Viticis Fructus

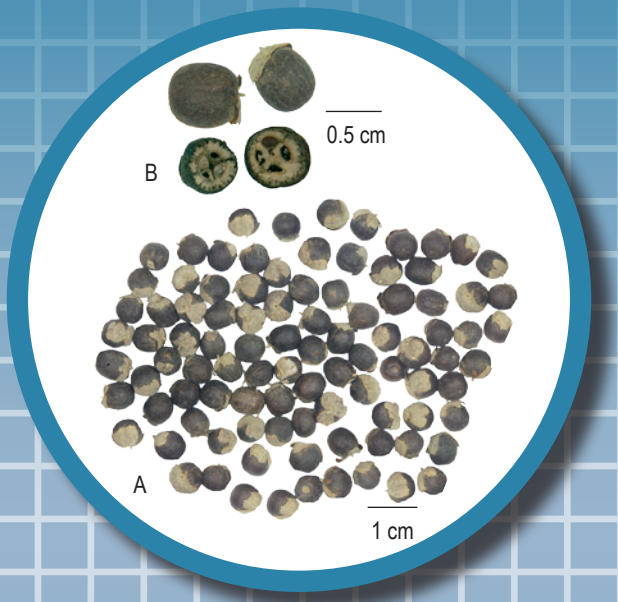

Figure 1 (i) A photograph of dried ripe fruit of *Vitex trifolia* L. var. *simplicifolia* Cham.

A. Viticis Fructus B. Magnified fruits and transverse section

Figure 1 (ii) A photograph of dried ripe fruit of *Vitex trifolia* L.

A. Viticis Fructus B. Magnified fruits and transverse section

1. NAMES

Official Name: Viticis Fructus

Chinese Name: 蔓荊子

Chinese Phonetic Name: Manjingzi

2. SOURCE

Viticis Fructus is the dried ripe fruit of *Vitex trifolia* L. var. *simplicifolia* Cham. or *Vitex trifolia* L. (Verbenaceae). The ripe fruit is collected in autumn, foreign matter removed, then dried under the sun to obtain Viticis Fructus.

3. DESCRIPTION

Spheroidal, 4-6 mm in diameter. Externally greyish-black to blackish-brown, bearing 4 longitudinal shallow furrows, covered with greyish-white frost-like hairs; apex slightly concave, with greyish-white persistent calyx and a short fruit stalk at base. Calyx 1/3 to 2/3 in length of fruit, 5-lobed, 2 of the lobes relatively deep and covered with densely pubescence. Texture hard and light in weight, uneasily broken. Transverse section shows 4 locules, each with 1 seed. Odour characteristic and aromatic; taste weak and slightly pungent [Fig. 1 (i) and (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Exocarp consists of 1 layer of flat cells with brown content inclusions, covered with cuticle, with glandular hairs. Mesocarp broad, cells subsquare to subrounded, walls slightly lignified, with distinct pits; small vascular bundles arranged in a ring. Endocarp consists of 3-8 layers of stone cells. The outer layer of testa consists of 1 layer of small flat parenchymatous cells, with 2-6 layers of larger cells beneath them, with reticulate striations [Fig. 2 (i) and (ii)].

Powder

Colour greyish-brown. Exocarp cells polygonal, with cuticular striations and scars of glandular and non-glandular hairs. Epidermal cells of calyx subrounded to rectangle, wall sinuous. Testa cells rounded to subrounded, 20-70 μm in diameter, with reticulate striation and lignified wall. Non-glandular hairs of calyx 2- to 3-celled, apical cell relatively wide with warty protrusions. Most of glandular hairs consist of 2- to 6-celled head and unicellular stalk, sometimes with unicellular head and 1- to 2-celled stalk. Mesocarp cells subsquare to subpolygonal to subrounded, wall slightly lignified, and with distinct pits. Stone cells of endocarp elliptical, rectangle or subsquare, 10-66 μm in diameter [Fig. 3 (i) and (ii)].

