Cuscutae Semen

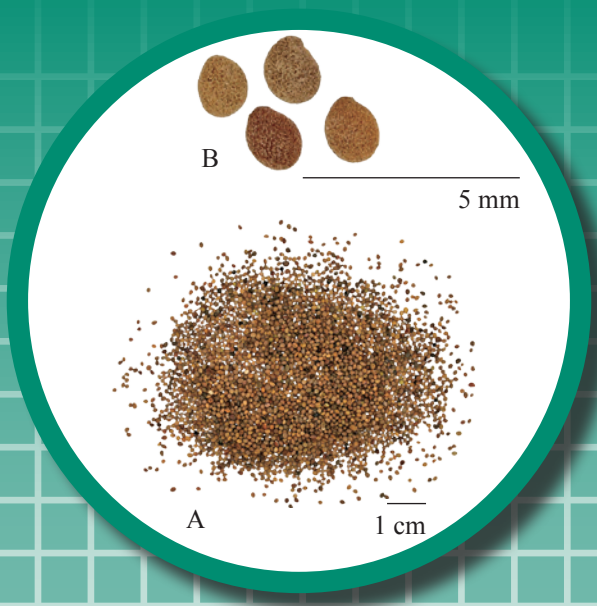


Figure 1 (i) A photograph of dried ripe seeds of *Cuscuta australis* R. Br.

A. Seeds B. Magnified seeds

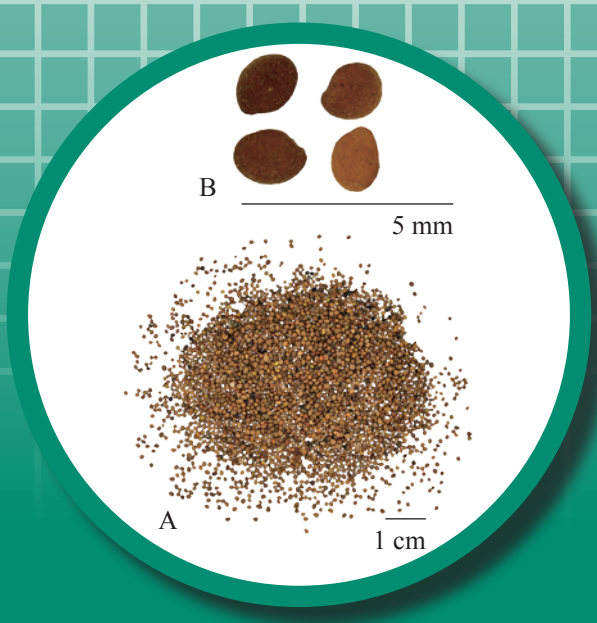


Figure 1(ii) A photograph of dried ripe seeds of *Cuscuta chinensis* Lam.

A. Seeds B. Magnified seeds

1. NAMES

Official Name: Cuscutae Semen

Chinese Name: 菟絲子

Chinese Phonetic Name: Tusizi

2. SOURCE

Cuscutae Semen is the dried ripe seed of *Cuscuta australis* R. Br. or *Cuscuta chinensis* Lam. (Convolvulaceae). The plant is collected in autumn when the fruit is ripe, dried under the sun, then threshed to gather the seeds and foreign matter removed to obtain Cuscutae Semen.

3. DESCRIPTION

Subspherical, 0.9-1.5 mm in diameter. Externally greyish-brown, yellowish-brown or reddish-brown, rough, with numerous fine protuberant dots; the hilum linear or oblate. Texture hard and compact. Odour slight; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermis consists of 1 layer of cells, subsquare to subrectangular, side wall thickened. Palisade tissue consists of 2 layers elongated cells; outer palisade cells shorter than the inner ones; light line locates at the upper half of the inner palisade cells. A narrow parenchyma, made up of mostly shrunken cells, lies underneath the inner layer of palisade cells. Endosperm cells polygonal to subrounded, with thickened cell wall. Cotyledons twisted, several fragments of cotyledon can be seen. Cells of cotyledon subsquare to subrounded, containing aleurone grains [Fig. 2 (i) and (ii)].

