Ligustici Rhizoma et Radix

Figure 1 (i) A photograph of dried rhizome and root of *Ligusticum sinense* Oliv.

Figure 1 (ii) A photograph of dried rhizome and root of *Ligusticum jeholense* Nakai et Kitag.

1. NAMES

Official Name: Ligustici Rhizoma et Radix

Chinese Name: 藁本

Chinese Phonetic Name: Gaoben

2. SOURCE

Ligustici Rhizoma et Radix is the dried rhizome and root of *Ligusticum sinense* Oliv. or *Ligusticum jeholense* Nakai et Kitag. (Apiaceae). The rhizome and root are collected in autumn when the stem and leaves withered or in the next spring when new growth started, soil removed, then dried under the sun or baked at temperature about 50ºC or lower to dryness to obtain Ligustici Rhizoma et Radix.

3. DESCRIPTION

***Ligusticum sinense* Oliv.:** Rhizome irregulary tubercular cylindrical, slightly twisted and branched, 3-12 cm long, 5-20 mm in diameter. Externally brown to dark brown, rough, with longitudinal wrinkles; the upper part consists of sunken remnants of several rounded stem bases, the lower part bearing numerous dotted, protuberant root scars and remnant roots. Texture relatively hard and light in weight, easily broken. Fracture yellowish-white, fibrous. Odour strongly aromatic; taste pungent, bitter, and slightly numbing [Fig. 1 (i)].

***Ligusticum jeholense* Nakai et Kitag.:** Rhizome relatively small, irregular masses or columnar, 1-10 cm long, 5-20 mm in diameter, with numerous, slender and curved roots [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root:

***Ligusticum sinense* Oliv.:** Cork consists of several layers to more than 10 layers of cells. Cortex relatively narrow, consisting of several layers of cells. Phloem broad. Oil cavity suborbicular, mainly scattered in phloem. Cambium in a ring. Xylem fibres abundant, mostly arranged in group [Fig. 2 (i)].

***Ligusticum jeholense* Nakai et Kitag.:** Small groups of fibres bundles scattered in xylem [Fig. 2 (ii)].

Rhizome:

***Ligusticum sinense* Oliv.:** Cork consists of several layers to more than 10 layers of cells. Cortex relatively narrow, several layers of cells. Phloem broad. Oil cavity suborbicular, 50-240 μm in diameter, mainly scattered in phloem and pith. Cambium in a ring. Xylem vessels mostly arranged singly or 2 to several vessels in a group, sparsely arranged in rhizome, surrounded by fibres or distributed near inner part of xylem fibres; xylem fibres abundant, arranged in group. Pith large [Fig. 3 (i)].

***Ligusticum jeholense* Nakai et Kitag.:** Oil cavity 35-210 μm in diameter. Xylem fibres bundles arranged with vessels alternately [Fig. 3 (ii)].

Powder

Colour pale brown. Cork cells yellowish-brown, polygonal, subsquare or rectangular. Vessels mainly reticulate, 10-80 μm in diameter. Fibres mostly in bundles. Most of oil cavities were broken, the fragment ocassionally found. Simple starch granules subround or elliptical, 4-17 μm in diameter; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-3 units [Fig. 4 (i) and (ii)].

