A photograph of Ampelopsis Radix

1. NAMES

Official Name: Ampelopsis Radix

Chinese Name: 白薇

Chinese Phonetic Name: Bailian

2. SOURCE

Ampelopsis Radix is the dried root tuber of *Ampelopsis japonica* (Thunb.) Makino (Vitaceae). The root tuber is collected in spring and autumn, stems and rootlets removed, cut into longitudinal segments or oblique slices, then dried under the sun to obtain Ampelopsis Radix.

3. DESCRIPTION

Mainly longitudinal segments, fusiform, oblong-ellipsoid or subglobularse, 1.5-13.4 cm long, 6-37 mm in diameter. Externally reddish-brown to dark brown, with longitudinal wrinkles, fine transverse striations and transversely elongated lenticels; bark easily fallen off, revealing pale reddish-brown surface. Cut surface whitish to pale reddish-brown, edges usually rolled inwards, with a protuberant ridge in the centre. Texture hard, fragile and light in weight, easily broken and dusting on breaking. Odour slight; taste sweet (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of cells. Cortex consists of parenchymatous cells, scattered with abundant mucilage cells. Raphides of calcium oxalate scattered singly or in bundles in mucilage cells. Clusters of calcium oxalate scattered mainly in parenchymatous cells in cortex. Phloem narrow, stripped. Cambium in a ring. Xylem vessels scattered sparsely, with few surrounded xylem fibres; mucilage cells with raphides of calcium oxalate scattered. Parenchymatous cells full of starch granules (Fig. 2).

Powder

Colour greyish-white to greyish-brown. Starch granules numerous, simple starch granules subrounded, reniform or ovate, 2-44 μm long, 2-25 μm in diameter; black and cruciate-shaped under the polarized microscope; compound starch granules occasionally found. Raphides of calcium oxalate abundant, scattered or grouped in bundles in mucilage cells, 23-179 μm long; polychromatic under the polarized microscope. Clusters of calcium oxalate 11-69 μm in diameter, with broad and large angles; polychromatic under the polarized microscope. Cork cells yellowish-brown, subrectangular or polygonal in surface view. Vessels mainly bordered-pitted, pits often scalariform arranged (Fig. 3).