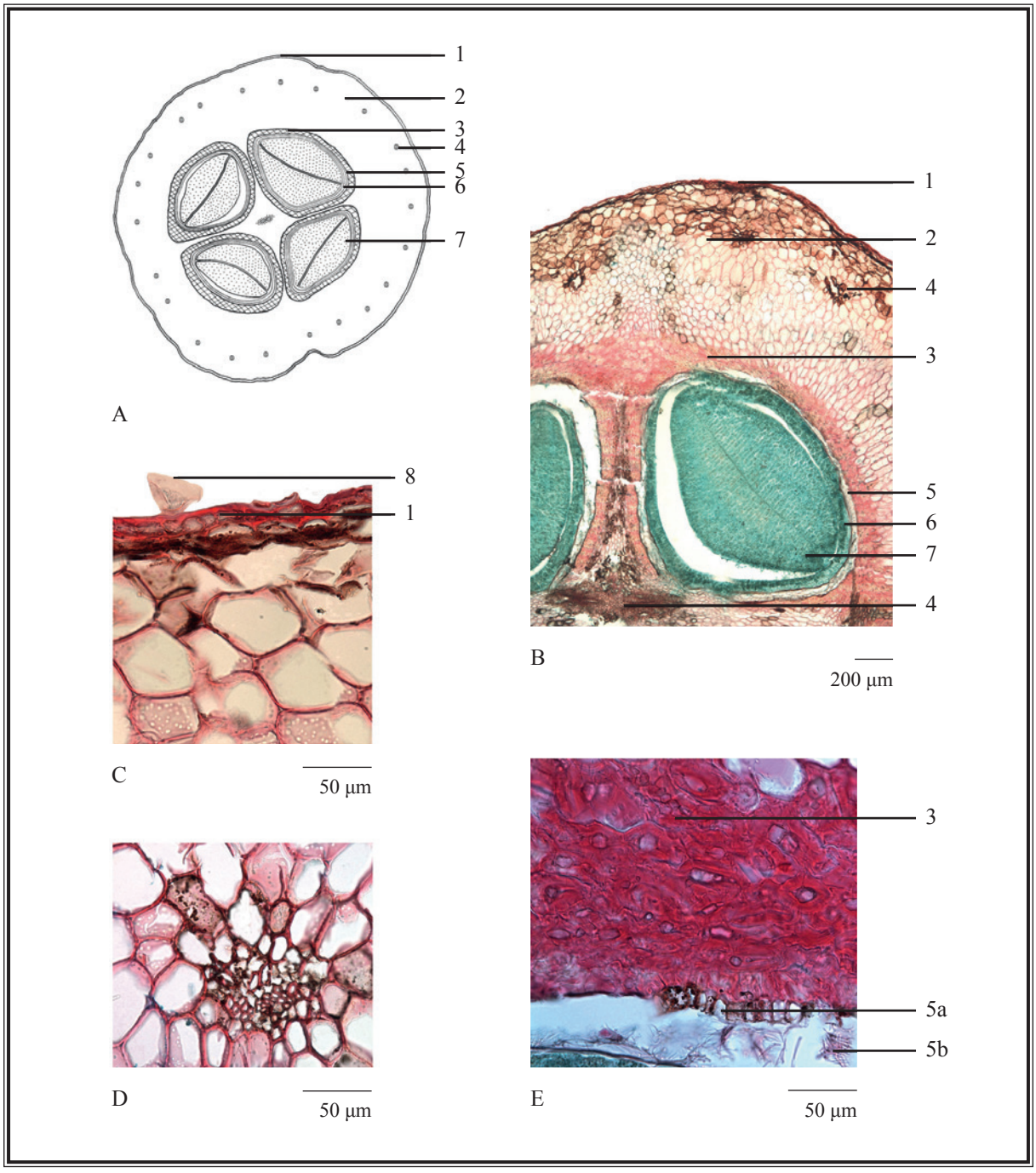

Figure 2 (i) Microscopic features of transverse section of fruit of *Vitex trifolia* L. var. *simplicifolia* Cham.