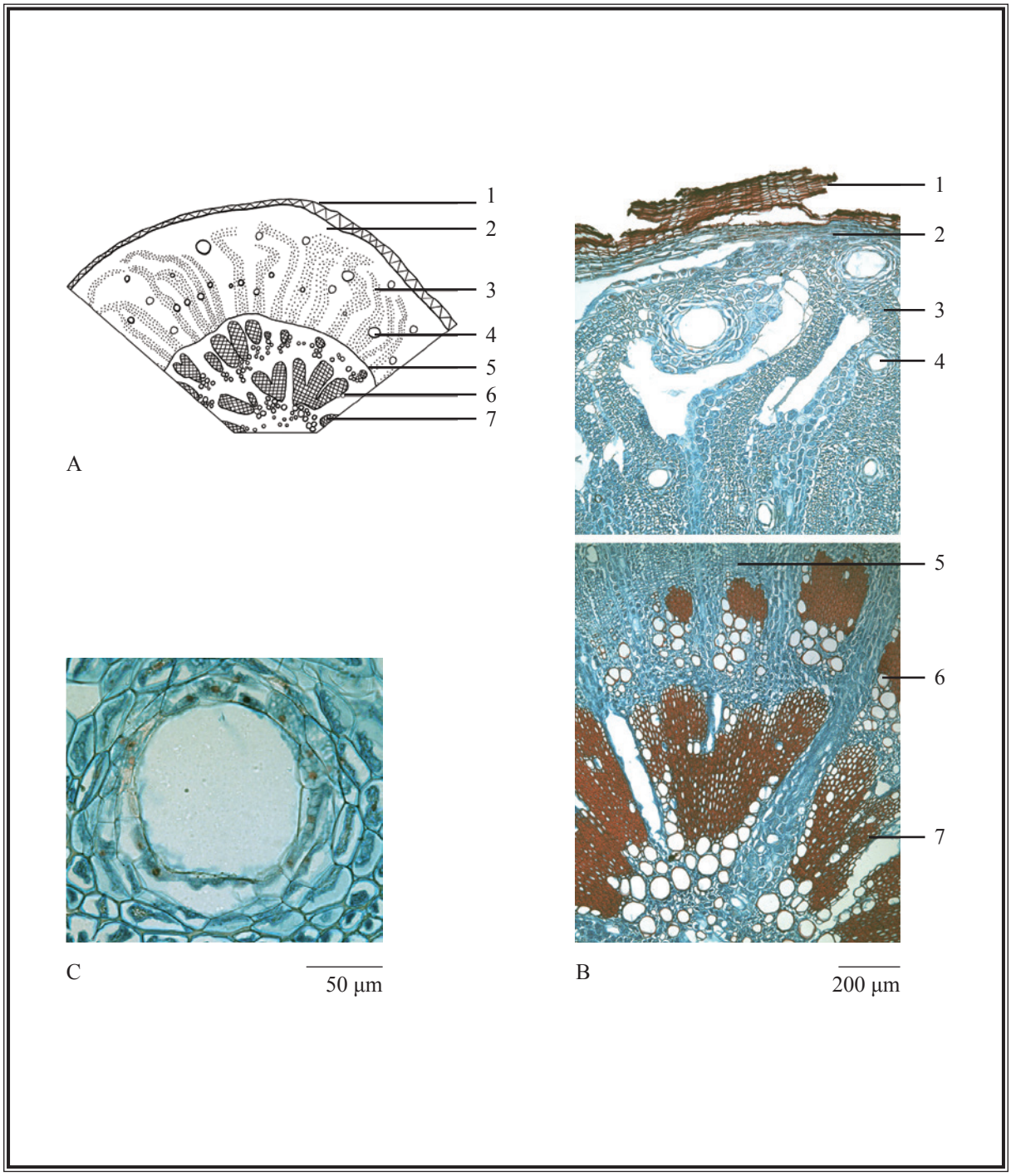

Figure 2 (i) Microscopic features of transverse section of dried root of *Ligusticum sinense* Oliv.

