Radix et Rhizoma Rhei

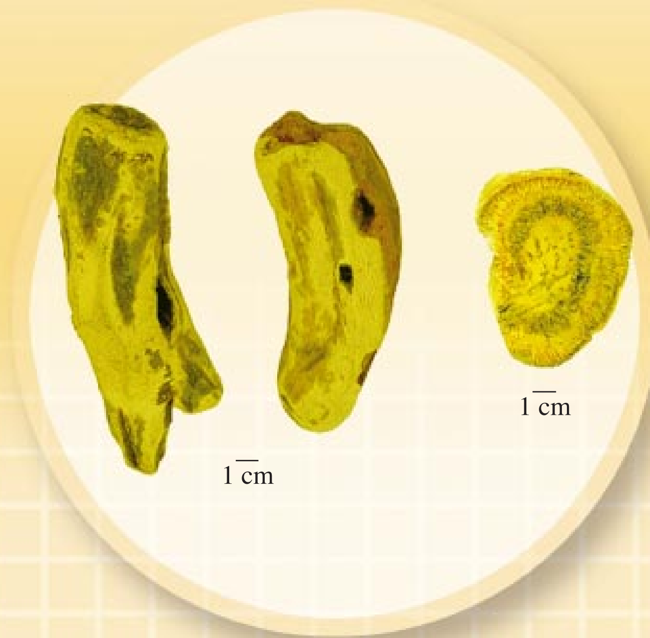

Figure 1(i) A photograph of dried root and rhizome of *Rheum palmatum* L.

Figure 1(ii) A photograph of dried root and rhizome of *Rheum tanguticum* Maxim. ex Balf.

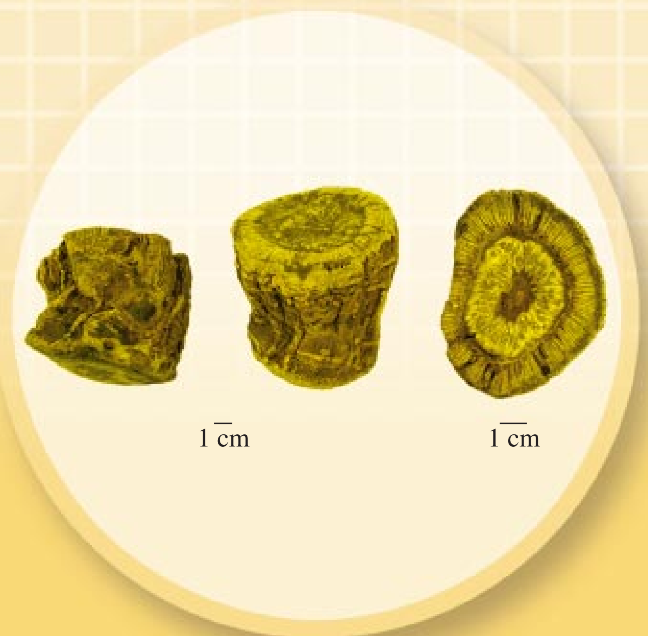

Figure 1(iii) A photograph of dried root and rhizome of *Rheum officinale* Baill.

1. NAMES

Official Name: Radix et Rhizoma Rhei

Chinese Name: 大黃

Chinese Phonetic Name: Dahuang

2. SOURCE

Radix et Rhizoma Rhei is the dried root and rhizome of *Rheum palmatum* L., *Rheum tanguticum* Maxim. ex Balf. or *Rheum officinale* Baill. (Polygonaceae). The root and rhizome are collected in late autumn when stem and leaves wither or in the following spring before germination. After removal of soil, rootlets or the outer bark, the root and rhizome are cut into segments or sections or processed into egg-shaped or cylindrical-shaped products, either kept in a string together to be dried or dried directly to obtain Radix et Rhizoma Rhei.

3. DESCRIPTION

Subcylindrical, conical, ovoid, hippocrepiform, or irregular slices or sections , 0.5-40 cm long or thick, 6-200 mm in diameter. Outer surface yellowish-brown to reddish-brown when peeled, sometimes the surface marked with whitish reticulations and scattered star spots (anomalous vascular bundles) or with remnants of brownish-black cork, mostly with coarse wrinkles. Hard and solid, sometimes loose and soft in the centre . When broken, the surface pale reddish-brown to yellowish-brown, granular. Pith of the rhizome broad; star spots in cross-section, appearing in rings or scattered. Cambium ring of root distinct; xylem well developed, with radial rays and no star spots. Odour delicately aromatic; taste bitter and slightly astringent, sticky and gritty on the chewing [Fig. 1(i), (ii) and (iii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

The cork and cortex have been mostly removed. The cork consists of several layers of flat cells;

while the remaining phelloderm contains a number of subround parenchyma cells, often with clefts. In the phloem, the parenchyma is well developed, with large mucilage cavities; phloem rays relatively dense, sometimes with clefts. Cambium distinct, in a ring. Xylem with relatively dense rays, 1-10 cells wide; vessels usually single or in group, sparsely arranged, unlignified. Parenchyma cells contain cluster crystals of calcium oxalate and abundant starch granules. The pith is broad, containing anomalous vascular bundles arranged in a more or less continuous ring(s) or scattered irregularly. The anomalous vascular bundles show a cambium in a ring, with the phloem inside and xylem outside, sometimes mucilage cavities visible near the cambium; the rays running radially from the centre of the ring towards the outside, forming whirls of tissues [Fig. 2(i), (ii) and (iii)].

Powder

Colour yellowish-brown to brown. Cluster crystals of calcium oxalate frequent, 12-200 µm in diameter. Reticulated vessels frequent, but bordered-pitted and spiral vessels are also visible, unlignified, 12-180 µm in diameter. Starch granules abundant, single granules rounded, subround or polygonal, 2-50 µm in diameter, hilum stellate, punctuate, herringbone-shaped, slit-shaped or crossed; compound granules consisting of 2-15 units [Fig. 3(i), (ii) and (iii)].

