Cinnamomi Ramulus

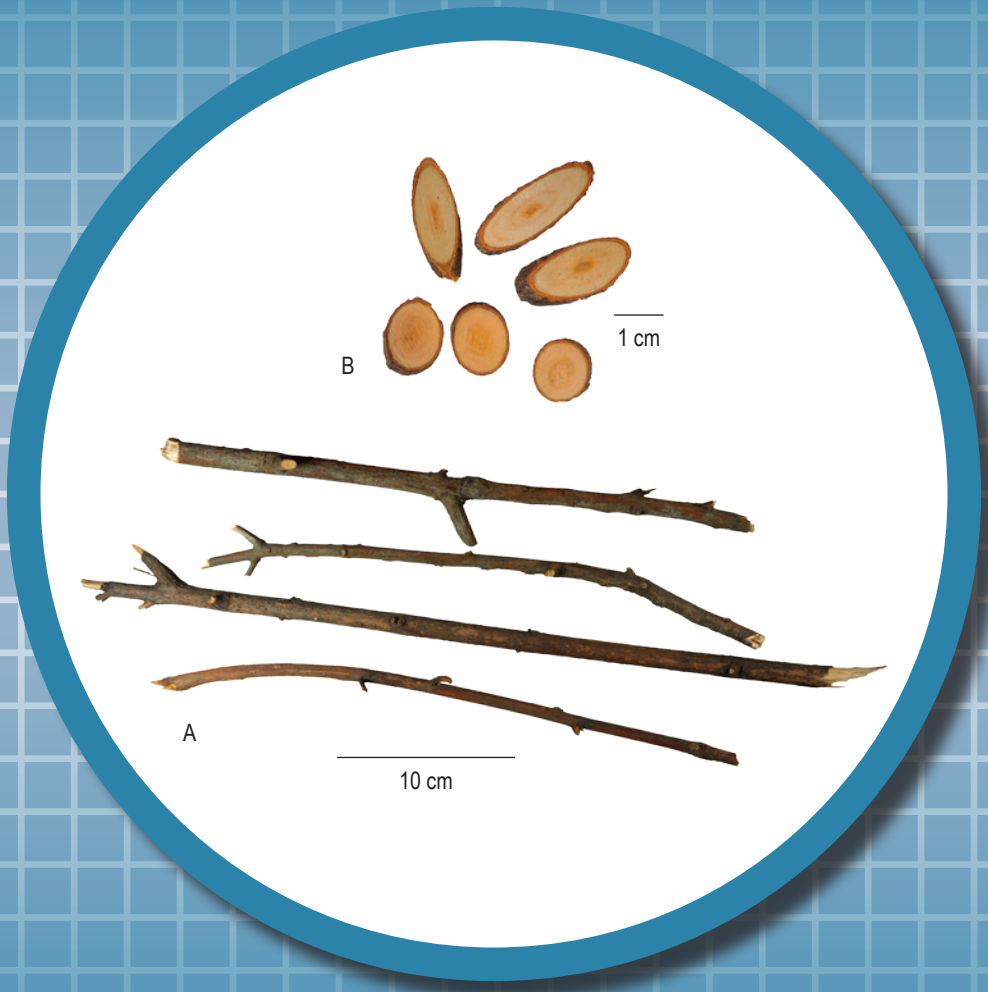


Figure 1 A photograph of Cinnamomi Ramulus

A. Cinnamomi Ramulus B. Slices

1. NAMES

Official Name: Cinnamomi Ramulus

Chinese Name: 桂枝

Chinese Phonetic Name: Guizhi

2. SOURCE

Cinnamomi Ramulus is the dried young branch of *Cinnamomum cassia* Presl (Lauraceae). The young branch is collected in spring and summer, leaves removed, then dried under the sun; or dried after sliced to obtain Cinnamomi Ramulus.