A. Sketch B. Section illustration C. Oil cavity

1. Cork 2. Cortex 3. Phloem 4. Oil cavity 5. Cambium
6. Vessel 7. Xylem fibre

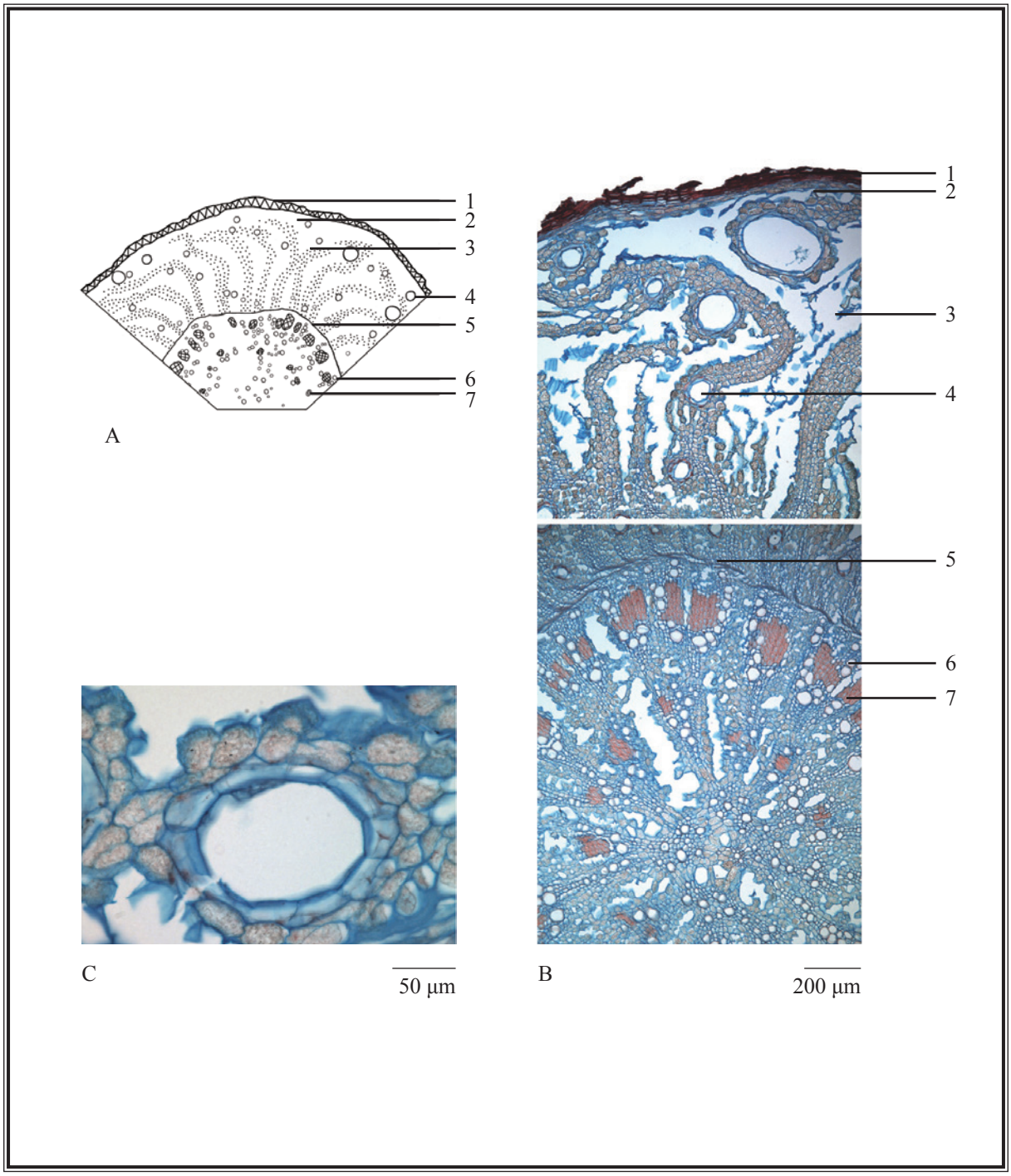

Figure 2 (ii) Microscopic features of transverse section of dried root of *Ligusticum jeholense* Nakai et Kitag.

