Rhizoma Curcumae

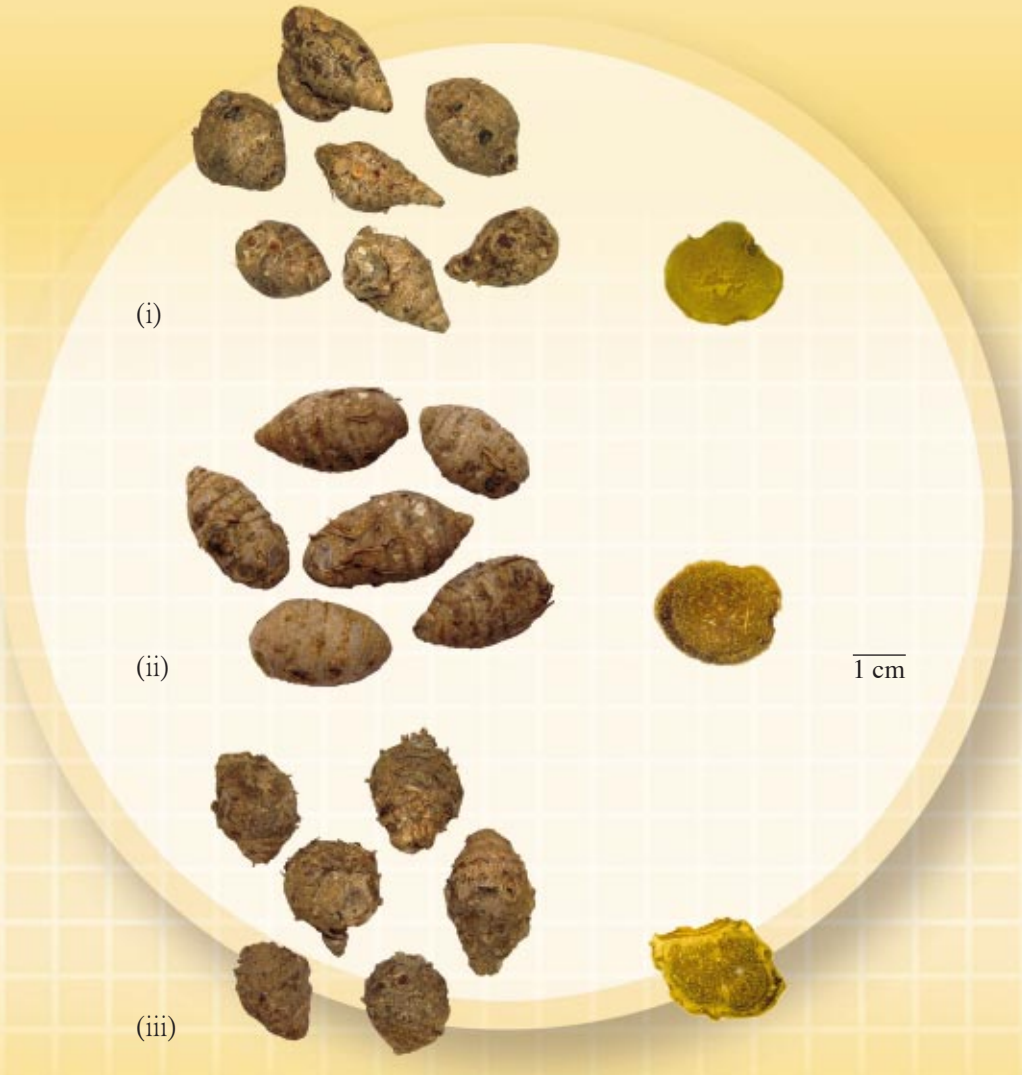


Figure 1 A photograph of Rhizoma Curcumae
(i) *Curcuma phaeocaulis* Val.
(ii) *Curcuma kwangsiensis* S. G. Lee et C. F. Liang
(iii) *Curcuma wenyujin* Y. H. Chen et C. Ling

1. NAMES

Official Name: Rhizoma Curcumae

Chinese Name: 莪术

Chinese Phonetic Name: Ezhu

2. SOURCE

Rhizoma Curcumae is the dried rhizome of *Curcuma phaeocaulis* Val., *Curcuma kwangsiensis* S. G. Lee et C. F. Liang or *Curcuma wenyujin* Y. H. Chen et C. Ling (Zingiberaceae). The rhizome is collected in winter when stem and leaves wither. After washing it clean, it is steamed or boiled thoroughly, then dried under the sun or in a shady and cool place, and once dried, fibrous root and foreign matter removed to obtain Rhizoma Curcumae.

3. DESCRIPTION

Ovoid, elongate-ovoid, conical or elongate-fusiform, 1.5-8.5 cm in length, 8-40 mm in diameter, the apex frequently obtuse, the base obtuse and rounded. Externally yellowish-white, greyish-yellow or pale brown, rough or slightly smooth. The upper part with conspicuous, raised–annulations, and with rounded and slightly dented rootlet scars, or with remaining rootlets, some exhibiting a row of concave bud scars and subrounded lateral rhizome scars on each of the two sides, respectively. Heavy, texture hard, fracture waxy, sometimes covered with pale yellow powder; cortex and stele easily detachable, endodermal ring distinct, dot-shaped or strip-shaped of vascular bundles scattered inside the endodermal ring. Odour slightly aromatic; taste slightly bitter and pungent.

***Curcuma phaeocaulis* Val. :** Rhizome 1.5-5.5 cm in length, 15-35 mm in diameter. Externally pale brown to greyish-yellow, slightly smooth. Fracture markedly waxy, dark green to green; endodermal ring pale yellow [Fig. 1(i)].

***Curcuma kwangsiensis* S. G. Lee et C. F. Liang :** Rhizome 2-8.5 cm in length, 12-40 mm in diameter. Externally pale brown, mostly rough. Fracture slightly waxy, dark brown; endodermal ring whitish [Fig. 1(ii)].