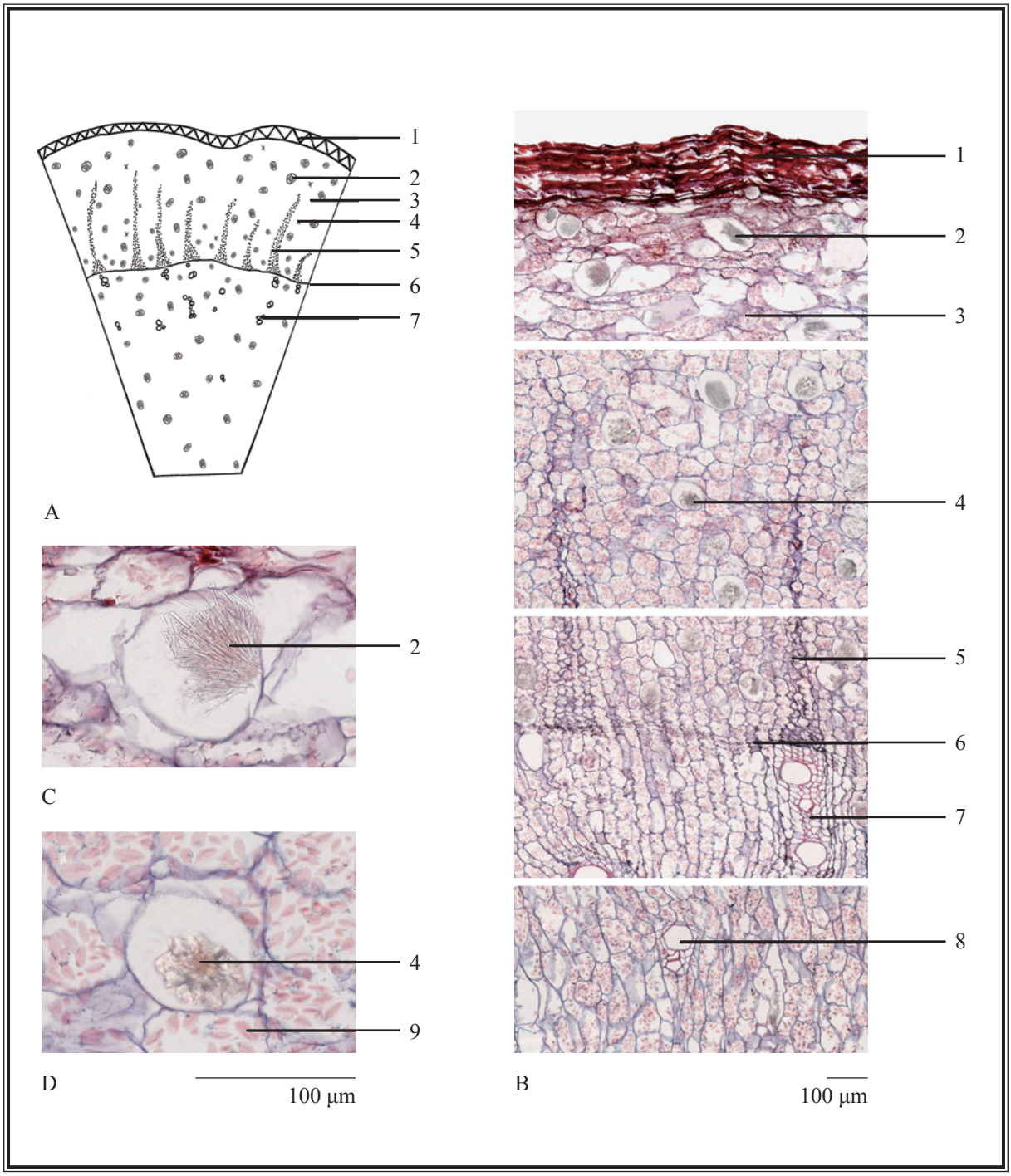


Figure 2 Microscopic features of transverse section of Ampelopsis Radix

A. Sketch B. Section illustration C. Raphides of calcium oxalate in mucilage cell
D. Clusters of calcium oxalate

1. Cork 2. Raphides of calcium oxalate 3. Cortex 4. Clusters of calcium oxalate
5. Phloem 6. Cambium 7. Xylem 8. Vessels 9. Starch granules

