Ganoderma

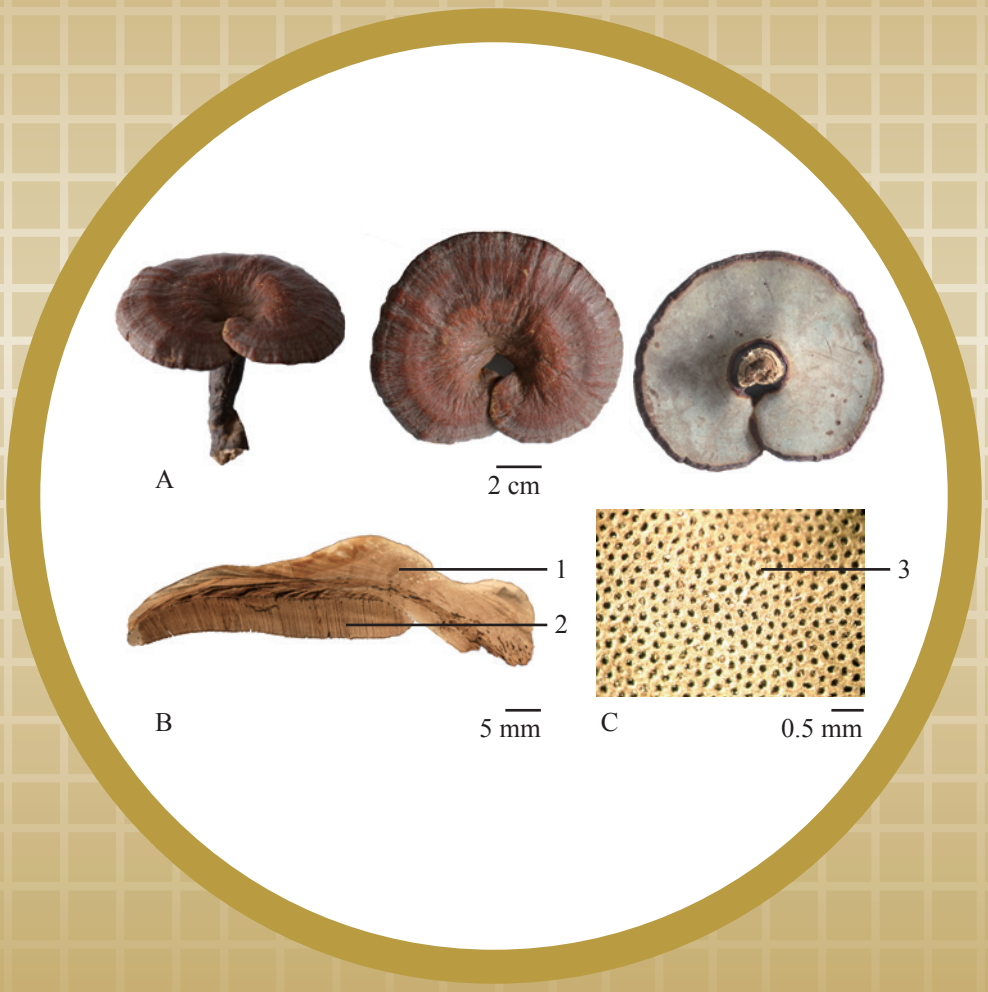

Figure 1 (i) A photograph of dried sporocarp of *Ganoderma lucidum* (Leyss. ex Fr.) Karst.

A. Sporocarps B. Longitudinal section of pileus

C. Magnified image of lower surface of pileus

1. Context 2. Spore-producing tube layer 3. Pores of tubes

Figure 1 (ii) A photograph of dried sporocarp of *Ganoderma sinense* Zhao, Xu et Zhang

A. Sporocarps B. Longitudinal section of pileus

C. Magnified image of lower surface of pileus

1. Context 2. Spore-producing tube layer 3. Pores of tubes

1. NAMES

Official Name: Ganoderma

Chinese Name: 靈芝

Chinese Phonetic Name: Lingzhi

2. SOURCE

Ganoderma is the dried sporocarp of *Ganoderma lucidum* (Leyss. ex Fr.) Karst. or *Ganoderma sinense* Zhao, Xu et Zhang (Polyporaceae). The sporocarp is collected all year round, foreign matter removed, cut off the lower end of the stipe with culture medium, then dried in the shade, baked at 40-50ºC or dried under the sun to obtain Ganoderma.

Part I Dried Sporocarp of *Ganoderma lucidum* (Leyss. ex Fr.) Karst.

3. DESCRIPTION

Cultivated species umbrella-shaped, consisting of pileus and stipe. Pileus subreniform, semicircular or suborbicular, 40-250 mm in diameter, 0.4-2.5 cm thick; upper surface yellowish-brown, brown to reddish-brown, lustrous, with concentric annular grooves and bands, also with distinct or indistinct radial wrinkles, margin thin and truncate, flat or incurved; context whitish to pale brown, thicker near the stipe, 1.6 cm at the thickest part, become thinner toward the margin; the spore-producing tube layer pale brown to brown, monolayer, the length of tube varies from short to long, 1.28 cm at the longest part; fine and dense pores of the tubes found on the lower surface, yellowish-brown, 4-7 pores per millimeter. Spores found on the raw ones, fine, brown. Stipe cylindrical, flat-cylindrical to subquadrangular, attachment of the stipe to the pileus varies from lateral to nearly central, occasionally eccentric, 2.5-20.2 cm long, 9-72 mm in diameter, same colour as pileus or purplish-brown, with lacquer-like lustre. Texture hard; odour slightly aromatic; taste slightly bitter and astringent [Fig. 1 (i)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Longitudinal section of pileus

Pileipellis consists of densely arranged palisade hyphae, hyphae clavate, pale yellowish-brown to reddish-brown. Context underneath pileipellis, consisting of interwoven, branched and non-septate hyphae, with 1-2 interrupted layers of pigmented hyphae. Pore-like clefts located near the middle of context. The spore-producing tube layer monolayer, tubes longitudinally arranged, 2.5-13.0 mm long, walls covered with the dark brown spores (Fig. 2).

Transverse section of tube layer

Pores of tubes subrounded or polygonal, 130-280 μm in diameter, dissepiment 25-275 μm wide. Spores mainly scattered near the tube walls or in the pores (Fig. 3).

Powder

Colour pale brown to brown. Spores brown, ovoid, apex truncate, with colourless sporophores, nearly truncate at the end, 8-12 μm long, 5-8 μm wide, double-walled, episporium smooth, colourless, endosporium pale brown, with warty protuberance, sometimes with oil droplets at the centre. Hyphae scattered or aggregated to form clumps, colourless or pale brown, slender, slightly curved, branched, 2-8 μm in diameter. Fragments of pileipellis pale yellowish-brown to reddish-brown, palisade-like hyphae densely arranged, clavate, apex enlarged, unbranched, 5-13 μm in diameter. Hyphae of pigment layer dark brown, consisting of hyphae and pigment (Fig. 4).