***Curcuma wenyujin* Y. H. Chen et C. Ling :** Rhizome 1.8-6.8 cm in length, 8-35 mm in diameter. Externally yellowish-brown, mostly rough. Slightly light, fracture slightly waxy, yellow to pale brown; endodermal ring dark brown [Fig. 1(iii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermal cells sometimes remains, mostly lignified, non-glandular hairs visible occasionally; cork consists of a number of layers of cells, strongly or slightly lignified or non-lignified; cortex occupying about 1/3-1/4 of the radius, showing scattered leaf-trace bundles; endodermis distinct, cells often crimpled. Stele broad, occupying about 2/3-3/4 of the radius, vascular bundles collateral and scattered, those along the pericycle relatively small and tightly arranged. Parenchyma cells filled with gelatinous starch masses; oil cells scattered in the parenchyma, containing golden oily matter [Fig. 2(i), (ii) and (iii)].

Powder

Colour pale yellow, yellowish-brown or pale brown. Gelatinous starch masses greyish-white, making up the major portion of the powder, the shape in rounded-ovate, oblong, subround or irregular. Oil cells elongated-subround, subround or rounded-ovate, containing brownish-yellow or brownish-red oily matter, 23-154 μm in length, 21-85 μm in diameter. Vessels of the spiral type frequent, scalariform or reticulate types also present, 18-95 μm in diameter. Cork cells brownish-yellow, consisting of several layers of cells, frequently overlapping, polygonal. Fibres occasional, obliquely sharp at the top, usually broken. Non-glandular hairs unicellular or 2-3 celled, some with sinuous wall, 11-55 μm in diameter, wall 2-17 μm thick [Fig. 3(i), (ii) and (iii)].

4.2 Physicochemical Identification

Reagent

Vanillin-sulphuric acid solution (0.5%, w/v)

Dissolve 0.5 g of vanillin in 100 mL of sulphuric acid.

Procedure

Weigh 1.0 g of the powdered sample and put into a 50-mL conical flask, then add 6 mL of ethyl acetate. Sonicate (240 W) the mixture for 20 min. Filter and transfer 0.5 mL of the filtrate to a test tube. Cautiously add 0.5 mL of vanillin-sulphuric acid solution (0.5%, w/v) along the inner wall of the tube. Allow to stand for 1 min. A dark purple layer is observed at the interface of the two solvent layers.

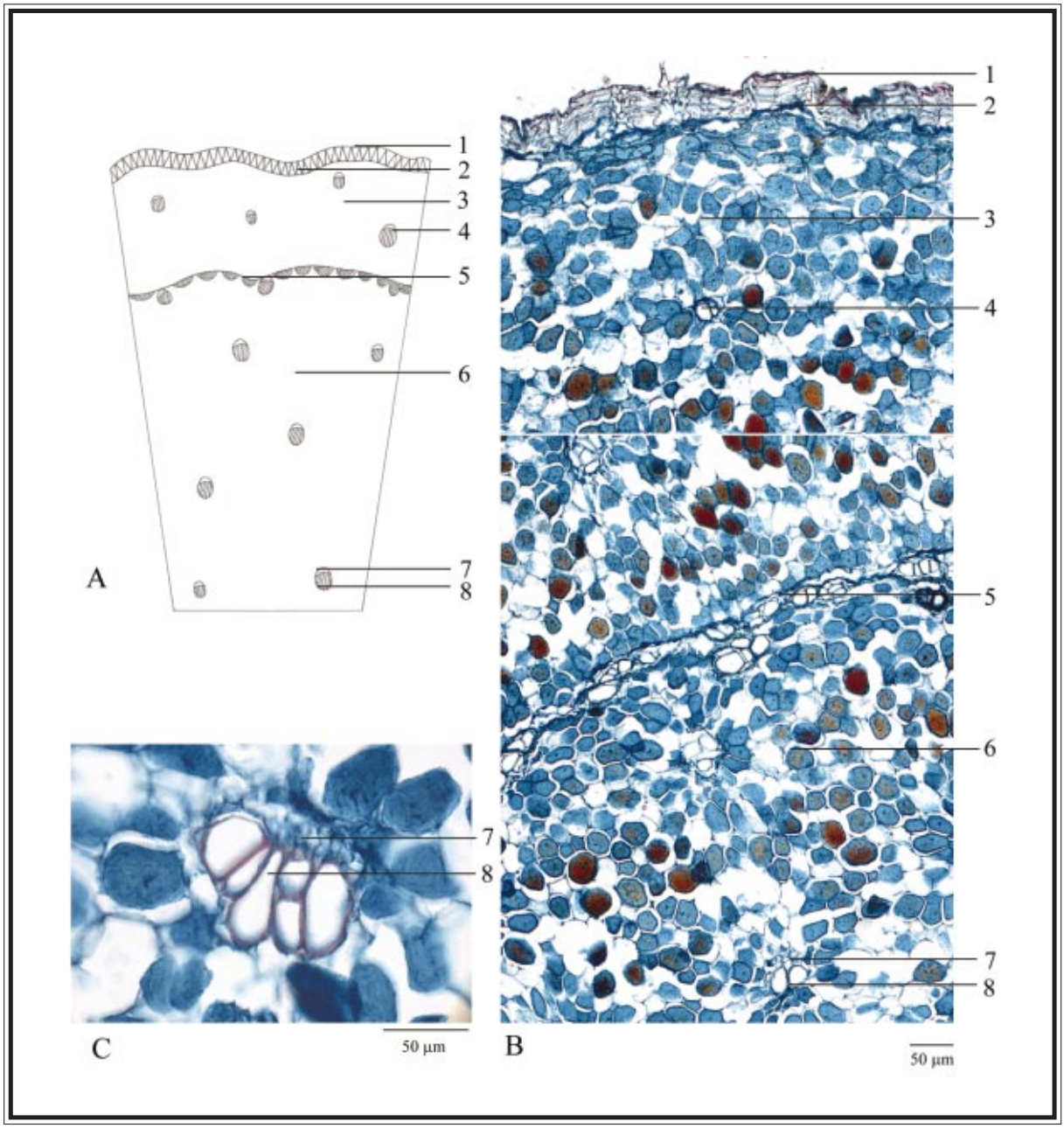


Figure 2(i) Microscopic features of transverse section of dried rhizome of *Curcuma phaeocaulis* Val.