3. DESCRIPTION

Long, mostly branched and cylindrical, 20.8-55.8 cm long, thick end 2-18 mm in diameter. Externally reddish-brown to brown, with longitudinal ridges, fine wrinkles, dotted lenticels, pimple-like leaf scars, branch scars and bud scars. Texture hard and fragile, easily broken. Slices 1-3 mm thick, bark of cut surface reddish-brown, wood yellowish-white to pale yellowish-brown; pith subsquare. Odour characteristic and aromatic; taste sweet and slightly pungent, relatively strong for bark (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermis consists of 1 layer of cells, with unicellular non-glandular hairs occasionally. Cork consists of 3-5 layers of cells, cells of the innermost layer with thickened outer wall. Cortex scattered with oil cells and stone cells. Groups of stone cells in pericycle arranged in a interrupted ring, accompanied by fibre bundles. Phloem scattered with secretory cells and fibres. Cambium distinct. Xylem vessels scattered singly or 2-4 in groups, arranged radially; xylem rays 1-2 cells wide, containing brown contents. Pith with slightly thickened and lignified cell wall (Fig. 2).

Powder

Colour reddish-brown. Stone cells abundant, scattered singly or in groups, colourless, pale yellow or brown, subsquare, rectangular, subrounded or short fusiform, 20-70 μm in diameter and 26-154 μm long, margins slightly uneven, wall thick, some with very thin wall on one side, striations rare, pit canals obvious. Phloem fibres scattered singly, colourless or brown, fusiform, slightly curved, with sharp, mucronate or obtuse-rounded endings, 9-39 μm in diameter, margins occasionally with toothlike projections, primary wall obvious, quite thick and lignified, pit canals indistinct. Cells of xylem ray subsquare or rectangular, wall thickened and beaded. Vessels mainly bordered-pitted, 5-59 μm in diameter; spiral vessels few. Cork cells yellowish-brown, subsquare to roundish-rectangular or polygonal in surface view, containing reddish-brown contents. Oil cells often broken, intact cells subrounded or elliptic, 41-104 μm in diameter, some containing pale brown oil droplets. Xylem fibres mainly in bundles, colourless to yellowish-brown (Fig. 3).