4.2 Physicochemical Identification

Procedure

Weigh 0.1 g of the powdered sample and put into a 100-mL conical flask, then add 50 mL of water. Heat the mixture on a water bath for 30 min. Cool to room temperature and then filter. Add 2 drops of hydrochloric acid to the filtrate, then extract twice each with 20 mL of diethyl ether and discard the ether layer. To the aqueous extract, add 5 mL of hydrochloric acid. Heat the mixture on a water bath for 30 min. Cool to room temperature. Extract with 20 mL of diethyl ether. Transfer 2 mL of the ether extract to a test tube, add 0.5 mL of sodium hydrogen carbonate solution (10%, w/v) and shake vigorously. A reddish-purple or reddish-brown colouration is observed in the lower layer.

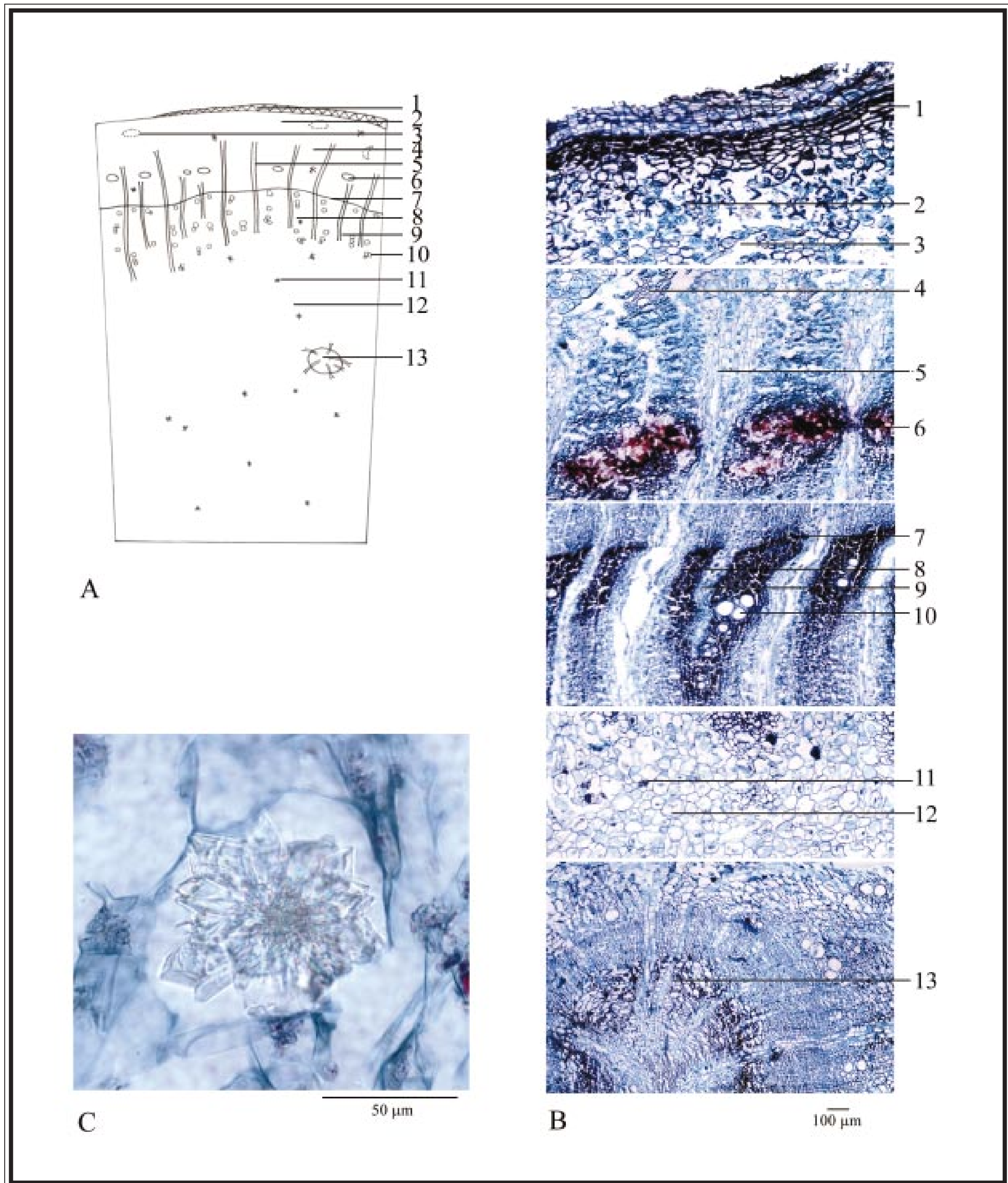

Figure 2(i) Microscopic features of transverse section of dried root and rhizome of *Rheum palmatum* L.

A. Sketch B. Section illustration C. Cluster of calcium oxalate

1. Cork 2. Cortex 3. Cleft 4. Phloem 5. Phloem ray 6. Mucilage cavities 7. Cambium 8. Xylem 9. Xylem ray 10. Vessels 11. Cluster of calcium oxalate 12. Pith 13. Anomalous vascular bundle

