Curcumae Radix

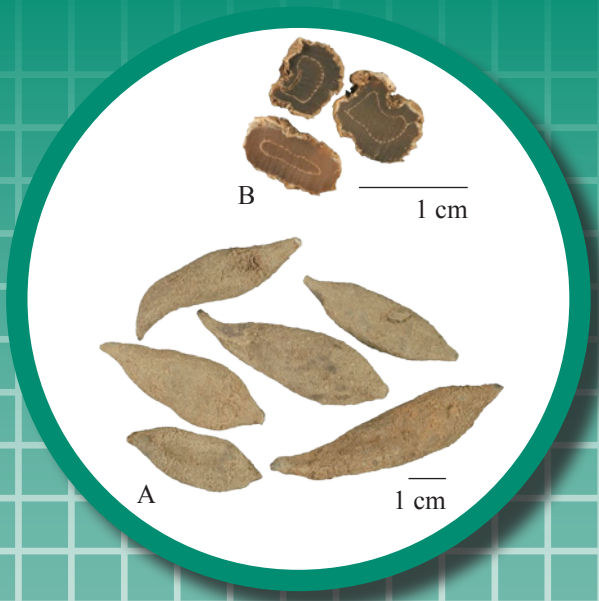

Figure 1 (i) A photograph of the dried root tubers of *Curcuma wenyujin* Y. H. Chen et C. Ling

A. Root tubers B. Magnified transverse section of root tubers

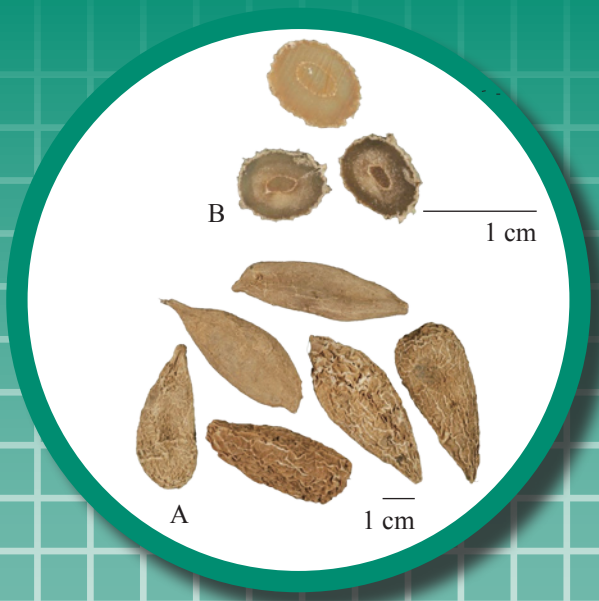

Figure 1 (ii) A photograph of the dried root tubers of *Curcuma kwangsiensis* S. G. Lee et C. F. Liang

A. Root tubers B. Magnified transverse section of root tubers

Figure 1 (iii) A photograph of the dried root tubers of *Curcuma phaeocaulis* Val.

A. Root tubers B. Magnified transverse section of root tubers

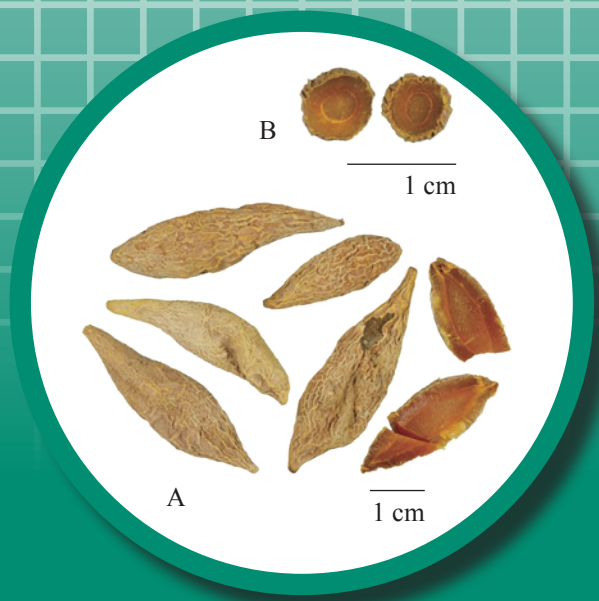

Figure 1 (iv) A photograph of the dried root tubers of *Curcuma longa* L.

A. Root tubers B. Magnified transverse section of root tubers

1. NAMES

Official Name: Curcumae Radix

Chinese Name: 鬱金

Chinese Phonetic Name: Yujin

2. SOURCE

Curcumae Radix is the dried root tuber of *Curcuma wenyujin* Y. H. Chen et C. Ling, *Curcuma longa* L., *Curcuma kwangsiensis* S. G. Lee et C. F. Liang, or *Curcuma phaeocaulis* Val. (Zingiberaceae). The root tuber derived from the former two are known as ‘‘Wenyujin’’ and ‘‘Huangsiyujin’’, respectively, and the root tuber derived from the others are known as ‘‘Guiyujin’’ or ‘‘Lusiyujin’’, respectively, according to differences in the appearance. The root tuber is collected in winter when stem and leaves wither, soil and rootlets removed, boiled thoroughly, then dried under the sun or baked at 60°C to obtain Curcumae Radix.

Part I Dried root tuber of *Curcuma wenyujin* Y. H. Chen et C. Ling, *Curcuma kwangsiensis* S. G. Lee et C. F. Liang and *Curcuma phaeocaulis* Val.

3. DESCRIPTION

***Curcuma wenyujin* Y. H. Chen et C. Ling:** Root tuber oblong or ovoid, slightly compressed or curved, both ends taper, 2.1-8.3 cm long, 6-19 mm in diameter. Externally pale brown or greyish-brown, with irregular longitudinal wrinkles, pale colour at the protuberant parts. Texture hard and compact; fracture greyish-brown and corneous; endodermis ring distinct. Odour slightly aromatic; taste slightly bitter [Fig. 1 (i)].

***Curcuma kwangsiensis* S. G. Lee et C. F. Liang:** Root tuber long conical or oblong, 2.1-7.6 cm long, 7-23 mm in diameter. Externally pale brown or reddish-brown, with sparse and shallow wrinkles or relatively tough reticulate wrinkles. Texture hard and compact; fracture greyish-brown or brown and corneous; endodermis ring distinct. Odour slight; taste slightly pungent and bitter [Fig. 1 (ii)].

***Curcuma phaeocaulis* Val.:** Root tuber long ellipsoid, relatively stout, 1.1-3.8 cm long, 7-15 mm in diameter. Externally grey or greyish-black, with wrinkles. Texture hard and compact; fracture brown or greyish-black, semi-corneous; endodermis ring distinct. Odour slight; taste bland [Fig. 1 (iii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root tuber:

***Curcuma wenyujin* Y. H. Chen et C. Ling:** Epidermal cells sometimes remain, the outer walls slightly thickened. Velamen narrow, consisting of 4-9 layers of cells with slightly sinuous and thin walls, arranged regularly. Cortex occupies about 1/2 of root. Oil cells rarely visible. Endodermis distinct. In stele, phloem bundles and xylem bundles each 35-52, arranged alternatively; each phloem bundle with 2-4 vessels, xylem fibres slightly lignified; vessels polygonal, with thin wall. Gelatinized starch granules in parenchymatous cells visible [Fig. 2 (i)].

***Curcuma kwangsiensis* S. G. Lee et C. F. Liang:** Velamen consists of 3-6 layers of cells, cell walls occasionally thickened, the inner side of velamen showing 1-2 layers of sclerenchymatous cells arranged in a ring, with distinct striation. In stele, phloem bundles and xylem bundles each 37-58, arranged alternatively; each phloem bundle with 2-3 subrounded vessels [Fig. 2 (ii)].

***Curcuma phaeocaulis* Val.:** Velamen consists of 3-6 layers of cells with thin walls. In stele, phloem bundles (shriveled) and xylem bundles each 42-62, arranged alternatively; each phloem bundle with 1 or 2-3 flattened vessels [Fig. 2 (iii)].

