Perillae Caulis

Figure 1 A photograph of Perillae Caulis

A. Perillae Caulis B. Slices

1. NAMES

Official Name: Perillae Caulis

Chinese Name: 紫蘇梗

Chinese Phonetic Name: Zisugeng

2. SOURCE

Perillae Caulis is the dried stem of *Perilla frutescens* (L.) Britt. (Lamiaceae). The stem is collected after fruit has ripened in autumn, foreign matter removed, then dried under the sun or cut into slices while still fresh, then dried under the sun to obtain Perillae Caulis.

3. DESCRIPTION

Quadrangular in transverse section, with four obtuse angles, the length varies, 5-15 mm in diameter. Externally purplish-green to purplish-brown or brownish-yellow, with longitudinal furrows and fine longitudinal striations, nodes slightly swollen, bearing opposite branch scars and leaf scars. Texture light and hard, fracture lobed. Stem slices usually rhomboidal, 2-5 mm thick, wood yellowish-white, radiate striations fine and dense, the pith white, lax or falling off. Odour slightly aromatic; taste weak (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermis consists of 1 layer of cells, tangentially elongated. Hypodermal collenchyma present at angular regions. Cortex consists of several layers of parenchymatous cells. Pericyclic fibre bundles arranged in an interrupted ring. Phloem narrow. Cambium indistinct. In the xylem, vessels are scattered singly or in groups, arranged radially; xylem rays consists of 1 to several rows of cells. Pith consists of large parenchymatous cells, with raphides of calcium oxalate occasionally present (Fig. 2).

Powder

Colour yellowish-white to greyish-green. Small raphides of calcium oxalate present mainly in parenchymatous cells, 2-16 μm long (occasionally up to 28 μm); bright white under the polarized microscope. Xylem fibres abundant, slender, 8-45 μm in diameter, with a large lumen; bright white under the polarized microscope. Pericyclic fibres fusiform, 10-46 μm in diameter, sometimes with distinct pit canals; white to orange or polychromatic under the polarized microscope. Parenchymatous cells of xylem rectangular, with distinct pits and pit canals, slightly lignified. Epidermal cells brownish-yellow, polygonal in surface view. Glandular hairs occasionally found, consisting of unicellular head and short stalk. Vessels mainly bordered-pitted and spiral, 8-81 μm in diameter (Fig. 3).

