Persicae Semen

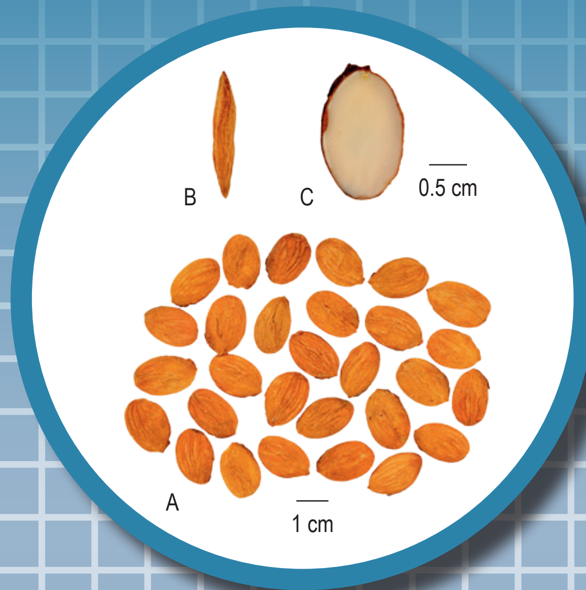

Figure 1 (i) A photograph of the seeds of *Prunus persica* (L.) Batsch

A. Seeds B. Lateral view of seed C. Cotyledon of seed

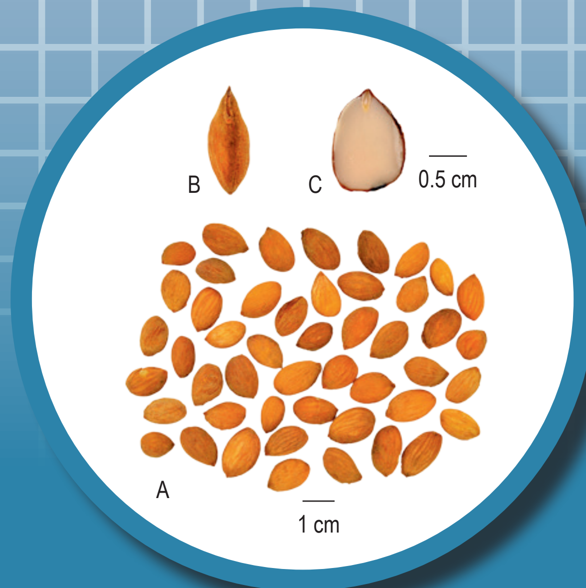

Figure 1 (ii) A photograph of the seeds of *Prunus davidiana* (Carr.) Franch.

A. Seeds B. Lateral view of seed C. Cotyledon of seed

1. NAMES

Official Name: Persicae Semen

Chinese Name: 桃仁

Chinese Phonetic Name: Taoren

2. SOURCE

Persicae Semen is the dried ripe seed of *Prunus persica* (L.) Batsch or *Prunus davidiana* (Carr.) Franch. (Rosaceae). The ripe fruit is collected, sarcocarp and endocarp are removed, the seed gathered and then dried under the sun to obtain Persicae Semen.

3. DESCRIPTION

***Prunus persica* (L.) Batsch:** Prolate-ovate, 1.1-2.1 cm long, 0.6-1.4 cm wide, 2-4 mm thick. Externally yellowish-brown to reddish-brown, with numerous granular protuberance. One end acute, expanded in the middle, the other end obtuse-rounded and slightly oblique, with relatively thin edge. A short linear hilum occurring at the apex and a relatively indistinct and slightly dark chalaza at the round end, with many longitudinal vascular bundles radiated from the chalaza. Testa thin, cotyledons 2, white and oily. Odour slight; taste slightly bitter [Fig. 1 (i)].

***Prunus davidiana* (Carr.) Franch.:** Subovoid, relatively smaller but thicker, 0.8-1.7 cm long, 0.6-1.1 cm wide and 4-7 mm thick [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Testa consists of several layers of parenchymatous cells, scattered with stone cells throughout. Vascular bundles pass through testa. Perisperm cells consist of 1 layer of cells, shrunken, underneath testa. Endosperm consists of 1 to several layers of rectangular to square cells, containing aleurone grains and oil droplets. Cotyledons consist of polygonal parenchymatous cells, containing aleurone grains, crystals of calcium oxalate and oil droplets. Primary vascular bundles scattered among cotyledons. Crystals of calcium oxalate, rosette-aggregated, scattered along testa and cotyledons [Fig. 2 (i) and (ii)].