A. Sketch B. Section illustration C. Vascular bundle

1. Epidermis 2. Cork 3. Cortex 4. Leaf-trace bundle 5. Endodermis 6. Stele 7. Phloem 8. Xylem

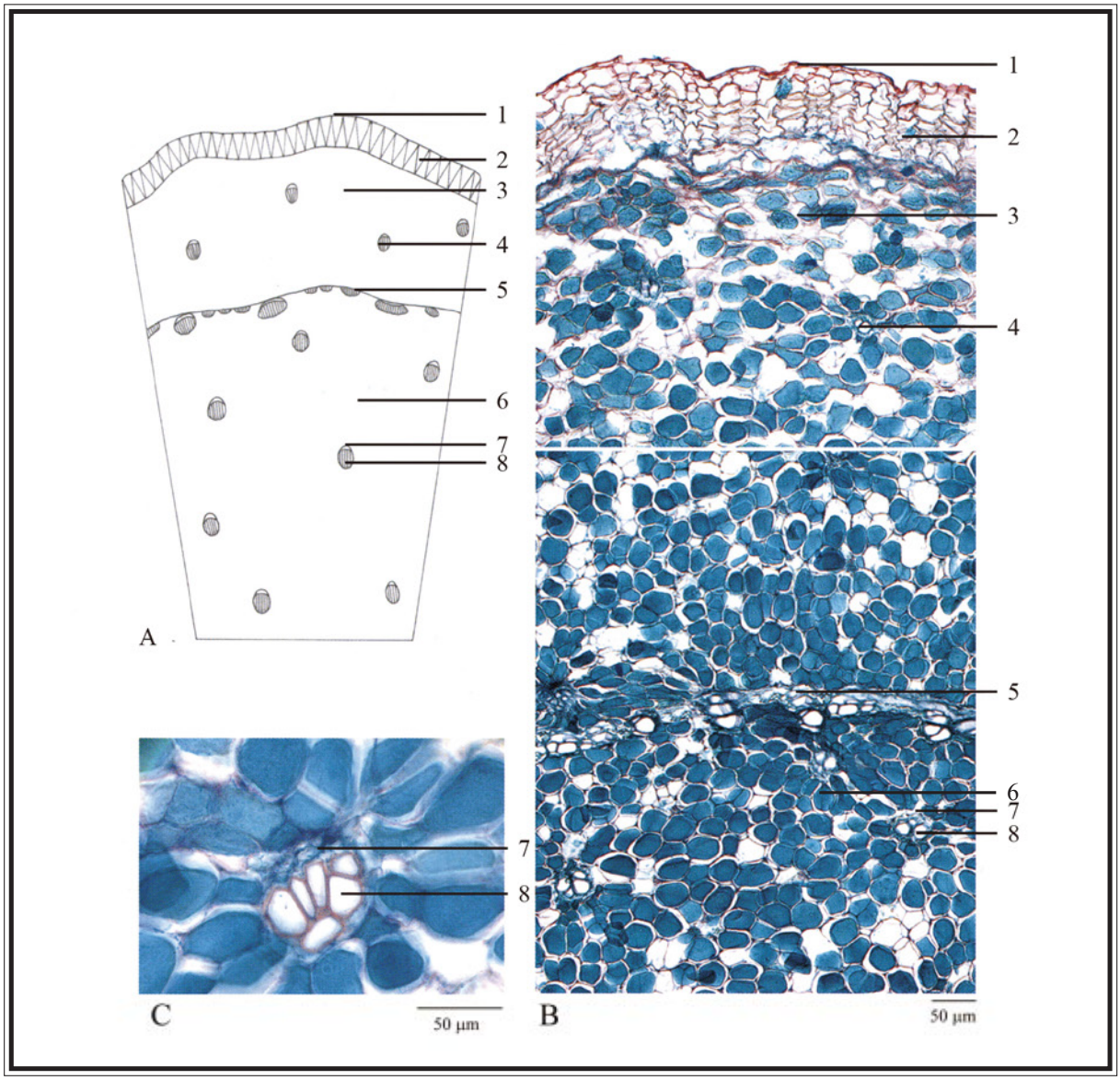


Figure 2(ii) Microscopic features of transverse section of dried rhizome of *Curcuma kwangsiensis* S. G. Lee et C. F. Liang