A. Sketch B. Section illustration C. Oil cavity

1. Cork 2. Cortex 3. Phloem 4. Oil cavity 5. Cambium
6. Vessel 7. Xylem fibre

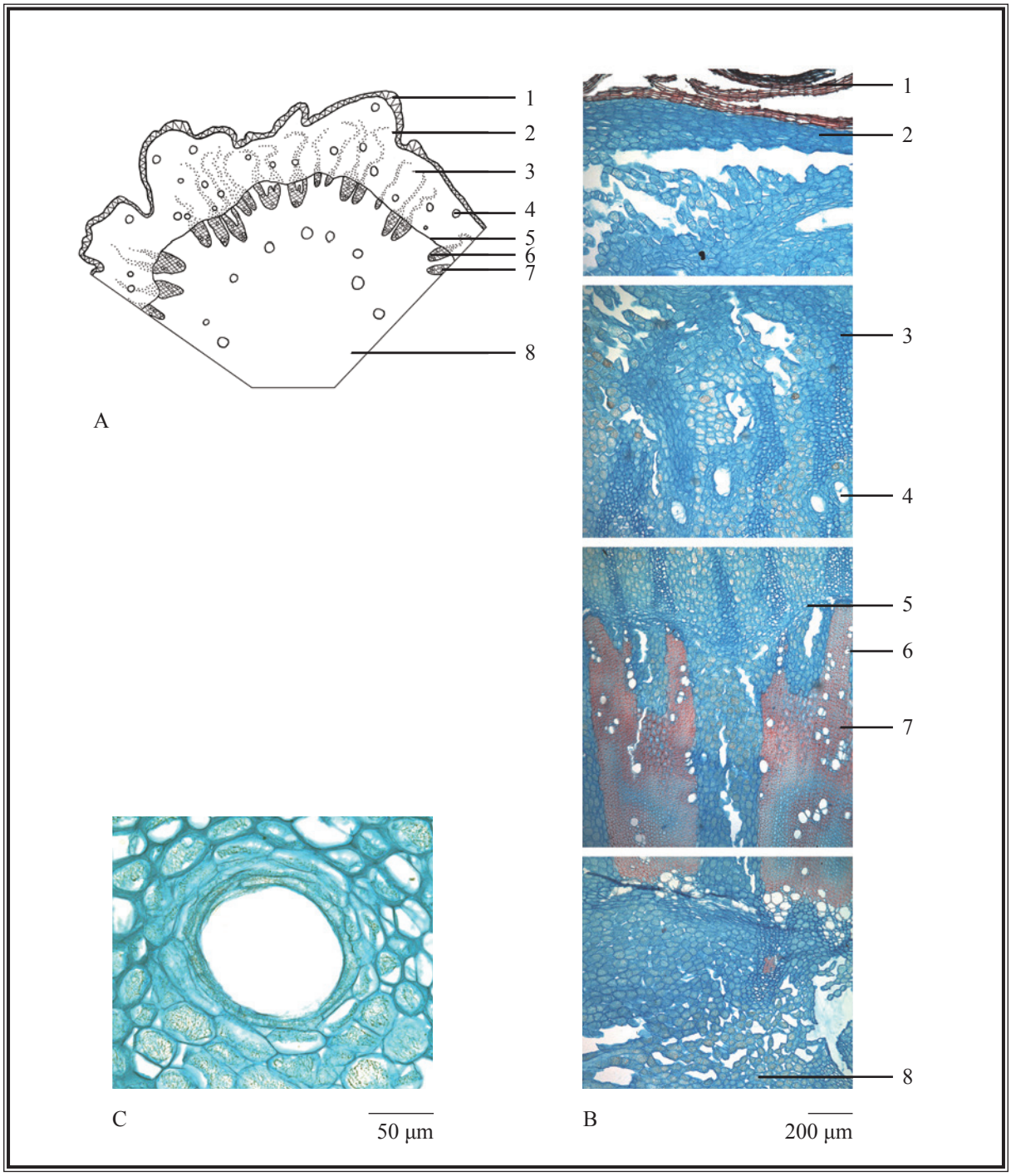

Figure 3 (i) Microscopic features of transverse section of dried rhizome of *Ligusticum sinense* Oliv.