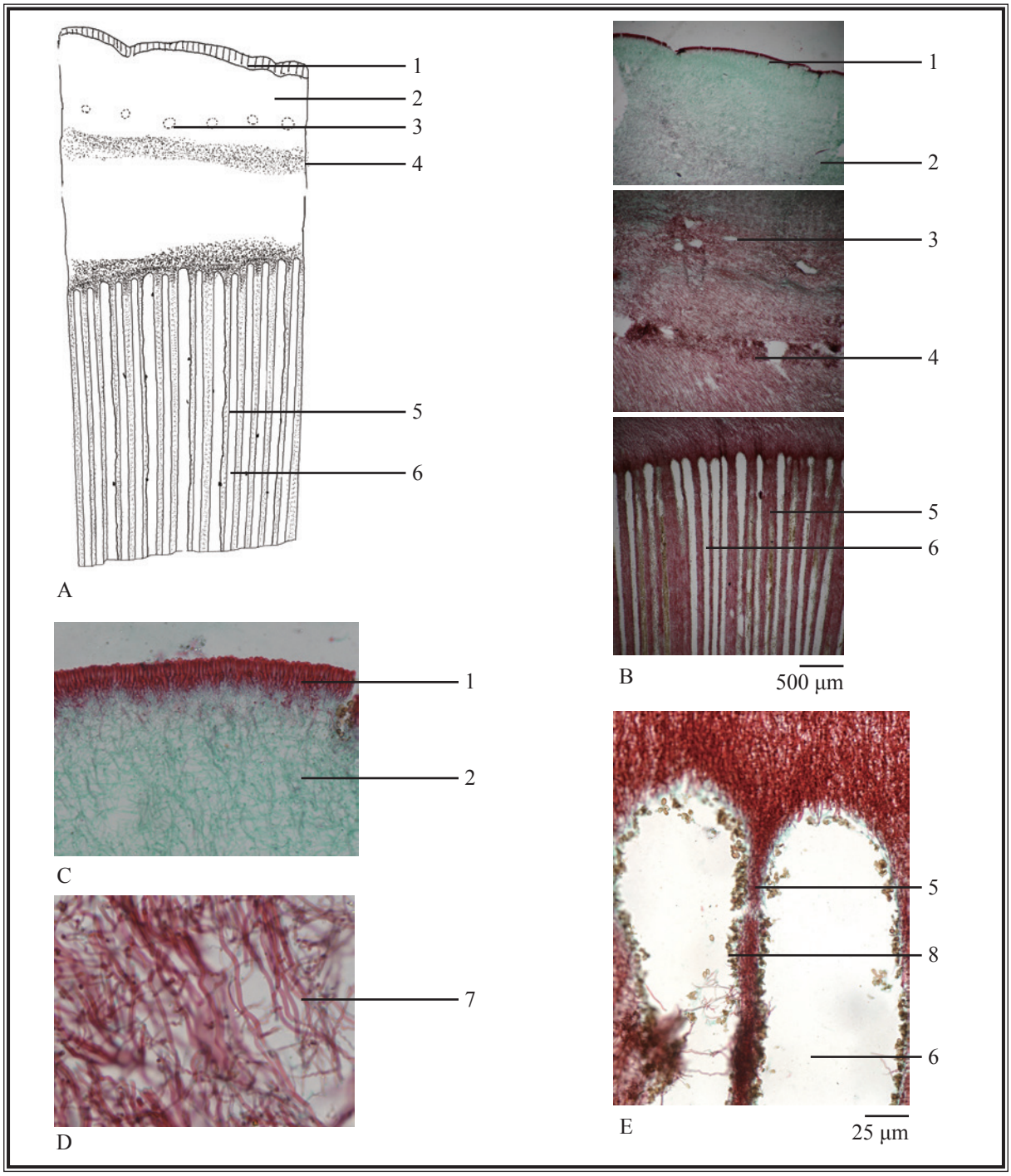

Figure 2 Microscopic features of longitudinal section of pileus of *Ganoderma lucidum* (Leyss. ex Fr.) Karst.

A. Sketch B. Section illustration C. Magnified image of pileipellis D. Magnified image of context E. Magnified image of tubes