Powder

***Curcuma wenyujin* Y. H. Chen et C. Ling:** Colour grey. Gelatinized starch granules scattered singly or in clumps, greyish-white, rounded, oblong or irregular polyhedral. Oil cells rare, subrounded or oblong, containing pale yellow or brownish-yellow oil droplets or secretions. Velamen cells greyish-brown, walls lignified and thickened. Vessels mainly spiral, scalariform or reticulate, 25-100 μm in diameter [Fig. 3 (i)].

***Curcuma kwangsiensis* S. G. Lee et C. F. Liang:** Colour greyish-brown. Gelatinized starch granules scattered singly or in clumps, greyish-white, rounded-ovate, oblong or subrounded-polyhedral. Oil cells rare, subrounded or oblong, containing brownish-red oil droplets or secretions. Velamen cells greyish-brown, consisting of several layers of cells, frequently overlapping. Vessels mainly spiral, scalariform or reticulate, up to 170 μm in diameter [Fig. 3 (ii)].

***Curcuma phaeocaulis* Val.:** Colour brown. Gelatinized starch granules scattered singly or in clumps, greyish-white, rounded-ovate, oblong or subrounded-polyhedral. Oil cells rare, elongated-subrounded, subrounded or oblong, containing brownish-red oil droplets or secretions. Velamen cells greyish-brown, consisting of several layers of cells, frequently overlapping. Vessels mainly spiral, scalariform or reticulate, 25-150 μm in diameter [Fig. 3 (iii)].

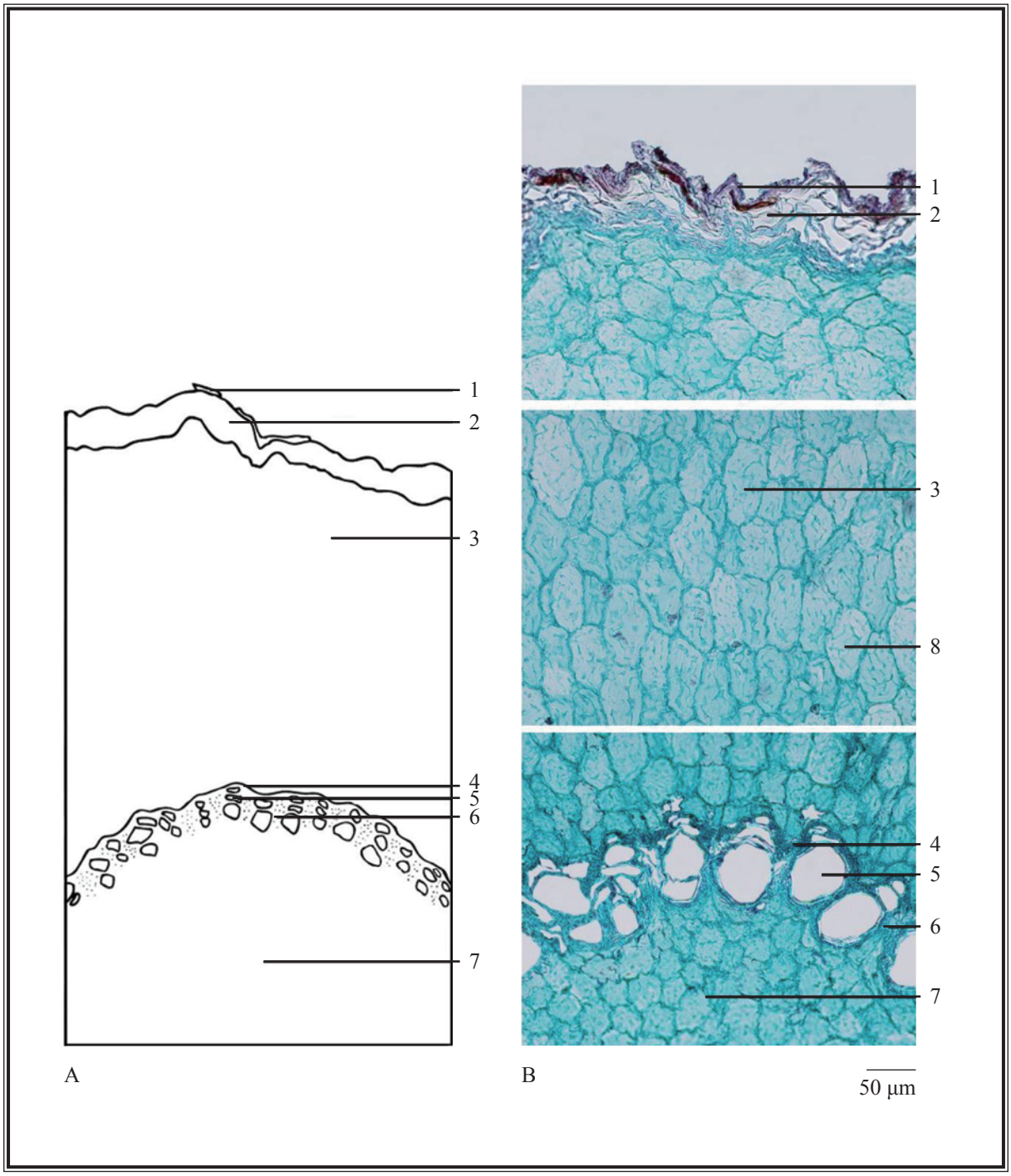

Figure 2 (i) Microscopic features of transverse section of dried root tuber of *Curcuma wenyujin* Y. H. Chen et C. Ling