Powder

***Prunus persica* (L.) Batsch:** Colour yellowish-white. Stone cells pale yellow to brownish-yellow, conchoidal, helmet-shaped, bow-shaped or elliptic in lateral view, 49-218 µm high, 32-208 µm wide at the base, with one side wall thicker and more densely striated than the other side, the thicker side of the cell wall about 8-40 µm thick; cell subrounded, rounded, polygonal or subsquare in surface view, with large and dense pits at the bottom wall. Testa cells orangish-red, subrounded to polygonal. Endosperm cells contain oil droplets, walls slightly thickened. Cotyledon cells contain small crystals of calcium oxalate, aleurone grains and oil droplets [Fig. 3 (i)].

***Prunus davidiana* (Carr.) Franch.:** Stone cells conchoidal, oblong, elliptic or stripe-shaped in lateral view, 43-233 µm high, 26-217 µm wide, with one side wall thicker and more densely striated than the other side, the thicker side of the wall 9-35 µm thick; cell subrounded, subhexagonal, prolately polygonal or subsquare in surface view, with smaller pits in the unevenly thickened bottom walls [Fig. 3 (ii)].

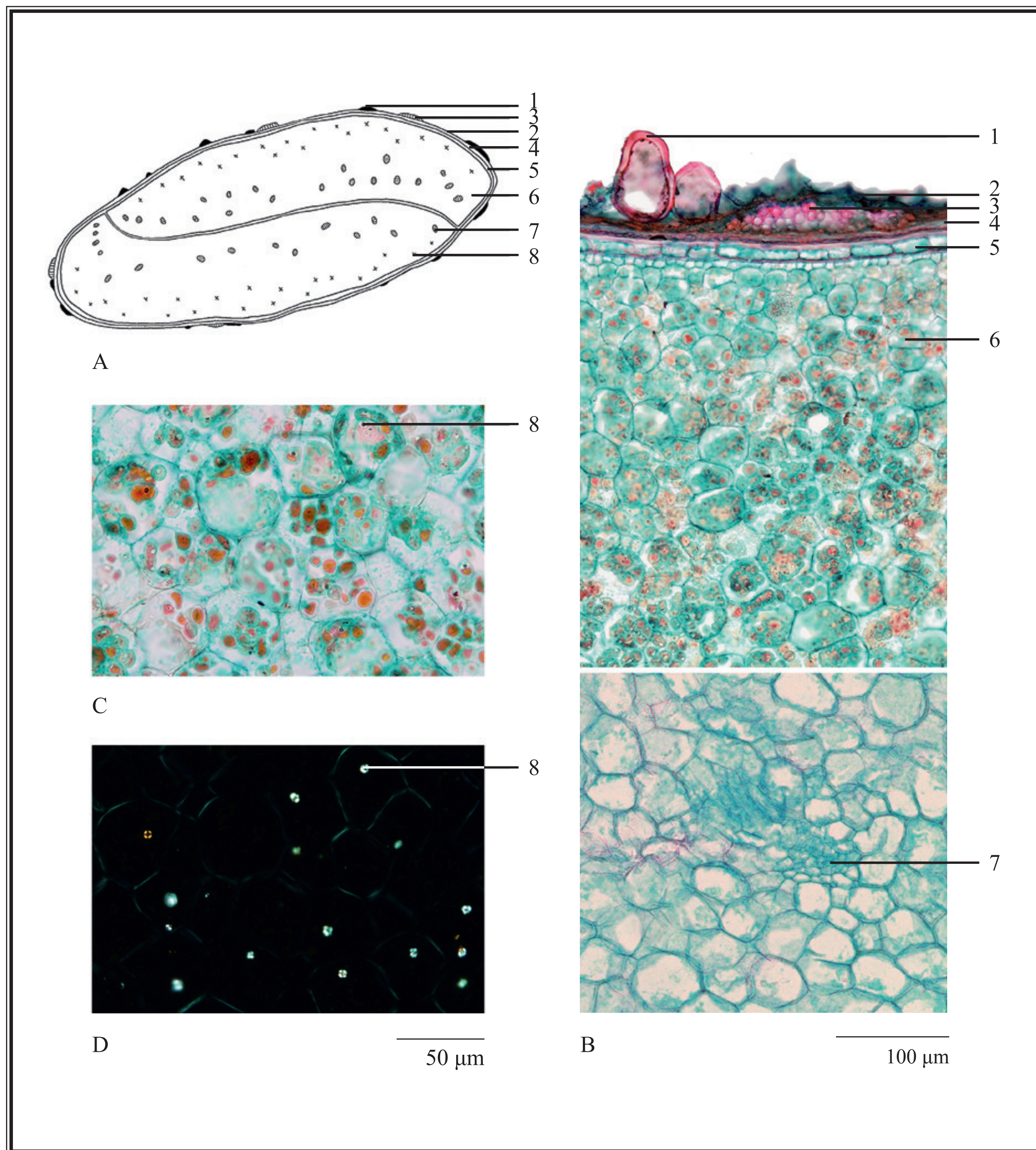

Figure 2 (i) Microscopic features of transverse section of dried ripe seed of *Prunus persica* (L.) Batsch

A. Sketch B. Section illustration C. Cotyledon
D. Cotyledon (under the polarized microscope)

1. Stone cell 2. Testa 3. Vascular bundles 4. Perisperm 5. Endosperm
6. Cotyledon 7. Primary vascular bundle 8. Crystal of calcium oxalate

