Platycladi Semen

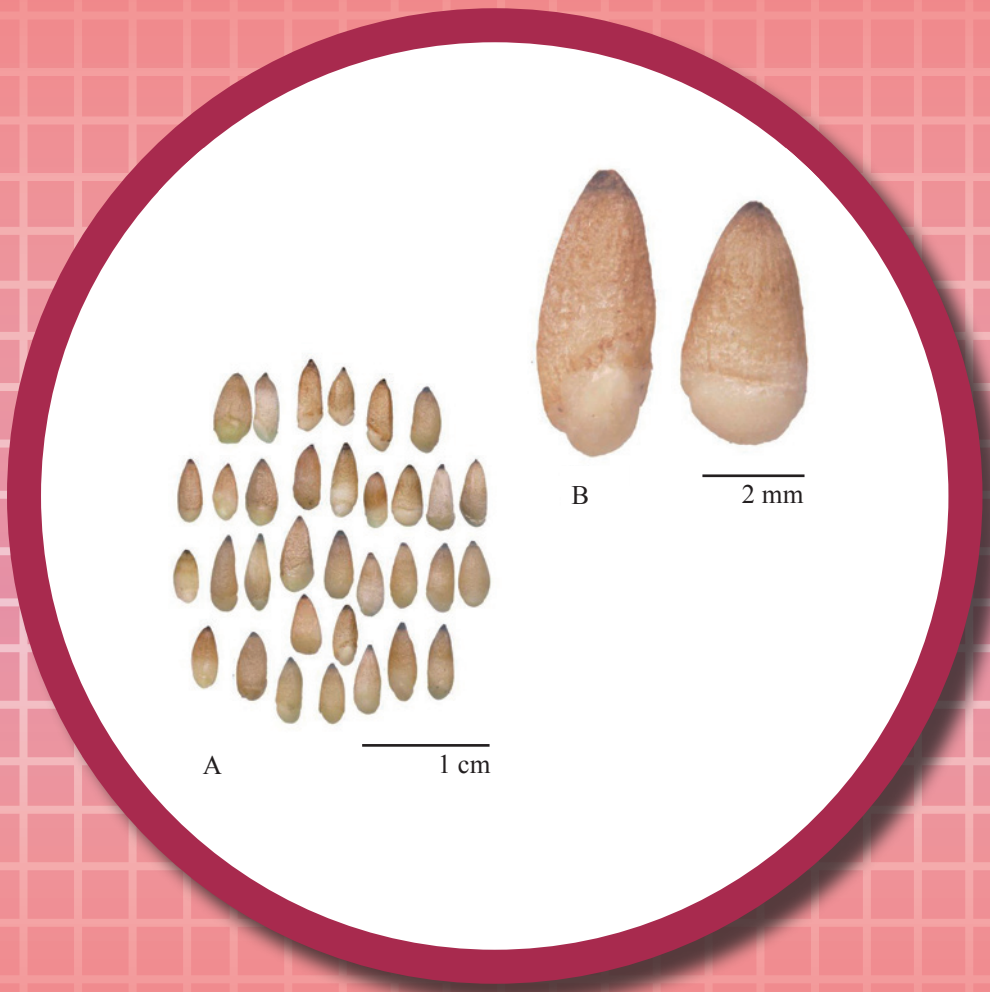


Figure 1 A photograph of Platycladi Semen

A. Platycladi Semen

B. Magnified image of ripe kernel

1. NAMES

Official Name: Platycladi Semen

Chinese Name: 柏子仁

Chinese Phonetic Name: Baiziren

2. SOURCE

Platycladi Semen is the dried ripe kernel of *Platycladus orientalis* (L.) Franco (Cupressaceae). The ripe seed is collected in autumn and winter, dried under the sun, then the testa removed to obtain Platycladi Semen.

3. DESCRIPTION

Long ovoid to elongate-ellipsoid, 0.4-0.7 cm long, 1.5-3 mm in diameter. Externally yellowish-white to pale yellowish-brown, covered with membranous tegmen, apex slightly acute, with a small dark brown dot, base obtusely rounded. Texture soft, oily. Odour slightly aromatic; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

Apex end: Endotesta shrunken, cell borders mostly indistinct. Endosperm developed, epidermal cells tangentially elongated. Epidermal cells of embryo subsquare to subpolygonal. Vascular bundles located in the middle of the embryo, cells relatively small. Endosperm and embryo cells filled with aleurone grains [Fig. 2 (i)].

Base end: Transverse section shows two pieces of cotyledon [Fig. 2 (ii)].