A. Sketch B. Section illustration

1. Epidermis 2. Velamen 3. Cortex 4. Endodermis 5. Xylem 6. Phloem
7. Pith 8. Gelatinized starch granules

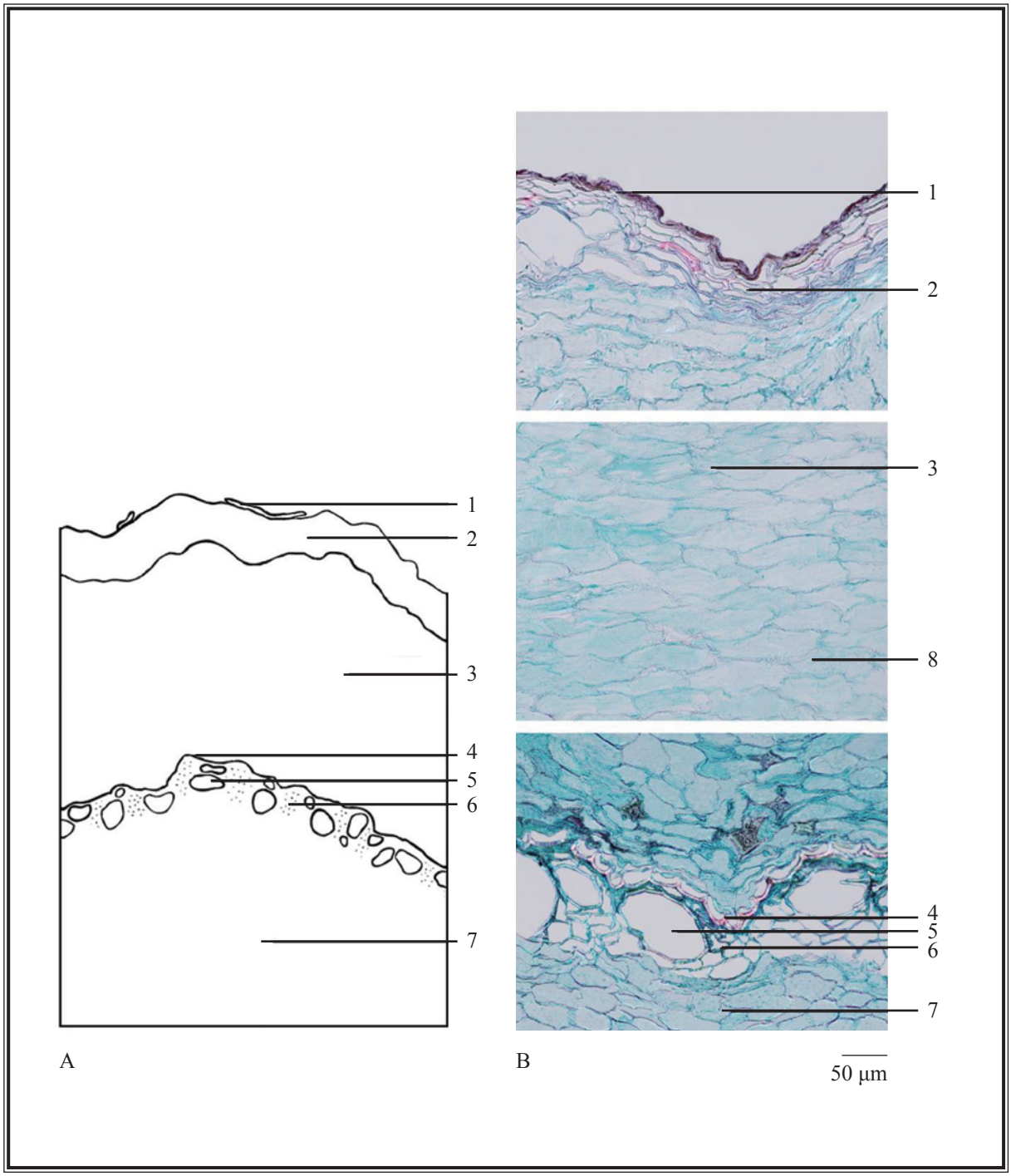

Figure 2 (ii) Microscopic features of transverse section of dried root tuber of *Curcuma kwangsiensis* S. G. Lee et C. F. Liang

A. Sketch B. Section illustration

1. Epidermis 2. Velamen 3. Cortex 4. Endodermis 5. Xylem 6. Phloem
7. Pith 8. Gelatinized starch granules

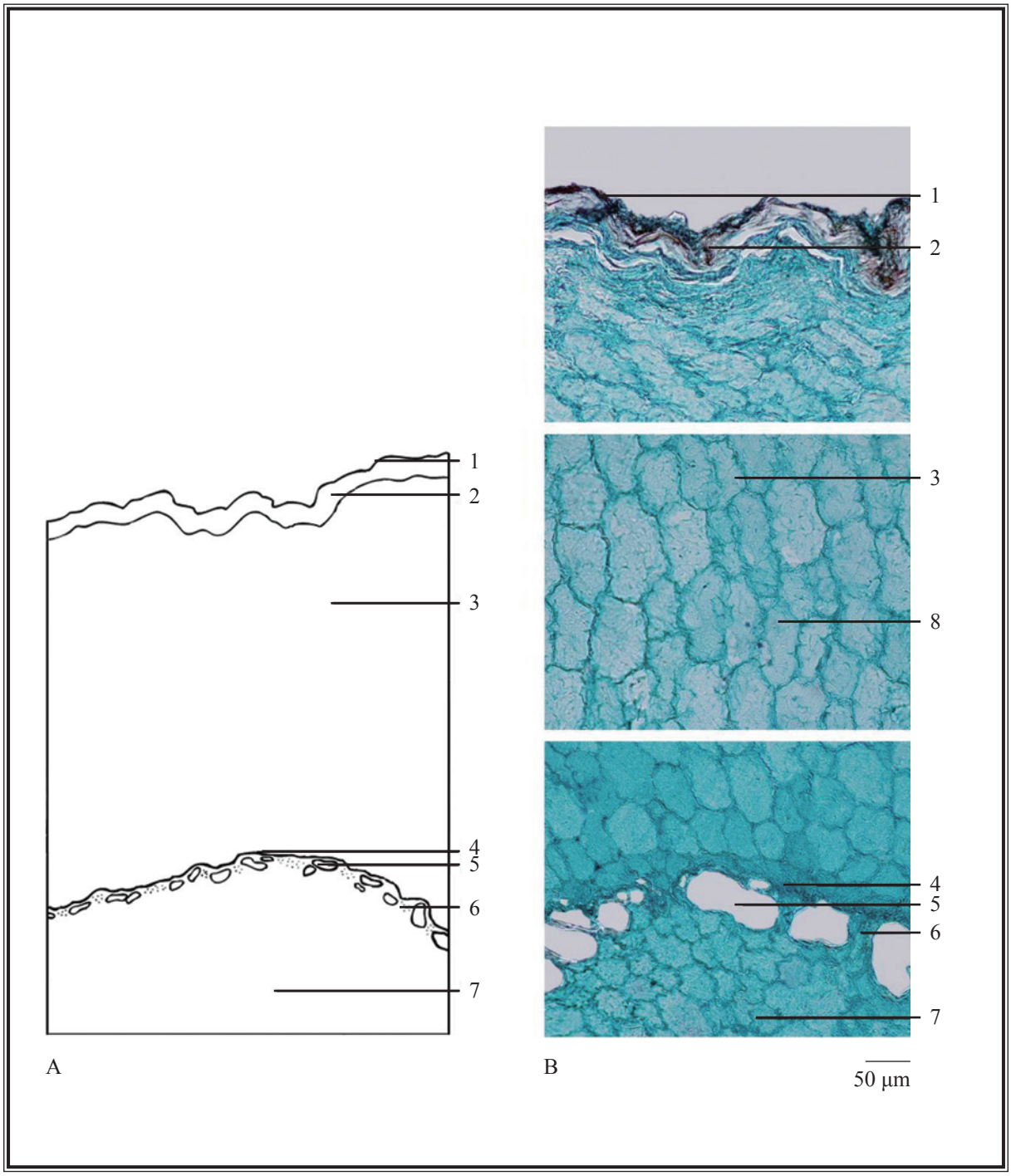

Figure 2 (iii) Microscopic features of transverse section of dried root tuber of *Curcuma phaeocaulis* Val.

