Atractylodis Rhizoma

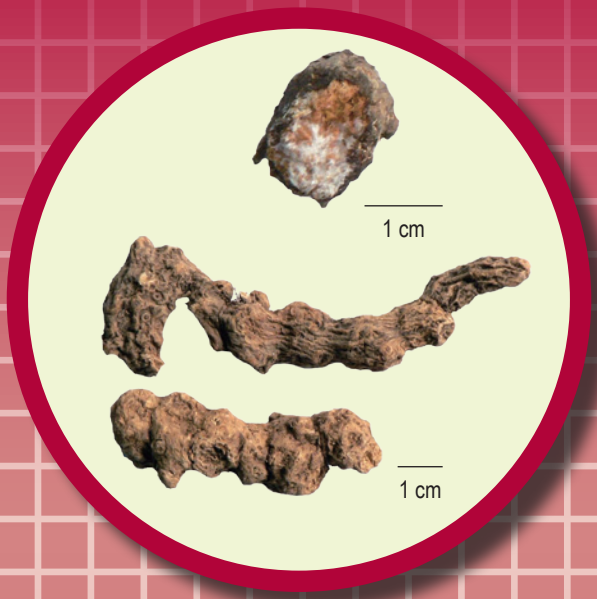


Figure 1 (i) A photograph of dried rhizome of *Atractylodes lancea* (Thunb.) DC.

Figure 1 (ii) A photograph of dried rhizome of *Atractylodes chinensis* (DC.) Koidz.

1. NAMES

Official Name: Atractylodis Rhizoma

Chinese Name: 蒼朮

Chinese Phonetic Name: Cangzhu

2. SOURCE

Atractylodis Rhizoma is the dried rhizome of *Atractylodes lancea* (Thunb.) DC. or *Atractylodes chinensis* (DC.) Koidz. (Asteraceae). The rhizome is collected in spring and autumn, freed of aerial part, dried under the sun, followed by the removal of fibrous roots to obtain Atractylodis Rhizoma.

3. DESCRIPTION

***Atractylodes lancea* (Thunb.) DC. :** Irregularly moniliform or nodular-cylindrical, slightly curved, occasionally branched, 2-9.3 cm long, 5-42 mm in diameter. Externally greyish-brown to dark brown, marked with wrinkle, remains of rootlets and stem scars. Texture compact, fracture fibrous-like, pale yellow or greyish-white, scattered with many reddish-brown oil spots, and white fine needle crystals appear after exposing for a long time. Odour aromatic; taste slightly sweetish, pungent and bitter [Fig. 1(i)].

***Atractylodes chinensis* (DC.) Koidz. :** Knotty-lumpy or nodular-cylindrical, slightly curved, occasionally branched. 3.2-11.6 cm long, 6-28 mm in diameter. Externally dark brown. Texture relatively loose, fracture fibrous-like, pale yellow or greyish-white, and scattered with yellowish-brown oil spots. Odour weakly aromatic; taste pungent and bitter [Fig. 1(ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

***Atractylodes lancea* (Thunb.) DC. :** Cork consists of 10 or more layers of cork cells, containing stone cells bands and composing of 1-3 layers of stone cells. Cortex relatively broad. Phloem narrow. Cambium in a ring. Fibres in bundles, mainly existing in xylem, large and mostly, usually alternate arrangement with vessels. Vessels singly or in group, arranged radially. Oil

cavities scattered in cortex, phloem, ray and pith, ellipsoid or subrounded, 75-525 μm in diameter. Parenchymatous cells contain small raphides of calcium oxalate [Fig. 2(i)].

***Atractylodes chinensis* (DC.) Koidz. :** Cortex broad. Fibres in bundles, mainly existing in the inner of xylem, relatively few, alternate arrangement with vessels. Oil cavities relatively few and large, 125-718 μm in diameter scattered in parenchyma; oil cavities are large in pith [Fig. 2(ii)].