Powder

Mount in chloral hydrate: Endotesta cells brownish-yellow, elongated rectangular. Endosperm cells nearly colourless, pale yellow to yellowish-green, subpolygonal, subrectangular or subrounded, thin-walled. Embryo cells near colourless, subrectangular to strip-shaped in lateral view, relatively evenly arranged, thin-walled. Oil droplets red observed after stained with Sudan III TS, relatively numerous, rounded.

Mount in liquid paraffin: Aleurone grains yellowish-brown observed after stained with iodine TS, relatively numerous, elliptic to subrounded, 3-9 μm in diameter (Fig. 3).

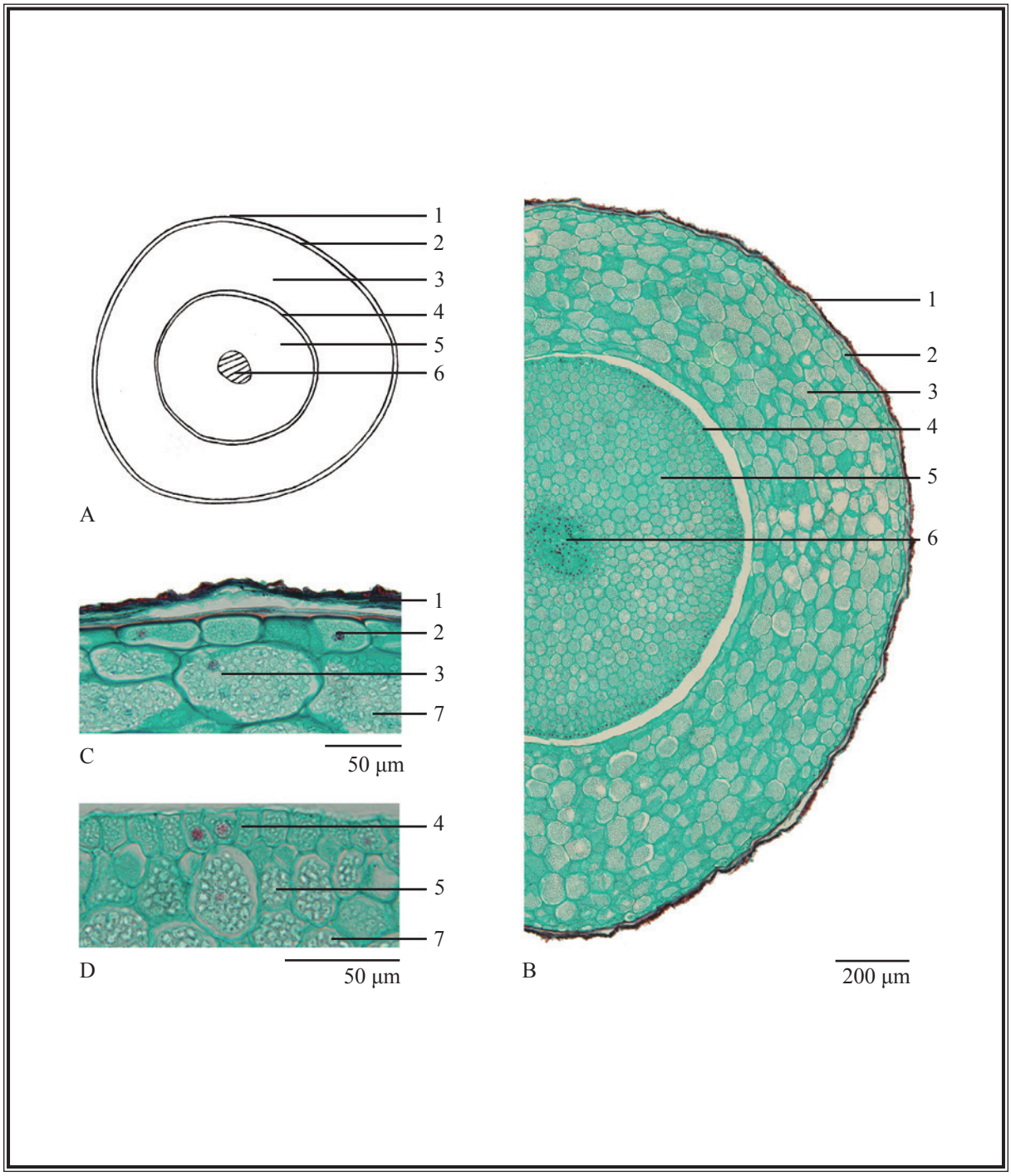


Figure 2 (i) Microscopic features of transverse section of apex end of Platycladi Semen