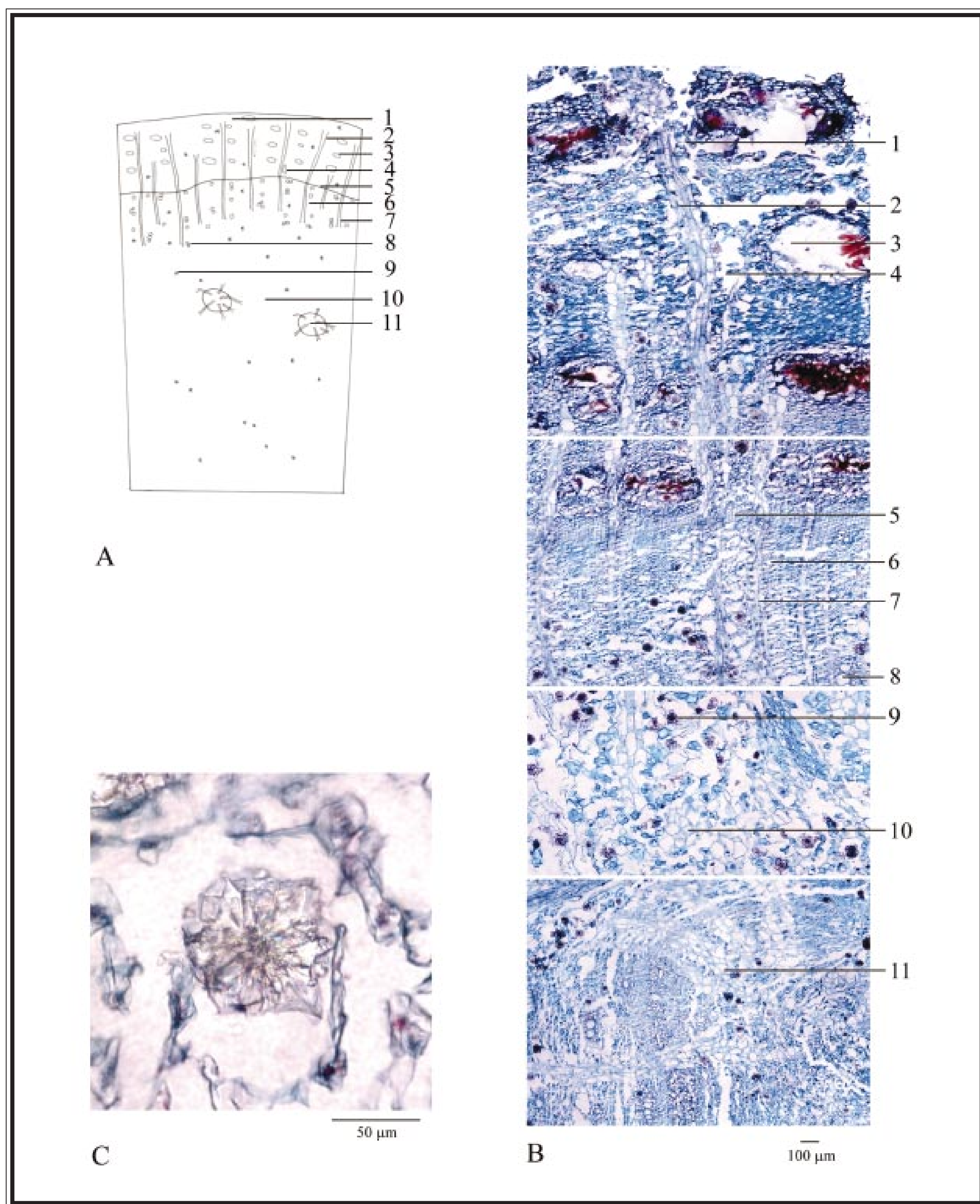

Figure 2(ii) Microscopic features of transverse section of dried root and rhizome of *Rheum tanguticum* Maxim. ex Balf.

A. Sketch B. Section illustration C. Cluster of calcium oxalate

1. Phloem 2. Phloem ray 3. Mucilage cavities 4. Cleft 5. Cambium 6. Xylem 7. Xylem ray 8. Vessels 9. Cluster of calcium oxalate 10. Pith 11. Anomalous vascular bundle

