

Citri Reticulatae Pericarpium

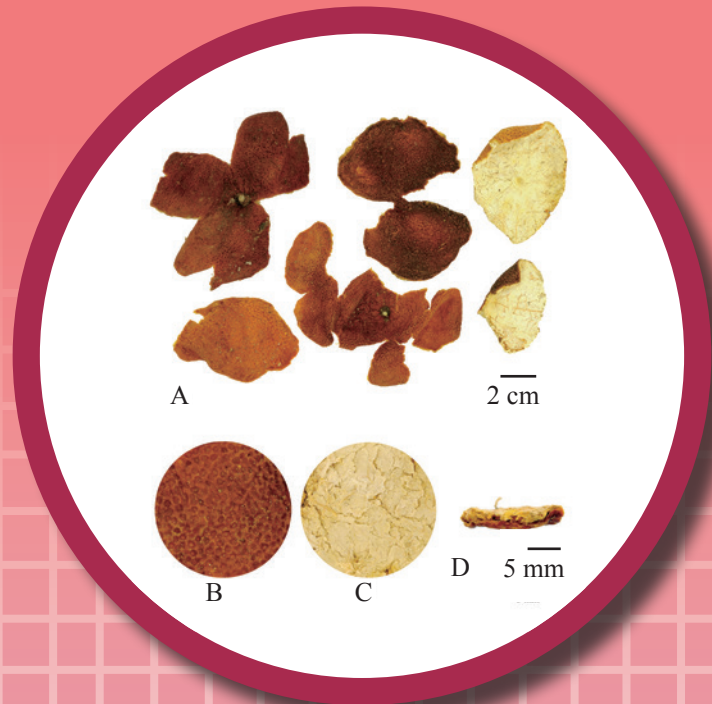


Figure 1 (i) A photograph of Citri Reticulatae Pericarpium – Chenpi

A. Chenpi B. Magnified image of outer surface
C. Magnified image of inner surface D. Lateral view of Chenpi

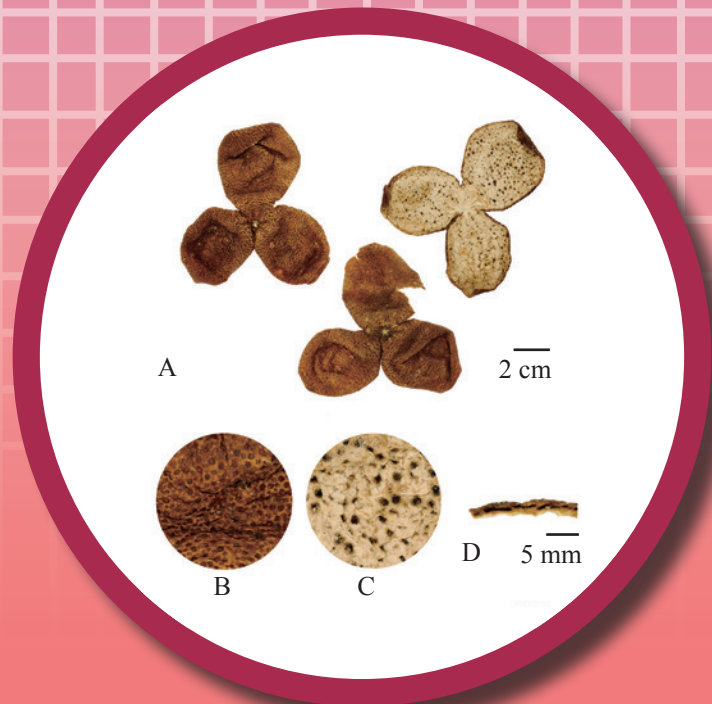


Figure 1 (ii) A photograph of Citri Reticulatae Pericarpium – Guang Chenpi

A. Guang Chenpi B. Magnified image of outer surface
C. Magnified image of inner surface D. Lateral view of Guang Chenpi

1. NAMES

Official Name: Citri Reticulatae Pericarpium

Chinese Name: 陳皮

Chinese Phonetic Name: Chenpi

2. SOURCE

Citri Reticulatae Pericarpium is the dried pericarp of the ripe fruit of *Citrus reticulata* Blanco and its cultivars (Rutaceae). The fruit is collected when it is ripe, the pericarp is peeled off and dried under the sun or at low temperature (below 60°C). The pericarp is subdivided into two types, known as “Chenpi” and “Guang Chenpi”. Guang Chenpi is mainly grown in Guangdong while Chenpi is grown in the other parts of China.

3. DESCRIPTION

Chenpi: Often peeled into several lobes connected at the base, or in singular irregular peel, 1-4 mm thick. Outer surface orange-red to reddish-brown, with fine wrinkles and dotted oil cavity, inner surface pale yellowish-white, rough, with yellowish-white or yellowish-brown vein-like vascular bundles. Texture slightly hard and fragile. Odour aromatic; taste pungent and bitter [Fig.1(i)].