A. Sketch B. Section illustration C-D. Section magnified

1. Endotesta 2. Epidermis of endosperm 3. Endosperm 4. Epidermis of embryo
5. Embryo 6. Vascular bundle 7. Aleurone grain

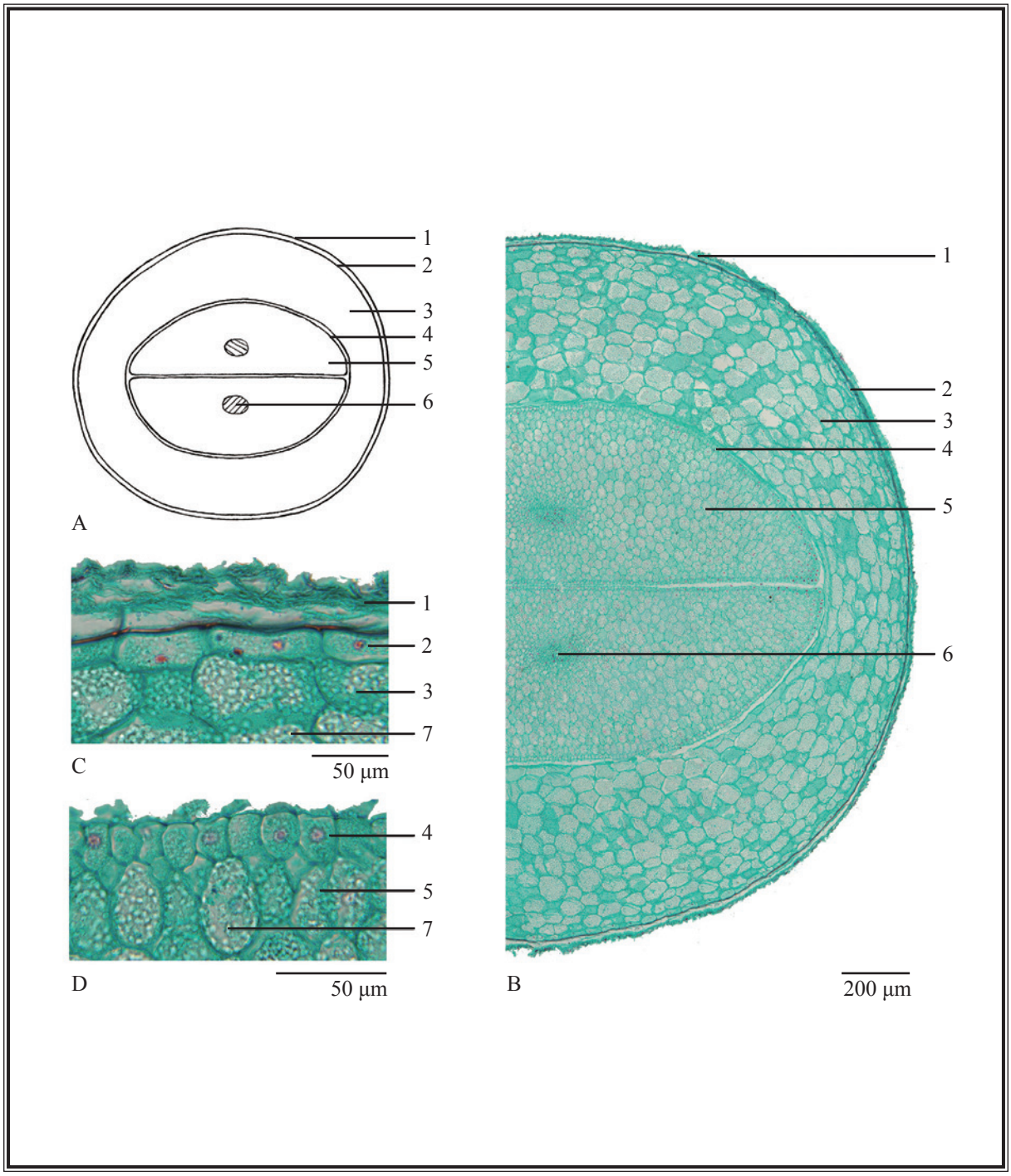


Figure 2 (ii) Microscopic features of transverse section of base end of Platycladi Semen

A. Sketch B. Section illustration C-D. Section magnified

1. Endotesta 2. Epidermis of endosperm 3. Endosperm 4. Epidermis of embryo
5. Embryo 6. Vascular bundle 7. Aleurone grain