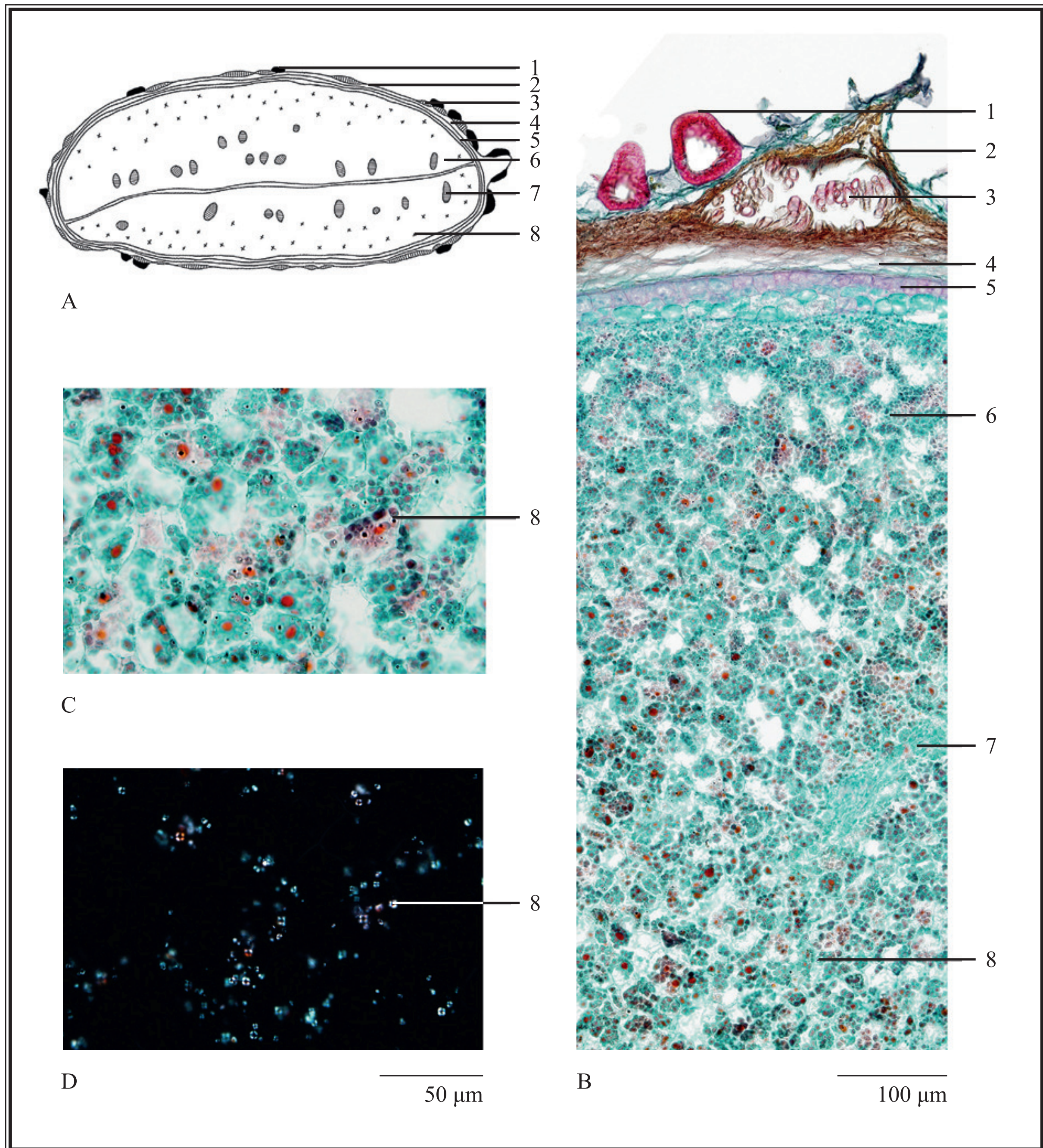

Figure 2 (ii) Microscopic features of transverse section of dried ripe seed of *Prunus davidiana* (Carr.) Franch.

A. Sketch B. Section illustration C. Cotyledon D. Cotyledon (under the polarized microscope)

1. Stone cell 2. Testa 3. Vascular bundles 4. Perisperm 5. Endosperm
6. Cotyledon 7. Primary vascular bundle 8. Crystal of calcium oxalate