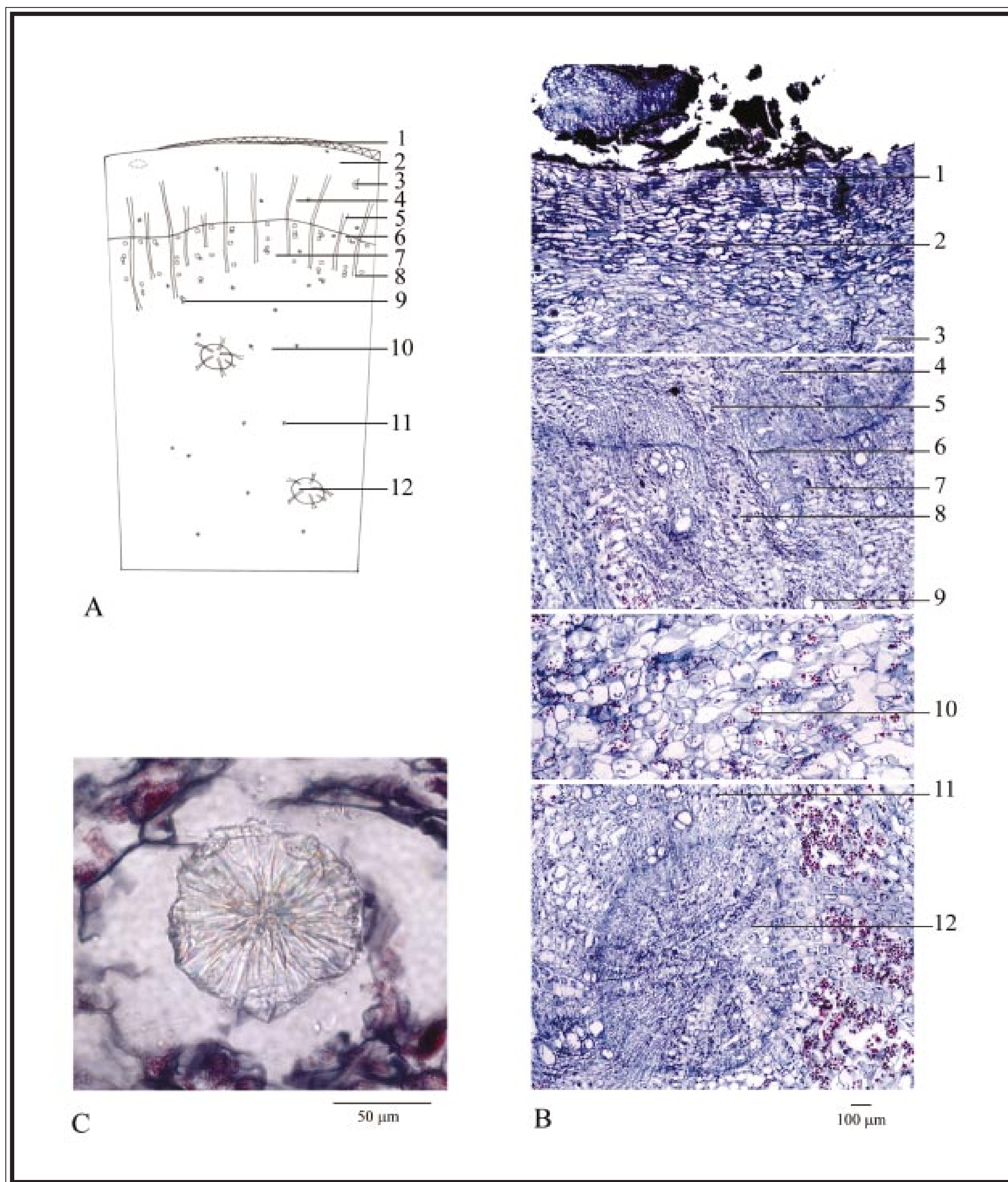

Figure 2(iii) Microscopic features of transverse section of dried root and rhizome of *Rheum officinale* Baill.

A. Sketch B. Section illustration C. Cluster of calcium oxalate

1. Cork 2. Cortex 3. Cleft 4. Phloem 5. Phloem ray 6. Cambium 7. Xylem 8. Xylem ray 9. Vessels 10. Pith 11. Cluster of calcium oxalate 12. Anomalous vascular bundle

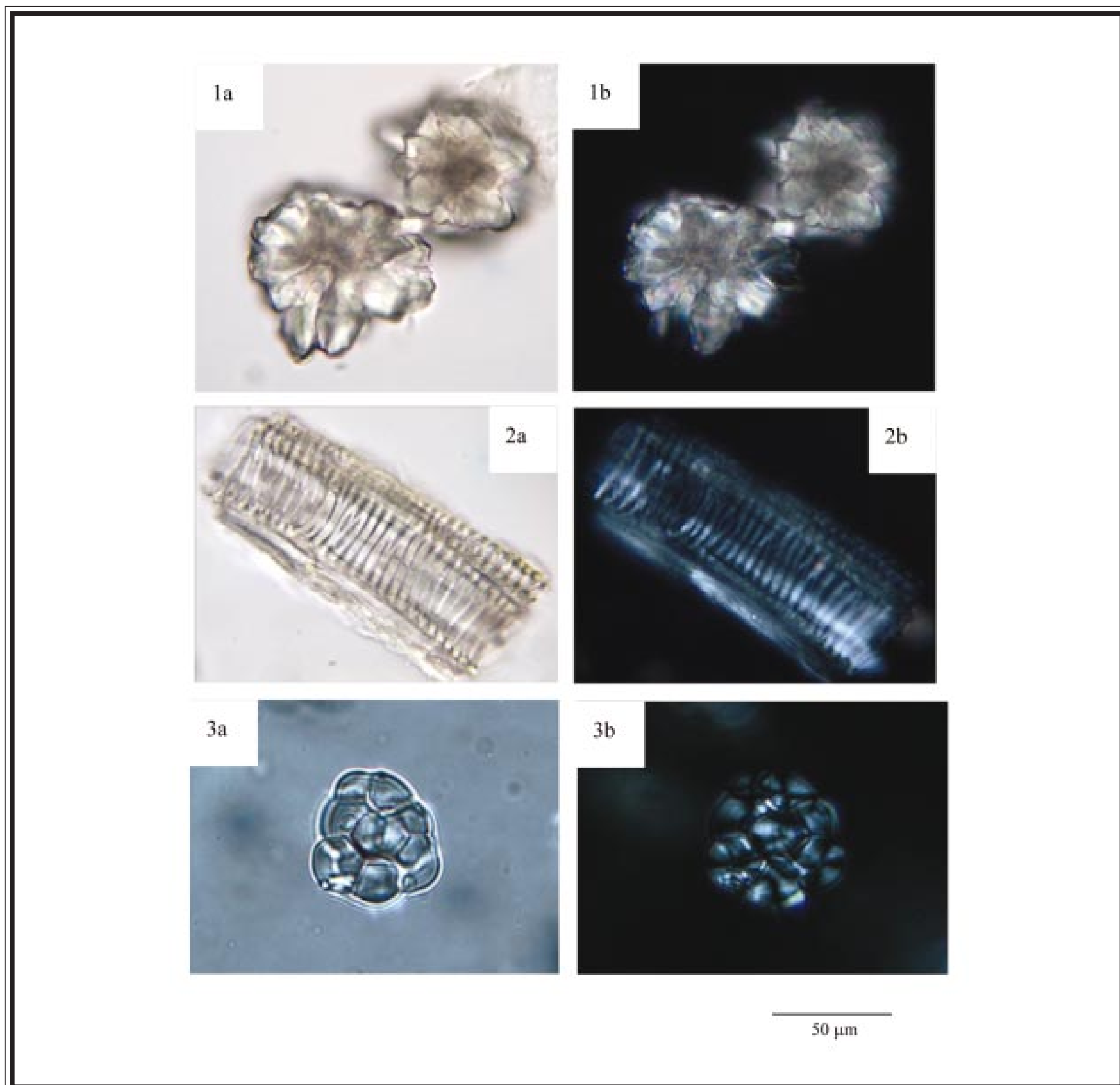

Figure 3(i) Microscopic features of powder of dried root and rhizome of *Rheum palmatum* L.