A. Sketch B. Section illustration C. Exocarp epidermal cells with glandular hair
D. Vascular bundle E. Endocarp stone cells and testa

1. Exocarp 2. Mesocarp 3. Endocarp 4. Vascular bundle
5. Testa (5a. Parenchymatous cells in outer layer of testa, 5b. Inner layers of testa cells with reticulate striation)
6. Endosperm 7. Cotyledon 8. Glandular hair

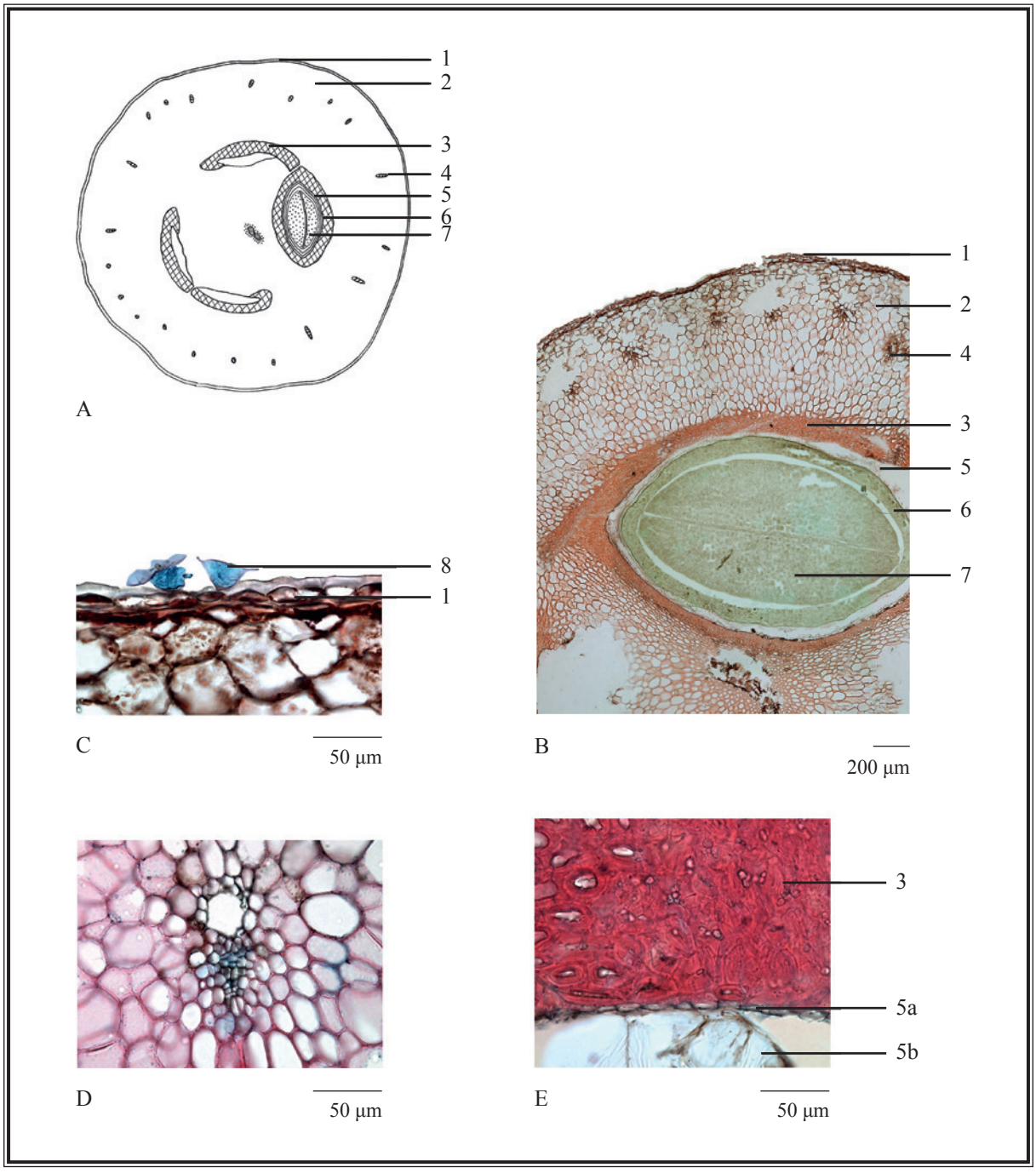

Figure 2 (ii) Microscopic features of transverse section of fruit of *Vitex trifolia* L.