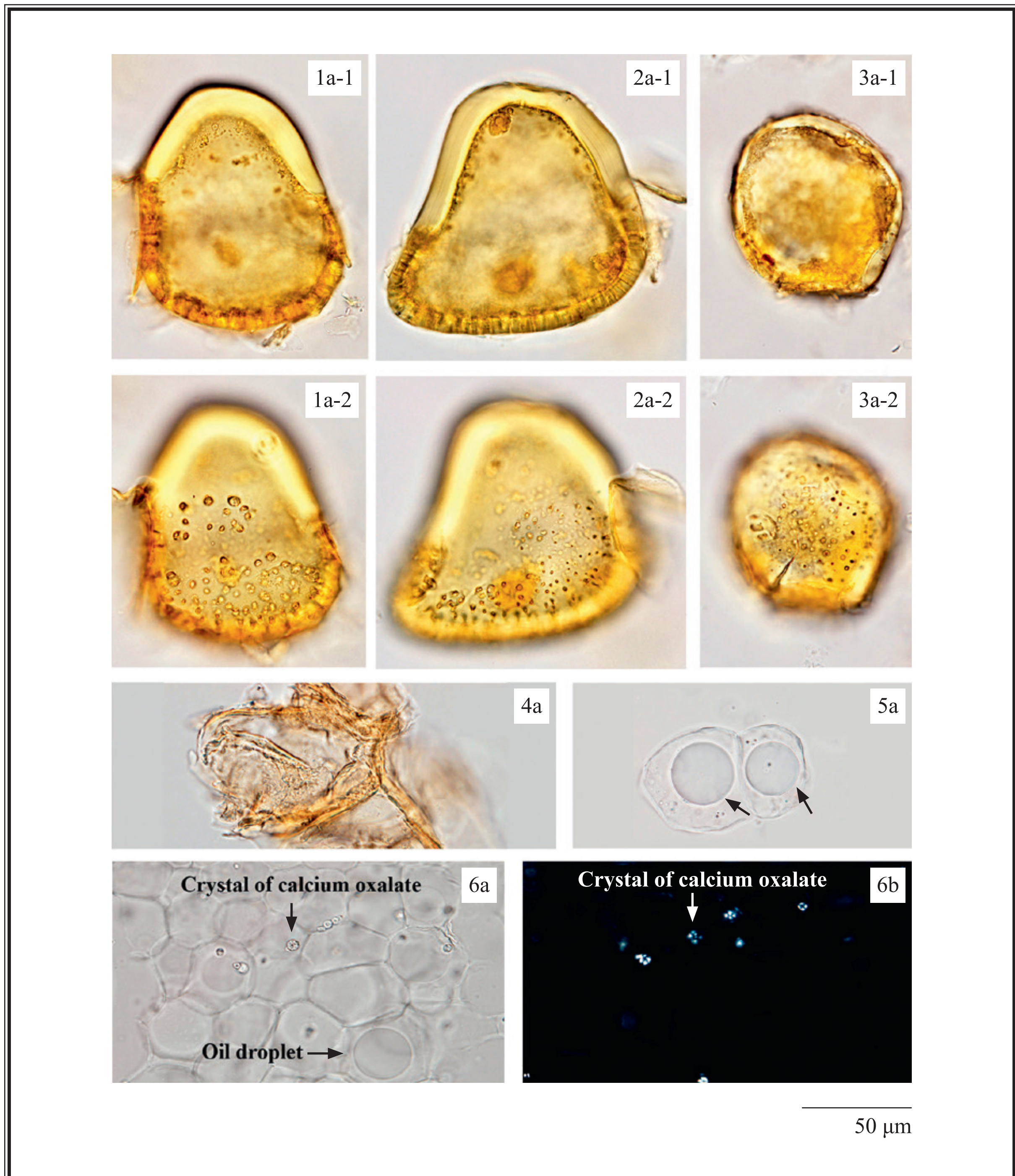

Figure 3 (i) Microscopic features of powder of dried ripe seed of *Prunus persica* (L.) Batsch

1. Stone cell (1-1 in lateral view, 1-2 with pits) 2. Stone cell (2-1 in lateral view, 2-2 with pits)
3. Stone cell (3-1 in base view, 3-2 with pits) 4. Testa cells
5. Endosperm cells containing oil droplets
6. Cotyledon cells containing crystals of calcium oxalate and oil droplets