A. Sketch B. Section illustration C. Vascular bundle

1. Epidermis 2. Cork 3. Cortex 4. Leaf-trace bundle 5. Endodermis 6. Stele 7. Phloem 8. Xylem

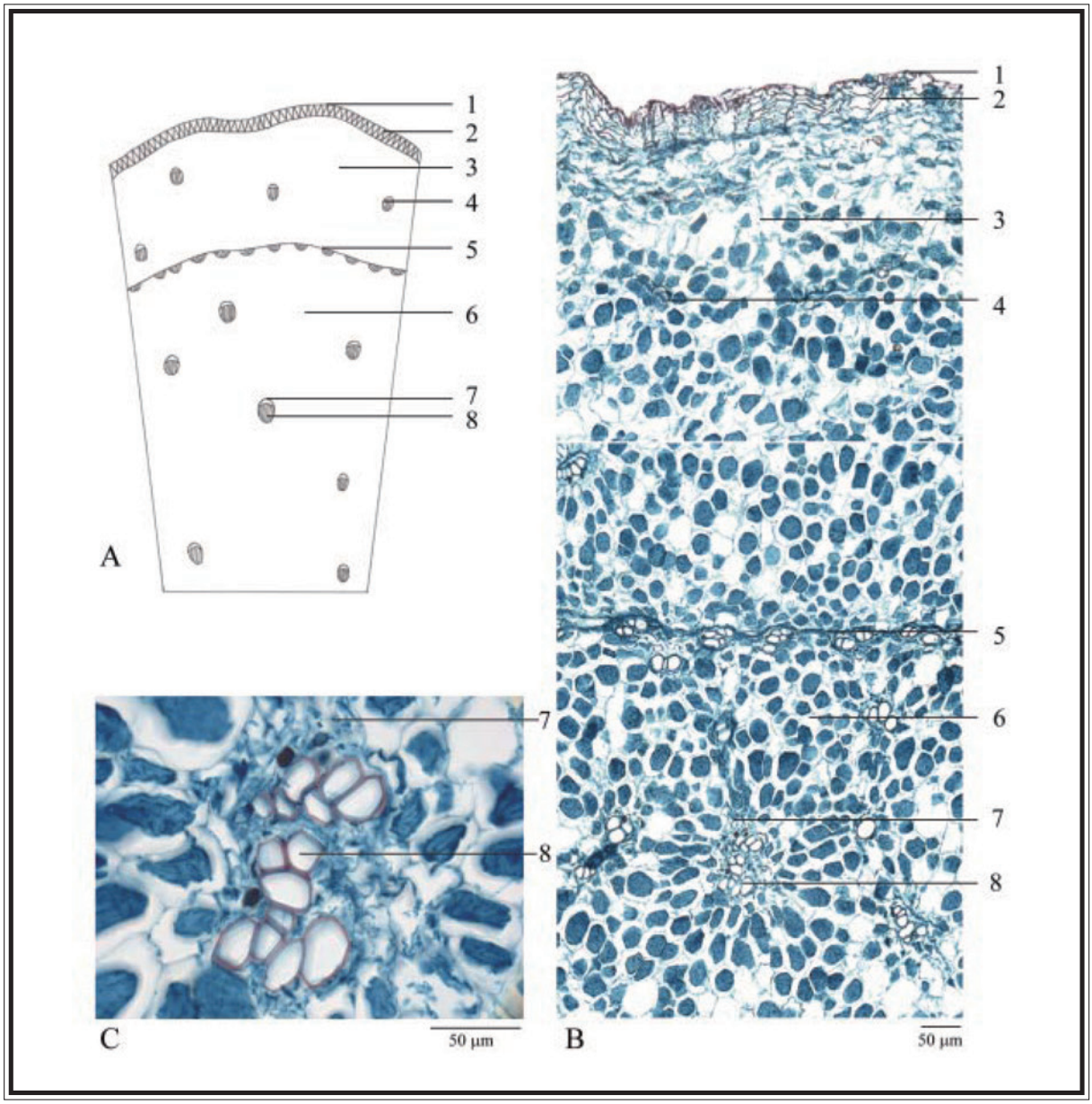


Figure 2(iii) Microscopic features of transverse section of dried rhizome of *Curcuma wenyujin* Y. H. Chen et C. Ling