Powder

Colour yellowish-brown to dark brown. Testa consists of epidermal cells and palisade cells. Epidermal cells of testa polygonal in surface view, walls thickened at corner; subsquare to subrectangular in lateral view, 15-55 μm wide, 26-71 μm high, side walls thickened. Palisade cells of testa 2 layers in lateral view, outer palisade cells shorter than inner ones; a light line presents at upper part of inner palisade cells; cells polygonal in surface view, walls thickened, shrunken. Endosperm cells polygonal or subrounded, walls thickened, containing aleurone grains. Cotyledon cells subrounded to subsquare, containing aleurone grains and oil droplets [Fig. 3 (i) and (ii)].

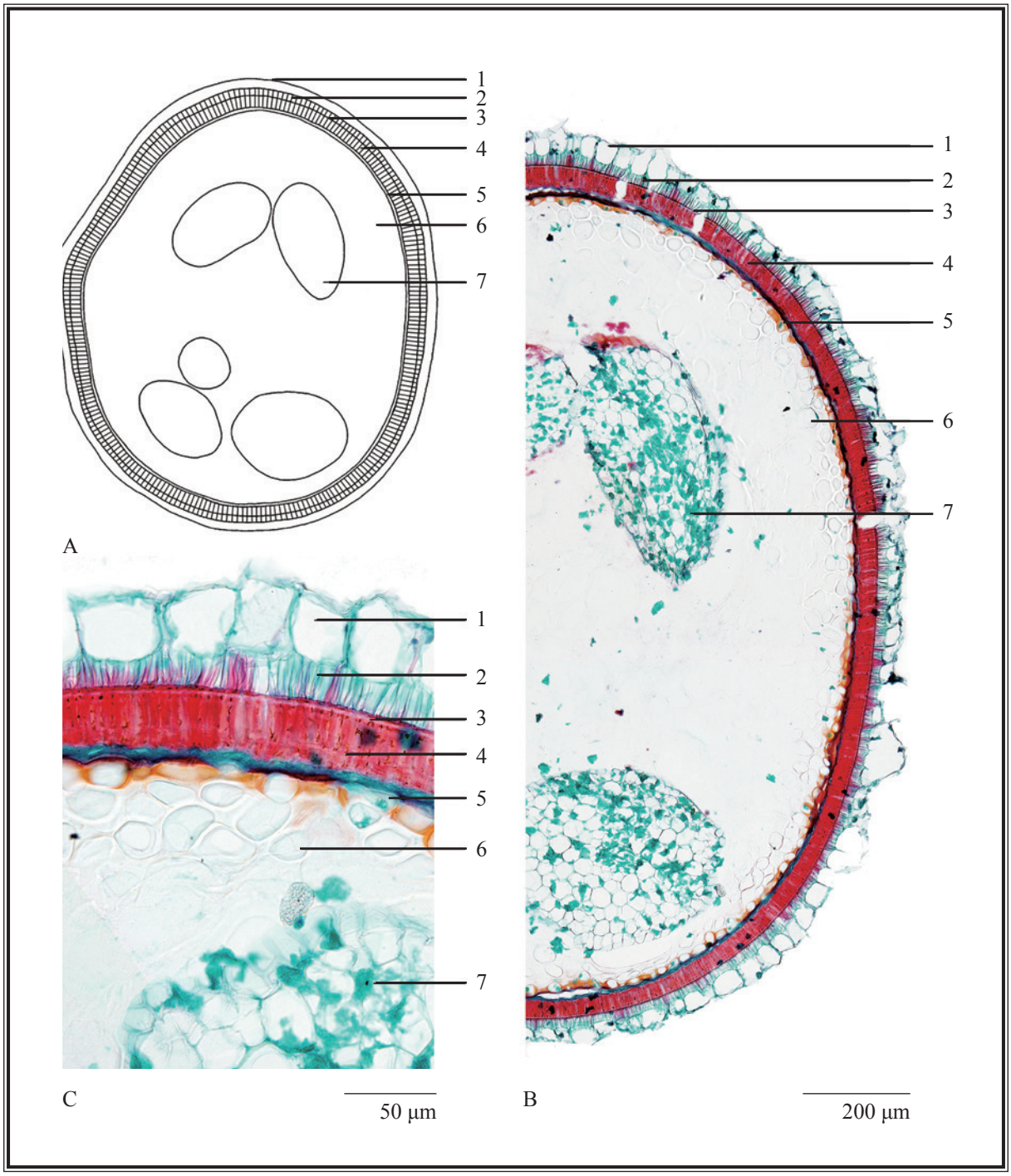


Figure 2 (i) Microscopic features of transverse section of dried ripe seed of *Cuscuta australis* R. Br.