Guang Chenpi: Often peeled into three lobes connected at the base, regular in shape, about 1 mm thick. The dotted oil cavity relatively obverse, transparent when observed against light. Texture slightly soft and fragile. Odour highly aromatic; taste slightly pungent and slightly bitter [Fig.1(ii)].

4. IDENTIFICATION

4.1 Microscopic Identification (*Appendix III*)

Transverse Section

Chenpi: Epidermis consists of 1 layer of small and subsquare cells, covered with cuticle. Mesocarp relatively thick, consisting of parenchymatous cells, with oil cavities, vascular bundles, prisms of calcium oxalate and crystals of hesperidin scattered throughout. Parenchymatous cells of mesocarp rectangular to subrounded, loosely arranged, with unevenly thickened cell wall, sometimes beaded or thickened at corners. Prisms of calcium oxalate numerous, scattered in mesocarp, polyhedral, rhombic or biconical. Oil cavities large, ovoid to ellipsoid, arranged irregularly, consisting 1-2 layers of cells. Vascular bundles small, collateral, randomly scattered. Crystals of hesperidin numerous, mostly presenting in parenchymatous cells, fan-shaped, rounded or as amorphous masses, some crystals with radial striations [Fig. 2(i)].

Guang Chenpi: Mesocarp relative thin. Crystals of hesperidin less abundant [Fig. 2(ii)].

Powder

Chenpi: Colour yellowish-white to yellowish-brown. Epidermal cells of pericarp polygonal, subsquare or rectangular, cell walls slightly thickened, stomata actinocytic, subrounded, 18-29 μm in diameter, subsidiary cells 6-8, indistinct. Parenchymatous cells of mesocarp numerous, cells irregular in shape, with unevenly thickened walls, sometimes beaded or thickened at corners. Crystals of hesperidin numerous, colourless or yellow, fan-shaped, rounded or as amorphous masses, some crystals with radial striations. Prisms of calcium oxalate numerous, polyhedral, rhombic or biconical, 3-32 μm in diameter, 7-41 μm long; sometimes two parallel polyhedral crystals or 3-5 prisms occur together in one cell. Spiral, pitted and reticulate vessels and tracheids small [Fig. 3(i)].

Guang Chenpi: Stomata actinocytic, subrounded, 18-28 μm in diameter, subsidiary cells 6-8, indistinct. Prisms of calcium oxalate numerous, polyhedral, rhombic or biconical, 4-33 μm in diameter, 6-38 μm long; sometimes two parallel polyhedral crystals or 3-5 prisms occur together in one cell [Fig. 3(ii)].