A. Sketch B. Section illustration

1. Epidermis 2. Velamen 3. Cortex 4. Endodermis 5. Xylem 6. Phloem 7. Pith
8. Gelatinized starch granules

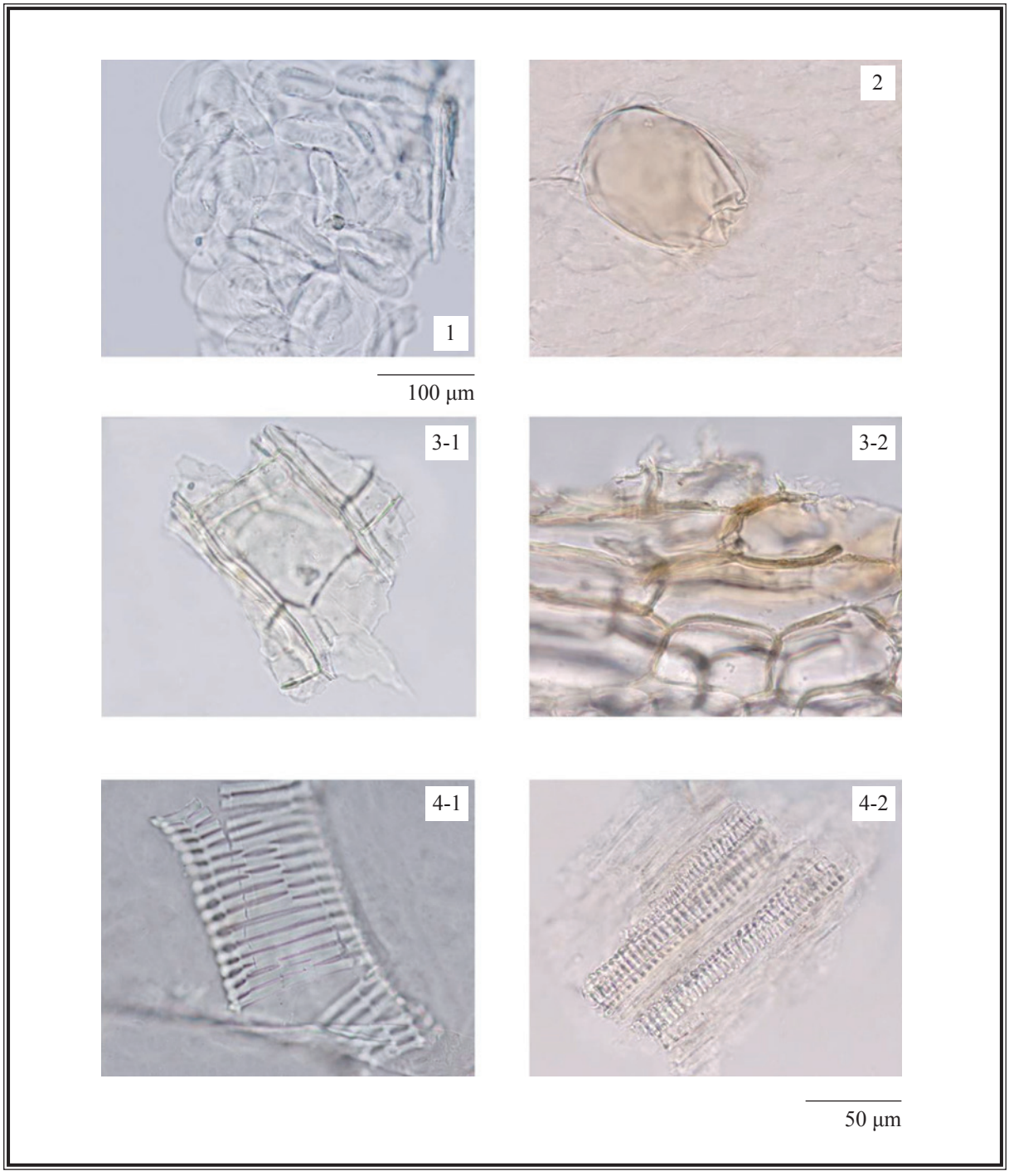

Figure 3 (i) Microscopic features of powder of dried root tuber of *Curcuma wenyujin* Y. H. Chen et C. Ling (under the light microscope)

1. Parenchymatous cells contain gelatinized starch granules 2. Oil cell
3. Velamen cells 4. Vessels (4-1 scalariform vessel, 4-2 spiral vessels)

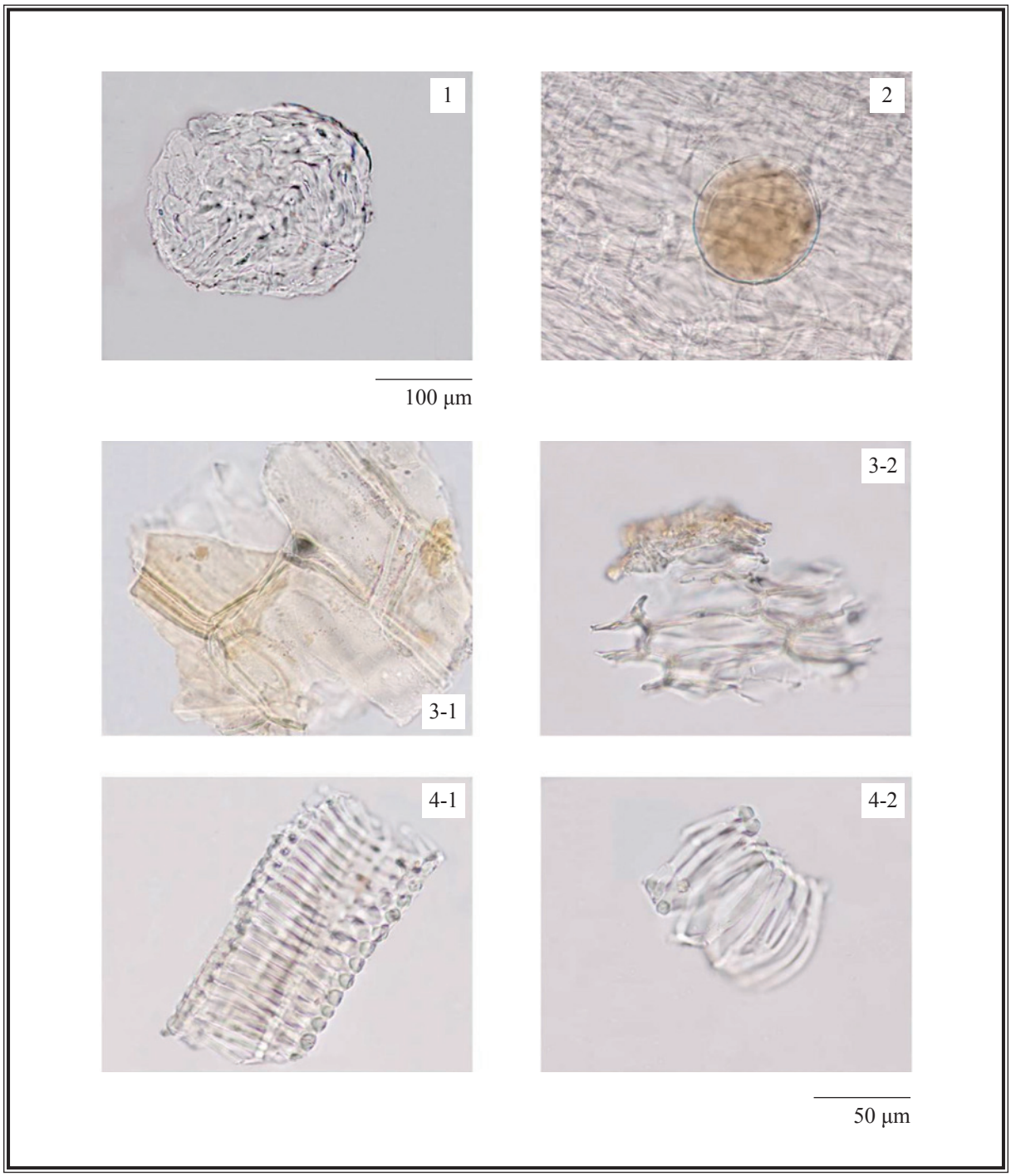

Figure 3 (ii) Microscopic features of powder of dried root tuber of *Curcuma kwangsiensis* S. G. Lee et C. F. Liang (under the light microscope)

1. Parenchymatous cells contain gelatinized starch granules 2. Oil cell
3. Velamen cells 4. Vessels (4-1 scalariform vessel, 4-2 spiral vessel)