A. Sketch B. Section illustration C. Section magnified

1. Epidermis 2. Outer palisade cells 3. Light line 4. Inner palisade cells
5. Parenchyma 6. Endosperm 7. Cotyledon

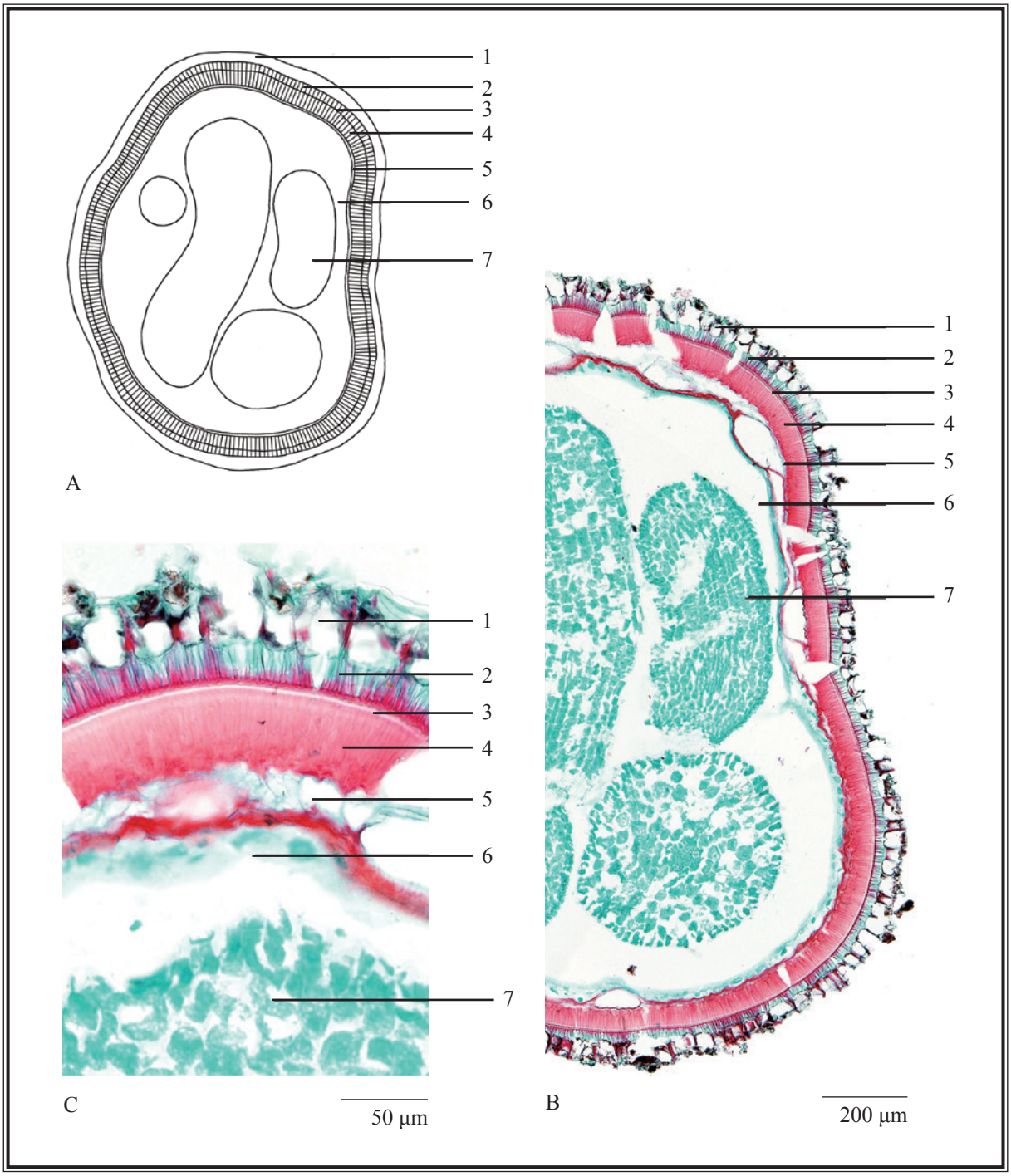


Figure 2 (ii) Microscopic features of transverse section of dried ripe seed of *Cuscuta chinensis* Lam.