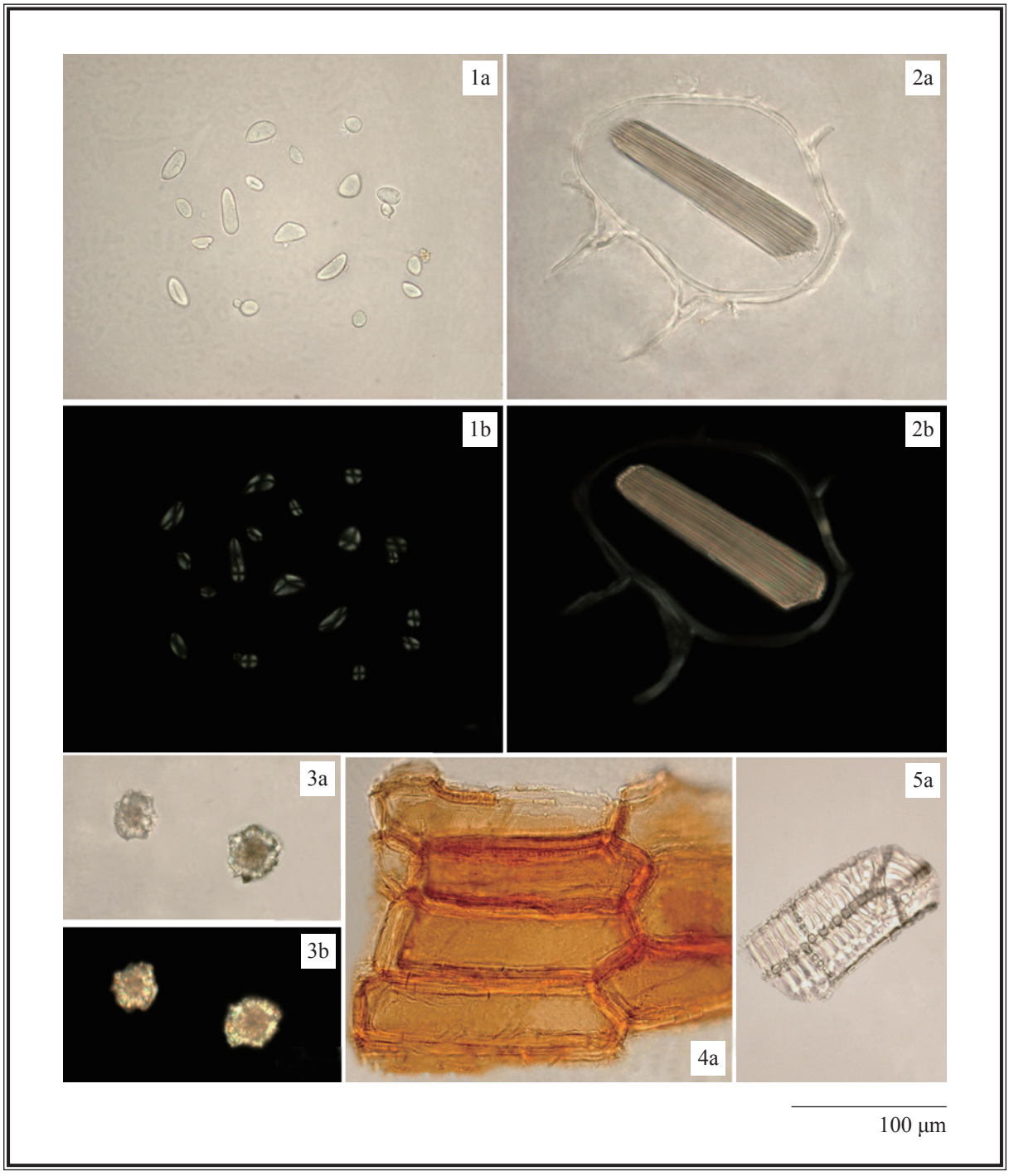


Figure 3 Microscopic features of powder of Ampelopsis Radix

1. Starch granules 2. Raphides of calcium oxalate 3. Clusters of calcium oxalate
4. Cork cells 5. Vessels

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

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

Standard solution

Gallic acid standard solution

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

Developing solvent system

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

Staining reagent

Iodine.

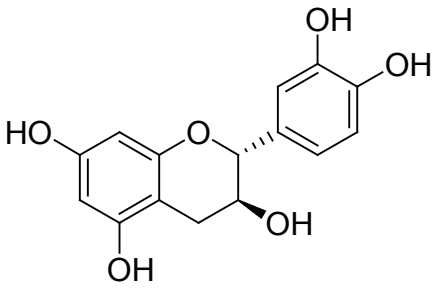
Test solution

Weigh 5.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol. Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Transfer the supernatant to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 10 mL of water. Transfer the solution to a 50-mL centrifuge tube. Add 10 mL of ethyl acetate. Centrifuge at about 2800 × *g* for 10 min. Transfer the upper layer to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol. 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 gallic acid standard solution (0.8 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 6 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Fumigate the plate with iodine vapour chamber for about 3-5 min until the spots or bands become visible. Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

(i)



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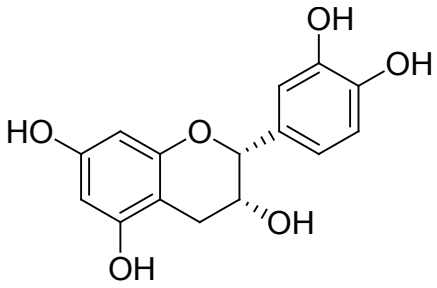

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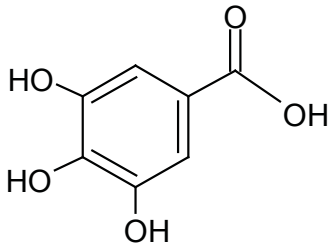