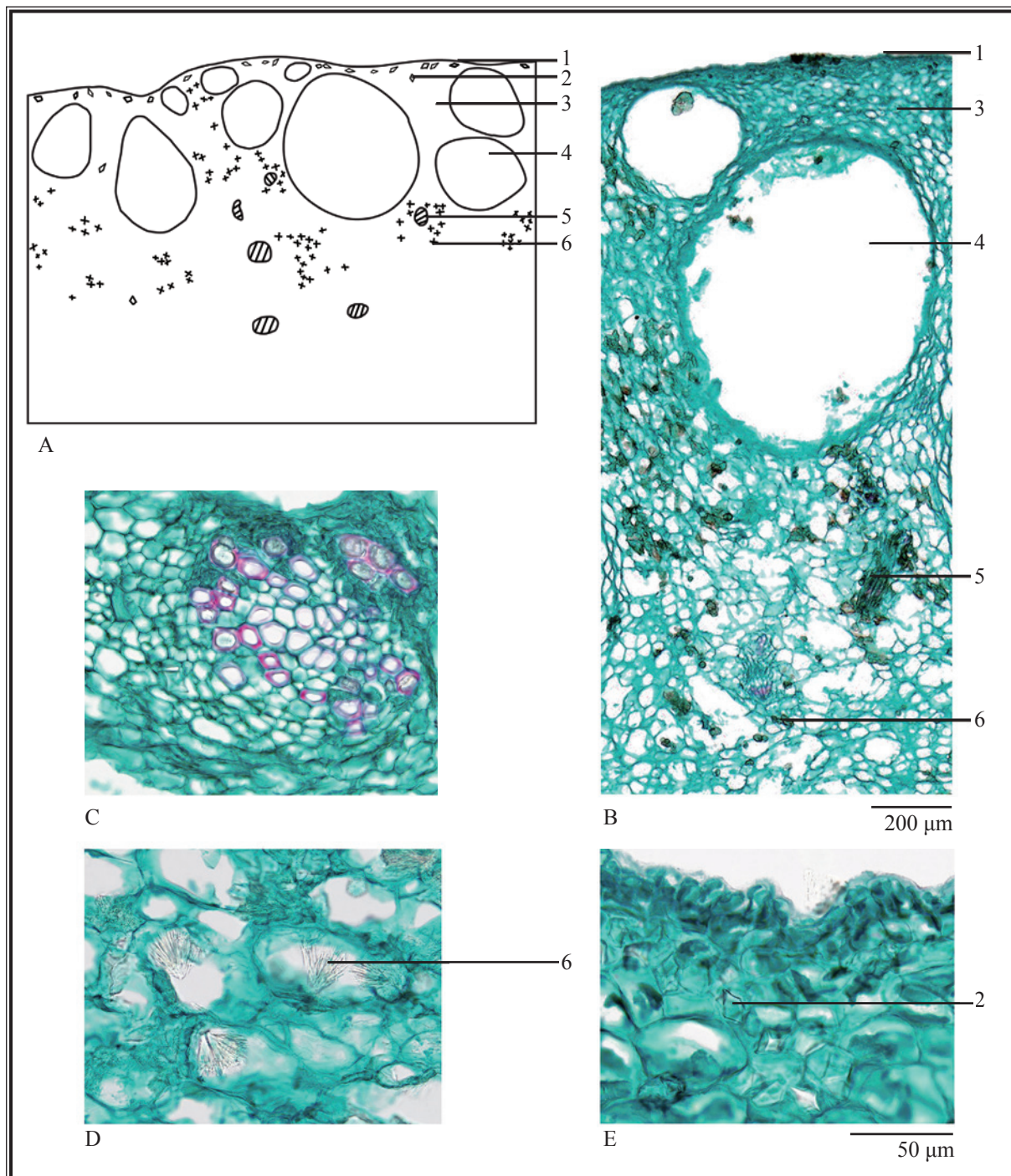


Figure 2 (i) Microscopic features of transverse section of Citri Reticulatae Pericarpium – Chenpi