A. Sketch B. Section illustration C. Section magnified

1. Epidermis 2. Outer palisade cells 3. Light line 4. Inner palisade cells
5. Parenchyma 6. Endosperm 7. Cotyledon

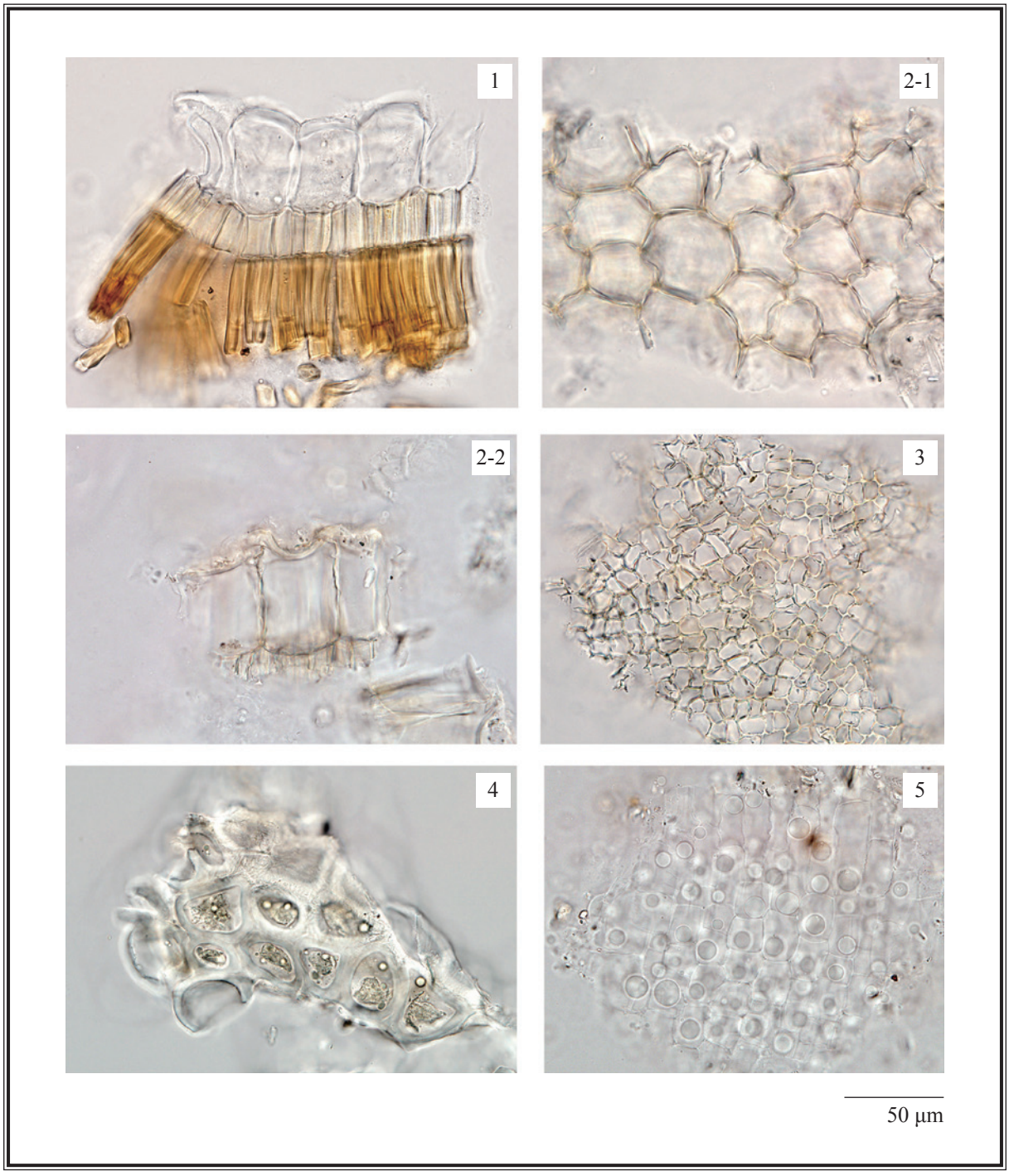


Figure 3 (i) Microscopic features of powder of dried ripe seed of *Cuscuta australis* R. Br. (under the light microscope)

1. Testa consists of epidermal cell and palisade cells (in lateral view)
2. Epidermal cells of testa (2-1 in surface view, 2-2 in lateral view)
3. Palisade cells of testa (in surface view) 4. Endosperm cells contain aleurone grains
5. Cotyledon cells contain aleurone grains and oil droplets