Powder

Colour yellowish-brown to brown. Raphides of calcium oxalate extremely abundant, minute, 4-29 μm long, irregularly filled in parenchymatous cells, often lean to one side of cell or scattered throughout; polychromatic under the polarized microscope. Stone cells fairly abundant, sometimes linking up with cork cells, polygonal, subrounded or subrectangular, 11-95 μm in diameter, with heavily thickened walls. Inulin frequently visible, mass or fan-shaped, with radial striations on surface; slightly bright blue under the polarized microscope. Mainly reticulate vessels or bordered pits vessels, 8-66 μm in diameter. Fibres singly scattered or dispersed in bundles, 9-60 μm in diameter, pit canals thin and dense, with rather thickened lignified walls; polychromatic under the polarized microscope. Cork cells subpolygonal or subrectangular in shape, sometimes linking up with stone cells. Fragment of oil cavities occasionally visible (Fig. 3).

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

Standard solution

Atractylodin standard solution

Weigh 1.2 mg of atractylodin CRS (Fig. 4) and place it in a 5-mL brown volumetric flask. Make up to the mark with n-hexane.

Developing solvent system

Prepare a mixture of n-hexane and ethyl acetate (10:0.8, v/v).

Spray reagent

Weigh 5 g of *p*-dimethylaminobenzaldehyde and dissolve in 100 mL of sulphuric acid (10%, v/v).

Test solution

Weigh 1.0 g of the freshly powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethyl acetate. Sonicate (90 W) the mixture for 15 min. Filter the mixture.