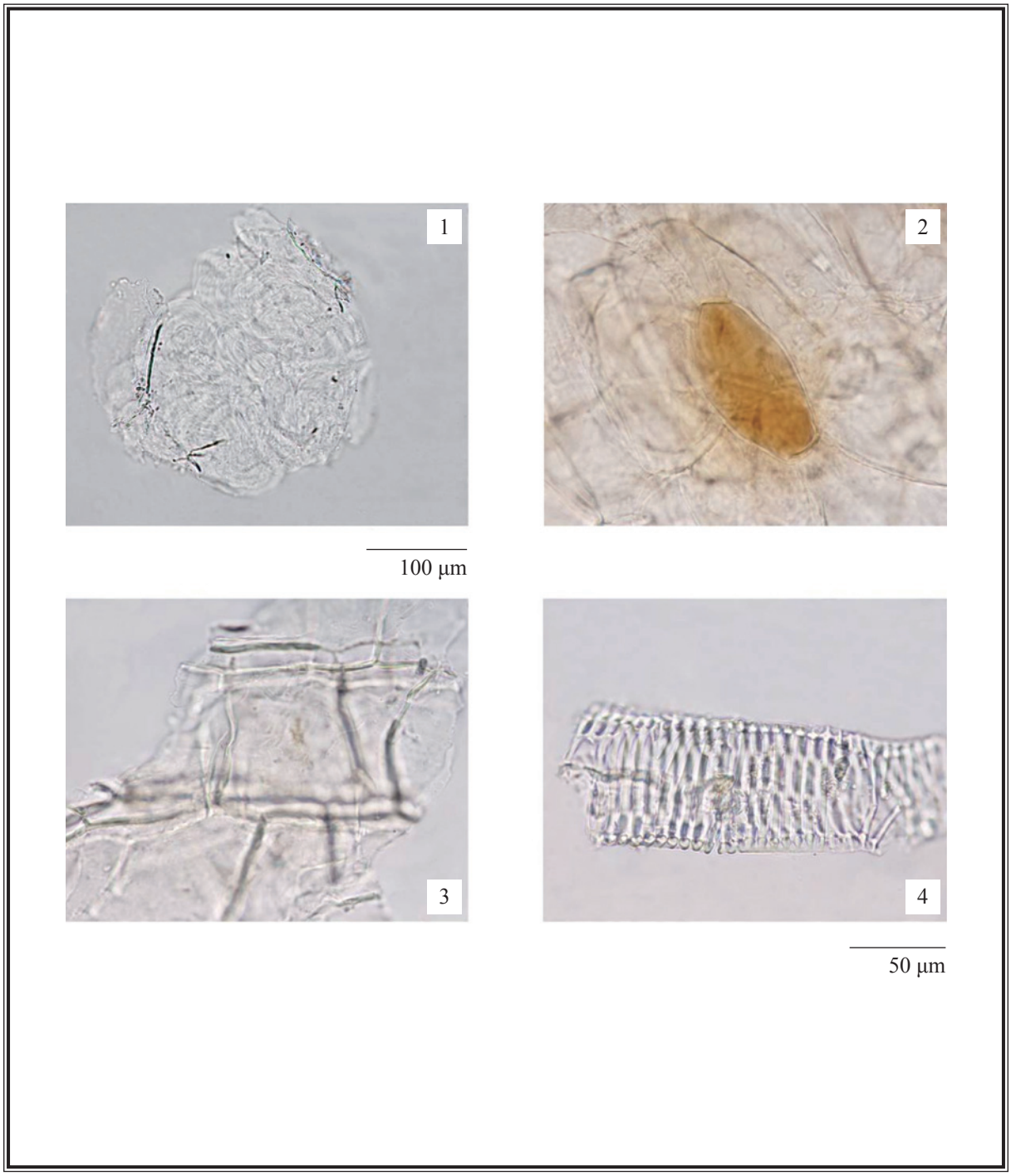

Figure 3 (iii) Microscopic features of powder of dried root tuber of *Curcuma phaeocaulis* Val. (under the light microscope)

1. Parenchymatous cells contain gelatinized starch granules 2. Oil cell
3. Velamen cells 4. Vessels

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

Standard solution

Germacrone standard solution

Weigh 2.0 mg of germacrone CRS (Fig. 4) and dissolve in 1 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC) and ethyl acetate (18:2, v/v).

Test solution

Weigh 10.0 g of the powdered sample and place it in a 100-mL conical flask, then add 50 mL of ethanol (95%). Sonicate (400 W) the mixture for 30 min. Filter and transfer the filtrate to a 150-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol (95%). Filter the solution.

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 germacrone standard solution (5 μL) and the test solution (5-8 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 value by using the equation as indicated in Appendix IV (A).

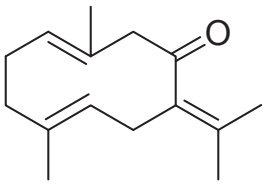

Figure 4 Chemical structure of germacrone

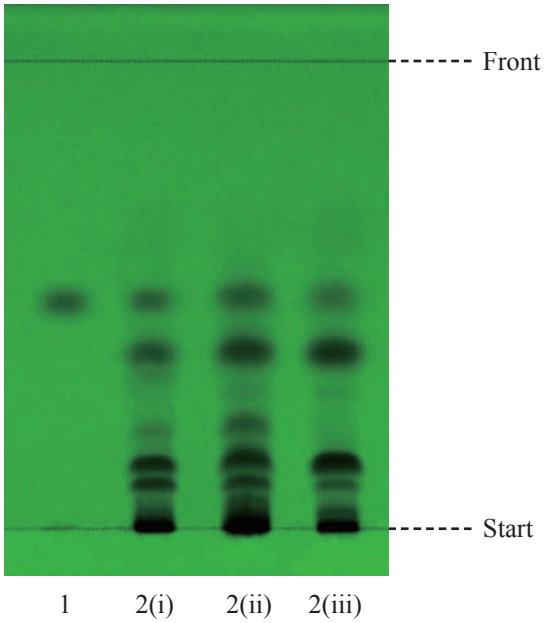

Figure 5 A reference HPTLC chromatogram of Curcumae Radix extract observed under UV light (254 nm)

1. Germacrone standard solution
2. Test solution of
(i) dried root tuber of *Curcuma wenyujin* Y. H. Chen et C. Ling
(ii) dried root tuber of *Curcuma kwangsiensis* S. G. Lee et C. F. Liang
(iii) dried root tuber of *Curcuma phaeocaulis* Val.

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 germacrone (Fig. 5).

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

Standard solution

Germacrone standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 1.0 mg of germacrone CRS and dissolve in 50 mL of methanol (50%).

Test solution

Weigh 2.5 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol (50%). Sonicate (400 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for two more times each with 15 mL of methanol (50%). Wash the residue with methanol (50%). Combine the solutions and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 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 – 35	51	49	isocratic
35 – 60	51 → 20	49 → 80	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of germacrone Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of germacrone should not be more than 5.0%; the RSD of the retention time of germacrone peak should not be more than 2.0%; the column efficiency determined from germacrone peak should not be less than 10000 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. 6 (i), (ii) or (iii)].

Procedure

Separately inject germacrone Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of germacrone peak in the chromatogram of germacrone Std-FP and the retention times of the four characteristic peaks [Fig. 6 (i), (ii) or (iii)] in the chromatogram of the test solution. Identify germacrone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of germacrone Std-FP. The retention times of germacrone 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 four characteristic peaks of dried root tuber of *Curcuma wenyujin* Y. H. Chen et C. Ling extract, *Curcuma kwangsiensis* S. G. Lee et C. F. Liang extract and *Curcuma phaeocaulis* Val. extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of dried root tuber of *Curcuma wenyujin* Y. H. Chen et C. Ling extract, *Curcuma kwangsiensis* S. G. Lee et C. F. Liang extract and *Curcuma phaeocaulis* Val. extract

Peak No.	RRT	Acceptable Range
1	0.62	± 0.03
2	0.76	± 0.03
3 (marker, germacrone)	1.00	-
4	1.70	± 0.03

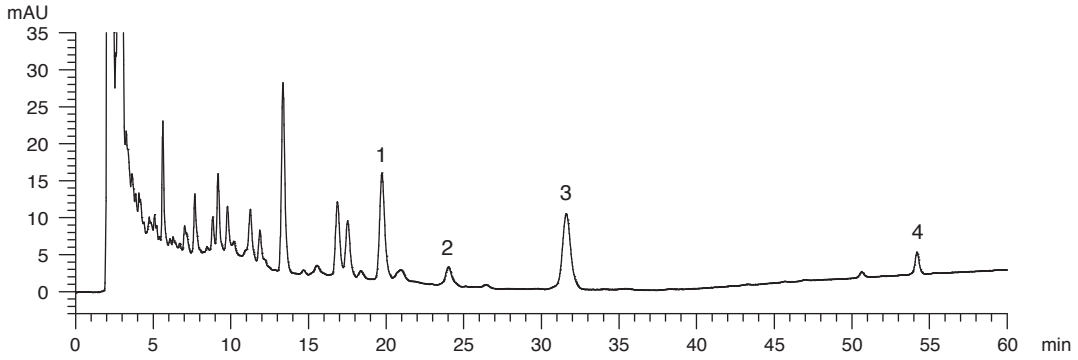

Figure 6 (i) A reference fingerprint chromatogram of dried root tuber of *Curcuma wenyujin* Y. H. Chen et C. Ling extract

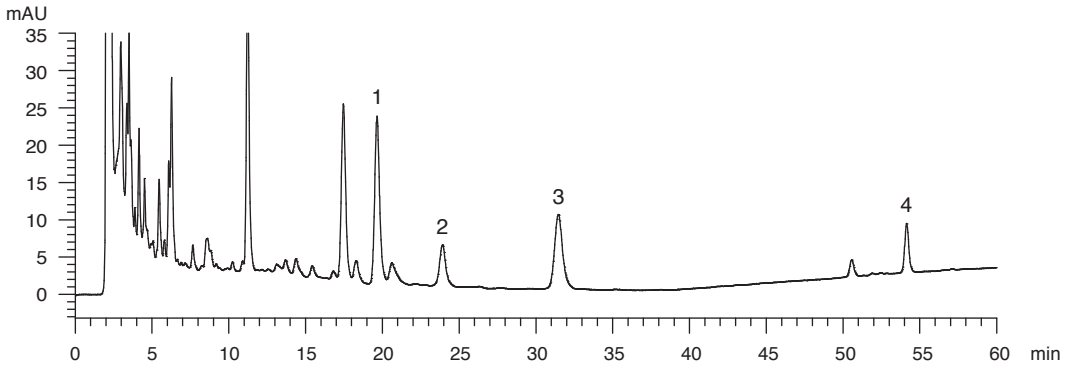

Figure 6 (ii) A reference fingerprint chromatogram of dried root tuber of *Curcuma kwangsiensis* S. G. Lee et C. F. Liang extract