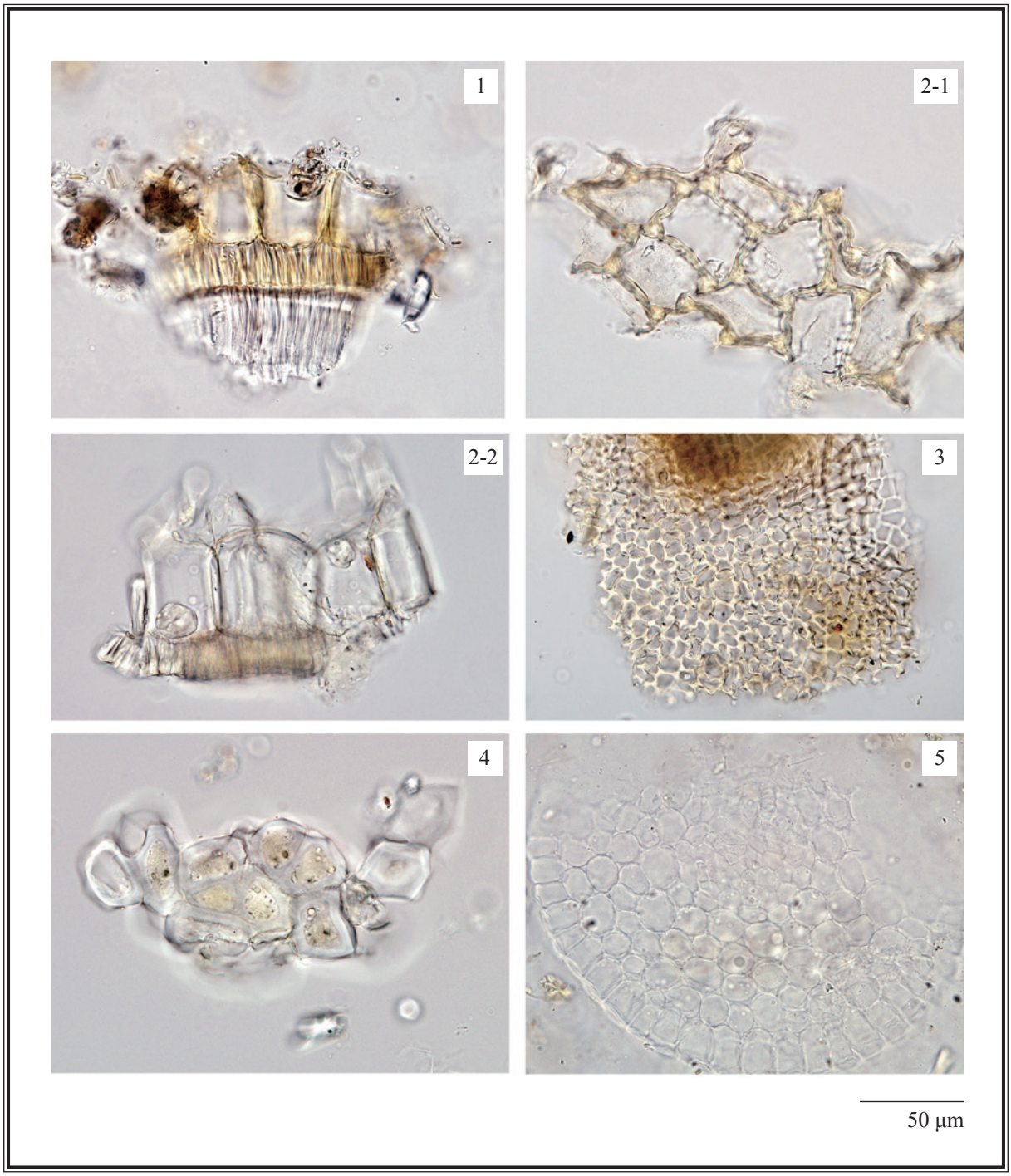


Figure 3 (ii) Microscopic features of powder of dried ripe seed of *Cuscuta chinensis* Lam. (under the light microscope)

1. Testa consists of epidermal cell and palisade cells (in lateral view)
2. Epidermal cells of testa (2-1 in surface view, 2-2 in lateral view)
3. Palisade cells of testa (in surface view) 4. Endosperm cells contain aleurone grains
5. Cotyledon cells contain aleurone grains and oil droplets

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

Standard solution

Hyperin standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, acetone, formic acid and water (25:2:2:1, v/v).

Spray reagent

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

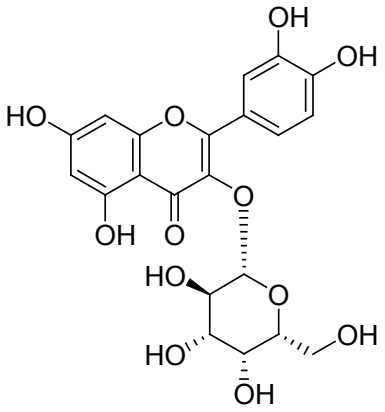
Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL conical flask, then add 40 mL of methanol. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 5 mL of 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 hyperin standard solution (1 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 dry in air. Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

(i)



(ii)