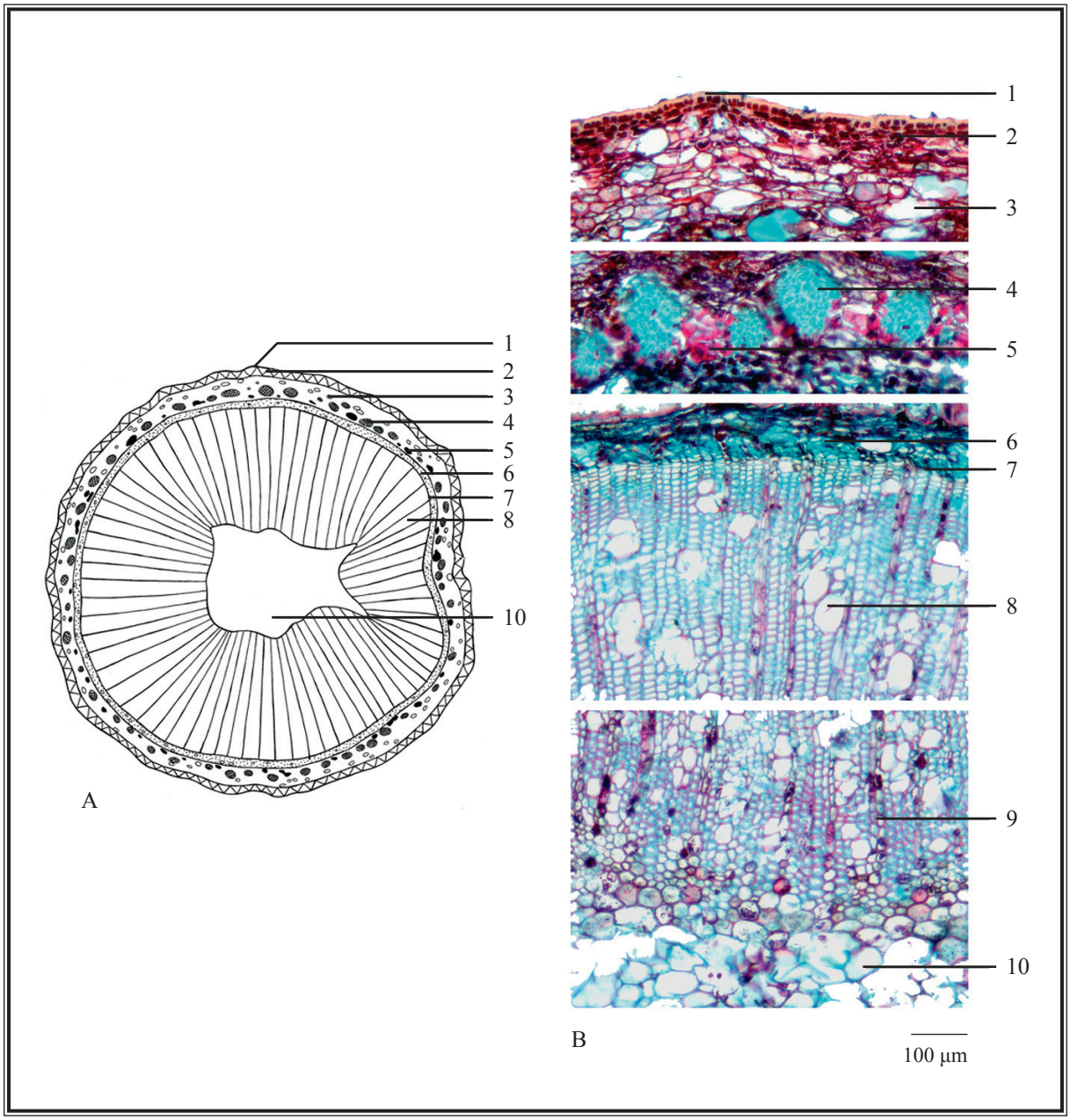


Figure 2 Microscopic features of transverse section of Cinnamomi Ramulus

A. Sketch B. Section illustration

1. Epidermis 2. Cork 3. Oil cell 4. Fibre bundles 5. Groups of stone cells
6. Phloem 7. Cambium 8. Xylem 9. Xylem ray 10. Pith