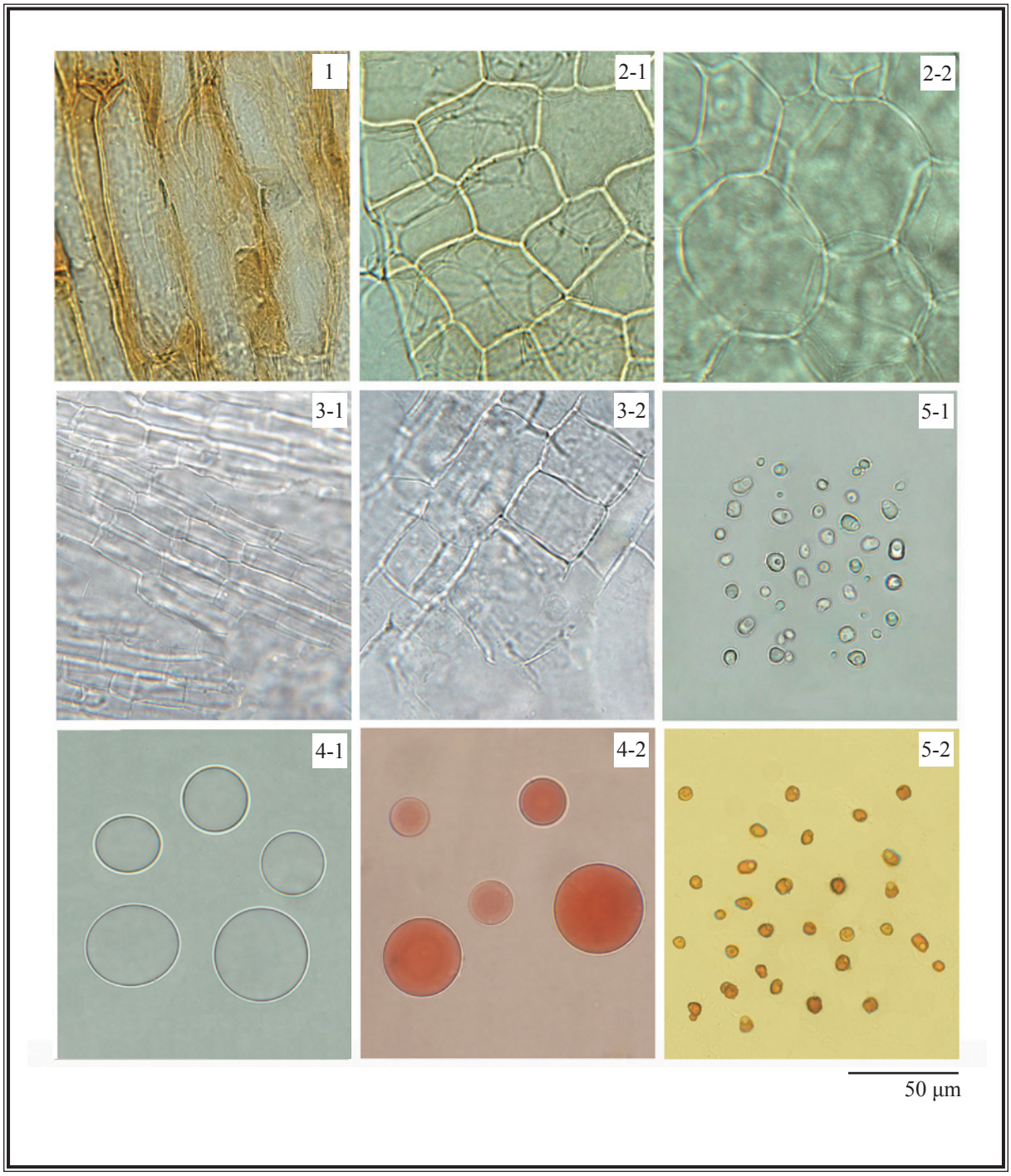


Figure 3 Microscopic features of powder of Platycladi Semen (under the light microscope)

1. Endotesta cells
2. Endosperm cells (2-1 epidermal cells of endosperm in lateral view, 2-2 core cells of endosperm)
3. Embryo cells (3-1 epidermal cells of embryo in lateral view, 3-2 core cells of embryo in lateral view)
4. Oil droplets (4-1 no stained, 4-2 stained with Sudan III TS)
5. Aleurone grains (5-1 no stained, 5-2 stained with iodine TS)

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

Standard solutions

Linoleic acid standard solution

Weigh 4.0 mg of linoleic acid CRS (Fig. 4) and dissolve in 1 mL of ethyl acetate.