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

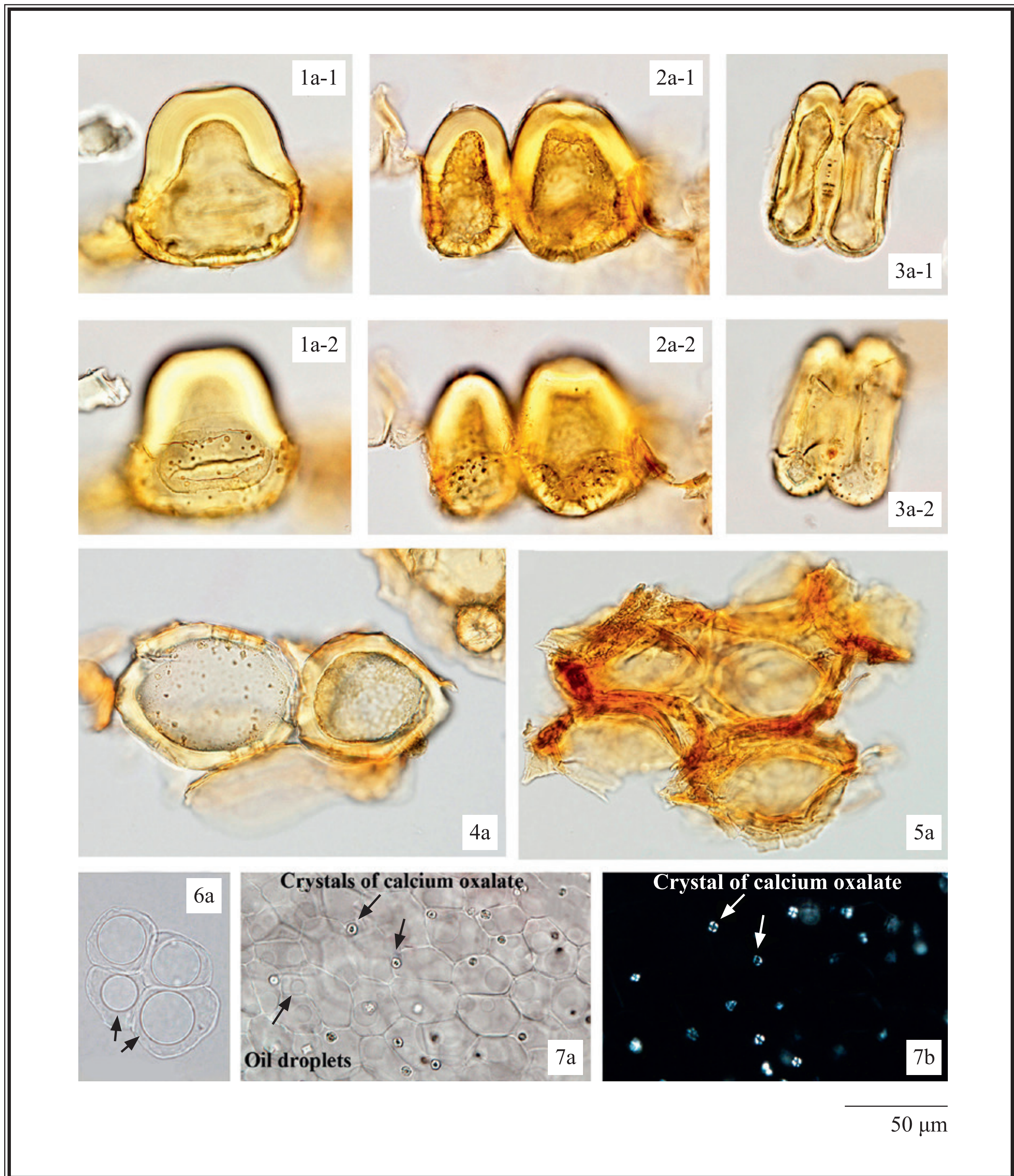

Figure 3 (ii) Microscopic features of powder of dried ripe seed of *Prunus davidiana* (Carr.) Franch.

1. Stone cell (1-1 in lateral view, 1-2 with pits) 2. Stone cells (2-1 in lateral view, 2-2 with pits)
3. Stone cells (3-1 in lateral view, 3-2 with pits) 4. Stone cells (in base view) 5. Testa cells
6. Endosperm cells containing oil droplets
7. Cotyledon cells containing crystals of calcium oxalate and oil droplets

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

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

Standard solution

Amygdalin standard solution

Weigh 2.0 mg of amygdalin CRS (Fig. 4) and dissolve in 2 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, methanol and water (40:15:6, v/v).

Spray reagent

Mix cautiously 25 mL of sulphuric acid (20%, v/v) into 25 mL of ice-cold glacial acetic acid. Add 2.5 mL of *p*-anisaldehyde. Add further 50 mL of sulphuric acid (20%, v/v).

Test solution

Weigh 1.0 g of the freshly powdered sample and place it in a 50-mL conical flask, then add 25 mL of petroleum ether (60-80ºC). Sonicate (220 W) the mixture for 1 h. Filter the mixture and wash the residue with 10 mL of petroleum ether. Discard the filtrate. Dry the residue in air. Transfer the dried residue to a 25-mL conical flask and add 10 mL of methanol. Sonicate (220 W) the mixture for 1 h. Filter and transfer the filtrate to a 10-mL volumetric flask. Make up to the mark with methanol.