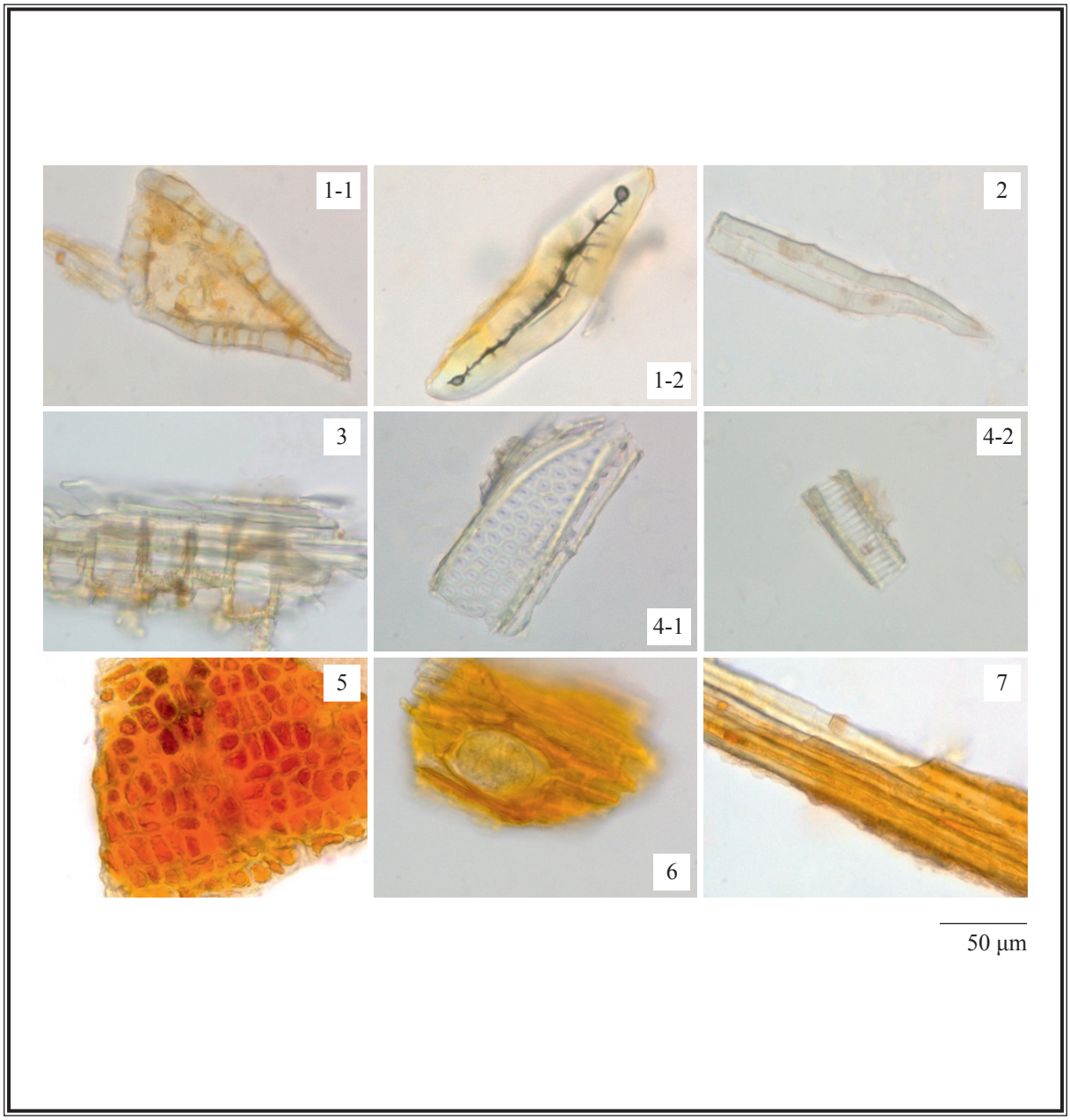


Figure 3 Microscopic features of powder of Cinnamomi Ramulus (under the light microscope)

1. Stone cells 2. Phloem fibre 3. Cells of xylem ray 4-1. Bordered-pitted vessel
4-2. Spiral vessel 5. Cork cells 6. Oil cell 7. Xylem fibres

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

Standard solutions

Cinnamaldehyde standard solution

Weigh 5.0 mg of cinnamaldehyde CRS (Fig. 4) and dissolve in 5 mL of ethanol (95%).

Cinnamic acid standard solution

Weigh 5.0 mg of cinnamic acid CRS (Fig. 4) and dissolve in 5 mL of ethanol (95%).

Developing solvent system

Prepare a mixture of petroleum ether (60 - 80°C), ethyl acetate and formic acid (8:2:0.2, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (95%). Sonicate (150 W) the mixture for 45 min. 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 cinnamaldehyde standard solution (6 µL), cinnamic acid standard solution (0.6 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 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of cinnamaldehyde and cinnamic acid.

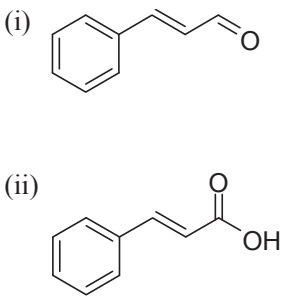


Figure 4 Chemical structures of (i) cinnamaldehyde and (ii) cinnamic acid

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

Standard solutions

Cinnamaldehyde standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 2.0 mg of cinnamaldehyde CRS and dissolve in 10 mL of ethanol (70%).

Cinnamic acid standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.1 mg of cinnamic acid CRS and dissolve in 10 mL of ethanol (70%).

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 3000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (250 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)	Acetonitrile (%, v/v)	0.5% Acetic acid (%, v/v)	Elution
0 – 10	28	72	isocratic
10 – 20	28 → 32	72 → 68	linear gradient
20 – 30	32 → 40	68 → 60	linear gradient
30 – 40	40 → 60	60 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of cinnamaldehyde Std-FP and cinnamic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of cinnamaldehyde and cinnamic acid should not be more than 5.0%; the RSD of the retention times of cinnamaldehyde and cinnamic acid peaks should not be more than 2.0%; the column efficiencies determined from cinnamaldehyde and cinnamic acid peaks should not be less than 25000 and 13000 theoretical plates respectively.