1. Pileipellis 2. Context 3. Cleft 4. Pigment layer 5. Dissepiment 6. Tube 7. Hyphae 8. Spore

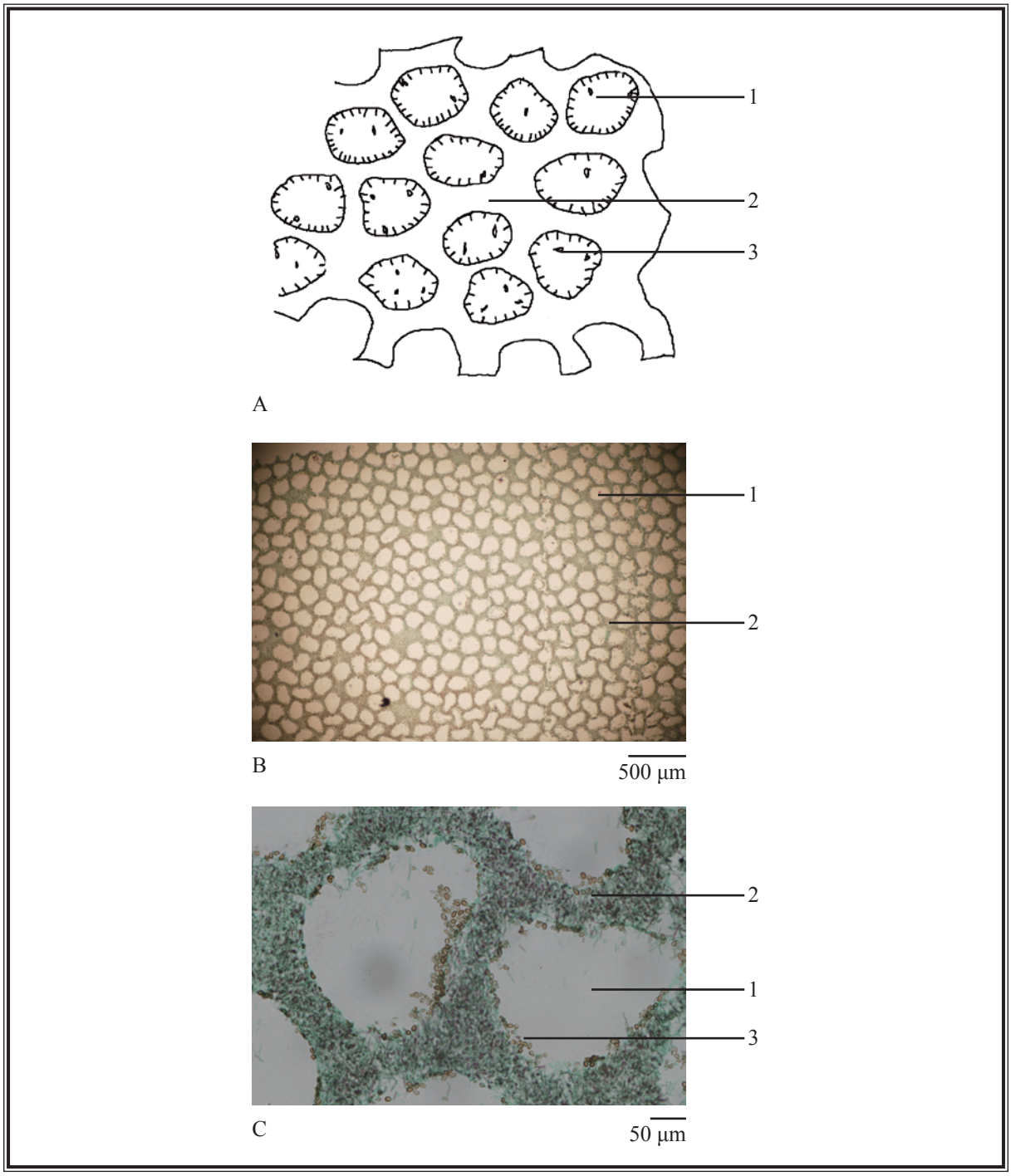

Figure 3 Microscopic features of transverse section of tube layer of *Ganoderma lucidum* (Leyss. ex Fr.) Karst.

A. Sketch B. Section illustration C. Magnified image of tubes

1. Tube 2. Dissepiment 3. Spore

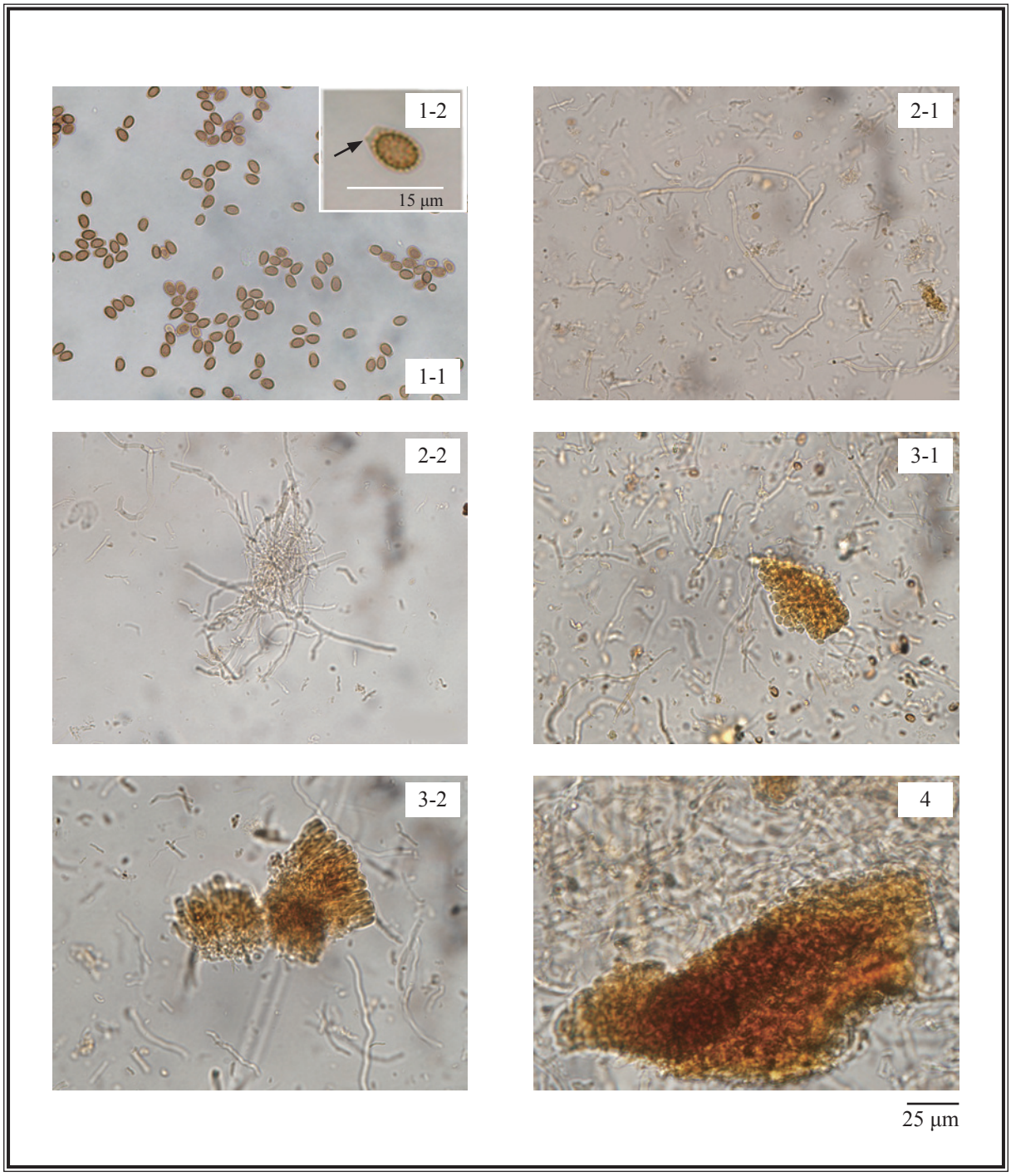

Figure 4 Microscopic features of powder of dried sporocarp of *Ganoderma lucidum* (Leyss. ex Fr.) Karst. (under the light microscope)

1. Spores (1-1 spores, 1-2 spore magnified under oil immersion lens; sporophores ⟶)
2. Hyphae (2-1 hyphae, 2-2 clump of hyphae)
3. Fragment of pileipellis (3-1 in top view, 3-2 in lateral view)
4. Hyphae of pigment layer

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

Standard solution

Ergosta-4,6,8(14),22-tetraen-3-one standard solution

Weigh 1.0 mg of ergosta-4,6,8(14),22-tetraen-3-one CRS (Fig. 5) and dissolve in 100 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of petroleum ether (60 – 80ºC), ethyl acetate and formic acid (15:5:1, v/v). Use the upper layer.

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 250-mL conical flask, then add 100 mL of ethanol (95%). Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethyl acetate. Filter through a 0.45-μm nylon filter.