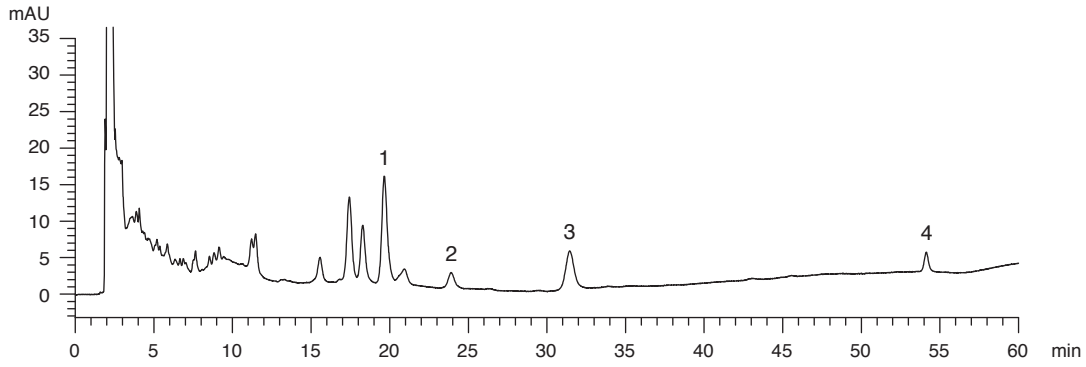

Figure 6 (iii) A reference fingerprint chromatogram of dried root tuber of *Curcuma phaeocaulis* Val. extract

For positive identification, the sample must give the above four characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the respective reference fingerprint chromatograms [Fig. 6 (i), (ii) or (iii)].

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 9.0%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 6.0%.
Ethanol-soluble extractives (cold extraction method): not less than 2.0%.

7. ASSAY

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

Standard solution

Germacrone standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 2.0 mg of germacrone CRS and dissolve in 10 mL of methanol (50%).

Germacrone standard solution for assay, Std-AS

Measure accurately the volume of the germacrone Std-Stock, dilute with methanol (50%) to produce a series of solutions of 1, 2, 5, 10, 20 mg/L for germacrone.

Test solution

Weigh accurately 2.5 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol (50%). Sonicate (400 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for two more times each with 15 mL of methanol (50%). Wash the residue with methanol (50%). Combine the solutions and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 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 water (55:45, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of germacrone Std-AS (5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of germacrone should not be more than 5.0%; the RSD of the retention time of germacrone peak should not be more than 2.0%; the column efficiency determined from germacrone peak should not be less than 7500 theoretical plates.

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

Limits

The dried root tuber of *Curcuma wenyujin* Y. H. Chen et C. Ling, *Curcuma kwangsiensis* S. G. Lee et C. F. Liang and *Curcuma phaeocaulis* Val. contains not less than 0.011% of germacrone ($C_{15}H_{22}O$), calculated with reference to the dried substance.

Part II Dried root tuber of *Curcuma longa* L.

3. DESCRIPTION

Root tuber fusiform, sometimes slender at one end, 1.8-6.2 cm long, 5-15 mm in diameter. Externally brownish-grey or greyish-yellow, with fine wrinkles. Texture hard and compact; fracture orange, the edge brownish-yellow to brownish-red. Odour aromatic; taste pungent [Fig. 1 (iv)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root tuber:

Epidermis sometimes remain. Velamen consists of 3-6 layers of cells, walls of the innermost layer of velamen cells thickened. Endodermis distinct. In stele, phloem bundles and xylem bundles each 17-34, arranged alternatively; each phloem bundle with 1 or 2-3 polygonal or subrounded vessels. Gelatinized starch granules in parenchymatous cells visible. Oil cells numerous, scattered throughout parenchyma (Fig. 7).

Powder

Colour brownish-yellow. Gelatinized starch granules scattered singly or in clumps, greyish-white, rounded-ovate, oblong or subrounded-polyhedral. Oil cells elongated-subrounded, subrounded or oblong, containing pale yellow or golden yellow oil droplets or secretions. Velamen cells pale yellow to greyish-brown, consisting of several layers of cells, frequently overlapping. Vessels mainly spiral, scalariform or reticulate, 20-100 µm in diameter (Fig. 8).

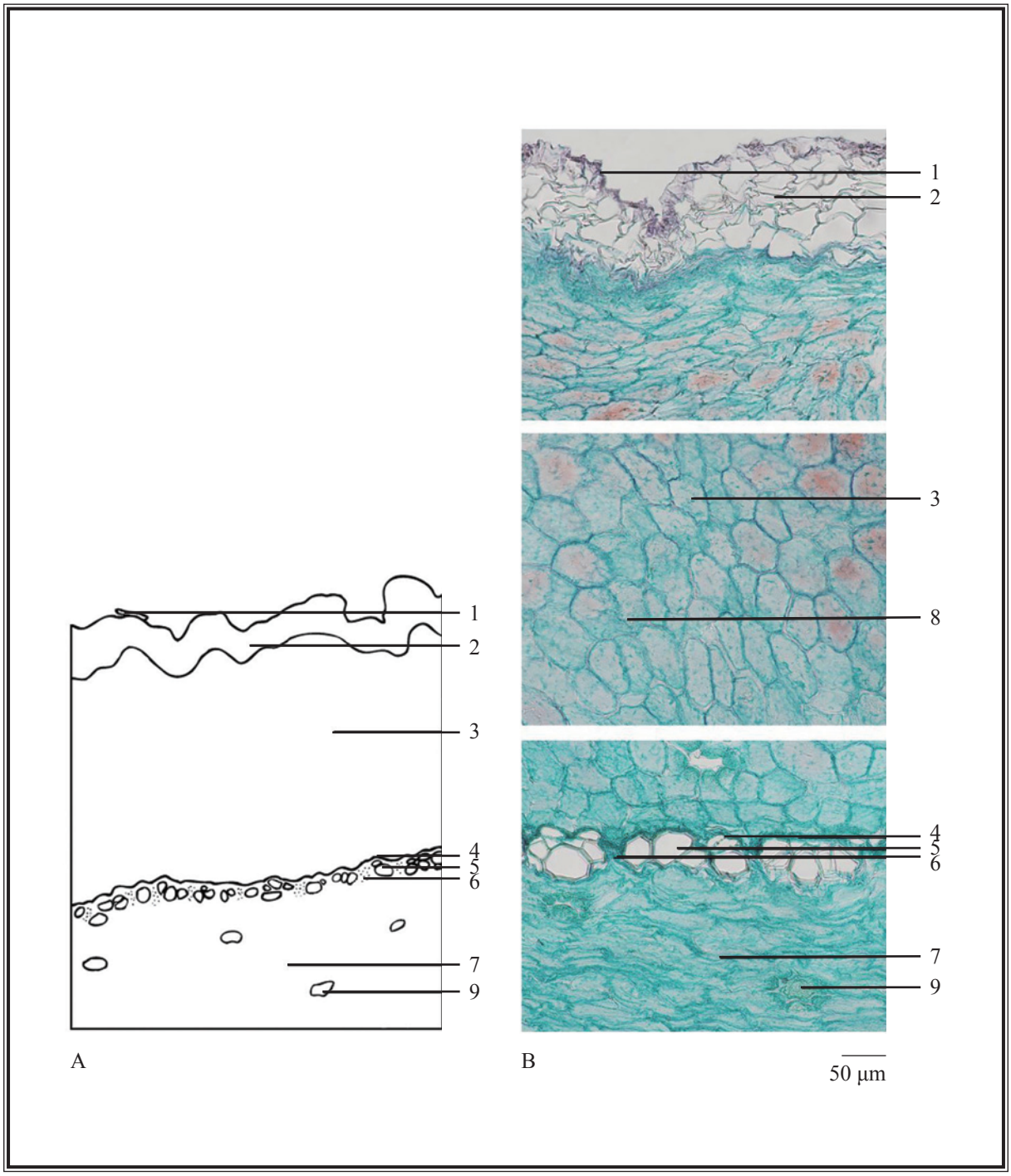

Figure 7 Microscopic features of transverse section of dried root tuber of *Curcuma longa* L.