A. Sketch B. Section illustration C. Vascular bundle

1. Epidermis 2. Cork 3. Cortex 4. Leaf-trace bundle 5. Endodermis 6. Stele 7. Phloem 8. Xylem

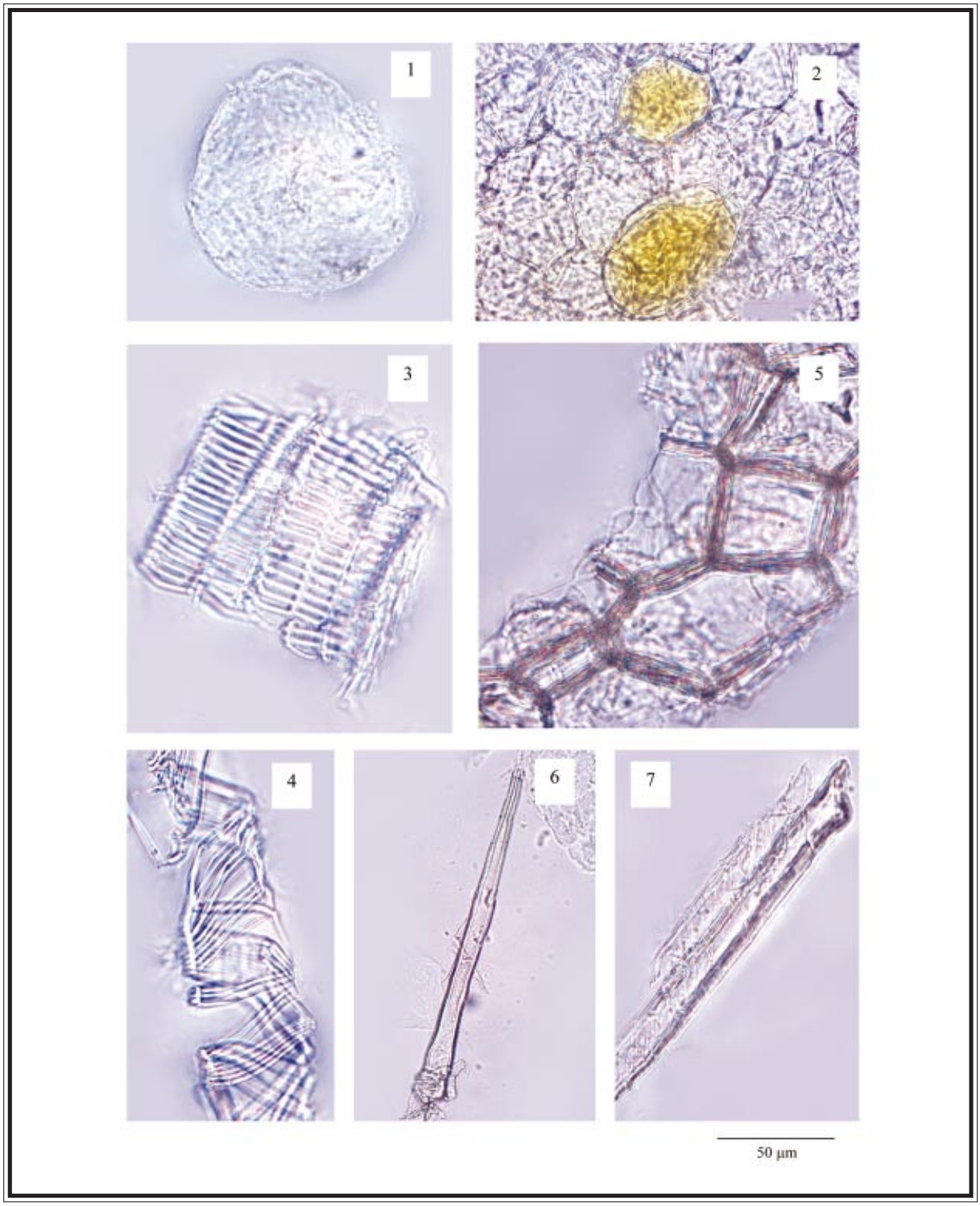


Figure 3(i) Microscopic features of powder of dried rhizome of *Curcuma phaeocaulis* Val. (under the light microscope)

1. Starch gelatinous masses 2. Oil cell 3. Scalariform vessels 4. Spiral vessels 5. Cork cells
6. Non-glandular hairs 7. Fibres

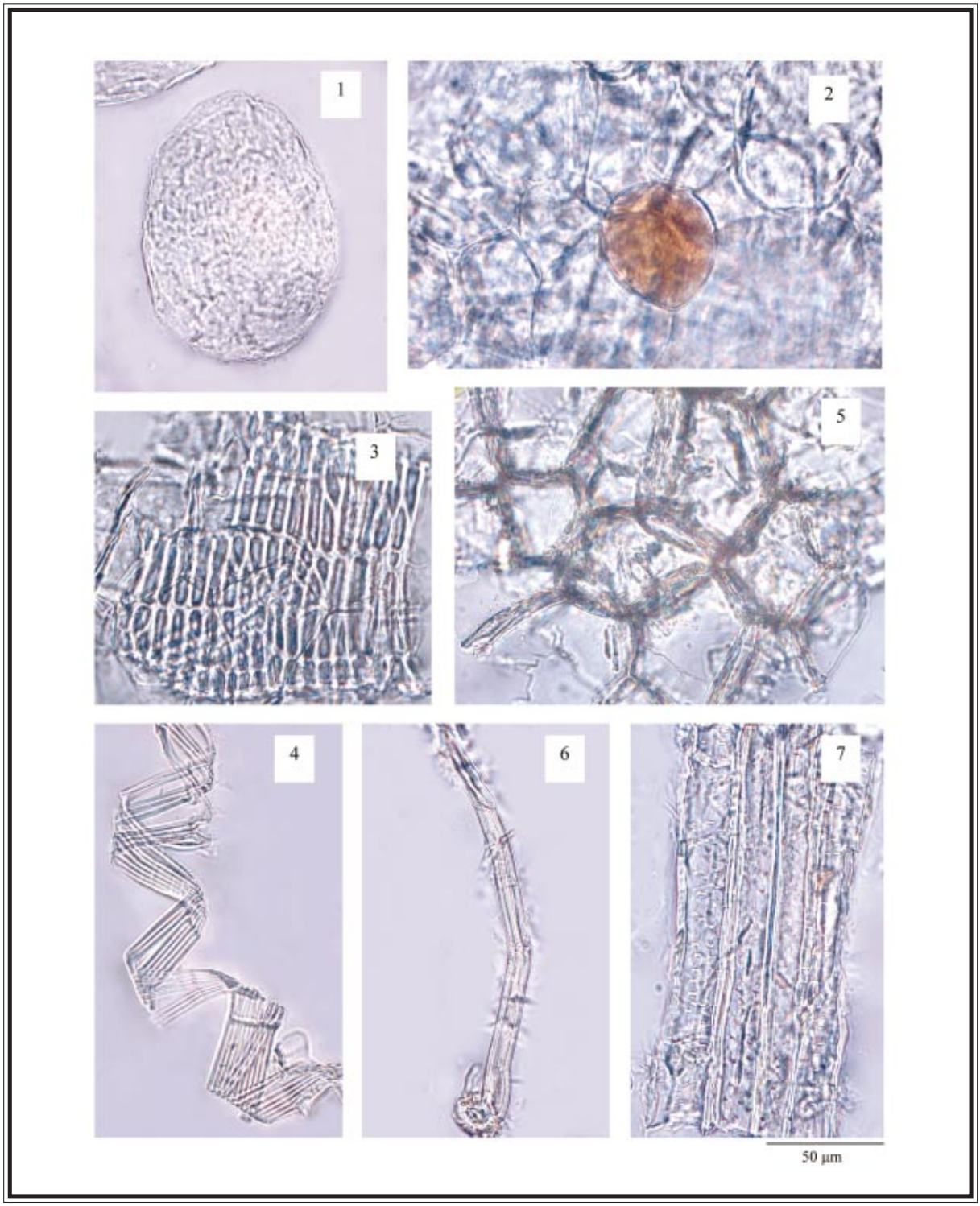


Figure 3(ii) Microscopic features of powder of dried rhizome of *Curcuma kwangsiensis* S. G. Lee et C. F. Liang (under the light microscope)

1. Starch gelatinous masses 2. Oil cell 3. Scalariform vessels 4. Spiral vessels 5. Cork cells
6. Non-glandular hairs 7. Fibres