α-Linolenic acid standard solution

Weigh 4.0 mg of α-linolenic acid CRS (Fig. 4) and dissolve in 1 mL of ethyl acetate.

Oleic acid standard solution

Weigh 4.0 mg of oleic acid CRS (Fig. 4) and dissolve in 1 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of *n*-hexane, ethyl acetate, glacial acetic acid and acetonitrile (6:2.5:0.1:0.25, v/v).

Spray reagent

Weigh 0.1 g of 2',7'-dichlorofluorescein and dissolve in 50 mL of ethanol.

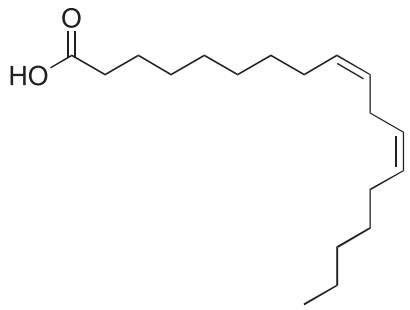
Test solution

Kieselguhr is added to the sample before powdering in the ratio of 1:1. Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of *n*-hexane. Sonicate (400 W) the mixture for 10 min. Filter and transfer 10 mL of 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 5 mL of sodium hydroxide in ethanol (4%, w/v). Heat the mixture in a water bath at 70ºC for 30 min. Cool down to room temperature. Add 1 mL of sulphuric acid in ethanol (50%, v/v). Transfer the solution to a separating funnel. Extract with 10 mL of *n*-hexane and 20 mL of boiled water at room temperature. Transfer the *n*-hexane layer to another separating funnel. Wash the *n*-hexane layer for three times each with 10 mL of boiled water at room temperature. Collect the *n*-hexane layer and add appropriate amount of anhydrous sodium sulphate. Filter the mixture.

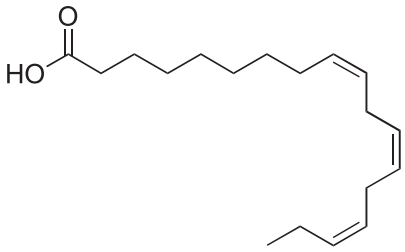
Procedure

Carry out the method by using a HPTLC plate of silica gel H with 15% silver nitrate and a freshly prepared developing solvent system as described above. Apply separately linoleic acid standard solution, α-linolenic acid standard solution, oleic acid standard solution and the test solution (3 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 dry in air. Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

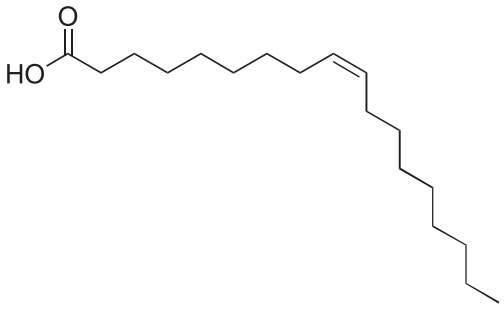
(i)



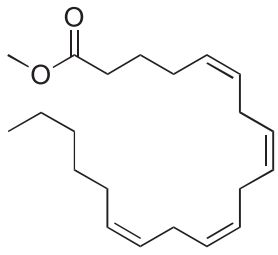
(ii)



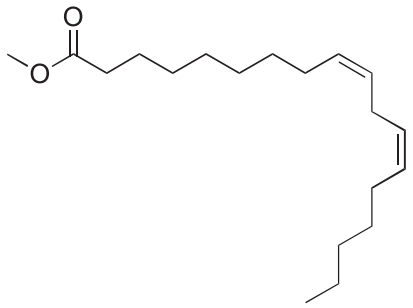
(iii)



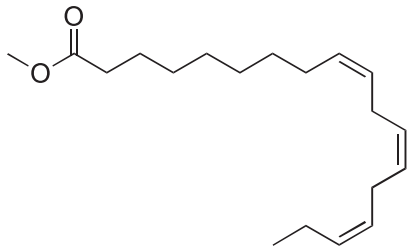
(iv)



(v)



(vi)



(vii)

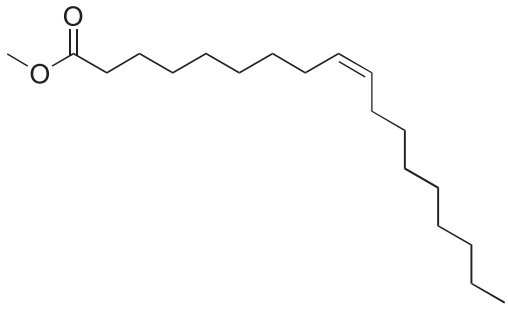


Figure 4 Chemical structures of (i) linoleic acid (ii) α-linolenic acid (iii) oleic acid (iv) methyl arachidonate (v) methyl linoleate (vi) methyl linolenate and (vii) methyl oleate