A. Sketch B. Section illustration C. Epidermal cells and glandular hairs
D. Vascular bundle E. Endocarp stone cells and testa

1. Exocarp 2. Mesocarp 3. Endocarp 4. Vascular bundle
5. Testa (5a. Parenchymatous cells in outer layer of testa, 5b. Inner layers of testa cells with reticulate striation)
6. Endosperm 7. Cotyledon 8. Glandular hair

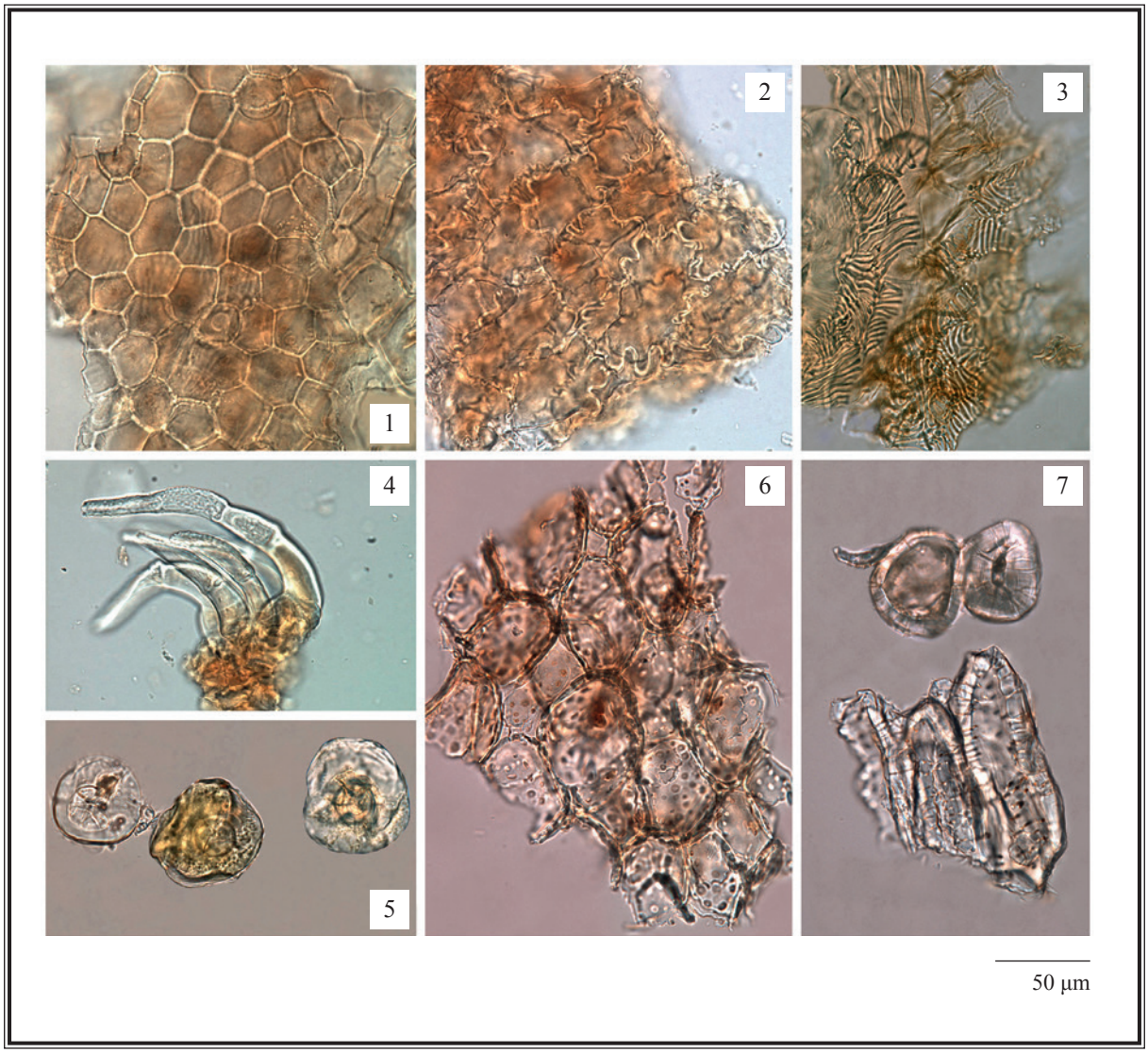

Figure 3 (i) Microscopic features of powder of fruit of *Vitex trifolia* L. var. *simplicifolia* Cham. (under the light microscope)