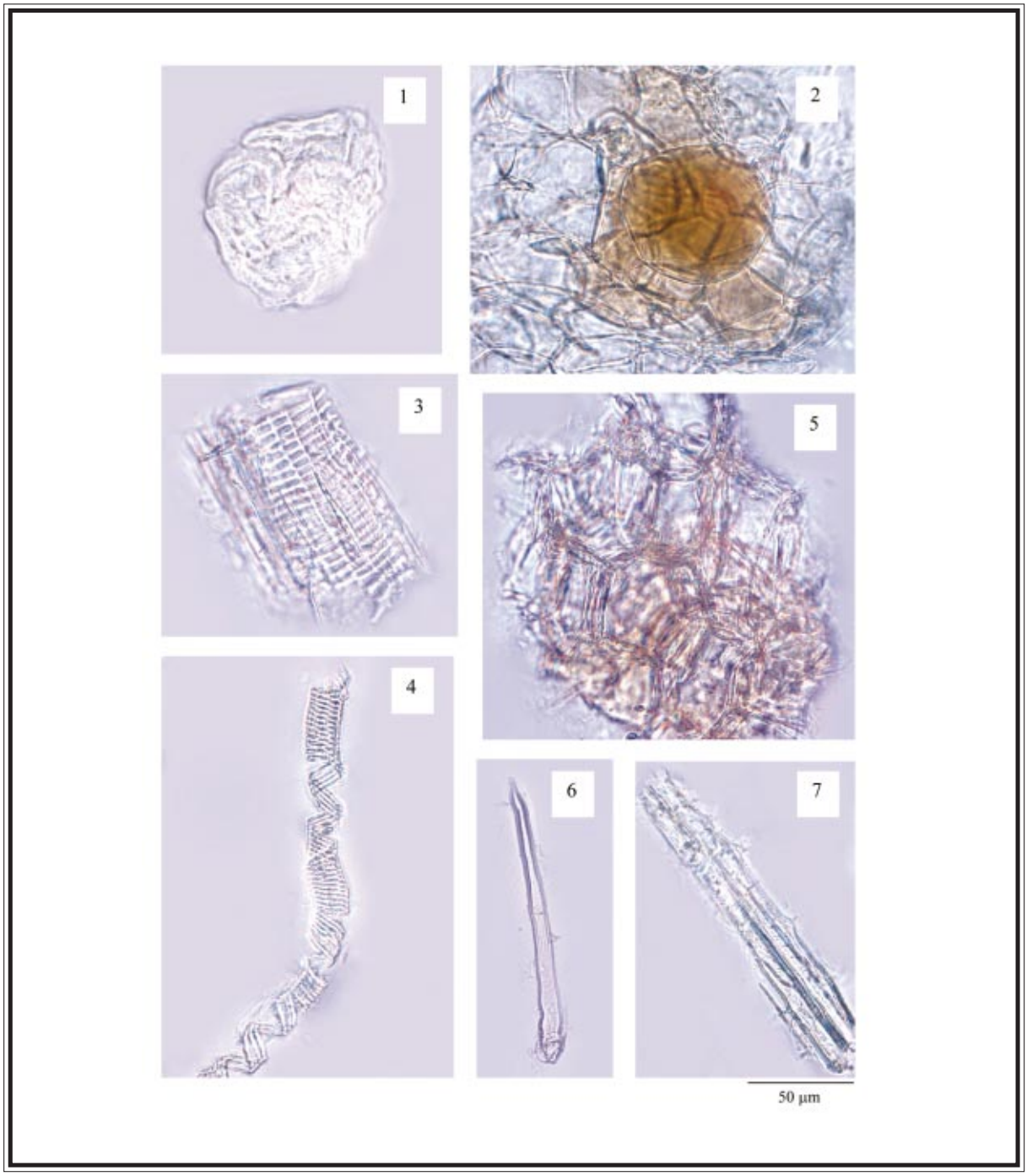


Figure 3(iii) Microscopic features of powder of dried rhizome of *Curcuma wenyujin* Y. H. Chen et C. Ling (under the light microscope)

1. Starch gelatinous masses 2. Oil cell 3. Scalariform vessels 4. Spiral vessels 5. Cork cells
6. Non-glandular hairs 7. Fibres

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

Standard solution

Germacrone standard solution

Weigh 1.0 mg of germacrone CRS (Fig. 4) and dissolve in 1 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of petroleum ether (60-80˚C) and ethyl acetate (96:4, v/v).

Test solution

Transfer 20 μL of the volatile oil (see 7.2 Assay of Volatile Oil) to a test tube and dissolve in 0.4 mL of ethyl acetate.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately germacrone standard solution and the test solution (2 μL each) to the plate. 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).

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.

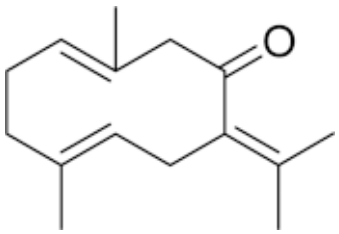


Figure 4 Chemical structure of germacrone

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

Standard solution

Germacrone standard stock solution, Std-Stock (1000 mg/L)

Weigh 2.0 mg of germacrone CRS and dissolve in 2 mL of methanol.

Germacrone standard solution for fingerprinting, Std-FP (100 mg/L)

Pipette 1.0 mL of germacrone Std-Stock into a 10-mL volumetric flask and make up to the mark with methanol.

Test solution

Weigh 0.5 g of the powdered sample and put into a 50-mL centrifugal tube, then add 20 mL of methanol. Sonicate (240 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Filter the supernatant through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (214 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 –

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 3	55	45	isocratic
3 – 30	55 → 35	45 → 65	linear gradient
30 – 38	35	65	isocratic
38 – 45	35 → 10	65 → 90	linear gradient
45 – 55	10 → 0	90 → 100	linear gradient
55 – 65	0	100	isocratic

System suitability requirements

Perform at least five replicate injections each with 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 1 and peak 2 in the chromatogram of the test solution should not be less than 1.5 [Fig. 5(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. 5(i), (ii) or (iii)] in the chromatogram of the test solution. Under the same HPLC conditions, 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 Rhizoma Curcumae extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the four characteristic peaks of Rhizoma Curcumae extract

Peak No.	RRT	Acceptable Range
1	0.68	±0.03
2	0.73	±0.03
3 (marker, germacrone)	1.00	-
4	1.32	±0.09