A. Sketch B. Section illustration

1. Epidermis 2. Velamen 3. Cortex 4. Endodermis 5. Xylem 6. Phloem
7. Pith 8. Gelatinized starch granules 9. Oil cell

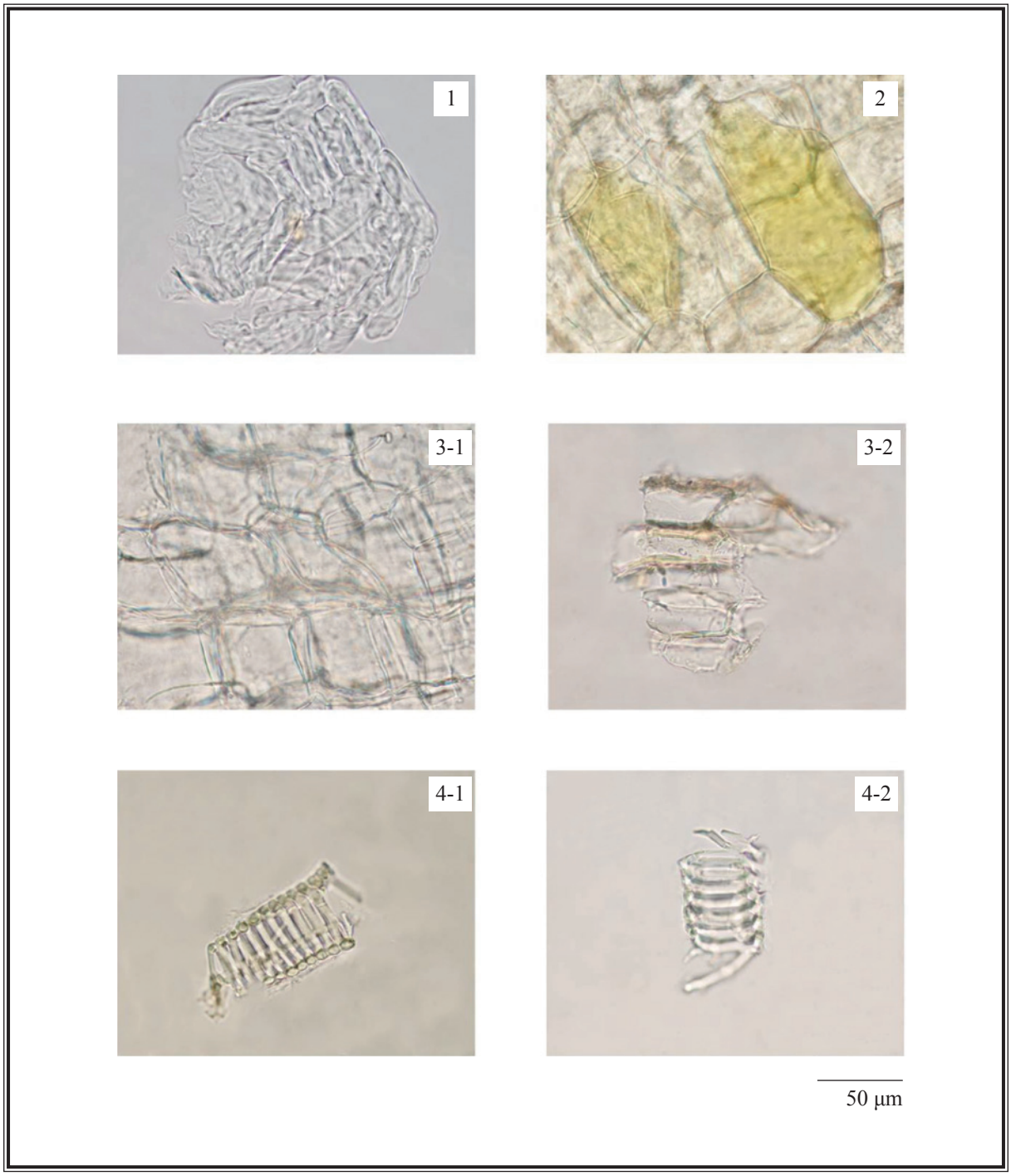

Figure 8 Microscopic features of powder of dried root tuber of *Curcuma longa* L. (under the light microscope)

1. Parenchymatous cells contain gelatinized starch granules 2. Oil cells
3. Velamen cells 4. Vessels (4-1 scalariform vessel, 4-2 spiral vessel)

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

Standard solutions

Bisdesmethoxycurcumin standard solution

Weigh 1.0 mg of bisdesmethoxycurcumin CRS (Fig. 9) and dissolve in 1 mL of methanol.

Curcumin standard solution

Weigh 1.0 mg of curcumin CRS (Fig. 9) and dissolve in 1 mL of methanol.

Desmethoxycurcumin standard solution

Weigh 1.0 mg of desmethoxycurcumin CRS (Fig. 9) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane, methanol and glacial acetic acid (9:1:0.1, v/v).

Test solution

Weigh 2.0 g of the powdered sample and place it in a 100-mL conical flask, then add 25 mL of ethanol (95%). Sonicate (400 W) the mixture for 30 min. Filter and transfer the filtrate to a 150-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol (95%). Filter the solution.

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 bisdesmethoxycurcumin standard solution (1 μL), curcumin standard solution (1 μL), desmethoxycurcumin 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 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 (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

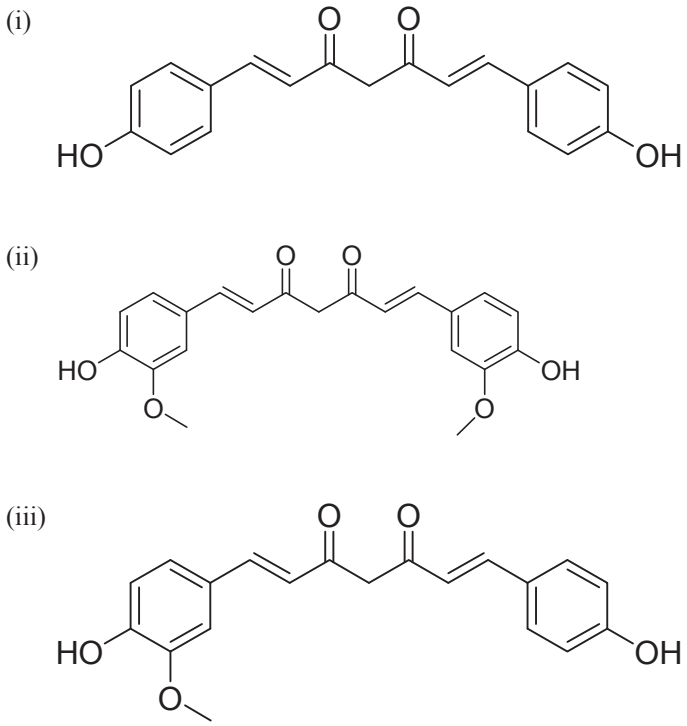

Figure 9 Chemical structures of (i) bisdesmethoxycurcumin (ii) curcumin and (iii) desmethoxycurcumin

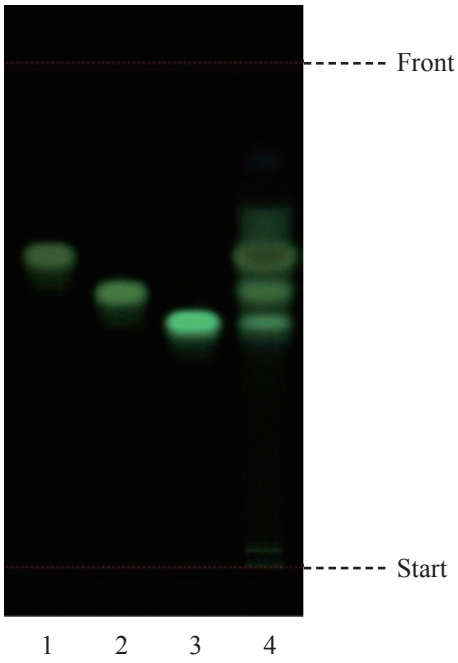

Figure 10 A reference HPTLC chromatogram of dried root tuber of *Curcuma longa* L. extract observed under UV light (366 nm)

1. Curcumin standard solution 2. Desmethoxycurcumin standard solution
3. Bisdesmethoxycurcumin standard solution 4. Test solution

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 bisdesmethoxycurcumin, curcumin and desmethoxycurcumin (Fig. 10).