1. Exocarp cells 2. Epidermal cells of calyx
3. Testa cells with reticulate striation 4. Non-glandular hairs
5. Glandular hairs 6. Mesocarp cells 7. Stone cells

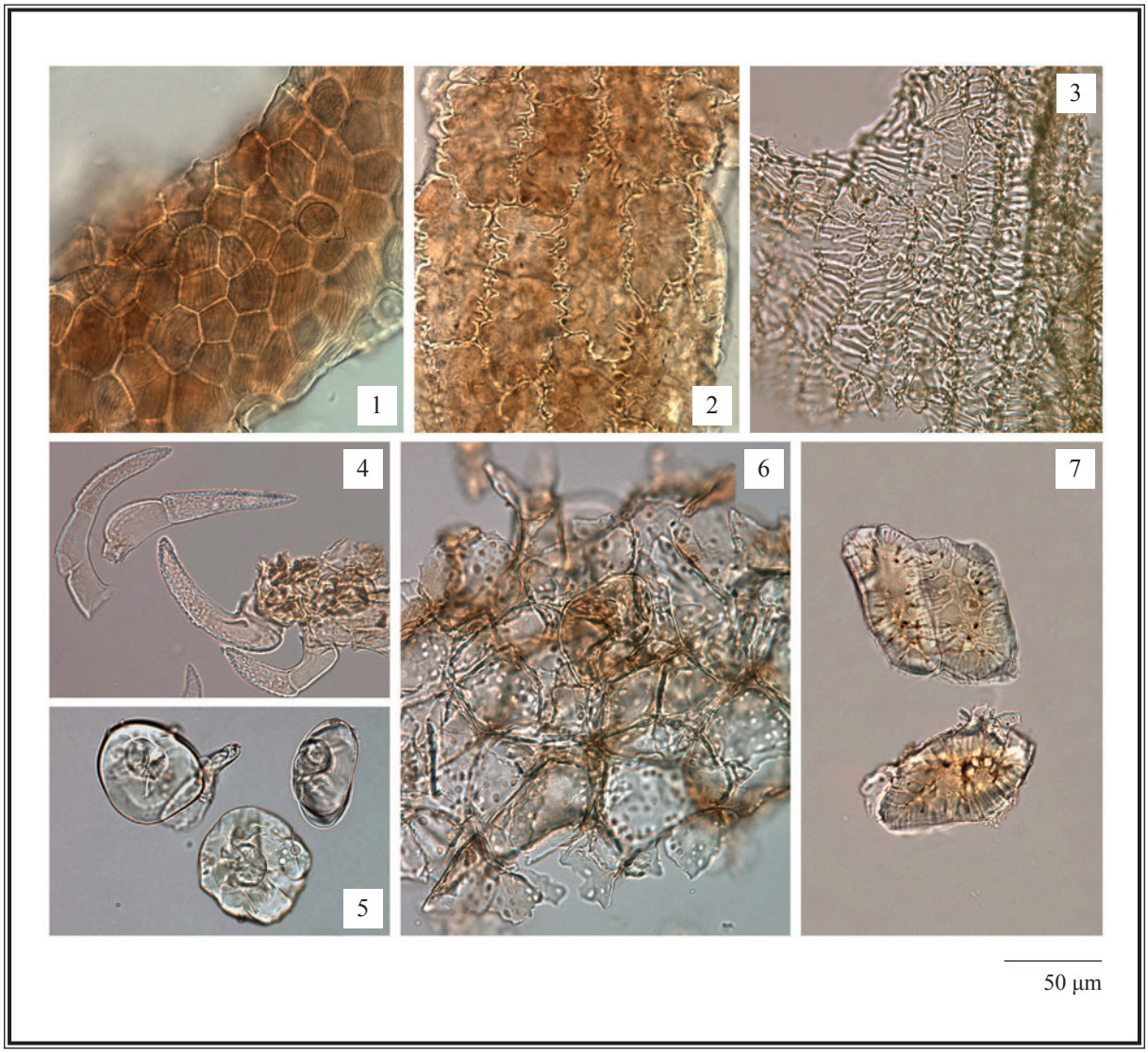

Figure 3 (ii) Microscopic features of powder of fruit of *Vitex trifolia* L. (under the light microscope)

1. Exocarp cells 2. Epidermal cells of calyx
3. Testa cells with reticulate striation 4. Non-glandular hairs
5. Glandular hairs 6. Mesocarp cells 7. Stone cells

Footnote: Microscopic features of powder have no significant differences between the fruit of *Vitex trifolia* L. var. *simplicifolia* Cham. and *Vitex trifolia* L.

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

Standard solution

Casticin standard solution

Weigh 1.0 mg of casticin CRS (Fig. 4) and dissolve in 1 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of petroleum ether (60-80˚C) and ethyl acetate (1:2, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethanol (70%). Sonicate (160 W) the mixture for 1 h. 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 0.5 mL of ethanol (70%).

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 casticin standard solution (2 µL) and the test solution (2-3 µL) to the plate. Develop over a path of about 5 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 casticin.

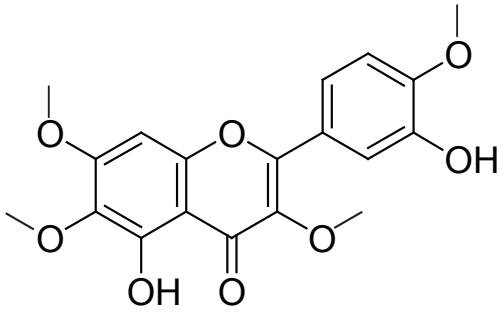

Figure 4 Chemical structure of casticin

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

Standard solution

Casticin standard solution for fingerprinting, Std-FP (35 mg/L)

Weigh 0.7 mg of casticin CRS and dissolve in 20 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (160 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (350 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)	0.4% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 40	75 → 20	25 → 80	linear gradient
40 – 60	20	80	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of casticin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of casticin should not be more than 5.0%; the RSD of the retention time of casticin peak should not be more than 2.0%; the column efficiency determined from casticin 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 casticin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of casticin peak in the chromatogram of casticin Std-FP and the retention times of the three characteristic peaks [Fig. 5 (i) or (ii)] in the chromatogram of the test solution. Identify casticin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of casticin Std-FP. The retention times of casticin 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 Viticis Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Viticis Fructus extract

Peak No.	RRT	Acceptable Range
1	0.46	± 0.03
2	0.89	± 0.03
3 (marker, casticin)	1.00	-