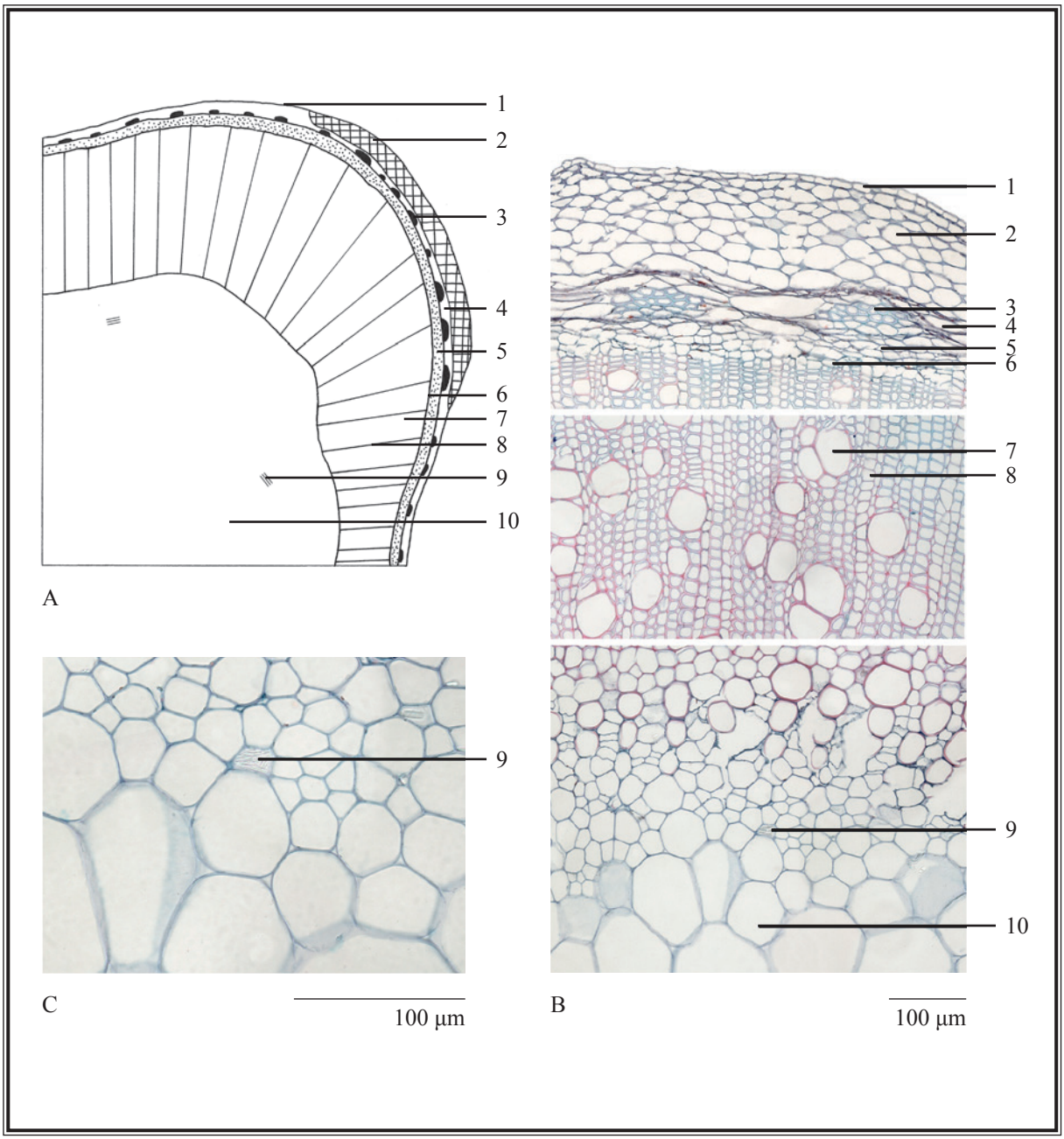

Figure 2 Microscopic features of transverse section of Perillae Caulis

A. Sketch B. Section illustration
C. Raphides of calcium oxalate in parenchyma

1. Epidermis 2. Hypodermal collenchyma 3. Pericyclic fibre bundle 4. Cortex
5. Phloem 6. Cambium 7. Xylem 8. Xylem ray 9. Raphides of calcium oxalate
10. Pith