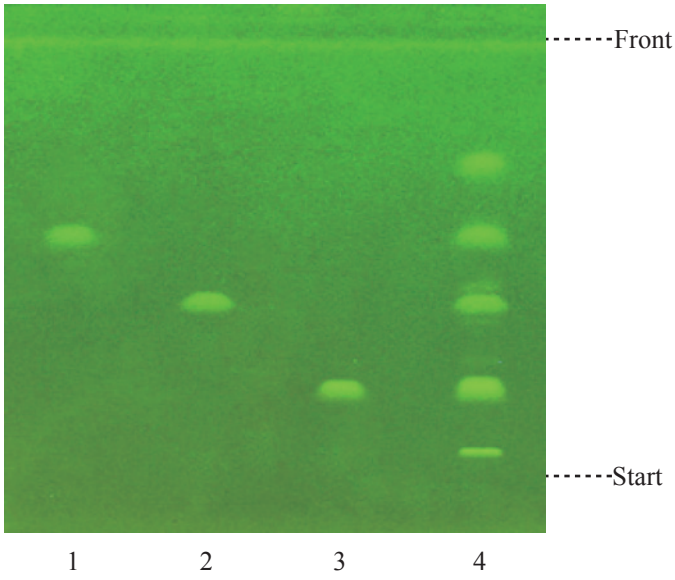


Figure 5 A reference HPTLC chromatogram of Platycladi Semen extract observed under UV light (366 nm) after staining

1. Oleic acid standard solution 2. Linoleic acid standard solution
3. α-Linolenic acid standard solution 4. Test solution

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 linoleic acid, α-linolenic acid and oleic acid (Fig. 5).

4.3 Gas Chromatographic Fingerprinting *(Appendix XII)*

Standard solutions

Methyl arachidonate standard solution for fingerprinting, Std-FP (23 mg/L)

Weigh 0.23 mg of methyl arachidonate CRS (Fig. 4) and dissolve in 10 mL of ethyl acetate.

Methyl linoleate standard solution for fingerprinting, Std-FP (500 mg/L)

Weigh 5.0 mg of methyl linoleate CRS (Fig. 4) and dissolve in 10 mL of ethyl acetate.

Methyl linolenate standard solution for fingerprinting, Std-FP (800 mg/L)

Weigh 8.0 mg of methyl linolenate CRS (Fig. 4) and dissolve in 10 mL of ethyl acetate.

Methyl oleate standard solution for fingerprinting, Std-FP (300 mg/L)

Weigh 3.0 mg of methyl oleate CRS (Fig. 4) and dissolve in 10 mL of ethyl acetate.

Test solution

Kieselguhr is added to the sample before powdering in the ratio of 1:1. Weigh 0.8 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethyl acetate. Sonicate (400 W) the mixture for 20 min. Centrifuge at about 690 × *g* for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Collect the filtrates and make up to the mark with ethyl acetate. Pipette 5 mL of the solution and transfer to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Add 3 mL of potassium hydroxide in methanol (2.8%, w/v) and heat at 45ºC for 30 min. Add 0.5 mL of sulphuric acid in methanol (50%, v/v) and 1 mL of boron trifluoride in methanol (14%, w/v). Mix well and heat at 45ºC for 15 min. Then, add 10 mL of isooctane and 20 mL of saturated sodium chloride solution. Transfer the solution to a 100-mL separating funnel. Shake well and collect the isooctane extract. Extract the aqueous layer for two times each with 5 mL of isooctane. Combine the isooctane extracts and add appropriate amount of anhydrous sodium sulphate. 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 isooctane. Transfer the solution to a 10-mL volumetric flask and make up to the mark with isooctane. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (VF-23 MS, 0.25 mm × 30 m) of which the internal wall is covered with cyanopropyl polysiloxane in a layer about 0.25 μm thick. The injection temperature is at 250ºC. The detector temperature is at 300ºC. The split injection mode at a ratio of 10:1 is used. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC /min)
0 – 9	80 → 170	10
9 – 24	170 → 200	2
24 – 26	200 → 250	25
26 – 31	250	-

System suitability requirements

Perform at least five replicate injections, each using 1 µL of methyl arachidonate Std-FP, methyl linoleate Std-FP, methyl linolenate Std-FP and methyl oleate Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate should not be more than 5.0%; the RSD of the retention times of methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate peaks should not be more than 2.0%; the column efficiencies determined from methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate peaks should not be less than 200000 theoretical plates.