Procedure

Carry out the method by using a HPTLC silica gel G60 plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately ergosta-4,6,8(14), 22-tetraen-3-one standard solution and the test solution (5 μL each) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the HPTLC plate in the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully tilt the chamber to allow sufficient solvent to pass from the trough containing the solvent to the other containing the HPTLC plate for development. 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. Spray the plate evenly with the spray reagent. Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

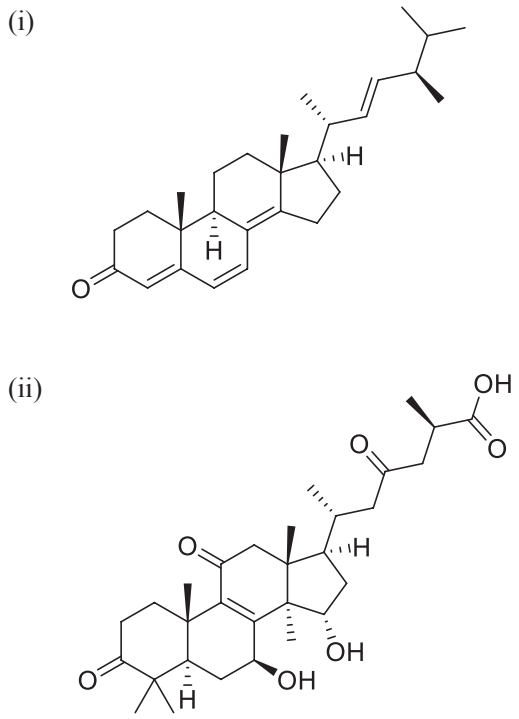

Figure 5 Chemical structures of (i) ergosta-4,6,8(14),22-tetraen-3-one and (ii) ganoderic acid A

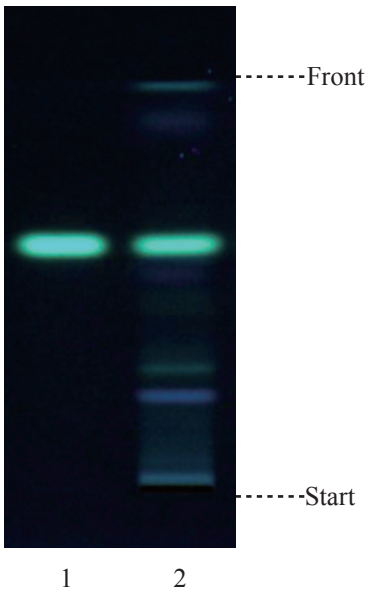

Figure 6 A reference HPTLC chromatogram of dried sporocarp of *Ganoderma lucidum* (Leyss. ex Fr.) Karst. extract observed under UV light (366 nm) after staining

1. Ergosta-4,6,8(14),22-tetraen-3-one standard solution
2. Test solution

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 ergosta-4,6,8(14),22-tetraen-3-one (Fig. 6).

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

Standard solution

Ganoderic acid A standard solution for fingerprinting, Std-FP (1000 mg/L)

Weigh 5.0 mg of ganoderic acid A CRS (Fig. 5) and dissolve in 5 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL conical flask, then add 50 mL of ethanol (95%). Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction for one more time. Combine the filtrates. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 5-mL volumetric flask 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 (254 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 35ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.04% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 50	70 → 62	30 → 38	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of ganoderic acid A Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of ganoderic acid A should not be more than 5.0%; the RSD of the retention time of ganoderic acid A peak should not be more than 2.0%; the column efficiency determined from ganoderic acid A peak should not be less than 10000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 7).

Procedure

Separately inject ganoderic acid A Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of ganoderic acid A peak in the chromatogram of ganoderic acid A Std-FP and the retention times of the four characteristic peaks (Fig. 7) in the chromatogram of the test solution. Identify ganoderic acid A peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of ganoderic acid A Std-FP. The retention times of ganoderic acid A 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 four characteristic peaks of dried sporocarp of *Ganoderma lucidum* (Leyss. ex Fr.) Karst. extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of dried sporocarp of *Ganoderma lucidum* (Leyss. ex Fr.) Karst. extract

Peak No.	RRT	Acceptable Range
1	0.77	± 0.03
2	0.80	± 0.03
3 (marker, ganoderic acid A)	1.00	-
4	1.41	± 0.03

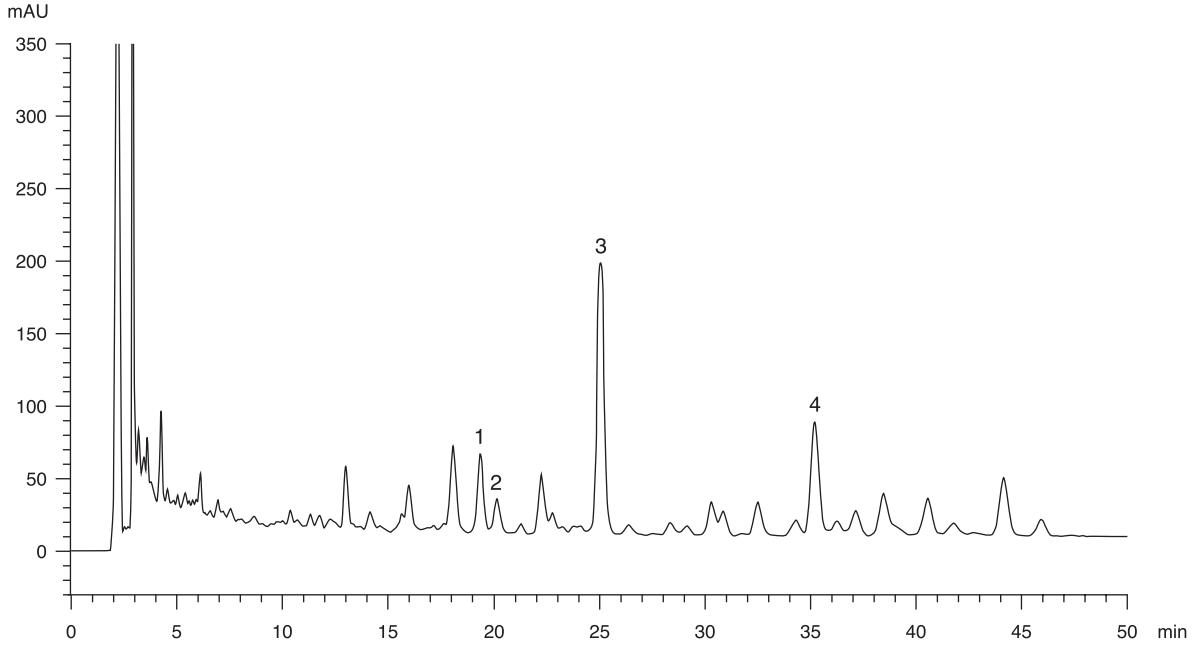

Figure 7 A reference fingerprint chromatogram of dried sporocarp of *Ganoderma lucidum* (Leyss. ex Fr.) Karst. extract

For positive identification, the sample must give the above four characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 7).

5. TESTS

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

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

5.3 Mycotoxins – Aflatoxins *(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 2.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 4.0%.
Ethanol-soluble extractives (hot extraction method): not less than 4.0%.

7. ASSAY

Carry out the method as directed in Appendix XIV.

Reagent
Anthrone sulphuric acid solution
Weigh accurately 0.1 g of anthrone and dissolve in 100 mL of sulphuric acid (98%, v/v).