A. Sketch B. Section illustration C. Oil cavity

1. Cork 2. Cortex 3. Phloem 4. Oil cavity 5. Cambium
6. Vessel 7. Xylem fibre 8. Pith

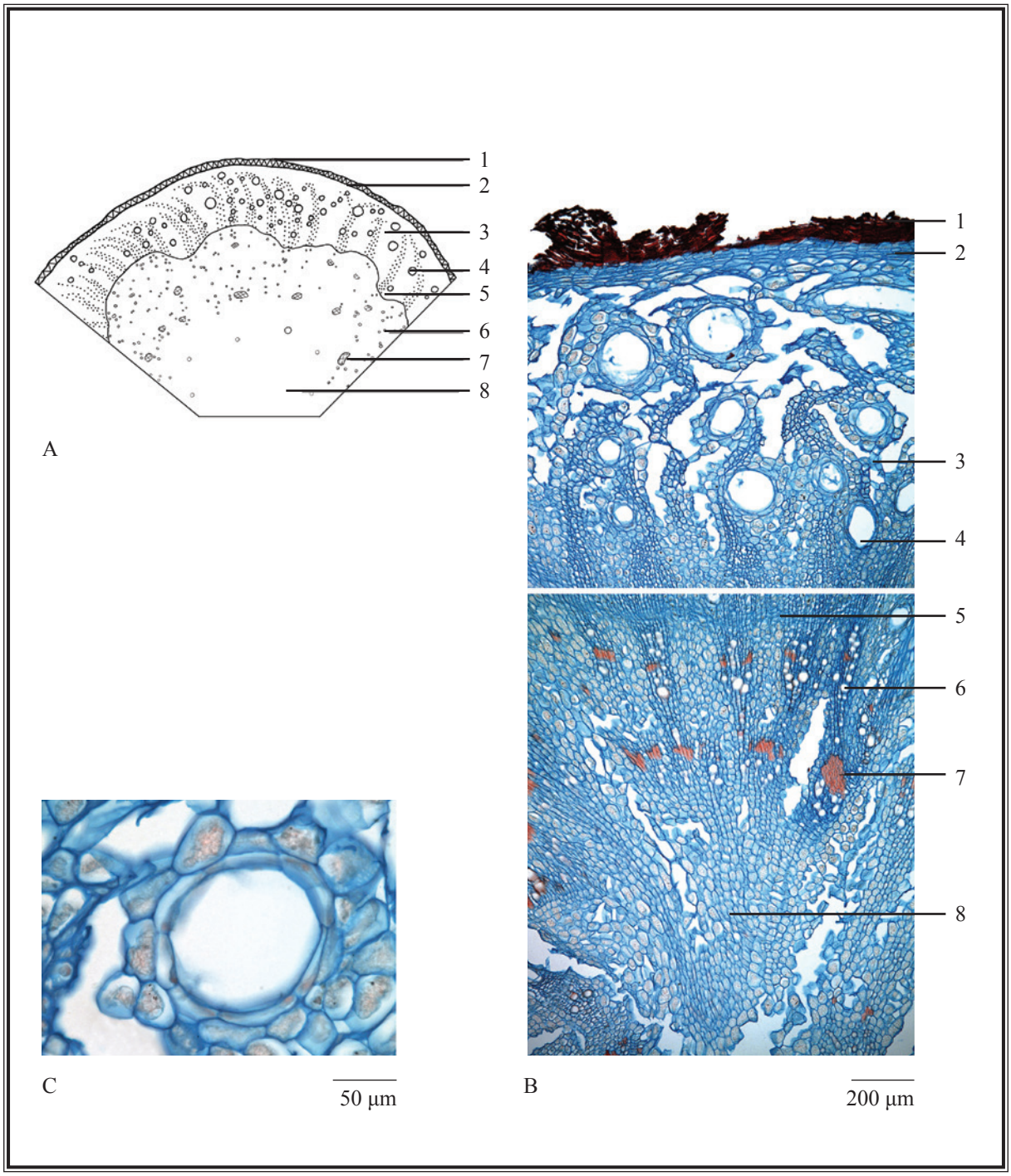

Figure 3 (ii) Microscopic features of transverse section of dried rhizome of *Ligusticum jeholense* Nakai et Kitag.

A. Sketch B. Section illustration C. Oil cavity

1. Cork 2. Cortex 3. Phloem 4. Oil cavity 5. Cambium
6. Vessel 7. Xylem fibre 8. Pith

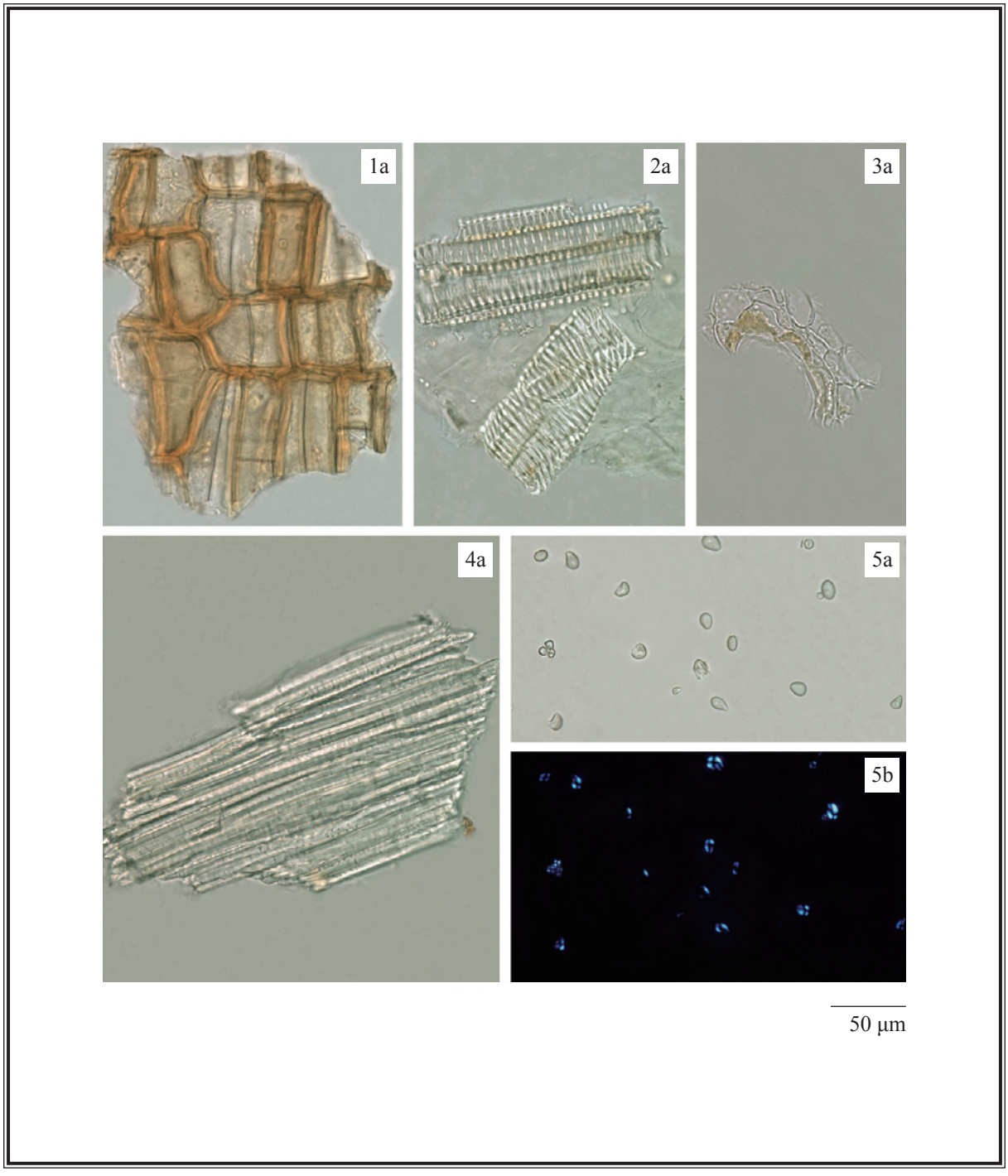

Figure 4 (i) Microscopic features of powder of dried rhizome and root of *Ligusticum sinense* Oliv.