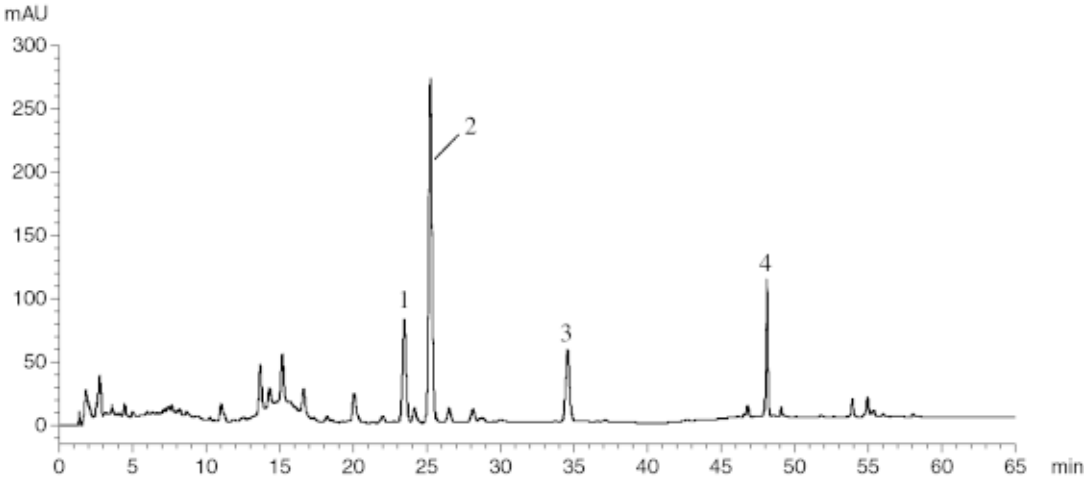


Figure 5(i) A reference fingerprint chromatogram of dried rhizome of *Curcuma phaeocaulis* Val. extract

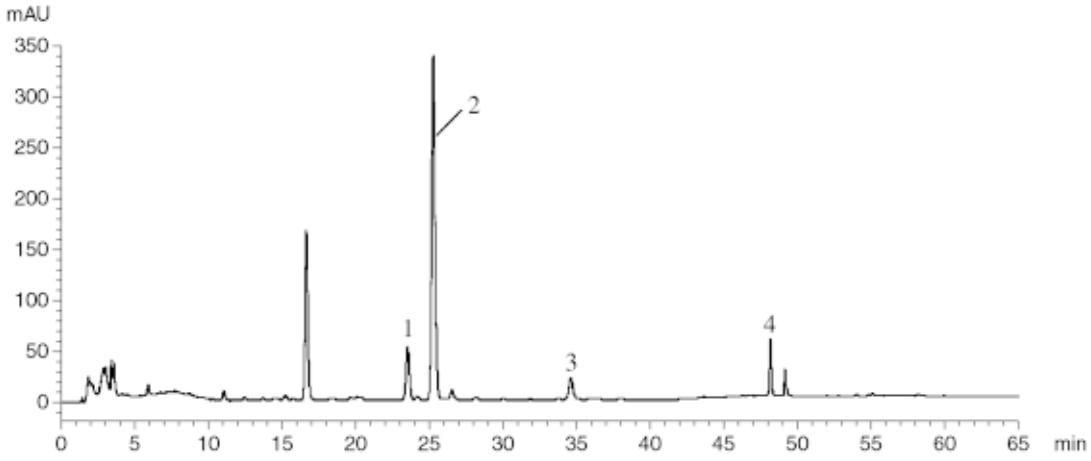


Figure 5(ii) A reference fingerprint chromatogram of dried rhizome of *Curcuma kwangsiensis* S. G. Lee et C. F. Liang extract

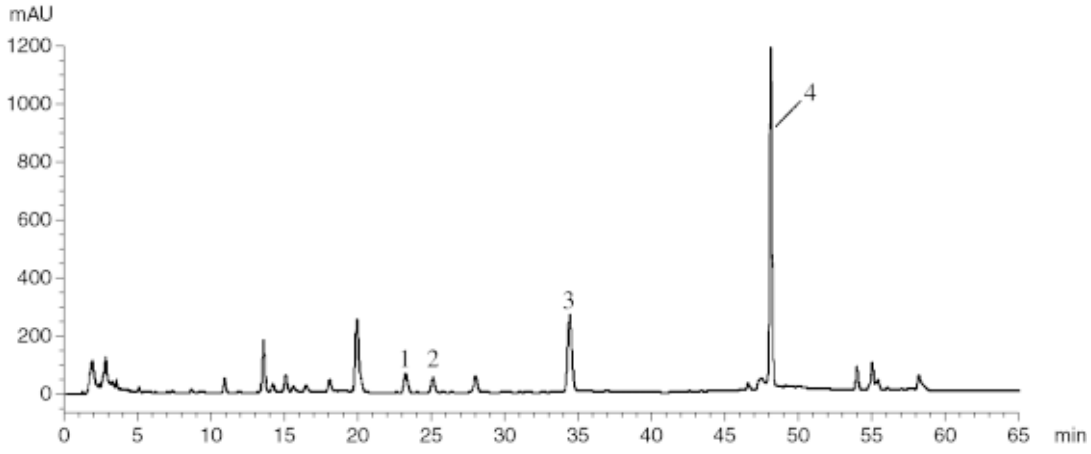


Figure 5(iii) A reference fingerprint chromatogram of dried rhizome of *Curcuma wenyujin* Y. H. Chen et C. Ling extract

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. 5(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 – 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 6.5%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*: not more than 16.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 5.0%.
Ethanol-soluble extractives (cold extraction method): not less than 3.0%.

7. ASSAY

7.1 Assay of Germacrone

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

Standard solution
Germacrone standard stock solution, Std-Stock (1000 mg/L)
Weigh accurately 5.0 mg of germacrone CRS and dissolve in 5 mL of methanol.
Germacrone standard solution for assay, Std-AS
Measure accurately the volume of the germacrone Std-Stock, dilute with methanol to produce a series of solutions of 10, 50, 100, 200, 300 mg/L for germacrone.