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

Standard solutions

Bisdesmethoxycurcumin standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 0.4 mg of bisdesmethoxycurcumin CRS and dissolve in 20 mL of ethanol (75%).

Curcumin standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 1.0 mg of curcumin CRS and dissolve in 20 mL of ethanol (75%).

Desmethoxycurcumin standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 0.4 mg of desmethoxycurcumin CRS and dissolve in 20 mL of ethanol (75%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 30 mL of ethanol (75%). Sonicate (400 W) the mixture for 30 min. Filter and transfer the filtrate to a 150-mL round-bottomed flask. Repeat the extraction for one more time. Wash the residue with 5 mL of ethanol (75%). Combine the solutions. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in ethanol (75%). Transfer the solution to a 20-mL volumetric flask and make up to the mark with ethanol (75%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (335 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)	Acetonitrile (%, v/v)	0.2% Formic acid (%, v/v)	Elution
0 – 5	40	60	isocratic
5 – 20	40 → 47	60 → 53	linear gradient
20 – 45	47	53	isocratic
45 – 60	47 → 85	53 → 15	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of bisdesmethoxycurcumin Std-FP, curcumin Std-FP and desmethoxycurcumin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of bisdesmethoxycurcumin, curcumin and desmethoxycurcumin should not be more than 5.0%; the RSD of the retention times of bisdesmethoxycurcumin, curcumin and desmethoxycurcumin peaks should not be more than 2.0%; the column efficiencies determined from bisdesmethoxycurcumin, curcumin and desmethoxycurcumin peaks should not be less than 25000, 10000 and 15000 theoretical plates respectively.

The *R* value between peak 2 and the closest peak; 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. 11).

Procedure

Separately inject bisdesmethoxycurcumin Std-FP, curcumin Std-FP, desmethoxycurcumin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of bisdesmethoxycurcumin, curcumin and desmethoxycurcumin peaks in the chromatograms of bisdesmethoxycurcumin Std-FP, curcumin Std-FP, desmethoxycurcumin Std-FP and the retention times of the four characteristic peaks (Fig. 11) in the chromatogram of the test solution. Identify bisdesmethoxycurcumin, curcumin and desmethoxycurcumin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of bisdesmethoxycurcumin Std-FP, curcumin Std-FP and desmethoxycurcumin Std-FP. The retention times of bisdesmethoxycurcumin, curcumin and desmethoxycurcumin 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 four characteristic peaks of dried root tuber of *Curcuma longa* L. extract are listed in Table 4.

Table 4 The RRTs and acceptable ranges of the four characteristic peaks of dried root tuber of *Curcuma longa* L. extract

Peak No.	RRT	Acceptable Range
1	0.78	± 0.03
2 (marker, curcumin)	1.00	-
3 (desmethoxycurcumin)	1.13	± 0.03
4 (bisdesmethoxycurcumin)	1.30	± 0.03

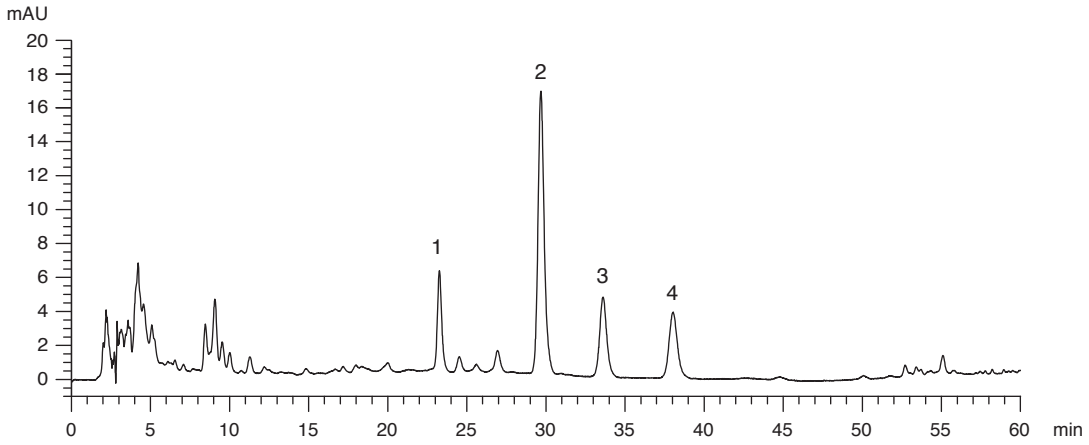

Figure 11 A reference fingerprint chromatogram of dried root tuber of *Curcuma longa* L. extract

For positive identification, the sample must give the above four characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 11).

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 9.0%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 6.0%.
Ethanol-soluble extractives (cold extraction method): not less than 2.0%.

7. ASSAY

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

Standard solution

Mixed bisdesmethoxycurcumin, curcumin and desmethoxycurcumin standard stock solution, Std-Stock (80 mg/L for bisdesmethoxycurcumin, 200 mg/L for curcumin and 80 mg/L for desmethoxycurcumin)

Weigh accurately 1.6 mg of bisdesmethoxycurcumin CRS, 4.0 mg of curcumin CRS and 1.6 mg of desmethoxycurcumin CRS, and dissolve in 20 mL of ethanol (75%).

Mixed bisdesmethoxycurcumin, curcumin and desmethoxycurcumin standard solution for assay, Std-AS

Measure accurately the volume of the mixed bisdesmethoxycurcumin, curcumin and desmethoxycurcumin Std-Stock, dilute with ethanol (75%) to produce a series of solutions of 0.2, 0.5, 1, 2, 5 mg/L for bisdesmethoxycurcumin, 2, 5, 10, 25, 50 mg/L for curcumin and 0.2, 0.5, 1, 2, 5 mg/L for desmethoxycurcumin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 30 mL of ethanol (75%). Sonicate (400 W) the mixture for 30 min. Filter and transfer the filtrate to a 150-mL round-bottomed flask. Repeat the extraction for one more time. Wash the residue with 5 mL of ethanol (75%). Combine the solutions. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in ethanol (75%). Transfer the solution to a 20-mL volumetric flask and make up to the mark with ethanol (75%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (430 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 0.2% formic acid and acetonitrile (45:55, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed bisdesmethoxycurcumin, curcumin and desmethoxycurcumin Std-AS (1 mg/L for bisdesmethoxycurcumin, 10 mg/L for curcumin and 1 mg/L for desmethoxycurcumin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of bisdesmethoxycurcumin, curcumin and desmethoxycurcumin should not be more than 5.0%; the RSD of the retention times of bisdesmethoxycurcumin, curcumin and desmethoxycurcumin peaks should not be more than 2.0%; the column efficiencies determined from bisdesmethoxycurcumin, curcumin and desmethoxycurcumin peaks should not be less than 5000, 3000 and 4000 theoretical plates respectively.

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

Limits

The dried root tuber of *Curcuma longa* L. contains not less than 0.052% of the total content of bisdesmethoxycurcumin ($C_{19}H_{16}O_4$), curcumin ($C_{21}H_{20}O_6$) and desmethoxycurcumin ($C_{20}H_{18}O_5$), calculated with reference to the dried substance.