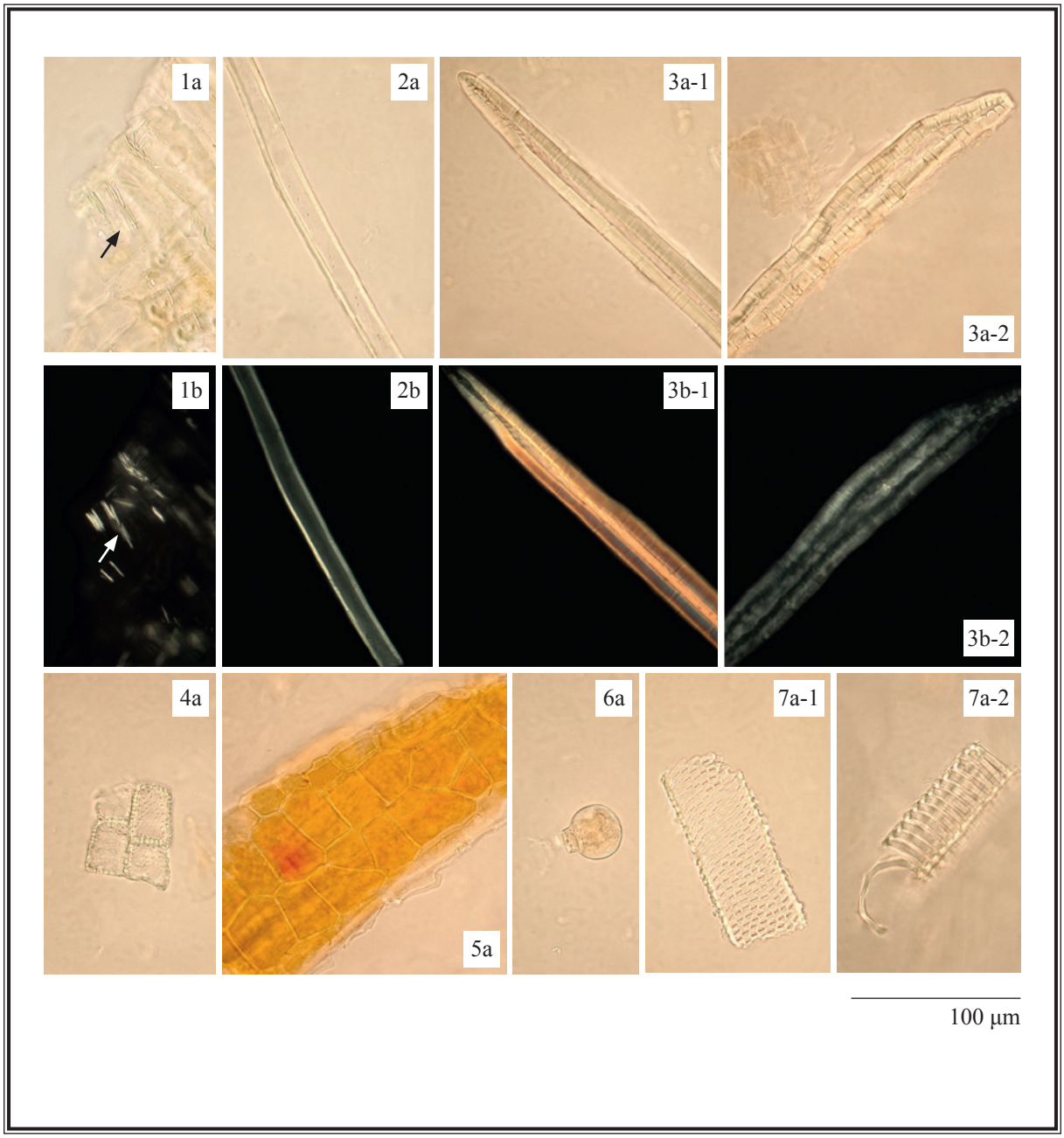

Figure 3 Microscopic features of powder of Perillae Caulis

1. Raphides of calcium oxalate 2. Xylem fibre 3. Pericyclic fibres
4. Parenchymatous cells of xylem 5. Epidermal cells 6. Glandular hair
7. Vessels (7-1 bordered-pitted vessel, 7-2 spiral vessel)

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

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

Standard solution

Rosmarinic acid standard solution

Weigh 0.5 mg of rosmarinic acid CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, petroleum ether (60-80°C) and formic acid (5:4:0.5, v/v).

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. Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Filter and transfer the filtrate to a 50-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 rosmarinic acid standard solution (1 μL) and the test solution (5 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. 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 rosmarinic acid.

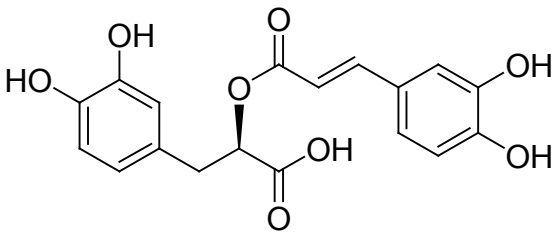

Figure 4 Chemical structure of rosmarinic acid

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

Standard solution

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

Weigh 1.0 mg of rosmarinic acid CRS and dissolve in 100 mL of methanol (70%).