Test solution

Weigh accurately 0.25 g of the powdered sample and put into a 100-mL conical flask, then add 20 mL of methanol. Sonicate (240 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Repeat the extraction twice. Combine the extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (214 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 –

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 3	55	45	isocratic
3 – 30	55 → 35	45 → 65	linear gradient
30 – 38	35	65	isocratic
38 – 45	35 → 10	65 → 90	linear gradient
45 – 55	10 → 0	90 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections each with 10 μL of germacrone Std-AS (100 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 10000 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 2.0%. 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 indicated in Appendix IV(B).

Limits

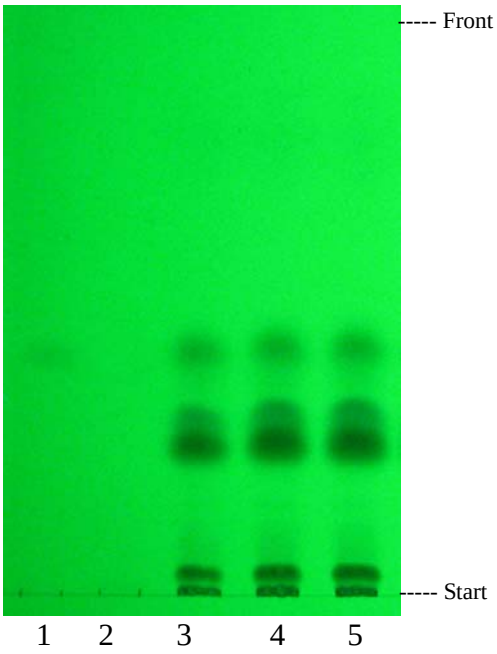
The sample contains not less than 0.062% of germacrone ($C_{15}H_{22}O$), calculated with reference to the dried substance.

7.2 Assay of Volatile Oil

Weigh accurately 30 g of the powdered sample and put into a 1000-mL round-bottomed flask. Add 500 mL of water and a few glass beads, shake and mix well. Carry out the method as directed in Appendix XV(Method A).

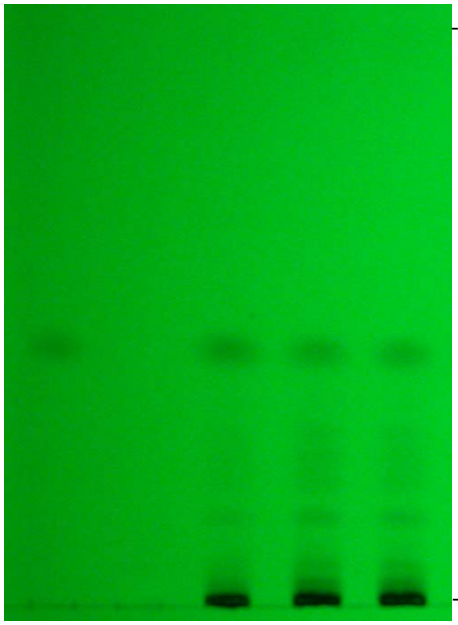
Limits

The sample contains not less than 1.5 % (v/w) of volatile oil.



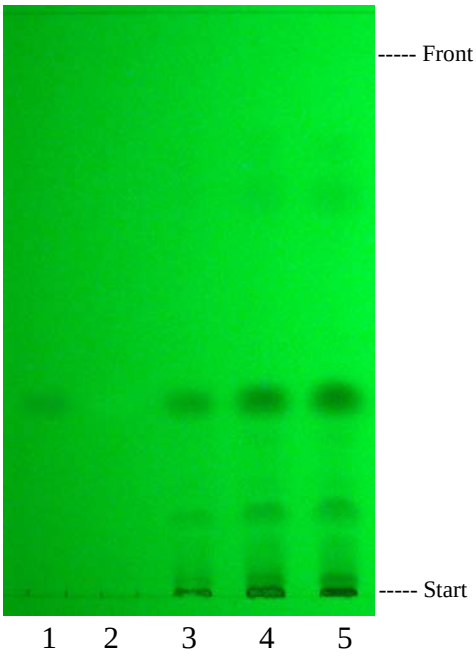
Lane	Sample	Results
1	Standard (Germacrone)	Germacrone positive
2	Blank (Ethyl acetate)	Negative
3	Spiked sample (Sample plus germacrone)	Germacrone positive
4	Sample (*Curcuma phaeocaulis* Val.)	Germacrone positive
5	Sample duplicate (*Curcuma phaeocaulis* Val.)	Germacrone positive

Figure 1 TLC results of dried rhizome of *Curcuma phaeocaulis* Val. extract observed under UV light (254 nm)



Lane	Sample	Results
1	Standard (Germacrone)	Germacrone positive
2	Blank (Ethyl acetate)	Negative
3	Spiked sample (Sample plus germacrone)	Germacrone positive
4	Sample (*Curcuma kwangsiensis* S. G. Lee et C. F. Liang)	Germacrone positive
5	Sample duplicate (*Curcuma kwangsiensis* S. G. Lee et C. F. Liang)	Germacrone positive

Figure 2 TLC results of dried rhizome of *Curcuma kwangsiensis* S. G. Lee et C. F. Liang extract observed under UV light (254 nm)



Lane	Sample	Results
1	Standard (Germacrone)	Germacrone positive
2	Blank (Ethyl acetate)	Negative
3	Spiked sample (Sample plus germacrone)	Germacrone positive
4	Sample (*Curcuma wenyujin* Y. H. Chen et C. Ling)	Germacrone positive
5	Sample duplicate (*Curcuma wenyujin* Y. H. Chen et C. Ling)	Germacrone positive

Figure 3 TLC results of dried rhizome of *Curcuma wenyujin* Y. H. Chen et C. Ling extract observed under UV light (254 nm)