A. Sketch B. Section illustration C. Vascular bundle D. Crystals of hesperidin
E. Prisms of calcium oxalate

1. Epidermis 2. Prism of calcium oxalate 3. Mesocarp 4. Oil cavity
5. Vascular bundle 6. Crystal of hesperidin

山豆根

Saururi Herba 三白草

牡荊葉

車前草

蓮鬚

Saussureae Involucratae Herba

Polygoni Perfoliati Herba

Lonicerae Flos

Plantaginis Herba

Bruceae Fructus 鴉膽子

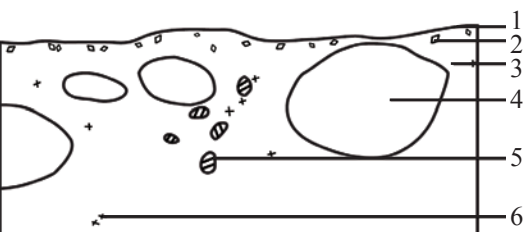
天山雪蓮

白花丹

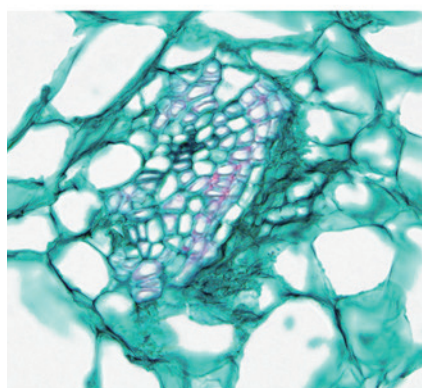
杠板歸

北豆根

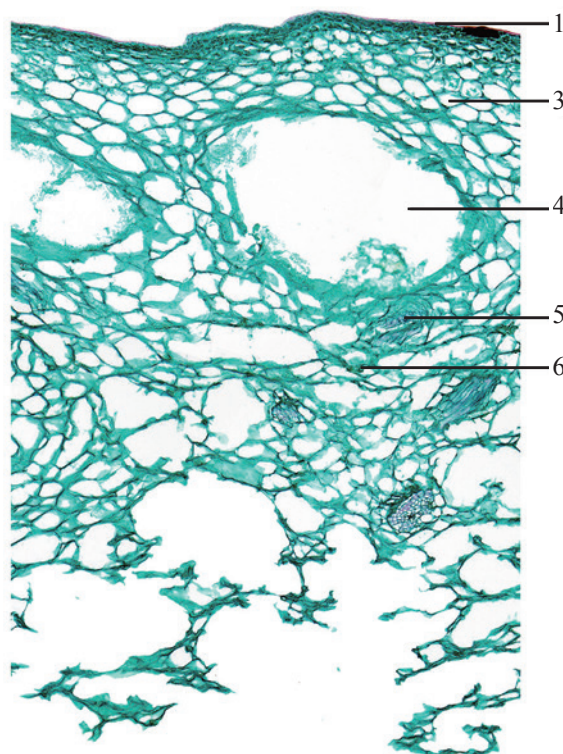
山銀花

Citri Reticulatae Pericarpium

A

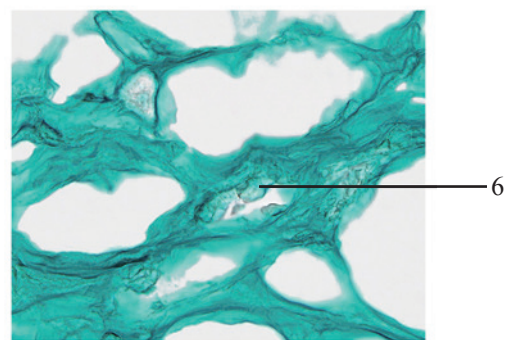


C



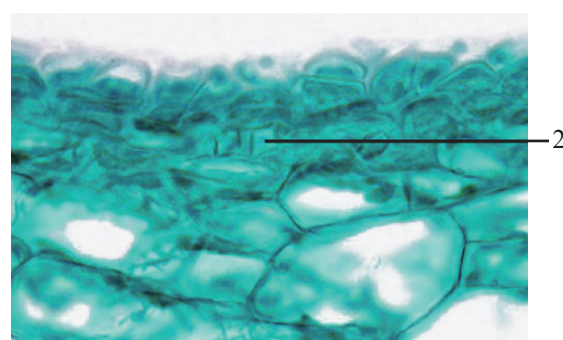
B

200 μm



D

50 μm



E

50 μm

Figure 2 (ii) Microscopic features of transverse section of *Citri Reticulatae Pericarpium* – Guang Chenpi

A. Sketch B. Section illustration C. Vascular bundle D. Crystals of hesperidin

E. Prisms of calcium oxalate

1. Epidermis 2. Prism of calcium oxalate 3. Mesocarp 4. Oil cavity

5. Vascular bundle 6. Crystal of hesperidin

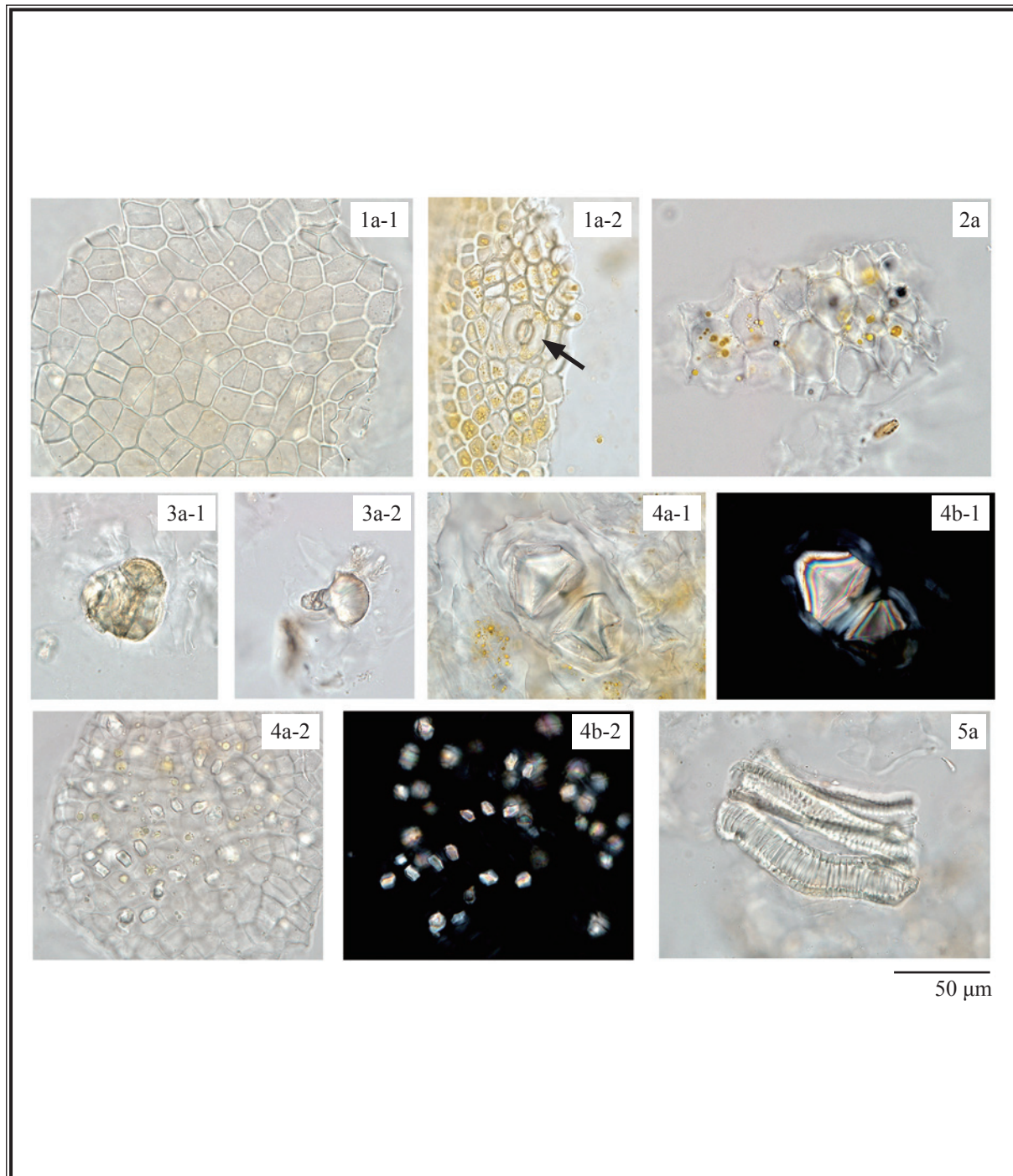


Figure 3 (i) Microscopic features of powder of Citri Reticulatae Pericarpium – Chenpi