Figure 4 Chemical structures of (i) (+)-catechin (ii) (-)-epicatechin and (iii) gallic acid

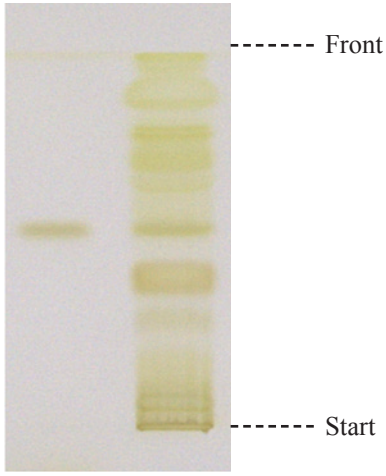

Figure 5 A reference HPTLC chromatogram of Ampelopsis Radix extract observed under visible light after staining

1. Gallic acid 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 gallic acid (Fig. 5).

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

Standard solutions

(+)-Catechin standard solution for fingerprinting, Std-FP (5 mg/L)

Weigh 0.5 mg of (+)-catechin CRS (Fig. 4) and dissolve in 100 mL of methanol (60%).

(-)-Epicatechin standard solution for fingerprinting, Std-FP (5 mg/L)

Weigh 0.5 mg of (-)-epicatechin CRS (Fig. 4) and dissolve in 100 mL of methanol (60%).

Gallic acid standard solution for fingerprinting, Std-FP (2.5 mg/L)

Weigh 0.25 mg of gallic acid CRS and dissolve in 100 mL of methanol (60%).

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 (60%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *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 (60%). Combine the solutions and make up to the mark with methanol (60%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (216 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 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)	Acetonitrile (%, v/v)	Elution
0 – 45	96 → 90	4 → 10	linear gradient
45 – 60	90 → 85	10 → 15	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of (+)-catechin Std-FP, (-)-epicatechin Std-FP and gallic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of (+)-catechin, (-)-epicatechin and gallic acid should not be more than 5.0%; the RSD of the retention times of (+)-catechin, (-)-epicatechin and gallic acid peaks should not be more than 2.0%; the column efficiencies determined from (+)-catechin, (-)-epicatechin and gallic acid peaks should not be less than 20000, 100000 and 10000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; the *R* value between peak 3 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 1.5 (Fig. 6).

Procedure

Separately inject (+)-catechin Std-FP, (-)-epicatechin Std-FP, gallic acid Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of (+)-catechin, (-)-epicatechin and gallic acid peaks in the chromatograms of (+)-catechin Std-FP, (-)-epicatechin Std-FP, gallic acid Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify (+)-catechin, (-)-epicatechin and gallic acid peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of (+)-catechin Std-FP, (-)-epicatechin Std-FP and gallic acid Std-FP. The retention times of (+)-catechin, (-)-epicatechin and gallic acid 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 Ampelopsis Radix extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Ampelopsis Radix extract

Peak No.	RRT	Acceptable Range
1 (gallic acid)	0.24	± 0.03
2	0.70	± 0.03
3 [marker, (+)-catechin]	1.00	-
4	1.12	± 0.03
5 [(-)-epicatechin]	1.41	± 0.03