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 amygdalin standard solution (5 μL) and the test solution (2 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 and heat at about 105°C until the spots or bands become visible (about 15 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the R_f value, corresponding to that of amygdalin.

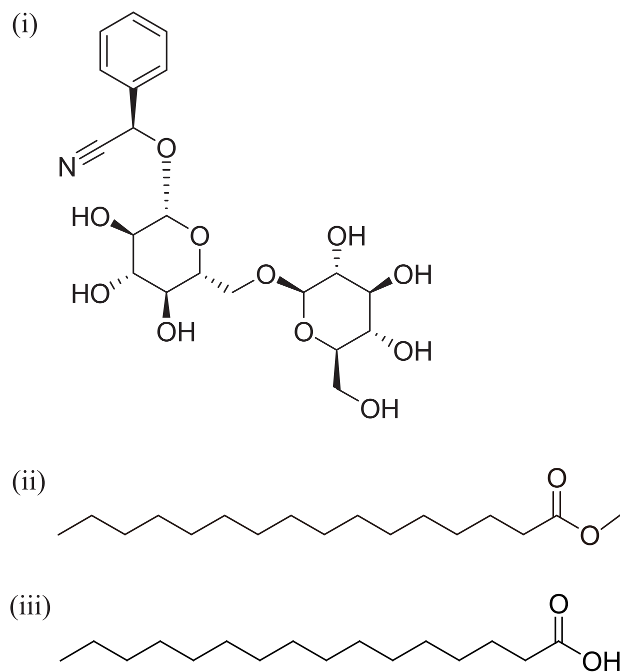

Figure 4 Chemical structures of (i) amygdalin (ii) methyl palmitate and (iii) palmitic acid

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

Standard solution

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

Weigh 1.0 mg of amygdalin CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.2 g of the freshly powdered sample and place it in a 50-mL conical flask, then add 20 mL of petroleum ether (60-80ºC). Sonicate (220 W) the mixture for 30 min. Filter the mixture and wash the residue with 5 mL of petroleum ether. Discard the filtrate. Dry the residue in air. Transfer the dried residue to a 25-mL conical flask and add 10 mL of methanol. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 10-mL volumetric flask. Make up to the mark with methanol. 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 0.5-1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	Water (%, v/v)	Flow rate (mL/min)	Elution
0 – 10	0 → 50	100 → 50	0.5	linear gradient
10 – 15	50	50	0.5 → 1.0	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 µL of amygdalin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of amygdalin should not be more than 5.0%; the RSD of the retention time of amygdalin peak should not be more than 2.0%; the column efficiency determined from amygdalin peak should not be less than 100000 theoretical plates.