1. Epidermal cells of pericarp (1-1 epidermal cells of pericarp, 1-2 epidermal cells of pericarp with stoma →)
2. Parenchymatous cells of mesocarp
3. Crystals of hesperidin (3-1 rounded, 3-2 fan-shaped)
4. Prisms of calcium oxalate (4-1 two parallel polyhedral crystals in a cell, 4-2 rhombic crystals)
5. Reticulate vessels

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

山豆根

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牡荊葉

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Saussureae Involucratae Herba

天山雪蓮

白花丹

Polygoni Perfoliati Herba

杠板歸

北豆根

Menispermi Rhizoma

Lonicerae Flos

山銀花

Plantaginis Herba

Bruceae Fructus 鴉膽子

Plumbaginis Zeylanicae Radix

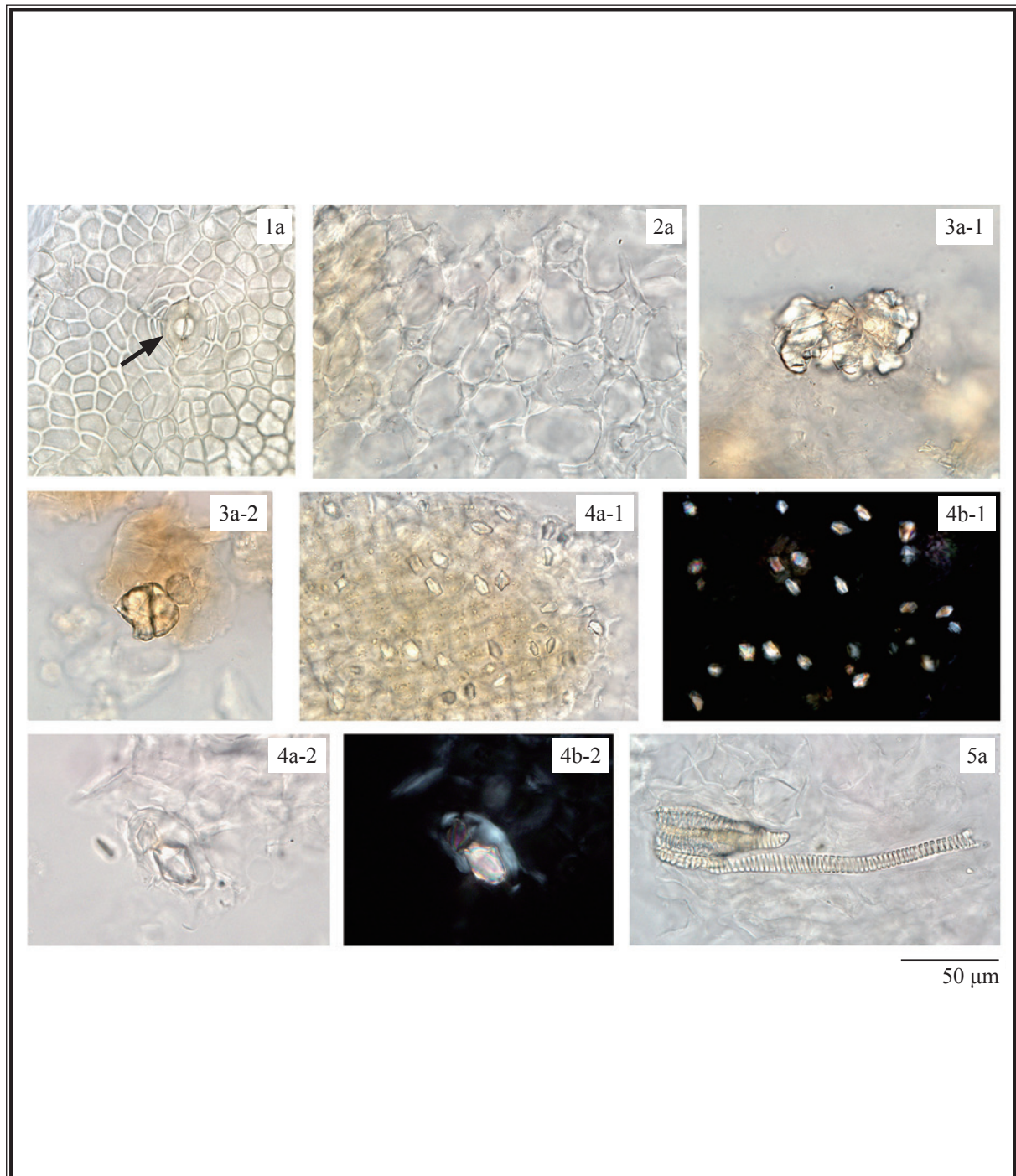
Citri Reticulatae Pericarpium

Figure 3 (ii) Microscopic features of powder of *Citri Reticulatae Pericarpium* – Guang Chenpi