1. Cork cells 2. Vessels 3. Oil cavity fragment 4. Fibres 5. Starch granules

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

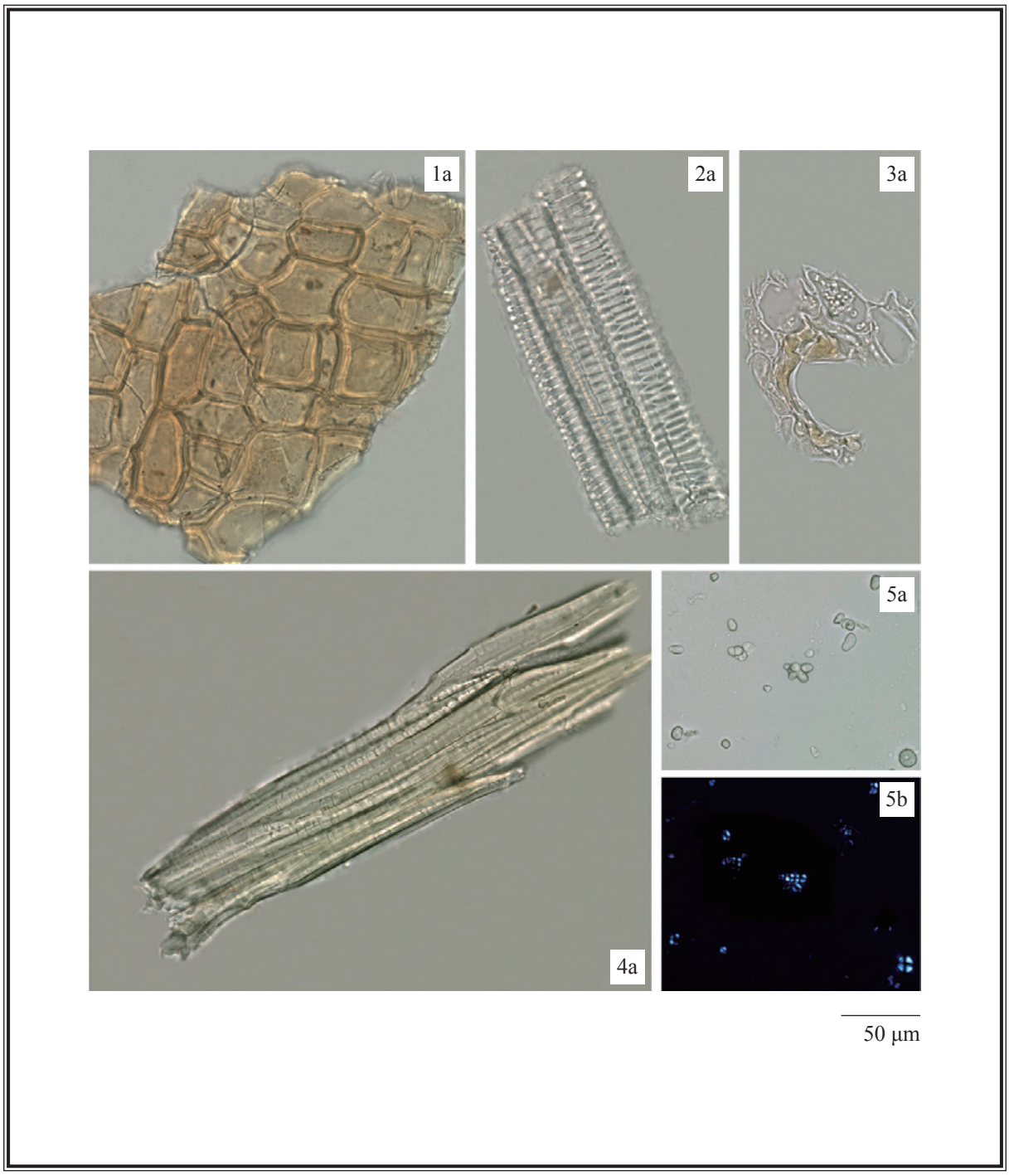

Figure 4 (ii) Microscopic features of powder of dried rhizome and root of *Ligusticum jeholense* Nakai et Kitag.