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

Procedure

Separately inject amygdalin Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention time of amygdalin peak in the chromatogram of amygdalin Std-FP and the retention times of the three characteristic peaks [Fig. 5 (i) or (ii)] in the chromatogram of the test solution. Identify amygdalin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of amygdalin Std-FP. The retention times of amygdalin peaks from the two chromatograms should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

The RRTs and acceptable ranges of the three characteristic peaks of Persicae Semen extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Persicae Semen extract

Peak No.	RRT	Acceptable Range
1	0.89	± 0.03
2	0.98	± 0.03
3 (marker, amygdalin)	1.00	-

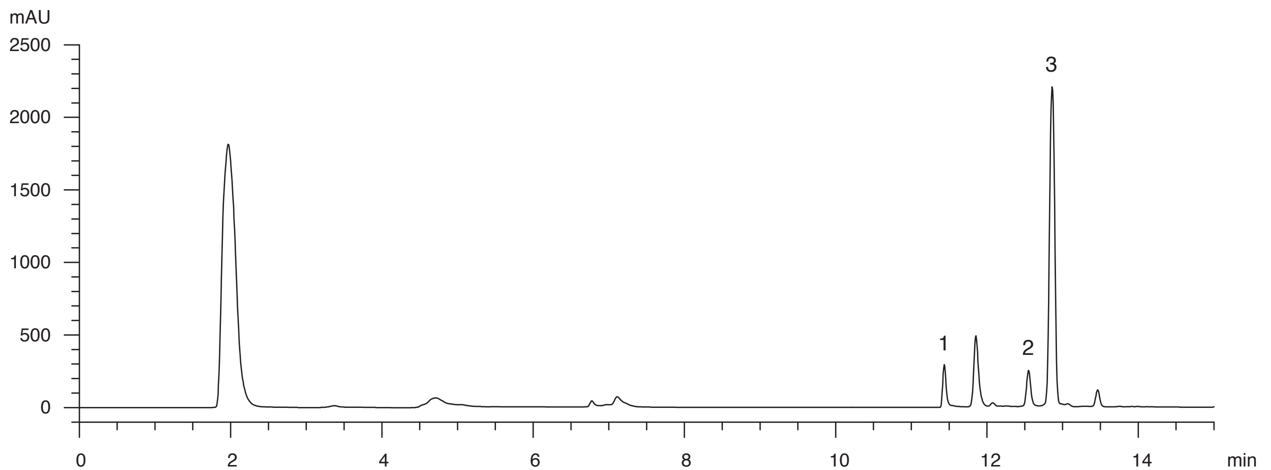

Figure 5 (i) A reference fingerprint chromatogram of dried ripe seed of *Prunus persica* (L.) Batsch extract