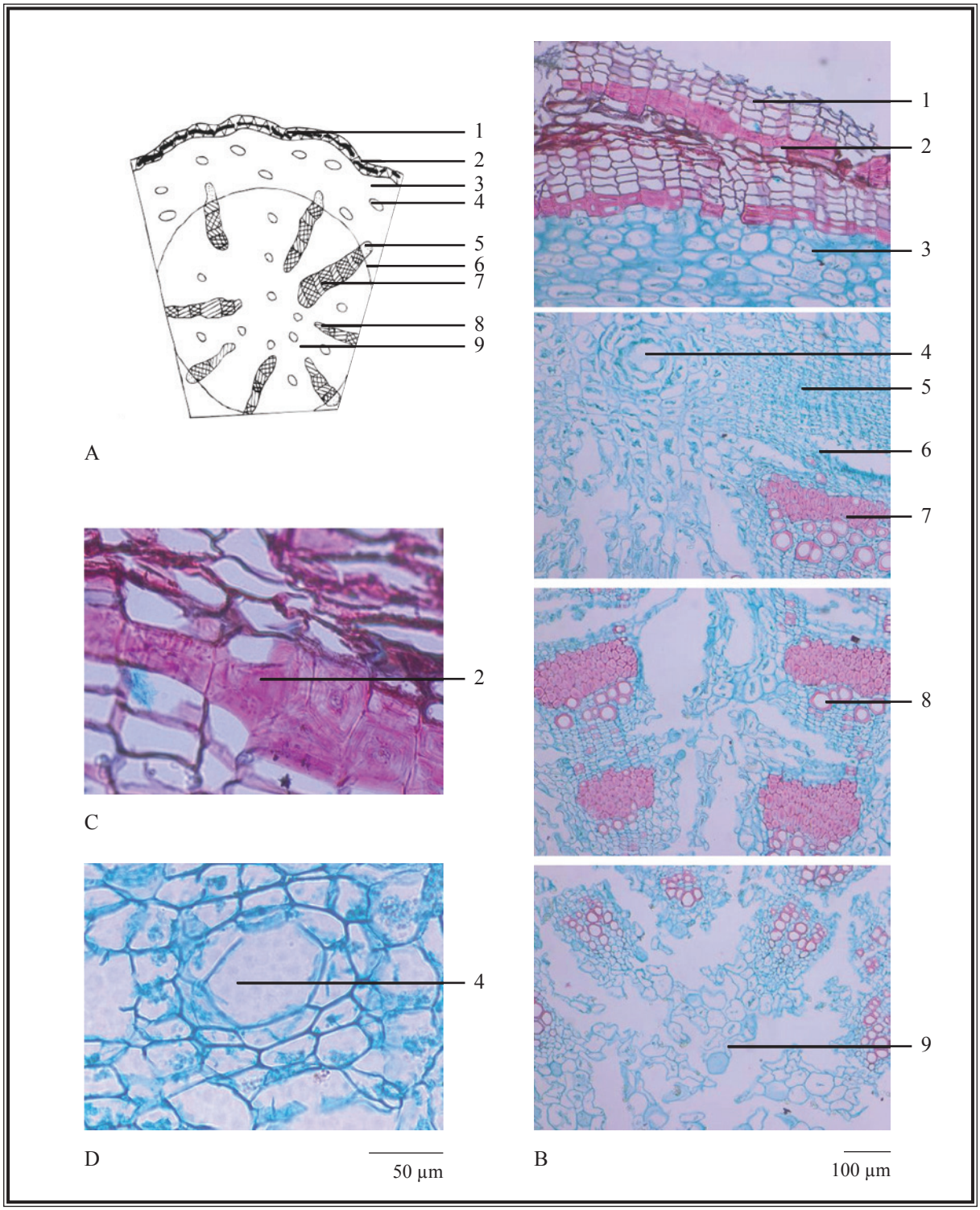


Figure 2(i) Microscopic features of transverse section of dried rhizome of *Atractylodes lancea* (Thunb.) DC.