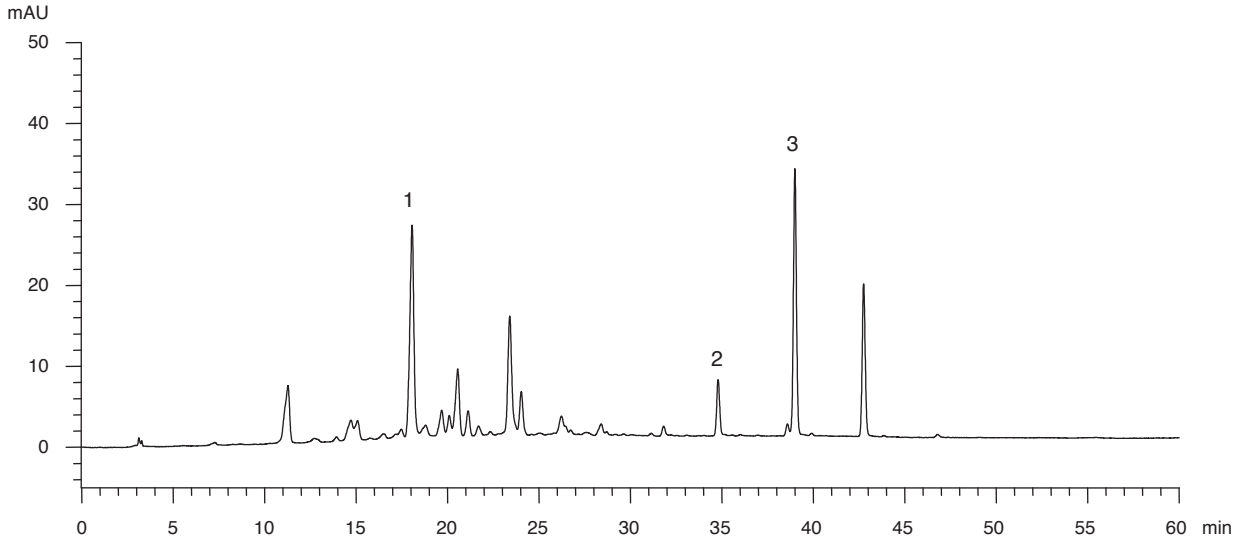

Figure 5 (i) A reference fingerprint chromatogram of dried ripe fruit of *Vitex trifolia* L. var. *simplicifolia* Cham. extract

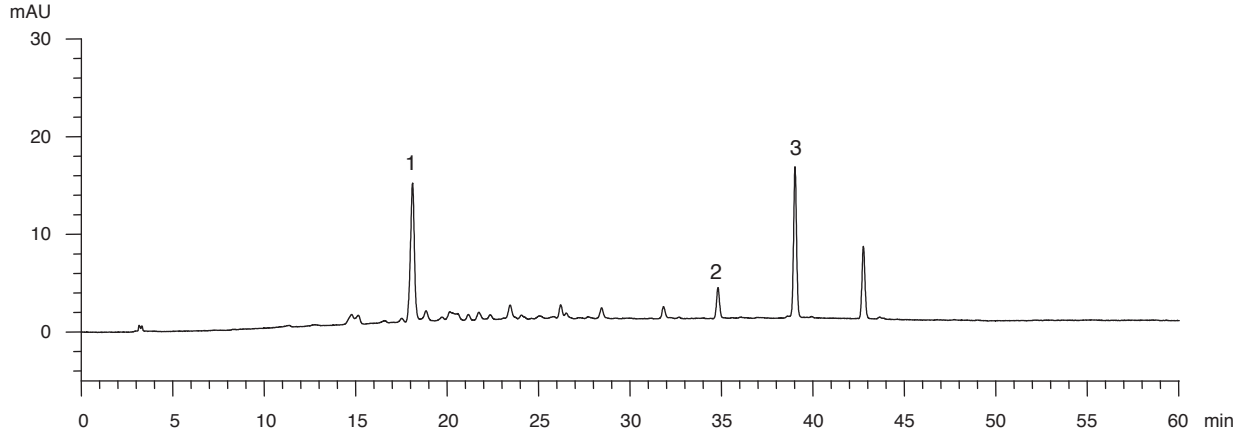

Figure 5 (ii) A reference fingerprint chromatogram of dried ripe fruit of *Vitex trifolia* L. extract

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 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 7.0%.
Ethanol-soluble extractives (hot extraction method): not less than 5.0%.

7. ASSAY

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

Standard solution

Casticin standard stock solution, Std-Stock (100 mg/L)

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

Casticin standard solution for assay, Std-AS

Measure accurately the volume of the casticin Std-Stock, dilute with methanol to produce a series of solutions of 1, 10, 20, 35, 50 mg/L for casticin.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (160 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (350 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)	0.4% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 40	75 → 20	25 → 80	linear gradient
40 – 60	20	80	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of casticin Std-AS (20 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of casticin should not be more than 5.0%; the RSD of the retention time of casticin peak should not be more than 2.0%; the column efficiency determined from casticin peak should not be less than 100000 theoretical plates.

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

Limits

The sample contains not less than 0.030% of casticin ($C_{19}H_{18}O_8$), calculated with reference to the dried substance.