Standard solution

Anhydrous glucose standard stock solution, Std-Stock (120 mg/L)

Weigh accurately 12.0 mg of anhydrous glucose CRS and dissolve in 100 mL of water.

Anhydrous glucose standard solution for assay, Std-AS

Measure accurately the volume of the anhydrous glucose Std-Stock, dilute with water to produce a series of solutions of 12, 24, 36, 48, 60 mg/L for anhydrous glucose.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 100 mL of water. Allow to stand for 1 h. Reflux the mixture for 4 h. Cool down to room temperature. Filter and transfer the filtrate to a 500-mL conical flask. Wash the residue and filter paper for two times each with 10 mL of hot water. Transfer the residue and filter paper to a 250-mL round-bottomed flask. Repeat the extraction for one more time without standing for 1 h. Combine and transfer the filtrates to an evaporating dish. Evaporate the solvent to dryness on a water bath. Dissolve the residue in 5 mL of water. Transfer the solution to a 250-mL round-bottomed flask and add 75 mL of ethanol. Place the mixture at 4ºC for 12 h. Transfer the mixture to a 100-mL centrifuge tube. Centrifuge at about 4000 × *g* for 10 min. Discard the supernatant. Dissolve the residue in hot water. Transfer the solution to a 50-mL volumetric flask and make up to the mark with water. Pipette 3 mL of the solution to a 50-mL volumetric flask and make up to the mark with water. The blank determination shall be performed.

Ultraviolet/ Visible spectrophotometric system

The spectrophotometer is set at 625 nm.

Colourimetric method

Pipette 2 mL of the standard solution or test solution into a 10-mL test tube, then pipette 6 mL of anthrone sulphuric acid solution. Allow to stand for 15 min. Cool the mixture in an ice water bath for 15 min. Using the corresponding anthrone sulphuric acid solution as the blank. Proceed to UV/Visible analysis at 625 nm.

System suitability requirements

Perform at least five replicate determinations, each using 2 mL of anhydrous glucose Std-AS (36 mg/L) by colourimetric method. The requirement of the system suitability parameter is as follows: the RSD of the absorbance of anhydrous glucose should not be more than 5.0%.

Calibration curve

Determine a series of anhydrous glucose Std-AS (2 mL each) in the ultraviolet/ visible spectrophotometric system and record the absorbance by colourimetric method. Plot the absorbances of anhydrous glucose against the corresponding concentrations of anhydrous glucose Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Measure the absorbance and calculate the concentration (in milligram per litre) of anhydrous glucose in the test solution, and calculate the percentage content of anhydrous glucose in the sample by using the equations as indicated in Appendix XIV.

Limits

The dried sporocarp of *Ganoderma lucidum* (Leyss. ex Fr.) Karst. contains not less than 3.6% of polysaccharides [calculated as anhydrous glucose ($C_6H_{12}O_6$)], calculated with reference to the dried substance.

Part II Dried sporocarp of *Ganoderma sinense* Zhao, Xu et Zhang

3. DESCRIPTION

Cultivated species umbrella-shaped, consisting of pileus and stipe. Pileus subreniform, suborbicular or semicircular, flatter, 50-262 mm in diameter, 0.3-2.7 cm thick; upper surface purplish-black to nearly black, with distinct concentric annular grooves and bands, also radial wrinkles, margin thin or obtuse, crisped. Context pale yellowish-brown to dark brown, thicker near the stipe, 1.6 cm at the thickest part, become thinner toward the margin; the spore-producing tube layer brown, dark brown to purplish-brown, the length of tube varies from short to long, 1.7 cm at the longest part; fine and dense pores of the tubes found on the lower surface, yellowish-brown, 5-7 pores per millimeter. Spores found on the raw ones, fine, brown. Stipe cylindrical, flat-cylindrical to sub-cylindrical, attachment of the stipe to the pileus mostly lateral, 1.5-20 cm long, 6-73 mm in diameter, same colour as pileus, with lacquer-like lustre. Texture hard; odour slightly aromatic; taste slightly astringent [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Longitudinal section of pileus

Pileipellis brown to purplish-brown. A pigmented hypha layer located beneath the pileipellis, coloured as pileipellis, varying in thickness. 2-3 layers of pigmented hyphae located in the inner side of context, thin gradually toward margin; some pigment layers not extend to the margin; colour of context between the pigment layers darker, with pore-like clefts. Tube layer monolayer, tubes 1.8-17.0 mm long (Fig. 8).

Transverse section of tube layer

Pores of tubes subrounded or polygonal, 130-350 μm in diameter, dissepiment 25-350 μm wide. Spores mainly scattered near the tube walls or in the pores (Fig. 9).

Powder

Colour purplish-brown. Spores pale brown to brown, ovoid, apex truncate, with colourless, acute sporophores, 8-13 μm long, 4.5-8.8 μm wide, double-walled, episporium smooth, colourless, endosporium pale brown, with distinct warty protuberance. Hyphae scattered or aggregated to form clumps, pale brown to brown or nearly colourless, slender, slightly curved, branched, 2-13 μm in diameter. Fragments of pileipellis purplish-brown to brown, palisade-like hyphae densely arranged, clavate, apex enlarged, unbranched, 6-15 μm in diameter. Hyphae of pigment layer purplish-brown, consisting of hyphae and pigment (Fig. 10).