A. Sketch B. Section illustration C. Stone cell band D. Oil cavity

1. Cork 2. Stone cell band 3. Cortex 4. Oil cavity 5. Phloem 6. Cambium 7. Fibres 8. Xylem 9. Pith

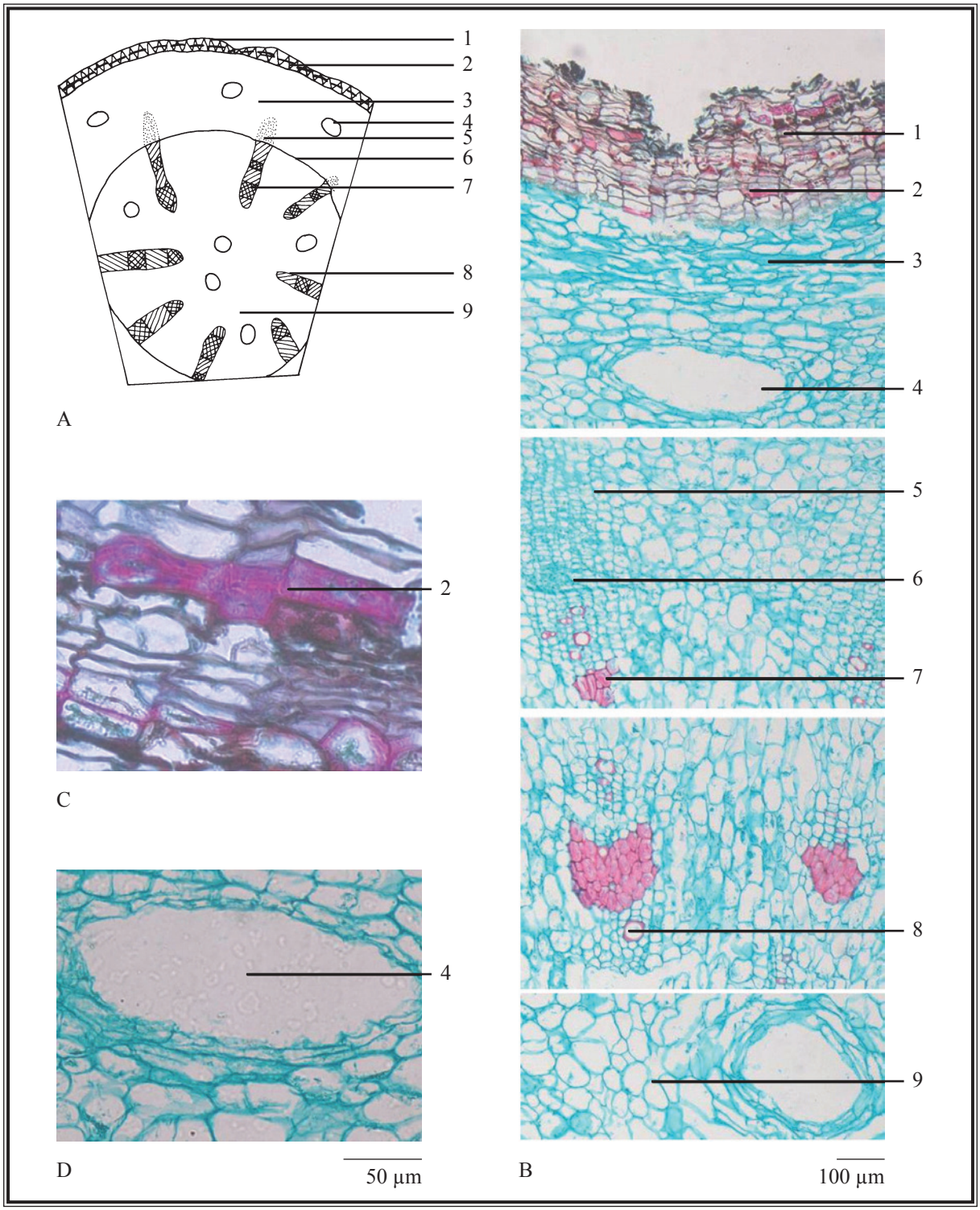


Figure 2(ii) Microscopic features of transverse section of dried rhizome of *Atractylodes chinensis* (DC.) Koidz.

A. Sketch B. Section illustration C. Stone cell band D. Oil cavity

1. Cork 2. Stone cell band 3. Cortex 4. Oil cavity 5. Phloem 6. Cambium
7. Fibres 8. Xylem 9. Pith

Figure 3(i) Microscopic features of powder of dried rhizome of *Atractylodes lancea* (Thunb.) DC.