1. Clusters of calcium oxalate 2. Reticulated vessels 3. Starch granules

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

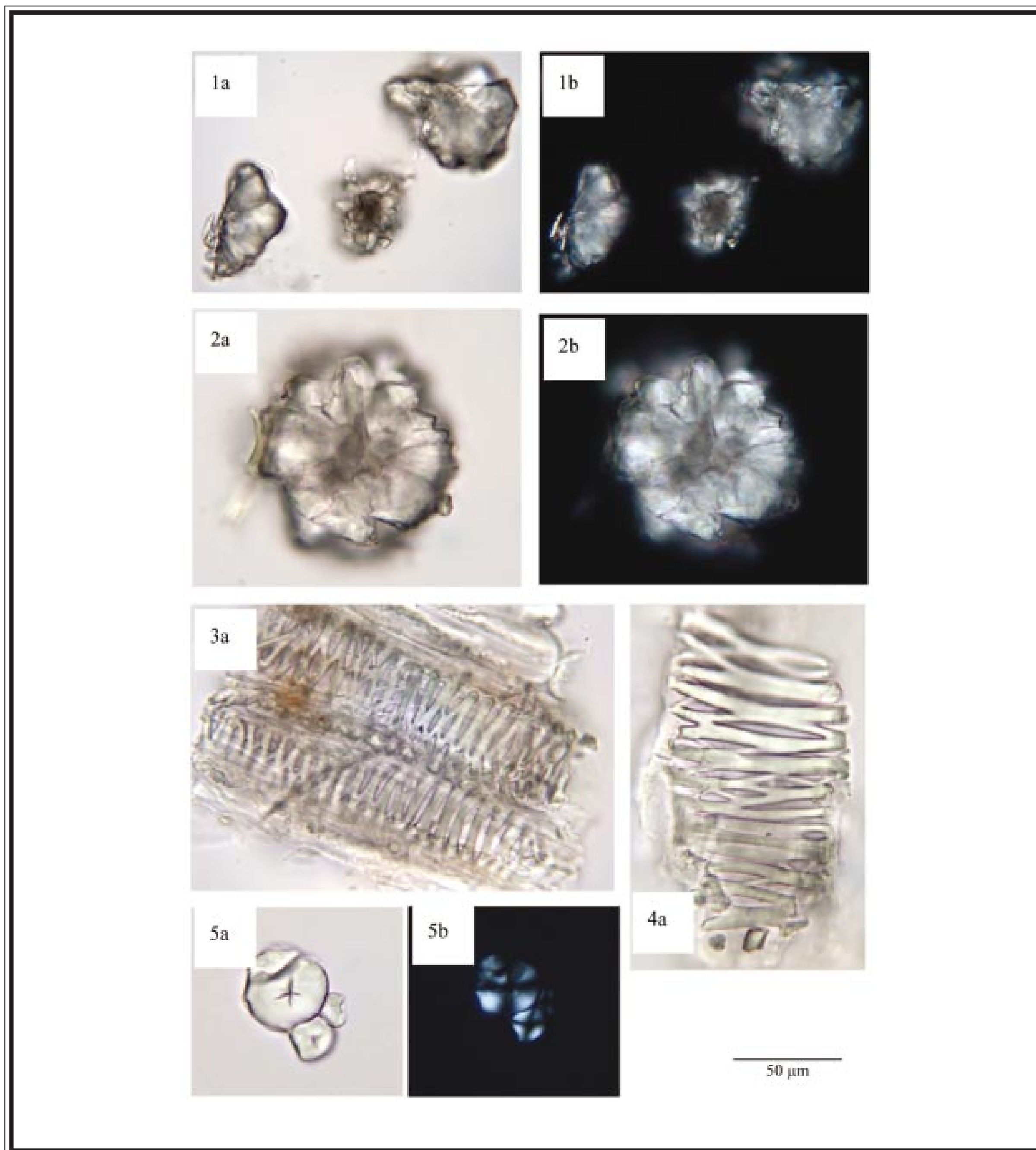

Figure 3(ii) Microscopic features of powder of dried root and rhizome of *Rheum tanguticum* Maxim. ex Balf.

1. Several clusters of calcium oxalate 2. Single cluster of calcium oxalate 3. Several reticulated vessels
4. Single reticulated vessel 5. Starch granules