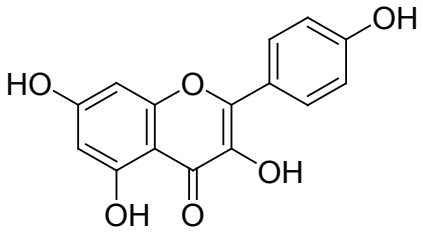


Figure 4 Chemical structures of (i) hyperin and (ii) kaempferol

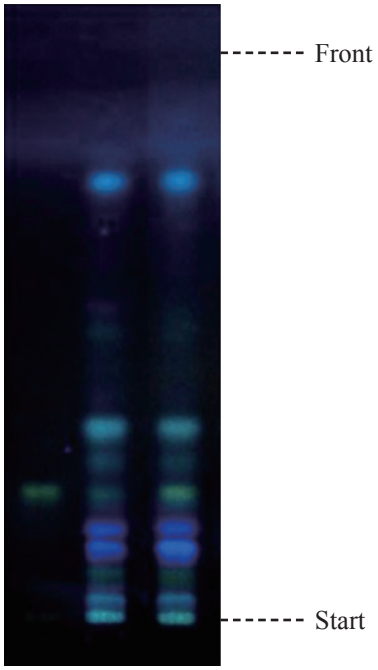


Figure 5 A reference HPTLC chromatogram of Cuscutae Semen extract observed under UV light (366 nm) after staining

1. Hyperin standard solution
2. Test solution of
(i) dried ripe seed of *Cuscuta australis* R. Br.
(ii) dried ripe seed of *Cuscuta chinensis* Lam.

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 hyperin (Fig. 5).

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

Standard solution

Hyperin standard solution for fingerprinting, Std-FP (25 mg/L)

Weigh 0.25 mg of hyperin CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then 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 (360 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	90 → 81	10 → 19	linear gradient
10 – 30	81	19	isocratic
30 – 60	81 → 45	19 → 55	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of hyperin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of hyperin should not be more than 5.0%; the RSD of the retention time of hyperin peak should not be more than 2.0%; the column efficiency determined from hyperin peak should not be less than 15000 theoretical plates.

The *R* value between peak 1 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 hyperin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of hyperin peak in the chromatogram of hyperin Std-FP and the retention times of the three characteristic peaks [Fig. 6 (i) or (ii)] in the chromatogram of the test solution. Identify hyperin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of hyperin Std-FP. The retention times of hyperin 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 Cuscutae Semen extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (marker, hyperin)	1.00	-
2	1.43	± 0.05
3 (kaempferol)	2.02	± 0.09

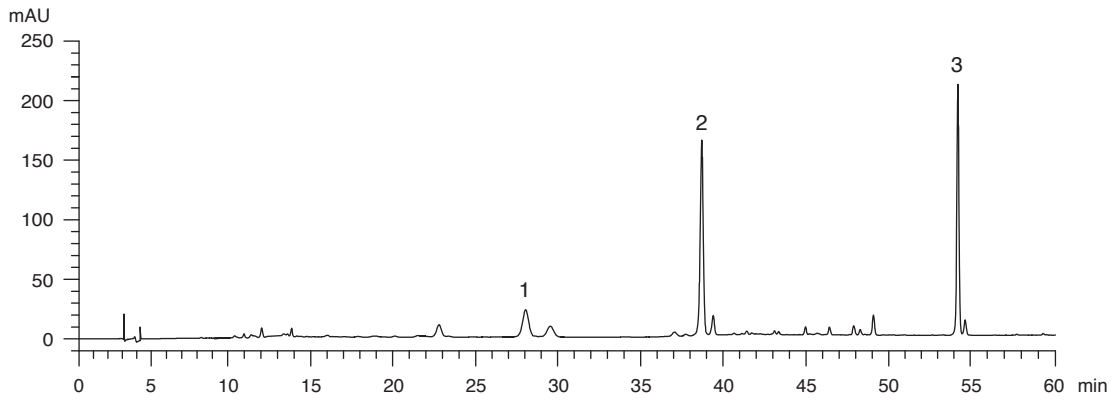


Figure 6 (i) A reference fingerprint chromatogram of dried ripe seed of *Cuscuta australis* R. Br. extract