1. Raphides of calcium oxalate 2. Stone cell 3. Inulin 4. Vessels 5. Fibre
6. Cork cells 7. Fragment of oil cavity

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

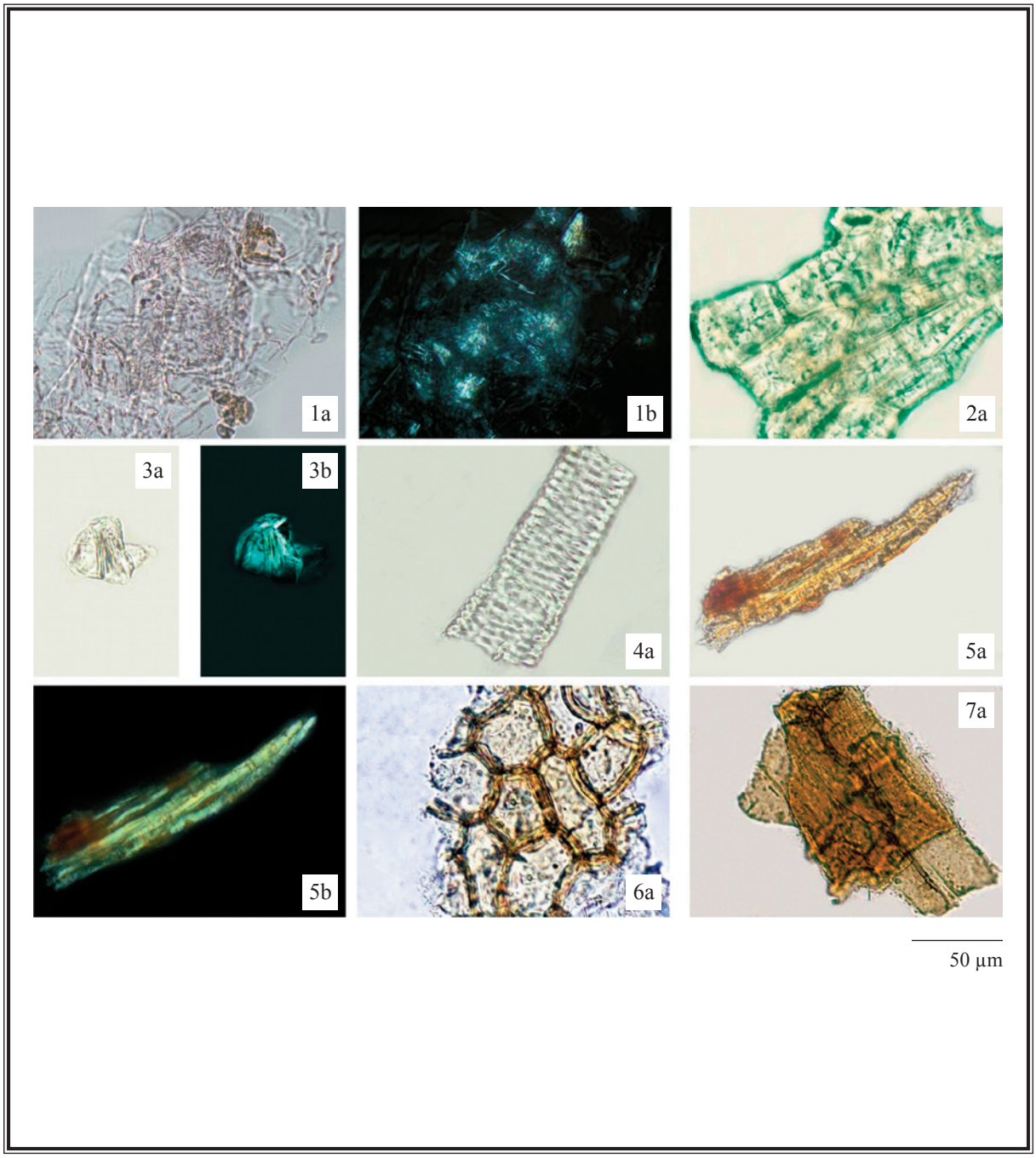


Figure 3(ii) Microscopic features of powder of dried rhizome of *Atractylodes chinensis* (DC.) Koidz.

1. Raphides of calcium oxalate 2. Stone cells 3. Inulin 4. Vessel 5. Fibres
6. Cork cells 7. Fragment of oil cavity

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

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 atractylodin standard solution and the test solution (10 µL each) 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 8.5 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 105°C until the spots or bands become visible (about 2 min). Examine the plate under visible light. 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 atractylodin.

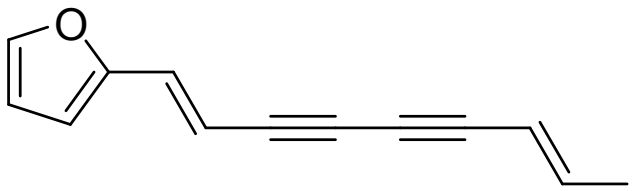


Figure 4 Chemical structure of atractylodin

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

Standard solution

Atractylodin standard solution for fingerprinting, Std-FP (18 mg/L)

Weigh 0.9 mg of atractylodin CRS and place it in a 50-mL brown volumetric flask. Make up to the mark with methanol.

Test solution

Weigh 0.1 g of freshly powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol. Sonicate (90 W) the mixture for 15 min. Centrifuge at about 1800 × *g* for 10 min. Filter through a 0.45-µm nylon 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)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 60	55 → 15	45 → 85	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of atractylodin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of atractylodin should not be more than 5.0%; the RSD of the retention time of atractylodin peak should not be more than 2.0%; the column efficiency determined from atractylodin peak should not be less than 50000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 [Fig. 5(i) or (ii)].

Procedure

Separately inject atractylodin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of atractylodin peak in the chromatogram of atractylodin Std-FP and the retention times of the three characteristic peaks [Fig. 5(i) or (ii)] in the chromatogram of the test solution. Identify atractylodin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of atractylodin Std-FP. The retention times of atractylodin 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 Atractylodis Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Atractylodis Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.76	± 0.03
2	0.83	± 0.03
3 (marker, atractylodin)	1.00	-