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

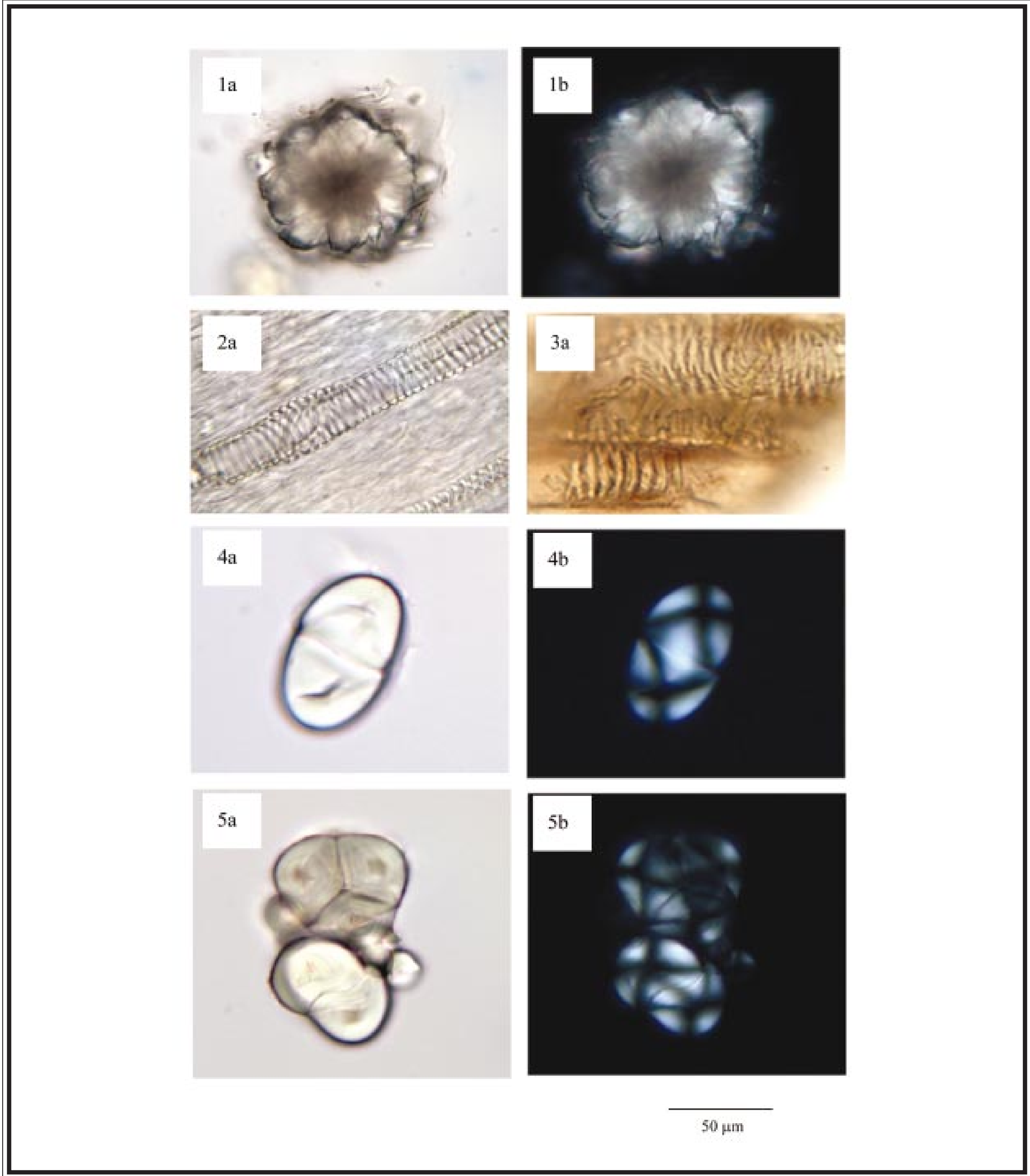

Figure 3(iii) Microscopic features of powder of dried root and rhizome of *Rheum officinale* Baill.

1. Cluster of calcium oxalate 2. Reticulated vessel and spiral vessel 3. Reticulated vessels
4. Compound starch granule 5. Starch granules

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

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

Standard solutions

Aloe-emodin standard solution

Weigh 1.0 mg of aloe-emodin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Chrysophanol standard solution

Weigh 1.0 mg of chrysophanol CRS (Fig. 4) and dissolve in 1 mL of methanol.

Emodin standard solution

Weigh 1.0 mg of emodin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Rhein standard solution

Weigh 1.0 mg of rhein CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of toluene, ethyl acetate and glacial acetic acid (15:5:0.3, v/v).

Spray reagent

Dissolve 10 g of potassium hydroxide in 100 mL of methanol.

Test solution

Weigh 0.1 g of the powdered sample and put into a 100-mL conical flask, then add 20 mL of methanol. Allow to stand for 1 h with occasional shaking. Filter and transfer 5 mL of filtrate to a 25-mL evaporating dish. Evaporate to dryness on a water bath at about 40°C. Add 10 mL of water, followed by 1 mL of hydrochloric acid. Heat the mixture on a water bath for 30 min. Cool immediately. Extract twice each with 20 mL of diethyl ether. Combine the ether extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of dichloromethane.

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 aloe-emodin standard solution, chrysophanol standard solution, emodin standard solution and rhein standard solution (4 µL each) and the test solution (6 µL) 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. Spray the plate evenly with the spray reagent. Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV(A).

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 aloe-emodin, chrysophanol, emodin and rhein.

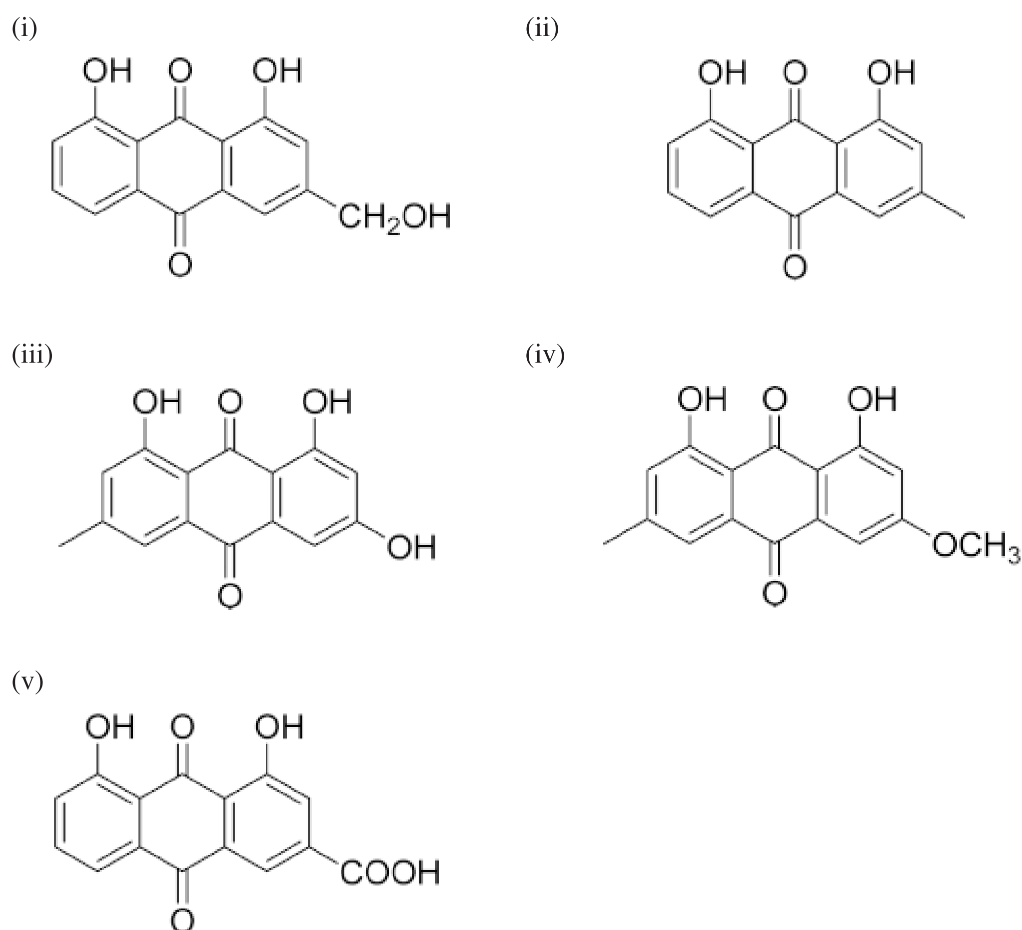

Figure 4 Chemical structures of (i) aloe-emodin (ii) chrysophanol (iii) emodin (iv) physcion and (v) rhein

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

Standard solution

Emodin standard solution for fingerprinting, Std-FP (40 mg/L)

Weigh 2.0 mg of emodin CRS and dissolve in 50 mL of methanol. Sonicate (220 W) to dissolve the standard.