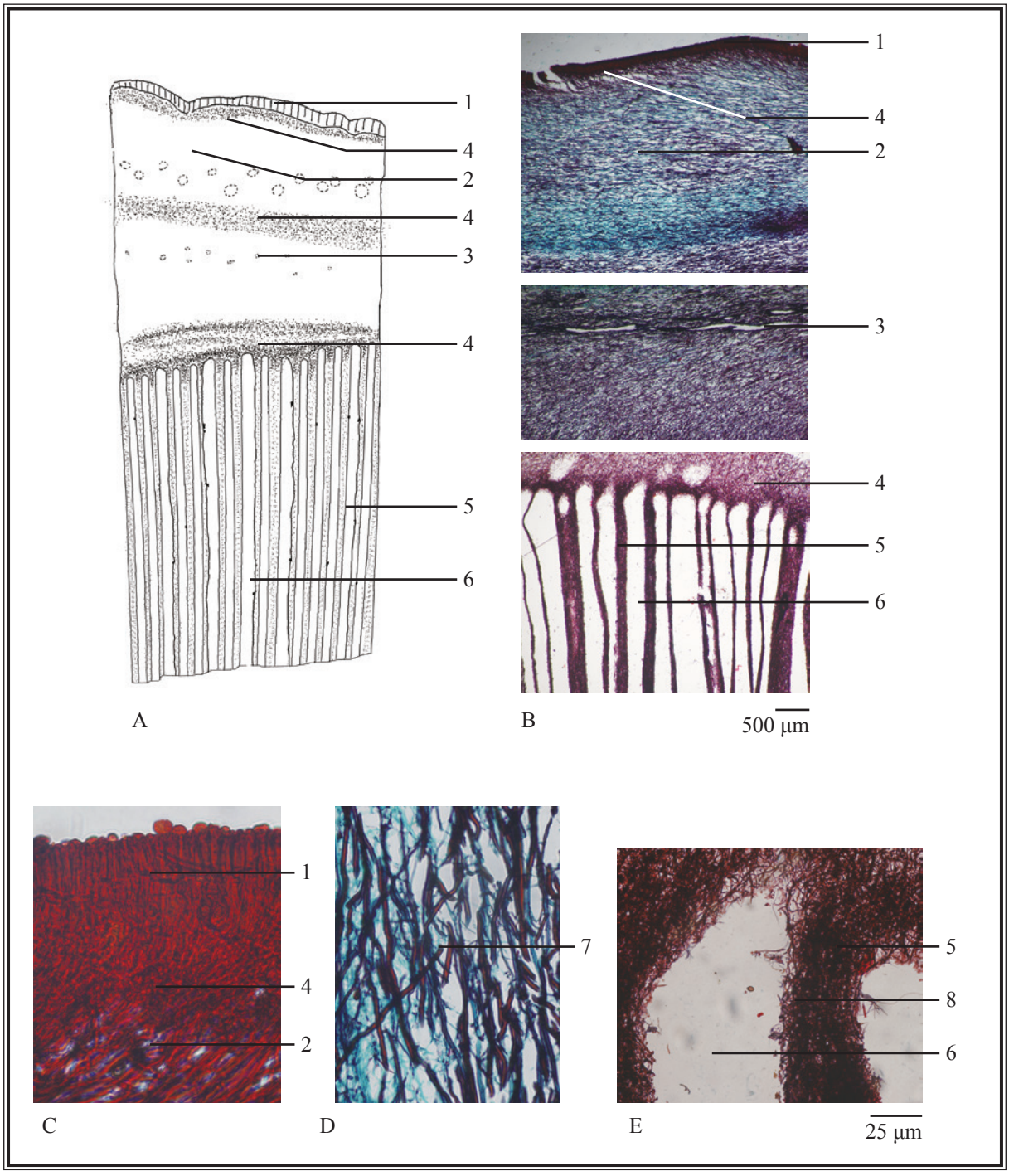

Figure 8 Microscopic features of longitudinal section of pileus of *Ganoderma sinense* Zhao, Xu et Zhang

A. Sketch B. Section illustration C. Magnified image of pileipellis D. Magnified image of context E. Magnified image of tubes

1. Pileipellis 2. Context 3. Cleft 4. Pigment layer 5. Dissepiment 6. Tube 7. Hyphae 8. Spore

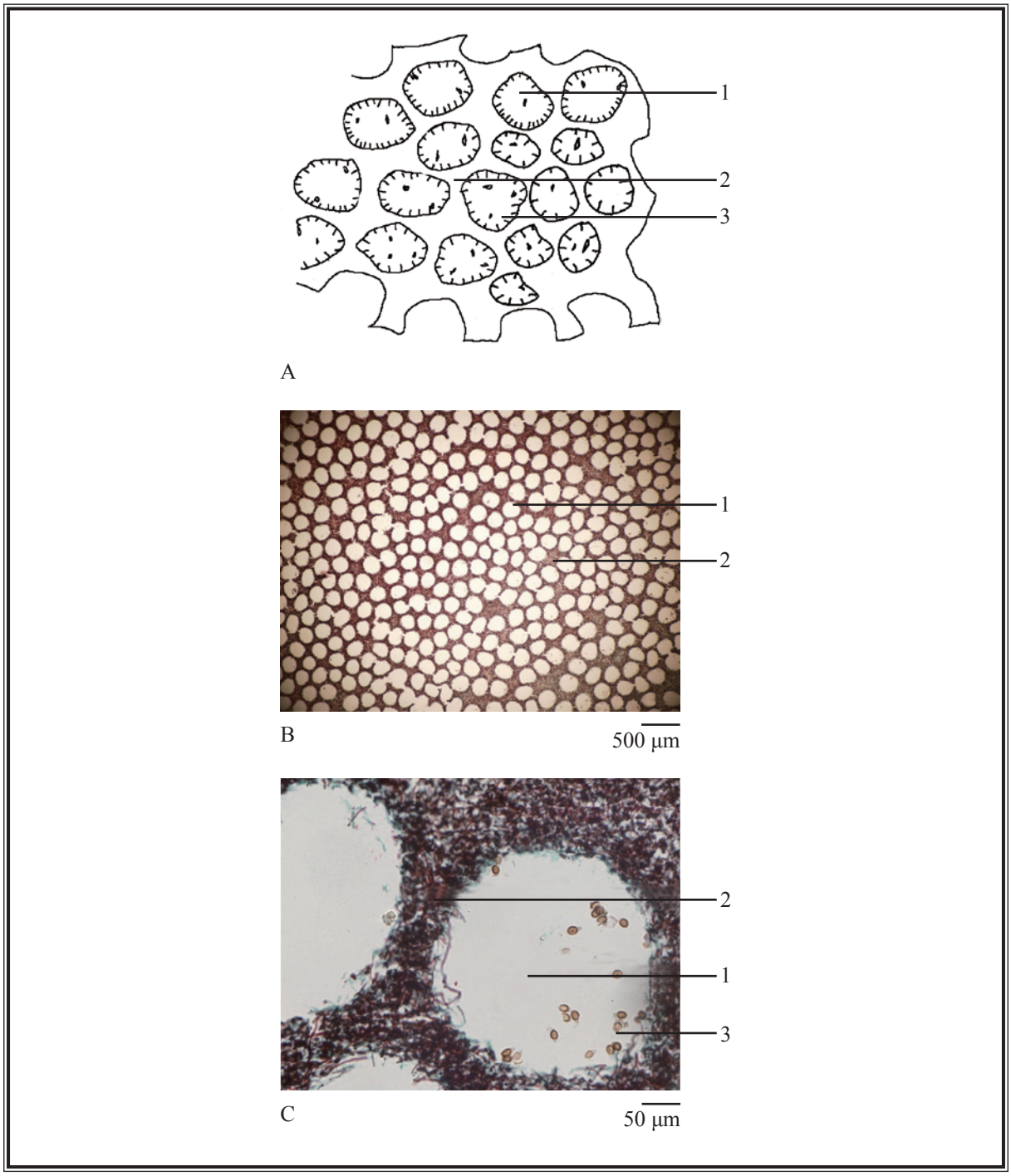

Figure 9 Microscopic features of transverse section of tube layer of *Ganoderma sinense* Zhao, Xu et Zhang