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 (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 3500 × *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 (70%). Centrifuge at about 3500 × *g* for 10 min. Combine the supernatants and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (330 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% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	88 → 70	12 → 30	linear gradient
30 – 60	70 → 40	30 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 µL of rosmarinic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of rosmarinic acid should not be more than 5.0%; the RSD of the retention time of rosmarinic acid peak should not be more than 2.0%; the column efficiency determined from rosmarinic acid peak should not be less than 90000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

Separately inject rosmarinic acid Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention time of rosmarinic acid peak in the chromatogram of rosmarinic acid Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify rosmarinic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of rosmarinic acid Std-FP. The retention times of rosmarinic acid 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 Perillae Caulis extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Perillae Caulis extract

Peak No.	RRT	Acceptable Range
1	0.48	± 0.03
2	0.80	± 0.03
3 (marker, rosmarinic acid)	1.00	-
4	1.53	± 0.03
5	1.60	± 0.03

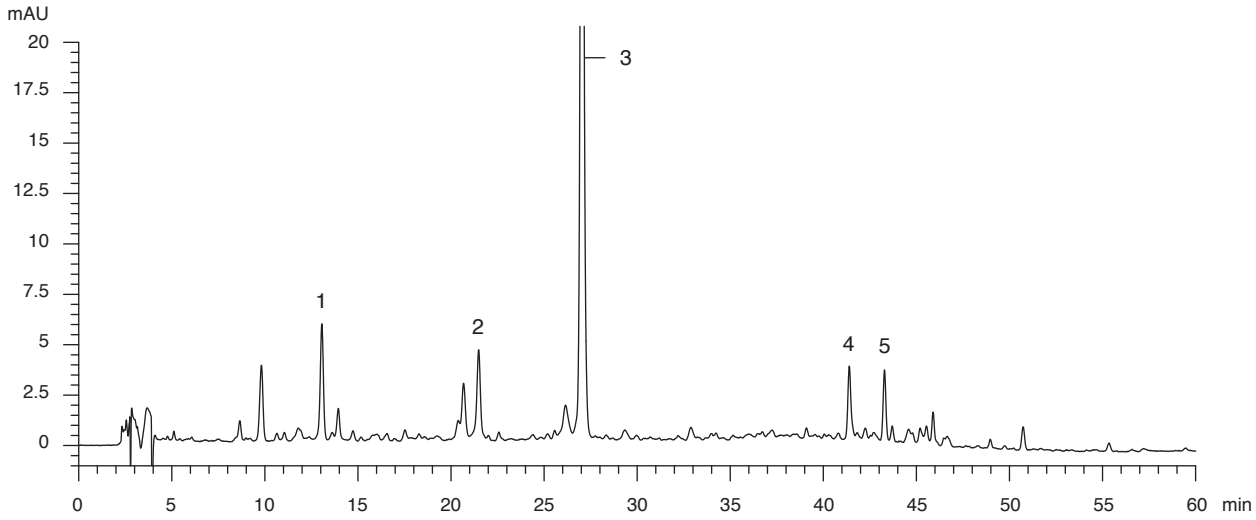

Figure 5 A reference fingerprint chromatogram of Perillae Caulis extract

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 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 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 4.0%.
Ethanol-soluble extractives (cold extraction method): not less than 3.0%.

7. ASSAY

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

Standard solution

Rosmarinic acid standard stock solution, Std-Stock (50 mg/L)

Weigh accurately 2.5 mg of rosmarinic acid CRS and dissolve in 50 mL of methanol (70%).

Rosmarinic acid standard solution for assay, Std-AS

Measure accurately the volume of the rosmarinic acid Std-Stock, dilute with methanol (70%) to produce a series of solutions of 0.5, 1, 2.5, 5, 25 mg/L for rosmarinic 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 (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 3500 × *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 (70%). Centrifuge at about 3500 × *g* for 10 min. Combine the supernatants and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (330 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% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	88 → 70	12 → 30	linear gradient
30 – 60	70 → 40	30 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 µL of rosmarinic acid Std-AS (2.5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of rosmarinic acid should not be more than 5.0%; the RSD of the retention time of rosmarinic acid peak should not be more than 2.0%; the column efficiency determined from rosmarinic acid peak should not be less than 90000 theoretical plates.

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

Limits

The sample contains not less than 0.012% of rosmarinic acid ($C_{18}H_{16}O_8$), calculated with reference to the dried substance.