Test solution

Weigh 0.1 g of the powdered sample and put into a 50-mL centrifugal tube, then add 20 mL of methanol. Sonicate (220 W) the mixture for 15 min. Centrifuge at about 3200 × *g* for 10 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. Add 33 mL of hydrochloric acid (3.4%, v/v). Reflux the mixture for 45 min. Cool immediately. Extract thrice each with 60 mL of diethyl ether. Combine the extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 10 mL of methanol. Transfer the solution to a 25-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 (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 –

Time (min)	Acetonitrile (%, v/v)	0.1% Phosphoric acid (%, v/v)	Elution
0 – 35	35 → 80	65 → 20	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 μL of emodin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of emodin should not be more than 5.0%; the RSD of the retention time of emodin peak should not be more than 5.0%; the column efficiency determined from emodin peak should not be less than 15000 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 emodin Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of emodin peak in the chromatogram of emodin Std-FP and the retention times of the five characteristic peaks [Fig. 5(i), (ii) or (iii)] in the chromatogram of the test solution. Under the same HPLC conditions, identify emodin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of emodin Std-FP. The retention times of emodin peaks from the two chromatograms should not differ by more than 5.0%. 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 Radix et Rhizoma Rhei extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the five characteristic peaks of Radix et Rhizoma Rhei extract

Peak No.	RRT	Acceptable Range
1 (aloe-emodin)	0.65	±0.03
2 (rhein)	0.70	±0.03
3 (marker, emodin)	1.00	-
4 (chrysophanol)	1.34	±0.03
5 (physcion)	1.49	±0.03

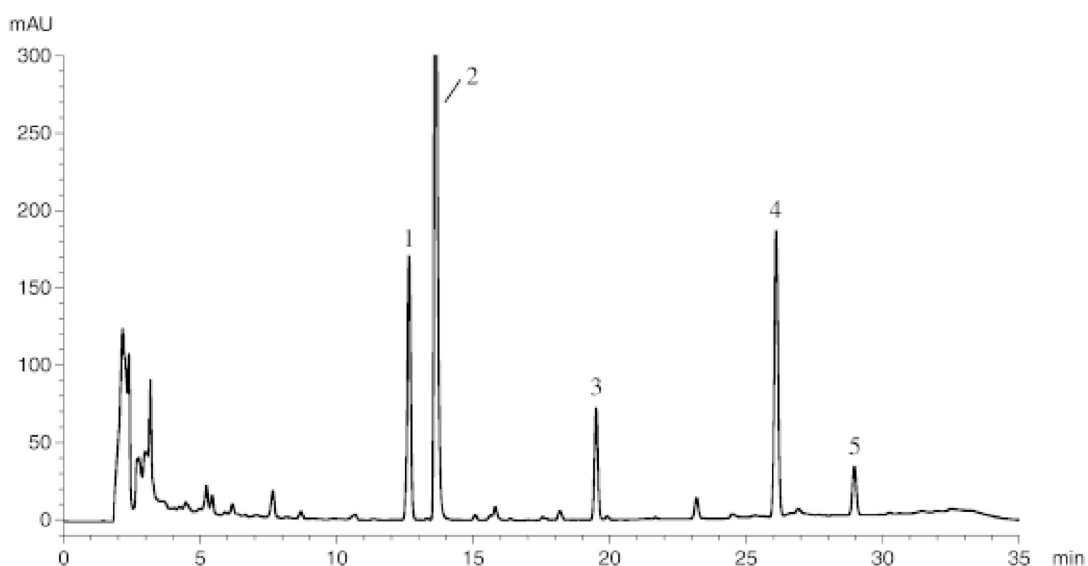

Figure 5(i) A reference fingerprint chromatogram of dried root and rhizome of *Rheum palmatum* L. extract

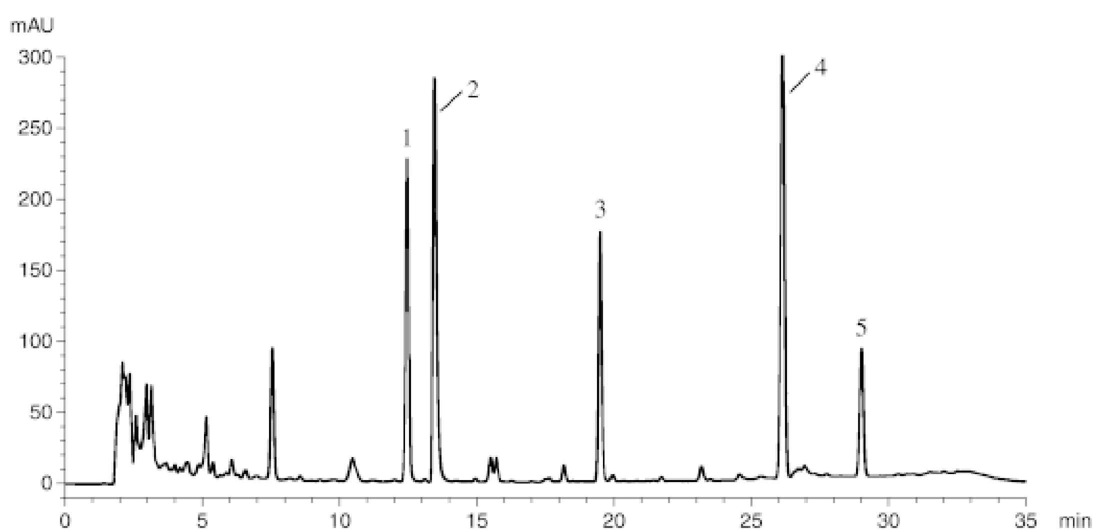

Figure 5(ii) A reference fingerprint chromatogram of dried root and rhizome of *Rheum tanguticum* Maxim. ex Balf. extract