The *R* value between peak 3 and the closest peak; the *R* value between peak 4 and the closest peak; the *R* value between peak 5 and the closest peak; and the *R* value between peak 10 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject methyl arachidonate Std-FP, methyl linoleate Std-FP, methyl linolenate Std-FP, methyl oleate Std-FP and the test solution (1 µL each) into the GC system and record the chromatograms. Measure the retention times of methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate peaks in the chromatograms of methyl arachidonate Std-FP, methyl linoleate Std-FP, methyl linolenate Std-FP, methyl oleate Std-FP and the retention times of the eleven characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of methyl arachidonate Std-FP, methyl linoleate Std-FP, methyl linolenate Std-FP and methyl oleate Std-FP. The retention times of methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate 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 eleven characteristic peaks of Platycladi Semen extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the eleven characteristic peaks of Platycladi Semen extract

Peak No.	RRT	Acceptable Range
1	0.69	± 0.03
2	0.82	± 0.03
3 (methyl oleate)	0.86	± 0.03
4 (methyl linoleate)	0.92	± 0.03
5 (marker, methyl linolenate)	1.00	-
6	1.04	± 0.03
7	1.07	± 0.03
8	1.11	± 0.03
9	1.15	± 0.03
10 (methyl arachidonate)	1.20	± 0.03
11	1.24	± 0.03

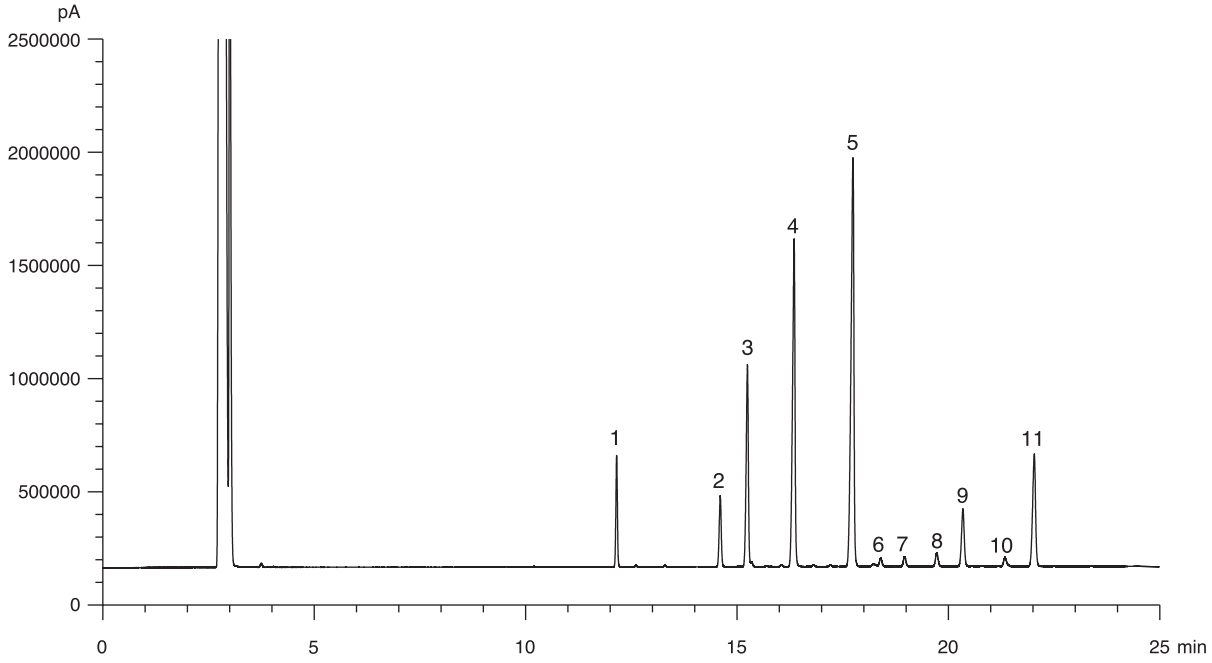


Figure 6 A reference GC fingerprint chromatogram of Platycladi Semen extract

For positive identification, the sample must give the above eleven characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference GC fingerprint chromatogram (Fig. 6).

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 6.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.5%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 6.0%.

5.8 Acid Value *(Appendix XIV)*: not more than 40.0.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 9.0%.
Ethanol-soluble extractives (hot extraction method): not less than 6.0%.