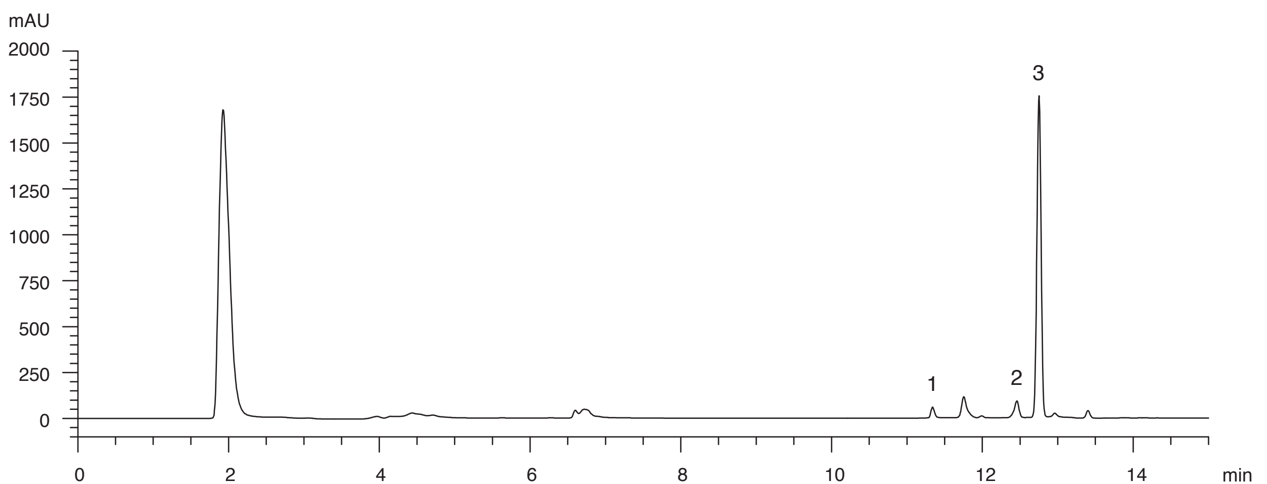

Figure 5 (ii) A reference fingerprint chromatogram of dried ripe seed of *Prunus davidiana* (Carr.) Franch. extract

For positive identification, the sample must give the above three characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the respective reference fingerprint chromatograms [Fig. 5 (i) or (ii)].

5. TESTS

5.1 Heavy Metals *(Appendix V)*: meet the requirements.

5.2 Pesticide Residues *(Appendix VI)*: meet the requirements.

5.3 Mycotoxins *(Appendix VII)*: meet the requirements.

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

5.5 Foreign Matter *(Appendix VIII)*: not more than 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 3.5%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

5.8 Acid Value *(Appendix XIV)*: not more than 10.0.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 17.0%.
Ethanol-soluble extractives (cold extraction method): not less than 8.0%.

7. ASSAY

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

Standard solution

Methyl palmitate standard stock solution, Std-Stock (5000 mg/L)
Weigh accurately 50.0 mg of methyl palmitate CRS (Fig. 4) and dissolve in 10 mL of diethyl ether.
Methyl palmitate standard solution for assay, Std-AS
Measure accurately the volume of the methyl palmitate Std-Stock, dilute with diethyl ether to produce a series of solutions of 20, 50, 100, 300, 500 mg/L for methyl palmitate.

Test solution

Weigh accurately 1.0 g of the freshly powdered sample and place it in a 250-mL conical flask, then add 100 mL of n-hexane. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 500-mL round-bottomed flask. Wash the residue for three times each with 10 mL of n-hexane. Repeat the extraction for one more time. Combine the extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 15 mL of methanol and 0.15 mL of hydrochloric acid. Reflux the mixture for 1.5 h. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 10 mL of water. Transfer the aqueous solution to a 250-mL separating funnel. Extract for three times each with 30 mL of diethyl ether. Combine the diethyl ether extracts. Add about 2.0 g of sodium sulphate and shake for a while. Filter and transfer the filtrate to a 100-mL volumetric flask. Make up to the mark with diethyl ether. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (HP-5, 0.32 mm × 30 m) of which the internal wall is covered with (5%-phenyl)-methylpolysiloxane in a layer about 0.25 µm thick. The injection temperature is at 250°C. The detector temperature is at 280°C. The split injection mode at a ratio of 30:1 is used. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC/min)
0 – 2	180	-
2 – 6	180 → 200	5
6 – 15	200	-
15 – 19	200 → 280	20
19 – 25	280	-

System suitability requirements

Perform at least five replicate injections, each using 1 μL of methyl palmitate Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of methyl palmitate should not be more than 5.0%; the RSD of the retention time of methyl palmitate peak should not be more than 2.0%; the column efficiency determined from methyl palmitate peak should not be less than 100000 theoretical plates.

The *R* value between methyl palmitate 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 methyl palmitate Std-AS (1 μL each) into the GC system and record the chromatograms. Plot the peak areas of methyl palmitate against the corresponding concentrations of methyl palmitate Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 1 μL of the test solution into the GC system and record the chromatogram. Identify methyl palmitate peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of methyl palmitate Std-AS. The retention times of methyl palmitate 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 methyl palmitate in the test solution, and calculate the percentage content of palmitic acid (the percentage content of methyl palmitate × 0.948) in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.87% of palmitic acid ($C_{16}H_{32}O_2$), calculated with reference to the dried substance.