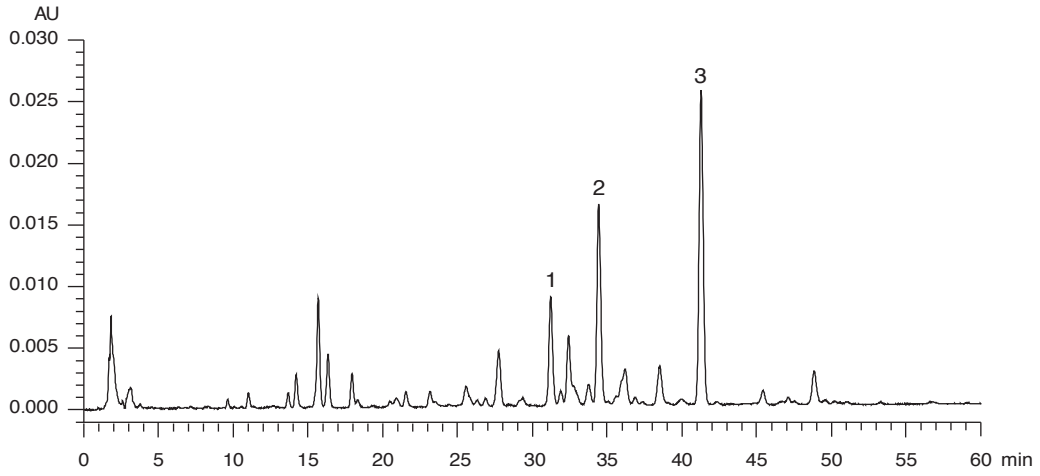


Figure 5 (i) A reference fingerprint chromatogram of dried rhizome of *Atractylodes lancea* (Thunb.) DC. extract

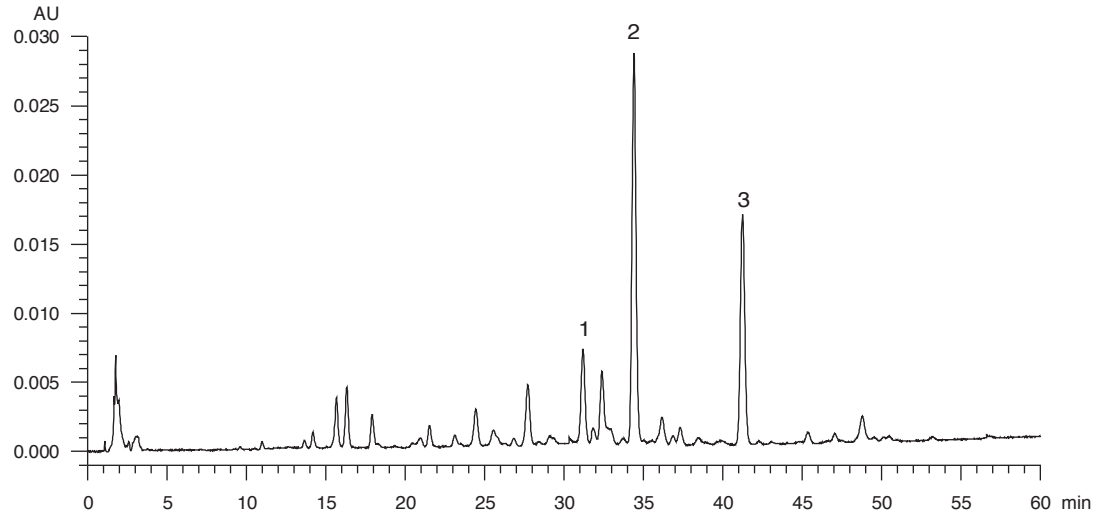


Figure 5 (ii) A reference fingerprint chromatogram of dried rhizome of *Atractylodes chinensis* (DC.) Koidz. extract

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. 5(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 – Aflatoxins *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XVIII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%

5.6 Ash *(Appendix IX)*

Total ash: not more than 7.0%
Acid-insoluble ash: not more than 1.0%

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 11.0%

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 35.0%.
Ethanol-soluble extractives (hot extraction method): not less than 25.0%.

7. ASSAY

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

Standard solution

Atractylodin standard stock solution, Std-Stock (60 mg/L)

Weigh accurately 1.5 mg of atractylodin CRS and place it in a 25-mL brown volumetric flask. Make up to the mark with methanol.

Atractylodin standard solution for assay, Std-AS

Measure accurately the volume of the atractylodin Std-Stock, dilute with methanol to produce a series of solutions of 6, 12, 18, 30, 42 mg/L for atractylodin.