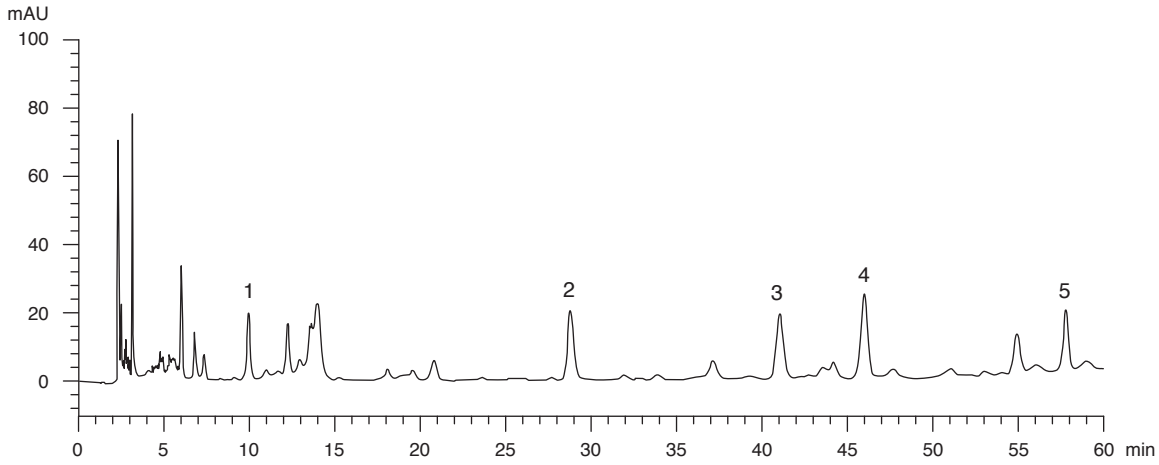

Figure 6 A reference fingerprint chromatogram of Ampelopsis Radix extract

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. 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 10.5%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 20.0%.
Ethanol-soluble extractives (hot extraction method): not less than 15.0%.

7. ASSAY

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

Standard solution

Mixed (+)-catechin, (-)-epicatechin and gallic acid standard stock solution, Std-Stock (20 mg/L for (+)-catechin, 20 mg/L for (-)-epicatechin and 10 mg/L for gallic acid)

Weigh accurately 1.0 mg of (+)-catechin CRS, 1.0 mg of (-)-epicatechin CRS and 0.5 mg of gallic acid, and dissolve in 50 mL of methanol (60%).

Mixed (+)-catechin, (-)-epicatechin and gallic acid standard solution for assay, Std-AS

Measure accurately the volume of the mixed (+)-catechin, (-)-epicatechin and gallic acid Std-Stock, dilute with methanol (60%) to produce a series of solutions of 1, 2, 5, 10, 20 mg/L for (+)-catechin, 1, 2, 5, 10, 20 mg/L for (-)-epicatechin and 0.5, 1, 2.5, 5, 10 mg/L for gallic acid.

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 (60%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *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 (60%). Combine the solutions and make up to the mark with methanol (60%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (202 nm for (+)-catechin and (-)-epicatechin; 216 nm for gallic acid) 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 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)	Acetonitrile (%, v/v)	Elution
0 – 45	96 → 90	4 → 10	linear gradient
45 – 60	90 → 85	10 → 15	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed (+)-catechin, (-)-epicatechin and gallic acid Std-AS (5 mg/L for (+)-catechin, 5 mg/L for (-)-epicatechin and 2.5 mg/L for gallic acid). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of (+)-catechin, (-)-epicatechin and gallic acid should not be more than 5.0%; the RSD of the retention times of (+)-catechin, (-)-epicatechin and gallic acid peaks should not be more than 2.0%; the column efficiencies determined from (+)-catechin, (-)-epicatechin and gallic acid peaks should not be less than 20000, 100000 and 10000 theoretical plates respectively.

The *R* value between (+)-catechin peak and the closest peak; the *R* value between (-)-epicatechin peak and the closest peak; and the *R* value between gallic acid 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 (+)-catechin, (-)-epicatechin and gallic acid Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of (+)-catechin, (-)-epicatechin and gallic acid against the corresponding concentrations of the mixed (+)-catechin, (-)-epicatechin and gallic acid 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 (+)-catechin, (-)-epicatechin and gallic acid peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed (+)-catechin, (-)-epicatechin and gallic acid Std-AS. The retention times of (+)-catechin, (-)-epicatechin and gallic acid 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 (+)-catechin, (-)-epicatechin and gallic acid in the test solution, and calculate the percentage contents of (+)-catechin, (-)-epicatechin and gallic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.044% of the total content of (+)-catechin ($C_{15}H_{14}O_6$) and (-)-epicatechin ($C_{15}H_{14}O_6$); and not less than 0.014% of gallic acid ($C_7H_6O_5$), calculated with reference to the dried substance.