7. ASSAY

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

Standard solution

Mixed methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate standard stock solution, Std-Stock (150 mg/L for methyl arachidonate, 2500 mg/L for methyl linoleate, 4000 mg/L for methyl linolenate and 1500 mg/L for methyl oleate)

Weigh accurately 1.5 mg of methyl arachidonate CRS, 25 mg of methyl linoleate CRS, 40 mg of methyl linolenate CRS and 15 mg of methyl oleate CRS, and dissolve in 10 mL of ethyl acetate.

Mixed methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate standard solution for assay, Std-AS

Measure accurately the volume of the mixed methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate Std-Stock, dilute with ethyl acetate to produce a series of solutions of 6, 12, 24, 48, 75 mg/L

for methyl arachidonate, 100, 250, 500, 1250, 2500 mg/L for methyl linoleate, 160, 400, 800, 2000, 4000 mg/L for methyl linolenate and 60, 150, 300, 750, 1500 mg/L for methyl oleate.

Test solution

Kieselguhr is added to the sample before powdering in the ratio of 1:1. Weigh accurately 0.8 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethyl acetate. Sonicate (400 W) the mixture for 20 min. Centrifuge at about 690 × *g* for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Collect the filtrates and make up to the mark with ethyl acetate. Pipette 5 mL of the solution and transfer to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Add 3 mL of potassium hydroxide in methanol (2.8%, w/v) and heat at 45ºC for 30 min. Add 0.5 mL of sulphuric acid in methanol (50%, v/v) and 1 mL of boron trifluoride in methanol (14%, w/v). Mix well and heat at 45ºC for 15 min. Then, add 10 mL of isooctane and 20 mL of saturated sodium chloride solution. Transfer the solution to a 100-mL separating funnel. Shake well and collect the isooctane extract. Extract the aqueous layer for two times each with 5 mL of isooctane. Combine the isooctane extracts and add appropriate amount of anhydrous sodium sulphate. 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 isooctane. Transfer the solution to a 10-mL volumetric flask and make up to the mark with isooctane. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (VF-23 MS, 0.25 mm × 30 m) of which the internal wall is covered with cyanopropyl polysiloxane in a layer about 0.25 μm thick. The injection temperature is at 250ºC. The detector temperature is at 300ºC. The split injection mode at a ratio of 10:1 is used. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC / min)
0 – 9	80 → 170	10
9 – 24	170 → 200	2
24 – 26	200 → 250	25
26 – 31	250	-

System suitability requirements

Perform at least five replicate injections, each using 1 μL of the mixed methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate Std-AS (24 mg/L for methyl arachidonate, 500 mg/L for methyl linoleate, 800 mg/L for methyl linolenate and 300 mg/L for methyl oleate). The requirements

of the system suitability parameters are as follows: the RSD of the peak areas of methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate should not be more than 5.0%; the RSD of the retention times of methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate peaks should not be more than 2.0%; the column efficiencies determined from methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate peaks should not be less than 200000 theoretical plates.

The *R* value between methyl arachidonate peak and the closest peak; the *R* value between methyl linoleate peak and the closest peak; the *R* value between methyl linolenate peak and the closest peak; and the *R* value between methyl oleate 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 methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate Std-AS (1 µL each) into the GC system and record the chromatograms. Plot the peak areas of methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate against the corresponding concentrations of the mixed methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 1 µL of the test solution into the GC system and record the chromatogram. Identify methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate Std-AS. The retention times of methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate 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 methyl arachidonate, methyl linoleate, methyl linolenate and methyl oleate in the test solution, and calculate the percentage contents of arachidonic acid (the percentage content of methyl arachidonate × 0.96, where 0.96 is the molar mass ratio of arachidonic acid and methyl arachidonate), linoleic acid (the percentage content of methyl linoleate × 0.95, where 0.95 is the molar mass ratio of linoleic acid and methyl linoleate), α-linolenic acid (the percentage content of methyl linolenate × 0.95, where 0.95 is the molar mass ratio of α-linolenic acid and methyl linolenate) and oleic acid (the percentage content of methyl oleate × 0.95, where 0.95 is the molar mass ratio of oleic acid and methyl oleate) in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 32% of the total content of arachidonic acid ($C_{20}H_{32}O_2$), linoleic acid ($C_{18}H_{32}O_2$), α-linolenic acid ($C_{18}H_{30}O_2$) and oleic acid ($C_{18}H_{34}O_2$), calculated with reference to the dried substance.