Test solution

Weigh accurately 0.1 g of the freshly powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol. Sonicate (90 W) the mixture for 15 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Make up to the mark with methanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (340 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 methanol and water (85:15, v/v). The elution time is about 15 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of atractylodin Std-AS (18 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of atractylodin should not be more than 5.0%; the RSD of the retention time of atractylodin peak should not be more than 2.0%; the column efficiency determined from atractylodin peak should not be less than 10000 theoretical plates.

The *R* value between atractylodin 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 atractylodin Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of atractylodin against the corresponding concentrations of atractylodin Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

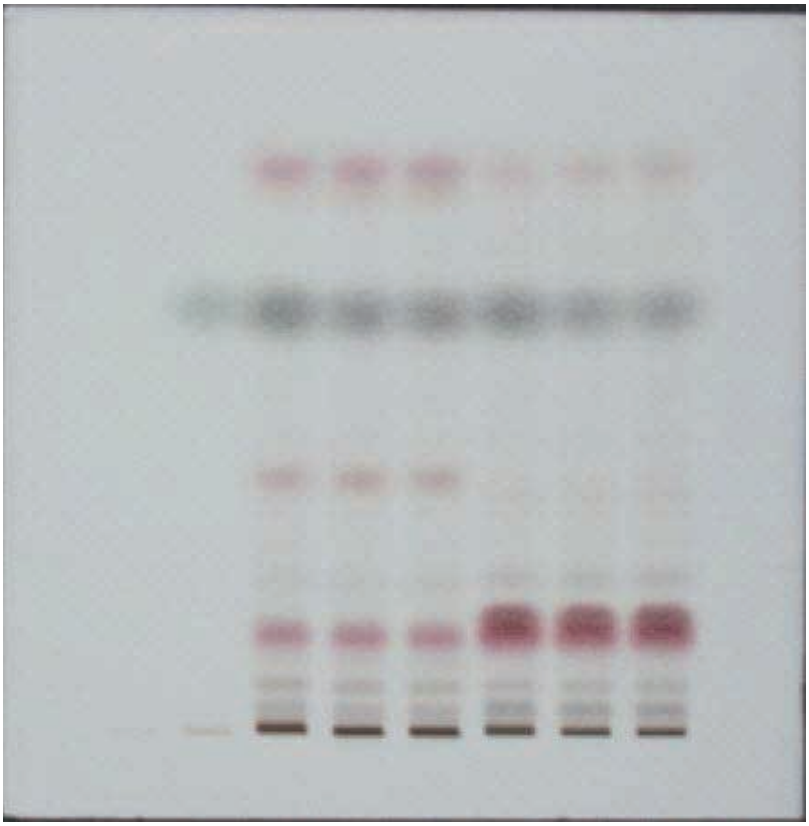
Procedure

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify atractylodin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of atractylodin Std-AS. The retention times of atractylodin 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 atractylodin in the test solution, and calculate the percentage content of atractylodin in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.32% of atractylodin ($C_{13}H_{10}O$), calculated with reference to the dried substance.

Atractylodis Rhizoma　(蒼朮)



Lane	Sample	Results
1	Blank (Ethyl acetate)	Negative
2	Standard (Atractylodin)	Atractylodin positive
3	Spiked sample [*Atractylodes chinensis* (DC.) Koidz. plus atractylodin]	Atractylodin positive
4	Sample [*Atractylodes chinensis* (DC.) Koidz.]	Atractylodin positive
5	Sample duplicate [*Atractylodes chinensis* (DC.) Koidz.]	Atractylodin positive
6	Spiked sample [*Atractylodes lancea* (Thunb.) DC. plus atractylodin]	Atractylodin positive

7	Sample [*Atractylodes lancea* (Thunb.) DC.]	Atractylodin positive
8	Sample duplicate [*Atractylodes lancea* (Thunb.) DC.]	Atractylodin positive

Figure 1 TLC results of the dried rhizome of *Atractylodes chinensis* (DC.) Koidz. and *Atractylodes lancea* (Thunb.) DC. extract observed under visible light after staining