A. Sketch B. Section illustration C. Magnified image of tubes

1. Tube 2. Dissepiment 3. Spore

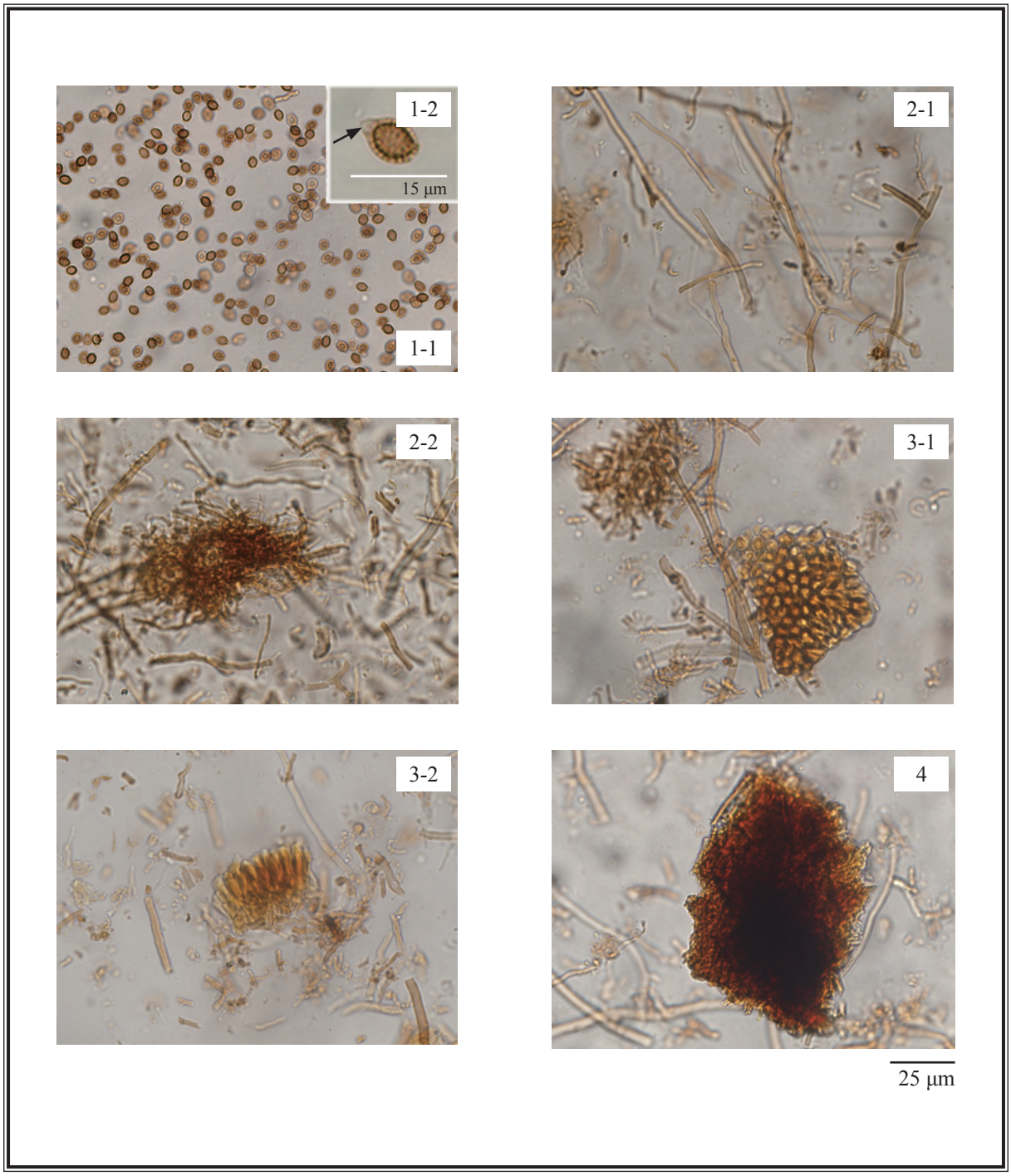

Figure 10 Microscopic features of powder of dried sporocarp of *Ganoderma sinense* Zhao, Xu et Zhang (under the light microscope)

1. Spores (1-1 spores, 1-2 spore magnified under oil immersion lens; sporophores ⟶)
2. Hyphae (2-1 hyphae, 2-2 clump of hyphae)
3. Fragment of pileipellis (3-1 in top view, 3-2 in lateral view)
4. Hyphae of pigment layer

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

Standard solution

Ergosta-4,6,8(14),22-tetraen-3-one standard solution

Weigh 1.0 mg of ergosta-4,6,8(14),22-tetraen-3-one CRS (Fig. 11) and dissolve in 100 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of petroleum ether (60 – 80ºC), ethyl acetate and formic acid (15:5:1, v/v). Use the upper layer.

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 250-mL conical flask, then add 100 mL of ethanol (95%). Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethyl acetate. Filter through a 0.45-μm nylon filter.

Procedure

Carry out the method by using a HPTLC silica gel G60 plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately ergosta-4,6,8(14), 22-tetraen-3-one standard solution and the test solution (5 μL each) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the HPTLC plate in the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully tilt the chamber to allow sufficient solvent to pass from the trough containing the solvent to the other containing the HPTLC plate for development. 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. Spray the plate evenly with the spray reagent. Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

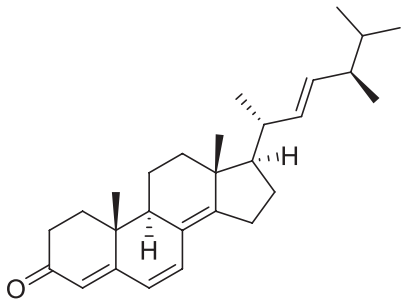

Figure 11 Chemical structure of ergosta-4,6,8(14),22-tetraen-3-one

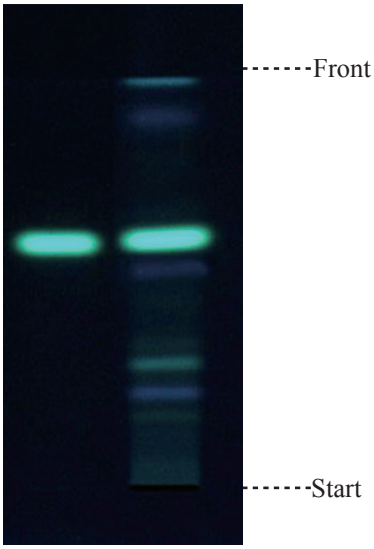

Figure 12 A reference HPTLC chromatogram of dried sporocarp of *Ganoderma sinense* Zhao, Xu et Zhang extract observed under UV light (366 nm) after staining

1. Ergosta-4,6,8(14),22-tetraen-3-one standard solution
2. Test solution

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 ergosta-4,6,8(14),22-tetraen-3-one (Fig. 12).

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

Standard solution

Ergosta-4,6,8(14),22-tetraen-3-one standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 5.0 mg of ergosta-4,6,8(14),22-tetraen-3-one CRS and dissolve in 50 mL of methanol.