1. Cork cells 2. Vessels 3. Oil cavity fragment 4. Fibres 5. Starch granules

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

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

Standard solution

(E)-ferulic acid standard solution

Weigh 1.0 mg of (*E*)-ferulic acid CRS (Fig. 5) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of cyclohexane, ethyl acetate and glacial acetic acid (6:3:0.4, 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. Sonicate (270 W) the mixture for 30 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 ethanol.

Procedure

Carry out the method by using a TLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately (*E*)-ferulic acid standard solution (2 μL) and the test solution (1 μL) to the plate. 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).

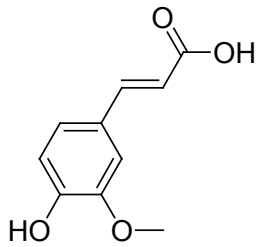

Figure 5 Chemical structure of (*E*)-ferulic acid

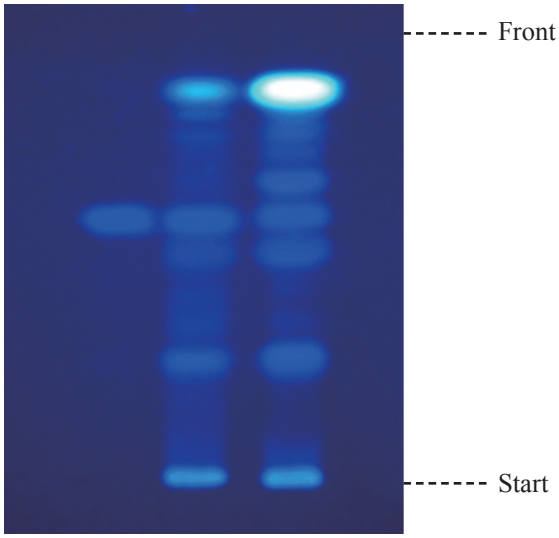

Figure 6 A reference TLC chromatogram of Ligustici Rhizoma et Radix extract observed under UV light (366 nm)

1. (*E*)-ferulic acid standard solution
2. Test solution of
(i) dried rhizome and root of *Ligusticum sinense* Oliv.
(ii) dried rhizome and root of *Ligusticum jeholense* Nakai et Kitag.

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 (E)-ferulic acid (Fig. 6).

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

Standard solution

(E)-ferulic acid standard solution for fingerprinting, Std-FP (25 mg/L)

Weigh 0.5 mg of (*E*)-ferulic acid CRS and dissolve in 20 mL of ethanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 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% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 60	95 → 27.5	5 → 72.5	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of (*E*)-ferulic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of (*E*)-ferulic acid should not be more than 5.0%; the RSD of the retention time of (*E*)-ferulic acid peak should not be more than 2.0%; the column efficiency determined from (*E*)-ferulic acid peak should not be less than 45000 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. 7 (i) or (ii)].

Procedure

Separately inject (*E*)-ferulic acid Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of (*E*)-ferulic acid peak in the chromatogram of (*E*)-ferulic acid Std-FP and the retention times of the three characteristic peaks [Fig. 7 (i) or (ii)] in the chromatogram of the test solution. Identify (*E*)-ferulic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of (*E*)-ferulic acid Std-FP. The retention times of (*E*)-ferulic 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 three characteristic peaks of Ligustici Rhizoma et Radix extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Ligustici Rhizoma et Radix extract

Peak No.	RRT	Acceptable Range
1 [marker, (*E*)-ferulic acid]	1.00	-
2	1.92	± 0.03
3	2.21	± 0.03