The *R* value between peak 3 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 cinnamaldehyde Std-FP, cinnamic acid Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of cinnamaldehyde and cinnamic acid peaks in the chromatograms of cinnamaldehyde Std-FP, cinnamic acid Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify cinnamaldehyde and cinnamic acid peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of cinnamaldehyde Std-FP and cinnamic acid Std-FP. The retention times of cinnamaldehyde and cinnamic 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 Cinnamomi Ramulus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Cinnamomi Ramulus extract

Peak No.	RRT	Acceptable Range
1 (coumarin)	0.78	± 0.03
2	0.87	± 0.03
3 (marker, cinnamic acid)	1.00	-
4 (cinnamaldehyde)	1.29	± 0.03
5	1.56	± 0.03

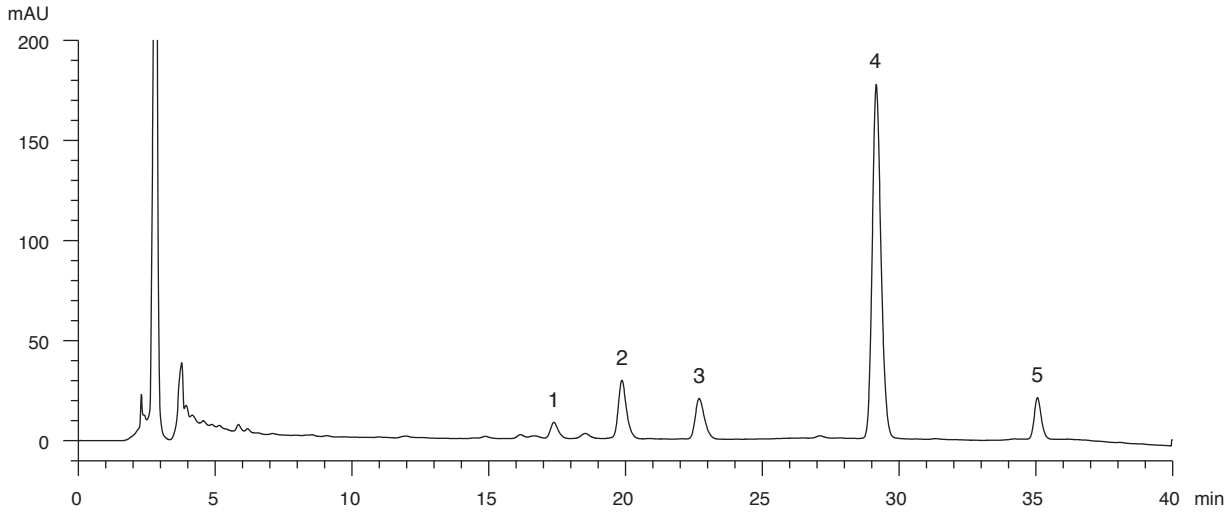


Figure 5 A reference fingerprint chromatogram of Cinnamomi Ramulus extract

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 – 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 2.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 6.0%.
Ethanol-soluble extractives (cold extraction method): not less than 6.0%.

7. ASSAY

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

Standard solution

Mixed cinnamaldehyde and cinnamic acid standard stock solution, Std-Stock (250 mg/L for cinnamaldehyde and 50 mg/L for cinnamic acid)

Weigh accurately 2.5 mg of cinnamaldehyde CRS and 0.5 mg of cinnamic acid CRS, and dissolve in 10 mL of ethanol (70%).

Mixed cinnamaldehyde and cinnamic acid standard solution for assay, Std-AS

Measure accurately the volume of the mixed cinnamaldehyde and cinnamic acid Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 5, 10, 25, 50, 100 mg/L for cinnamaldehyde and 0.5, 1, 2, 5, 10 mg/L for cinnamic acid.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (70%). Sonicate (200 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with ethanol (70%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (290 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. The mobile phase is a mixture of acetonitrile and 0.5% acetic acid (34:66, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 5 μL of the mixed cinnamaldehyde and cinnamic acid Std-AS (25 mg/L for cinnamaldehyde and 2 mg/L for cinnamic acid). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of cinnamaldehyde and cinnamic acid should not be more than 5.0%; the RSD of the retention times of cinnamaldehyde and cinnamic acid peaks should not be more than 2.0%; the column efficiencies determined from cinnamaldehyde and cinnamic acid peaks should not be less than 11000 theoretical plates.