1. Epidermal cells of pericarp with stoma (→)
2. Parenchymatous cells of mesocarp
3. Crystals of hesperidin (3-1 amorphous, 3-2 rounded)
4. Prisms of calcium oxalate (4-1 rhombic crystals, 4-2 two rhombic crystals in a cell)
5. Spiral vessels

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

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

Standard solution

Hesperidin standard solution

Weigh 2.0 mg of hesperidin CRS (Fig. 4) and dissolve in 5 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane, methanol and water (13:7:2, v/v). Shake well and use the lower layer.

Spray reagent

Weigh 1 g of aluminium trichloride and dissolve in 100 mL of ethanol.

Test solution

Weigh 0.3 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (180 W) the mixture for 30 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F₂₅₄ plate and a freshly prepared developing solvent system as described above. Apply separately hesperidin standard solution and the test solution (1 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. Spray the plate evenly with the spray reagent and heat at about 105°C (about 5 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

山豆根

Saururi Herba 三白草

牡荊葉

車前草

蓮鬚

Saussureae Involucratae Herba

Polygoni Perfoliati Herba

北豆根

Lonicerae Flos

Plantaginis Herba

Bruceae Fructus 鴉膽子

天山雪蓮

白花丹

杠板歸

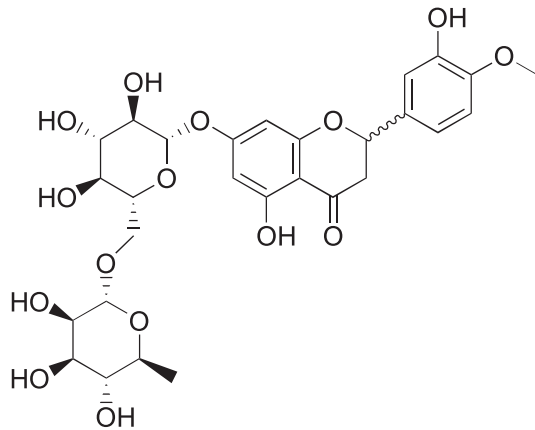
Menispermī Rhizoma

山銀花

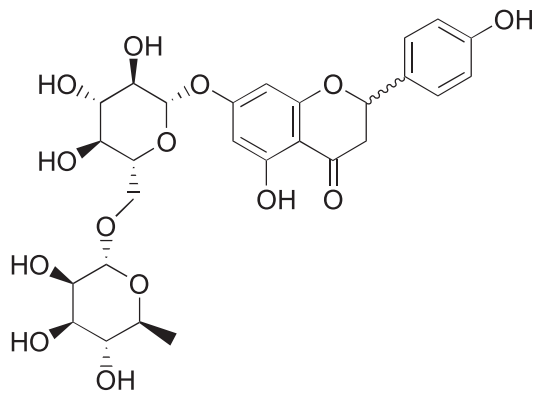
Citri Reticulatae Pericarpium

Plumbaginis Zeylanicae Radix

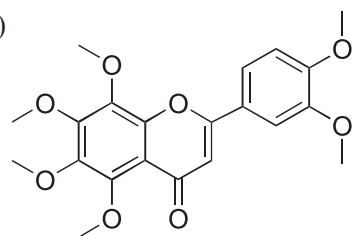
(i)



(ii)



(iii)



(iv)

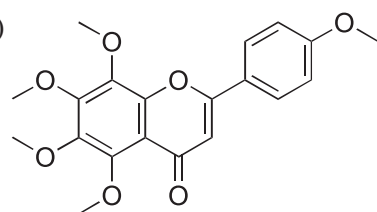


Figure 4 Chemical structures of (i) hesperidin (ii) narirutin (iii) nobiletin and (iv) tangeretin

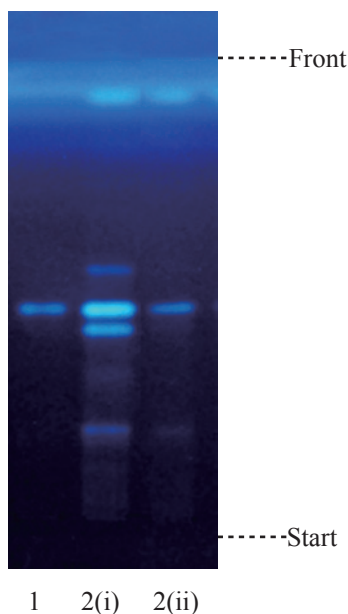


Figure 5 A reference HPTLC chromatogram of Citri Reticulatae Pericarpium extract observed under UV light (366 nm) after staining

1. Hesperidin standard solution
2. Test solution of
 - (i) Chenpi
 - (ii) Guang Chenpi

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 hesperidin (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solutions

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

Weigh 1.0 mg of hesperidin CRS and dissolve in 20 mL of methanol.