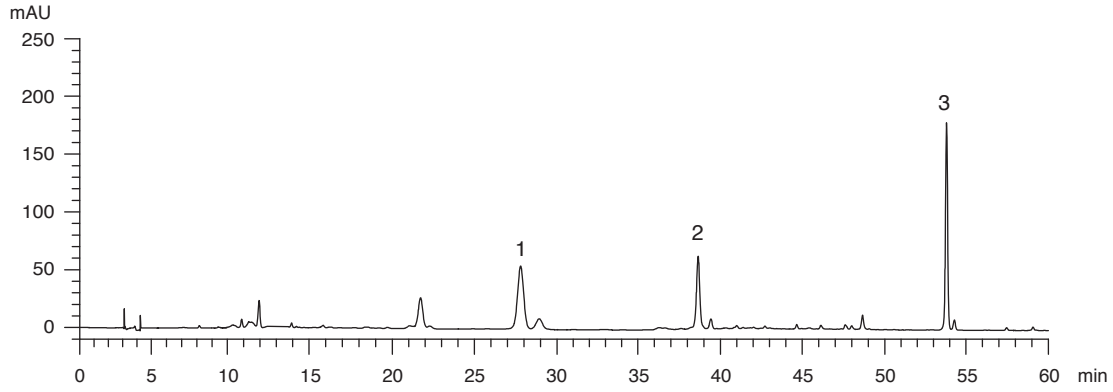


Figure 6 (ii) A reference fingerprint chromatogram of dried ripe seed of *Cuscuta chinensis* Lam. extract

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. 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 XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 8.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 10.0%.
Acid-insoluble ash: not more than 4.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 15.0%.
Ethanol-soluble extractives (cold extraction method): not less than 9.0%.

7. ASSAY

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

7.1 Assay of Hyperin

Standard solution

Hyperin standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 2.0 mg of hyperin CRS and dissolve in 10 mL of methanol (50%).

Hyperin standard solution for assay, Std-AS

Measure accurately the volume of the hyperin Std-Stock, dilute with methanol (50%) to produce a series of solutions of 2.5, 5, 10, 25, 50 mg/L for hyperin.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 90 mL of methanol (50%). 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 two times each with 5 mL of methanol (50%). Combine the solutions and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (354 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. The mobile phase is a mixture of 0.1% phosphoric acid and acetonitrile (83:17, v/v). The elution time is about 50 min.

System suitability requirements

Perform at least five replicate injections, each using 20 μL of hyperin Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of hyperin should not be more than 5.0%; the RSD of the retention time of hyperin peak should not be more than 2.0%; the column efficiency determined from hyperin peak should not be less than 10000 theoretical plates.

The *R* value between hyperin 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 hyperin Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of hyperin against the corresponding concentrations of hyperin Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify hyperin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of hyperin Std-AS. The retention times of hyperin 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 hyperin in the test solution, and calculate the percentage content of hyperin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.10% of hyperin ($C_{21}H_{20}O_{12}$), calculated with reference to the dried substance.

7.2 Assay of Kaempferol

Standard solution

Kaempferol standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 2.0 mg of kaempferol CRS (Fig. 4) and dissolve in 10 mL of methanol (80%).

Kaempferol standard solution for assay, Std-AS

Measure accurately the volume of the kaempferol Std-Stock, dilute with methanol (80%) to produce a series of solutions of 0.8, 2.5, 5, 10, 15 mg/L for kaempferol.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 30 mL of methanol (80%). Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Repeat the extraction for two more times. Combine the filtrates and make up to the mark with methanol (80%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (365 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. The mobile phase is a mixture of 0.1% phosphoric acid and methanol (40:60, v/v). The elution time is about 25 min.

System suitability requirements

Perform at least five replicate injections, each using 10 µL of kaempferol Std-AS (5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of kaempferol should not be more than 5.0%; the RSD of the retention time of kaempferol peak should not be more than 2.0%; the column efficiency determined from kaempferol peak should not be less than 8000 theoretical plates.

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

Limits

The sample contains not less than 0.020% of kaempferol ($C_{15}H_{10}O_6$), calculated with reference to the dried substance.