The *R* value between cinnamaldehyde peak and the closest peak; and the *R* value between cinnamic 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 cinnamaldehyde and cinnamic acid Std-AS (5 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of cinnamaldehyde and cinnamic acid against the corresponding concentrations of the mixed cinnamaldehyde and cinnamic acid Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

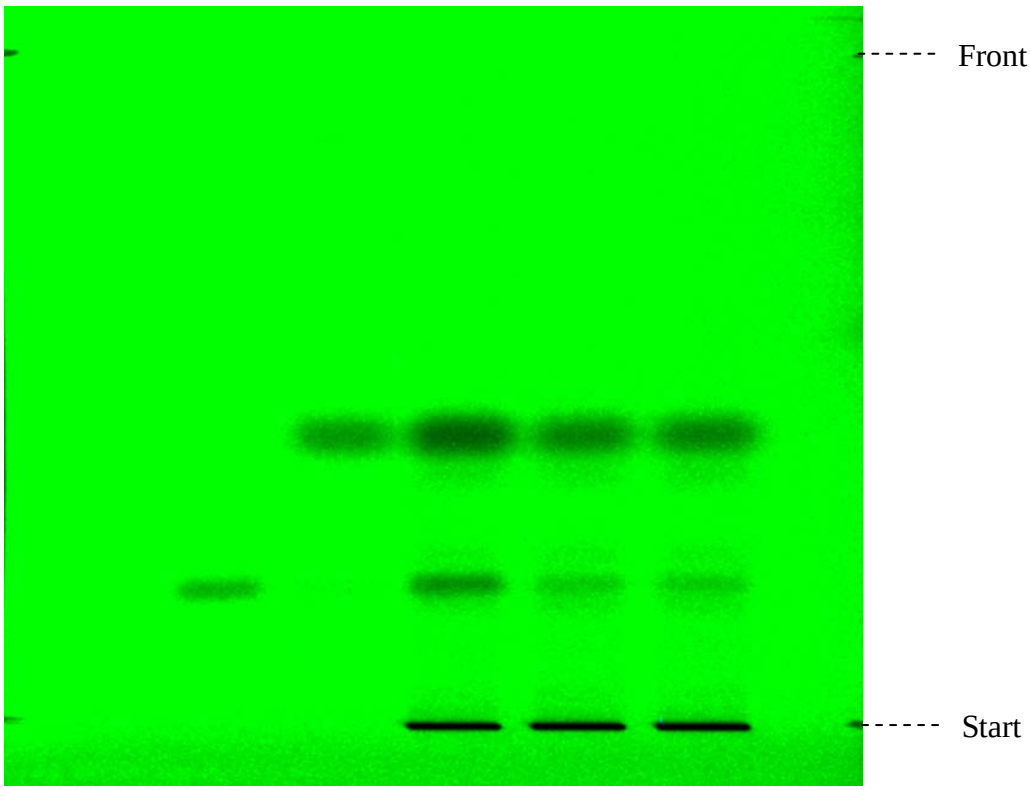
Procedure

Inject 5 μL of the test solution into the HPLC system and record the chromatogram. Identify cinnamaldehyde and cinnamic acid peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed cinnamaldehyde and cinnamic acid Std-AS. The retention times of cinnamaldehyde and cinnamic 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 cinnamaldehyde and cinnamic acid in the test solution, and calculate the percentage contents of cinnamaldehyde and cinnamic acid in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.63% of the total content of cinnamaldehyde (C_9H_8O) and cinnamic acid ($C_9H_8O_2$), calculated with reference to the dried substance.

Cinnamomi Ramulus (桂枝)



Lane	Sample	Results
1	Blank (95% Ethanol)	Negative
2	Standard (Cinnamic acid)	Cinnamic acid positive
3	Standard (Cinnamaldehyde)	Cinnamaldehyde positive
4	Spiked sample (Sample plus cinnamic acid and cinnamaldehyde)	Cinnamic acid and cinnamaldehyde positive
5	Sample (Cinnamomi Ramulus)	Cinnamic acid and cinnamaldehyde positive
6	Sample duplicate (Cinnamomi Ramulus)	Cinnamic acid and cinnamaldehyde positive

Figure 1 TLC results of Cinnamomi Ramulus extract observed under UV light (254 nm)