Narirutin standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.2 mg of narirutin CRS (Fig. 4) and dissolve in 20 mL of methanol.

Nobiletin standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.2 mg of nobiletin CRS (Fig. 4) and dissolve in 20 mL of methanol.

Tangeretin standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.2 mg of tangeretin CRS (Fig. 4) and dissolve in 20 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 90 mL of methanol. Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL volumetric flask. Wash the residue for three times each with 3 mL of methanol. Combine the solutions and make up to the mark with methanol. Filter through a 0.45- μ m PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (283 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μ m 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 – 20	79	21	isocratic
20 – 30	79 \rightarrow 55	21 \rightarrow 45	linear gradient
30 – 60	55	45	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μ L of hesperidin Std-FP, narirutin Std-FP, nobiletin Std-FP and tangeretin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of hesperidin, narirutin, nobiletin and tangeretin should not be more than 5.0%; the RSD of the retention times of hesperidin, narirutin, nobiletin and tangeretin peaks should not be more than 2.0%; the column efficiencies determined from hesperidin, narirutin, nobiletin and tangeretin peaks should not be less than 4000, 4000, 50000 and 35000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; the *R* value between peak 2 and the closest peak; the *R* value between peak 4 and the closest peak; and the *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 [Fig. 6 (i) or (ii)].

Procedure

Separately inject hesperidin Std-FP, narirutin Std-FP, nobiletin Std-FP, tangeretin Std-FP and the test solution (10 μ L each) into the HPLC system and record the chromatograms. Measure the retention times of hesperidin, narirutin, nobiletin and tangeretin peaks in the chromatograms of hesperidin Std-FP, narirutin Std-FP, nobiletin Std-FP, tangeretin Std-FP and the retention times of the five characteristic peaks [Fig. 6 (i) or (ii)] in the chromatogram of the test solution. Identify

hesperidin, narirutin, nobiletin and tangeretin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of hesperidin Std-FP, narirutin Std-FP, nobiletin Std-FP and tangeretin Std-FP. The retention times of hesperidin, narirutin, nobiletin and tangeretin 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 five characteristic peaks of Citri Reticulatae Pericarpium extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Citri Reticulatae Pericarpium extract

Peak No.	RRT	Acceptable Range
1 (narirutin)	0.30	± 0.03
2 (hesperidin)	0.39	± 0.04
3	0.65	± 0.03
4 (marker, nobiletin)	1.00	-
5 (tangeretin)	1.19	± 0.03

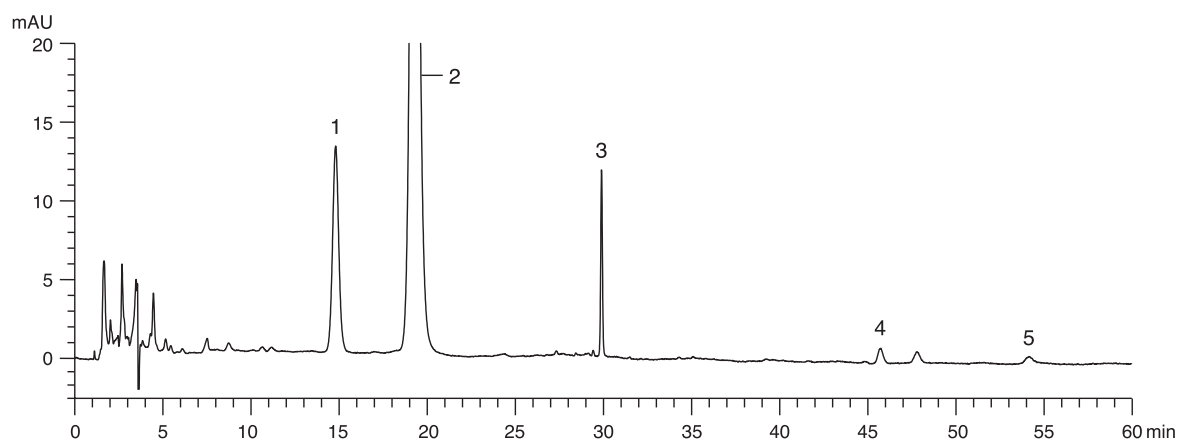


Figure 6 (i) A reference fingerprint chromatogram of Citri Reticulatae Pericarpium – Chenpi extract

山豆根

Saururi Herba 三白草

牡荊葉

車前草

蓮鬚

Saussureae Involucratae Herba

Polygoni Perfoliati Herba

Loniceræ Flos

Plantaginis Herba

Bruceae Fructus 鴉膽子

天山雪蓮

白花丹

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Plumbaginis Zeylanicae Radix