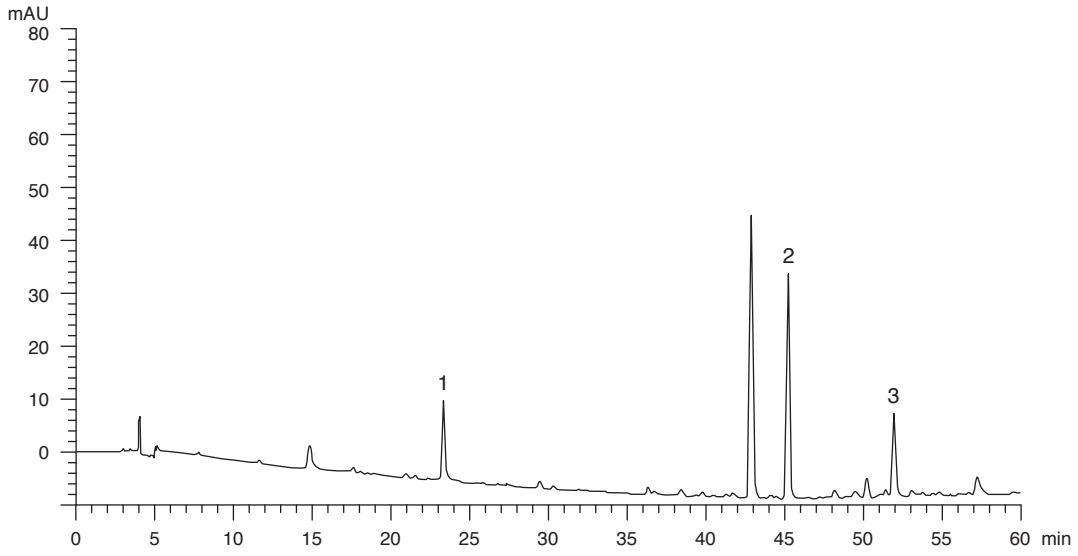

Figure 7 (i) A reference fingerprint chromatogram of dried rhizome and root of *Ligusticum sinense* Oliv. extract

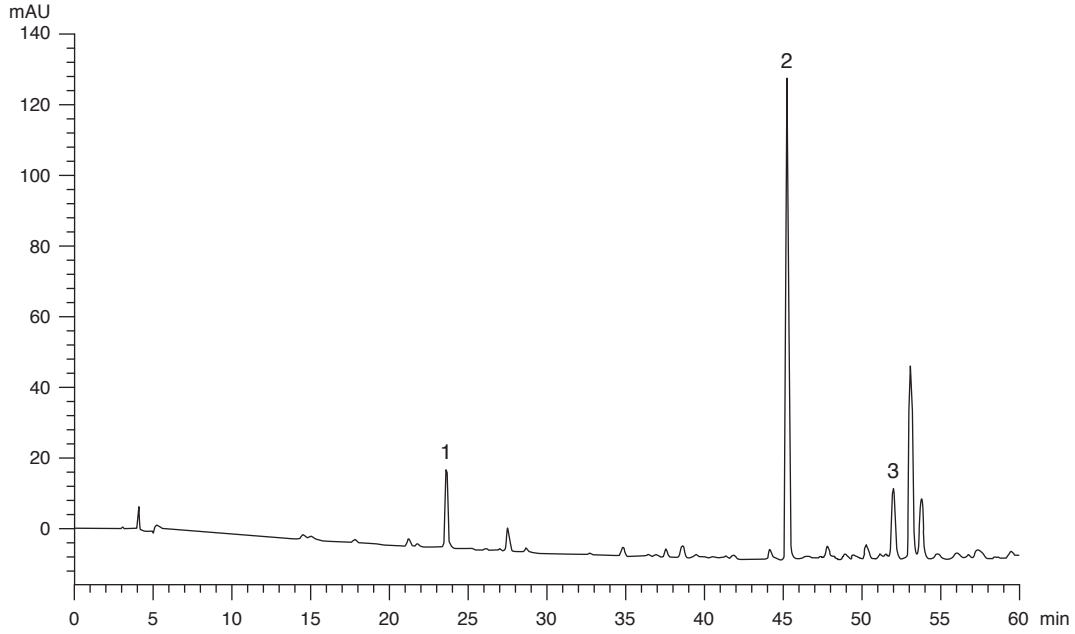

Figure 7 (ii) A reference fingerprint chromatogram of dried rhizome and root of *Ligusticum jeholense* Nakai et Kitag. extract

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 respective reference fingerprint chromatograms [Fig. 7 (i) or (ii)].

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 6.5%.
Acid-insoluble ash: not more than 2.5%.

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

7. ASSAY

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

Standard solution

(E)-ferulic acid standard stock solution, Std-Stock (300 mg/L)

Weigh accurately 3.0 mg of (*E*)-ferulic acid CRS and dissolve in 10 mL of ethanol.

(E)-ferulic acid standard solution for assay, Std-AS

Measure accurately the volume of the (*E*)-ferulic acid Std-Stock, dilute with ethanol to produce a series of solutions of 1.25, 5, 12.5, 25, 50 mg/L for (*E*)-ferulic 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 ethanol. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (320 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% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 60	95 → 27.5	5 → 72.5	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of (*E*)-ferulic acid Std-AS (12.5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of (*E*)-ferulic acid should not be more than 5.0%; the RSD of the retention time of (*E*)-ferulic acid peak should not be more than 2.0%; the column efficiency determined from (*E*)-ferulic acid peak should not be less than 45000 theoretical plates.

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

Limits

The sample contains not less than 0.050% of (*E*)-ferulic acid ($C_{10}H_{10}O_4$), calculated with reference to the dried substance.