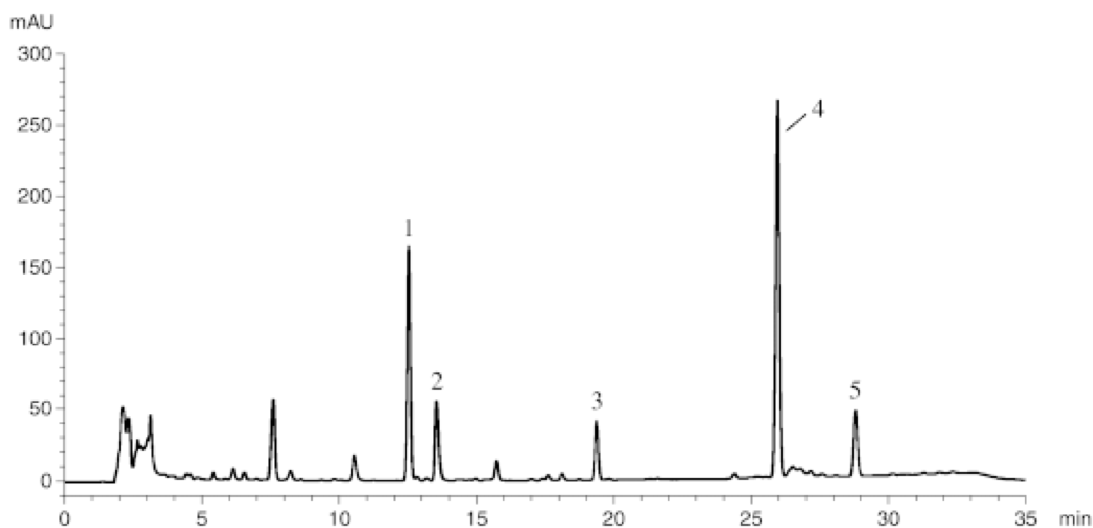

Figure 5(iii) A reference fingerprint chromatogram of dried root and rhizome of *Rheum officinale* Baill. extract

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 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 *(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 13.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*: not more than 12.0%.

5.8 Rhaponticin

Procedure

Weigh 0.2 g of powdered sample and put into a 20-mL test tube, then add 2 mL of methanol. Immerse the mixture in a water bath at about 45°C for 10 min. Cool to room temperature. Pipette 10 μL of supernatant and spot onto a filter paper. Dry in air. Allow to stand for 10 min. Apply 10 μL of ethanol (45%) to the dried spot. Examine the spot under UV light (365 nm). No persistent bright violet fluorescence should be observed.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 27.0%.
Ethanol-soluble extractives (cold extraction method): not less than 20.0%.

7. ASSAY

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

Standard solution

Mixed aloe-emodin, chrysophanol, emodin, physcion and rhein standard stock solution, Std-Stock (40 mg/L each)

Weigh accurately 2.0 mg each of aloe-emodin CRS, chrysophanol CRS, emodin CRS, physcion CRS (Fig. 4) and rhein CRS and dissolve in 50 mL of methanol. Warm and sonicate (220 W) the mixture to dissolve the standards.

Mixed aloe-emodin, chrysophanol, emodin, physcion and rhein standard solution for assay, Std-AS

Measure accurately the volume of the mixed aloe-emodin, chrysophanol, emodin, physcion and rhein Std-Stock, dilute with methanol to produce a series of solutions of 5, 7.5, 10, 20, 30 mg/L for aloe-emodin, chrysophanol, emodin, physcion and rhein.

Test solution

Weigh accurately 0.1 g of the powdered sample and put into a 50-mL centrifugal tube, then add 20 mL of methanol. Sonicate (220 W) the mixture for 15 min. Centrifuge at about 3200× *g* for 10 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. Add 33 mL of hydrochloric acid (3.4%, v/v). Reflux the mixture for 45 min. Cool immediately. Extract thrice each with 60 mL of

diethyl ether. Combine the extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 10 mL of methanol. Transfer the solution to a 25-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 (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 –

Time (min)	Acetonitrile (%, v/v)	0.1% Phosphoric acid (%, v/v)	Elution
0 – 35	35 → 80	65 → 20	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 µL of the mixed aloe-emodin, chrysophanol, emodin, physcion and rhein Std-Stock (40 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of aloe-emodin, chrysophanol, emodin, physcion and rhein should not be more than 5.0%; the RSD of the retention times of aloe-emodin peak, chrysophanol peak, emodin peak, physcion peak and rhein peak should not be more than 2.0%; the column efficiencies determined from aloe-emodin peak, chrysophanol peak, emodin peak, physcion peak and rhein peak should not be less than 15000 theoretical plates.

The *R* value between aloe-emodin peak and rhein peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed aloe-emodin, chrysophanol, emodin, physcion and rhein Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of aloe-emodin, chrysophanol, emodin, physcion and rhein against the corresponding concentrations of the mixed aloe-emodin, chrysophanol, emodin, physcion and rhein Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify the peaks of aloe-emodin, chrysophanol, emodin, physcion and rhein in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed aloe-emodin, chrysophanol, emodin, physcion and rhein Std-AS. The retention times of the peaks of aloe-emodin, chrysophanol,

emodin, physcion and rhein in both chromatograms should not differ from their counterparts by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of aloe-emodin, chrysophanol, emodin, physcion and rhein in the test solution, and calculate the percentage contents of aloe-emodin, chrysophanol, emodin, physcion and rhein in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 1.5% of the total content of aloe-emodin ($C_{15}H_{10}O_5$), chrysophanol ($C_{15}H_{10}O_4$), emodin ($C_{15}H_{10}O_5$), physcion ($C_{16}H_{12}O_5$) and rhein ($C_{15}H_8O_6$), calculated with reference to the dried substance.