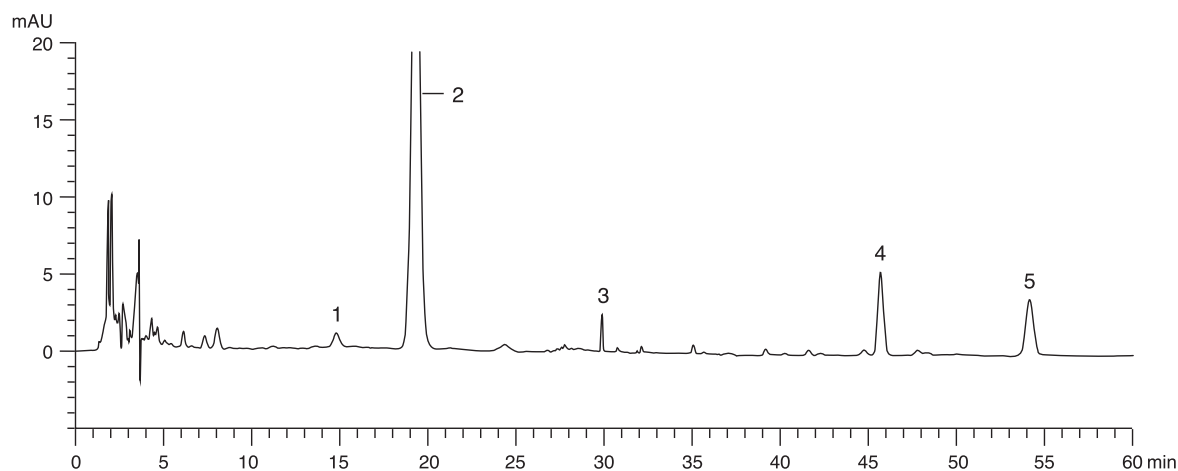


Figure 6 (ii) A reference fingerprint chromatogram of *Citri Reticulatae Pericarpium* – Guang Chenpi extract

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. 6 (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 XVI*): meet the requirements.

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

5.6 Ash (*Appendix IX*)

Total ash: not more than 4.0%.

Acid-insoluble ash: not more than 1.0%.

5.7 Water Content (*Appendix X*)

Toluene distillation method: not more than 13.0%.

6. EXTRACTIVES (Appendix XI)

Water-soluble extractives (hot extraction method): not less than 39.0%.

Ethanol-soluble extractives (hot extraction method): not less than 29.0%.

7. ASSAY

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

Standard solution

Mixed hesperidin, narirutin, nobiletin and tangeretin standard stock solution, Std-Stock (500 mg/L each)

Weigh accurately 2.5 mg of hesperidin CRS, 2.5 mg of narirutin CRS, 2.5 mg of nobiletin CRS and 2.5 mg of tangeretin CRS, and dissolve in 5 mL of methanol.

Mixed hesperidin, narirutin, nobiletin and tangeretin standard solution for assay, Std-AS

Measure accurately the volume of the mixed hesperidin, narirutin, nobiletin and tangeretin Std-Stock, dilute with methanol to produce a series of solutions of 5, 25, 50, 100, 250 mg/L for hesperidin, 0.5, 1, 5, 20, 50 mg/L for narirutin, 0.5, 1, 5, 20, 50 mg/L for nobiletin and 0.5, 1, 5, 20, 50 mg/L for tangeretin.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 90 mL of methanol. Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL volumetric flask. Wash the residue for three times each with 3 mL of methanol. Combine the solutions and make up to the mark with methanol. Filter through a 0.45- μ m PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (283 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μ m 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)	Water (% v/v)	Acetonitrile (% v/v)	Elution
0 – 20	79	21	isocratic
20 – 30	79 \rightarrow 55	21 \rightarrow 45	linear gradient
30 – 60	55	45	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed hesperidin, narirutin, nobiletin and tangeretin Std-AS (50 mg/L for hesperidin, 5 mg/L for narirutin, 5 mg/L for nobiletin and 5 mg/L for tangeretin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of hesperidin, narirutin, nobiletin and tangeretin should not be more than 5.0%; the RSD of the retention times of hesperidin, narirutin, nobiletin and tangeretin peaks should not be more than 2.0%; the column efficiencies determined from hesperidin, narirutin, nobiletin and tangeretin peaks should not be less than 4000, 4000, 50000 and 35000 theoretical plates respectively.

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

Limits

The sample contains not less than 2.9% of the total content of hesperidin ($\text{C}_{28}\text{H}_{34}\text{O}_{15}$) and narirutin ($\text{C}_{27}\text{H}_{32}\text{O}_{14}$); and not less than 0.071% of the total content of nobiletin ($\text{C}_{21}\text{H}_{22}\text{O}_8$) and tangeretin ($\text{C}_{20}\text{H}_{20}\text{O}_7$), calculated with reference to the dried substance.