Test solution

Weigh 10.0 g of the powdered sample and place it in a 100-mL conical flask, then add 50 mL of ethanol (95%). Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction for one more time. Combine the filtrates. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 5-mL volumetric flask 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 (210 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 40ºC during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of methanol and water (92:8, v/v). The elution time is about 60 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of ergosta-4,6,8(14),22-tetraen-3-one Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of ergosta-4,6,8(14),22-tetraen-3-one should not be more than 5.0%; the RSD of the retention time of ergosta-4,6,8(14),22-tetraen-3-one peak should not be more than 2.0%; the column efficiency determined from ergosta-4,6,8(14),22-tetraen-3-one peak should not be less than 10000 theoretical plates.

The R value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 13).

Procedure

Separately inject ergosta-4,6,8(14),22-tetraen-3-one Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of ergosta-4,6,8(14),22-tetraen-3-one peak in the chromatogram of ergosta-4,6,8(14),22-tetraen-3-one Std-FP and the retention times of the five characteristic peaks (Fig. 13) in the chromatogram

of the test solution. Identify ergosta-4,6,8(14),22-tetraen-3-one peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of ergosta-4,6,8(14),22-tetraen-3-one Std-FP. The retention times of ergosta-4,6,8(14),22-tetraen-3-one 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 five characteristic peaks of dried sporocarp of *Ganoderma sinense* Zhao, Xu et Zhang extract are listed in Table 3.

Table 3 The RRTs and acceptable ranges of the five characteristic peaks of dried sporocarp of *Ganoderma sinense* Zhao, Xu et Zhang extract

Peak No.	RRT	Acceptable Range
1	0.92	± 0.03
2 [marker, ergosta-4,6,8(14),22-tetraen-3-one]	1.00	-
3	1.09	± 0.03
4	1.34	± 0.03
5	1.48	± 0.04

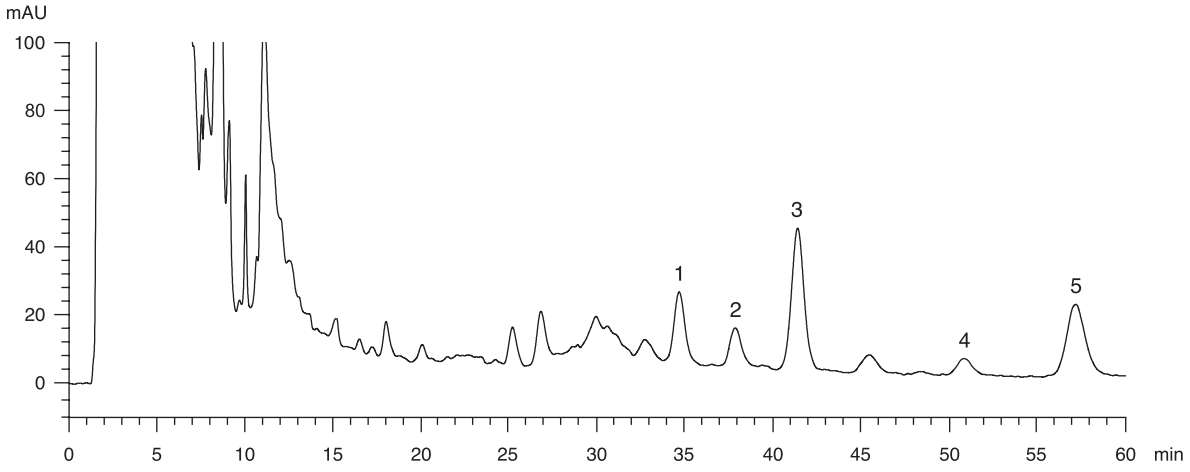

Figure 13 A reference fingerprint chromatogram of dried sporocarp of *Ganoderma sinense* Zhao, Xu et Zhang extract

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 reference fingerprint chromatogram (Fig. 13).

5. TESTS

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

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

5.3 Mycotoxins – Aflatoxins *(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 2.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 4.0%.
Ethanol-soluble extractives (hot extraction method): not less than 3.0%.

7. ASSAY

Carry out the method as directed in Appendix XIV.

Reagent

Anthrone sulphuric acid solution
Weigh accurately 0.1 g of anthrone and dissolve in 100 mL of sulphuric acid (98%, v/v).

Standard solution

Anhydrous glucose standard stock solution, Std-Stock (120 mg/L)
Weigh accurately 12.0 mg of anhydrous glucose CRS and dissolve in 100 mL of water.
Anhydrous glucose standard solution for assay, Std-AS
Measure accurately the volume of the anhydrous glucose Std-Stock, dilute with water to produce a series of solutions of 12, 24, 36, 48, 60 mg/L for anhydrous glucose.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 100 mL of water. Allow to stand for 1 h. Reflux the mixture for 4 h. Cool down to room temperature. Filter and transfer the filtrate to a 500-mL conical flask. Wash the residue and filter paper for two times each with 10 mL of hot water. Transfer the residue and filter paper to a 250-mL round-bottomed flask. Repeat the extraction for one more time without standing for 1 h. Combine and transfer the filtrates to an evaporating dish. Evaporate the solvent to dryness on a water bath. Dissolve the residue in 5 mL of water. Transfer the solution to a 250-mL round-bottomed flask and add 75 mL of ethanol. Place the mixture at 4ºC for 12 h. Transfer the mixture to a 100-mL centrifuge tube. Centrifuge at about 4000 × *g* for 10 min. Discard the supernatant. Dissolve the residue in hot water. Transfer the solution to a 50-mL volumetric flask and make up to the mark with water. Pipette 3 mL of the solution to a 50-mL volumetric flask and make up to the mark with water. The blank determination shall be performed.

Ultraviolet/ Visible spectrophotometric system

The spectrophotometer is set at 625 nm.

Colourimetric method

Pipette 2 mL of the standard solution or test solution into a 10-mL test tube, then pipette 6 mL of anthrone sulphuric acid solution. Allow to stand for 15 min. Cool the mixture in an ice water bath for 15 min. Using the corresponding anthrone sulphuric acid solution as the blank. Proceed to UV/Visible analysis at 625 nm.

System suitability requirements

Perform at least five replicate determinations, each using 2 mL of anhydrous glucose Std-AS (36 mg/L) by colourimetric method. The requirement of the system suitability parameter is as follows: the RSD of the absorbance of anhydrous glucose should not be more than 5.0%.

Calibration curve

Determine a series of anhydrous glucose Std-AS (2 mL each) in the ultraviolet/ visible spectrophotometric system and record the absorbance by colourimetric method. Plot the absorbances of anhydrous glucose against the corresponding concentrations of anhydrous glucose Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Measure the absorbance and calculate the concentration (in milligram per litre) of anhydrous glucose in the test solution, and calculate the percentage content of anhydrous glucose in the sample by using the equations as indicated in Appendix XIV.

Limits

The dried sporocarp of *Ganoderma sinense* Zhao, Xu et Zhang contains not less than 3.8% of polysaccharides [calculated as anhydrous glucose ($C_6H_{12}O_6$)], calculated with reference to the dried substance.