Smilacis Glabrae Rhizoma

Figure 1 A photograph of Smilacis Glabrae Rhizoma

1. NAMES

Official Name: Smilacis Glabrae Rhizoma

Chinese Name: 土茯苓

Chinese Phonetic Name: Tufuling

2. SOURCE

Smilacis Glabrae Rhizoma is the dried rhizome of *Smilax glabra* Roxb. (Liliaceae). The rhizome is collected in summer or autumn. After fibrous roots have been removed, the rhizome is washed clean, then dried to yield the intact form. Alternatively, the rhizome is sliced while fresh, and dried immediately to obtain the sliced form of Smilacis Glabrae Rhizoma.

3. DESCRIPTION

Subcylindrical, slightly flattened or irregularly strip-shaped, with knob-like outgrowths and short branches, 2-26 cm long, 20-80 mm in diameter. Externally yellowish-brown, uneven, with stiff remains of fibrous roots, and with rounded bud scars at the top. Some outer bark irregularly fissured, fallen off easily and exhibiting remains of scales. Texture hard, uneasily broken, fracture whitish to pale reddish-brown, starchy; dotted vascular bundles and numerous small light spots visible, frequently distributed near the centre. Fracture texture slightly tough, dusting on breaking, viscous and slippery when moistened. Odourless; taste slightly sweet and astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermis consists of 3-10 layers of cells, yellowish-brown, arranged densely, the wall relatively thick, slightly lignified. Cortex scattered large mucilage cells, containing raphides of calcium oxalate in mucilage cells. Parenchymatous cells radially elongated in vascular cylinder, collateral vascular bundles scattered, relatively densely near centre. Xylem usually contains 2 large vessels and numbers of small vessels; fibres occasionally visible outside of phloem. Parenchymatous cells contain numerous starch granules (Fig. 2).

Powder

Colour pale brown. Starch granules abundant, simple granules subrounded, polygonal or subsquare, 9-49 µm in diameter, hilum cleft, stellate, Y-shaped or pointed, striations visible in large granules; compound granules composed of 2-4 units; black and cruciate in shape under the polarized microscope. Stone cells abundant, subsquare, subellipitic or triangular, 24-126 µm in diameter, with fine and close pit canals; deep brown stone cell long-striped, walls heavily thickened on the three sides and thin on one side. Raphides of calcium oxalate clustered in bundles or scattered singly, 40-133 µm long; polychromatic under the polarized microscope. Mainly bordered-pitted vessels and tracheids, pits mostly horizontal extension, reticulate vessels occasionally visible. Fibres few, scattered singly, with extremely thick walls, pit distinct, lumina narrow to form slit-shaped, 11-53 µm in diameter; polychromatic under the polarized microscope (Fig. 3).

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

Standard solution

Astilbin standard solution

Weigh 1.0 mg of astilbin CRS (Fig. 4) and dissolve in 2 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, water and formic acid (10:0.8:1, v/v).

Spray reagent

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

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (90 W) the mixture for 15 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately astilbin standard solution (2 µL) and the test solution (10 µL) 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 8.5 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 and heat at about 105°C until the spots or bands become visible (about 5 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV(A).

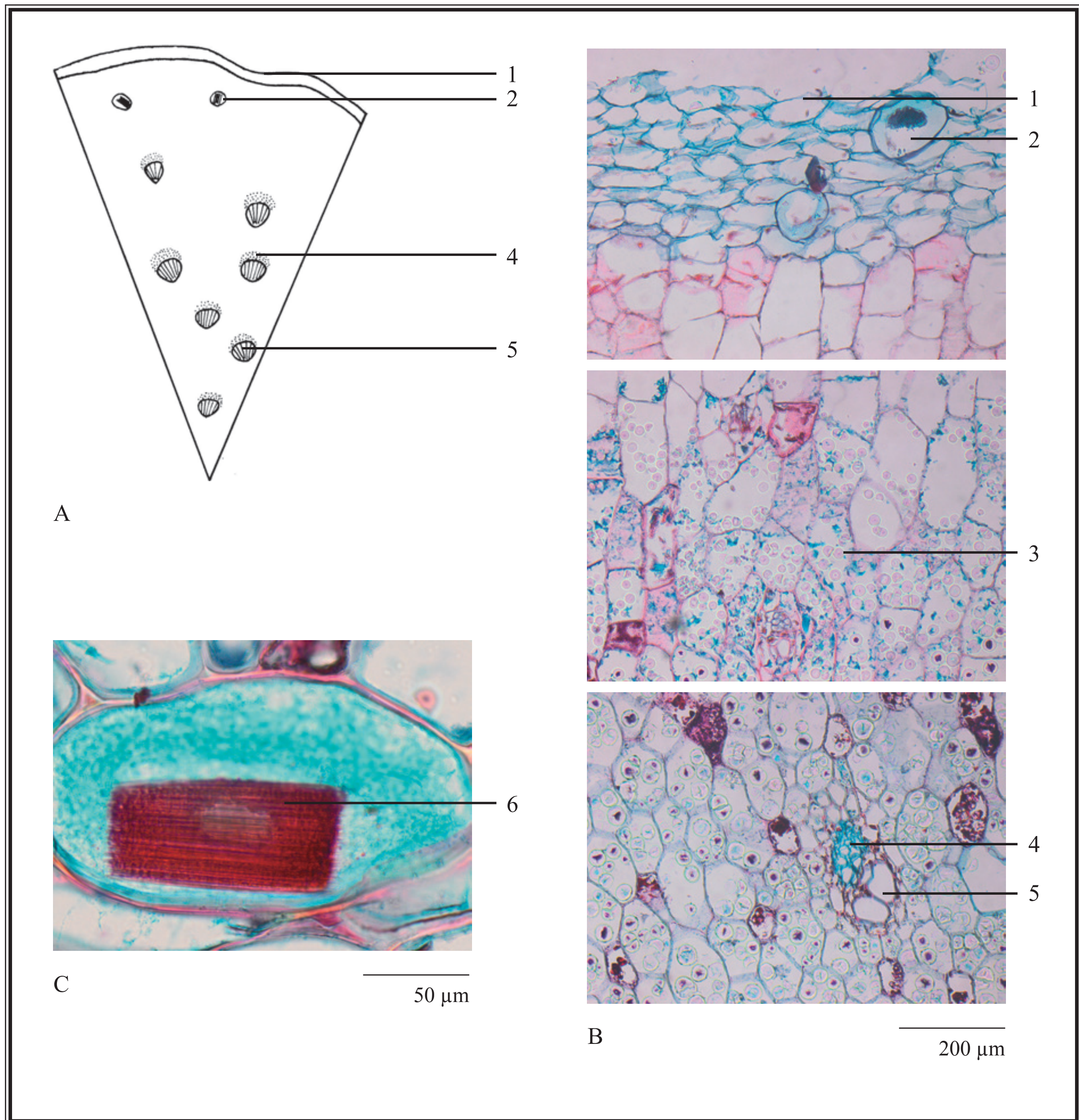

Figure 2 Microscopic features of transverse section of Smilacis Glabrae Rhizoma

A. Sketch B. Section illustration C. Raphides of calcium oxalate in mucilage cell

1. Epidermis 2. Mucilage cells 3. Starch granules 4. Phloem 5. Xylem 6. Raphides of calcium oxalate

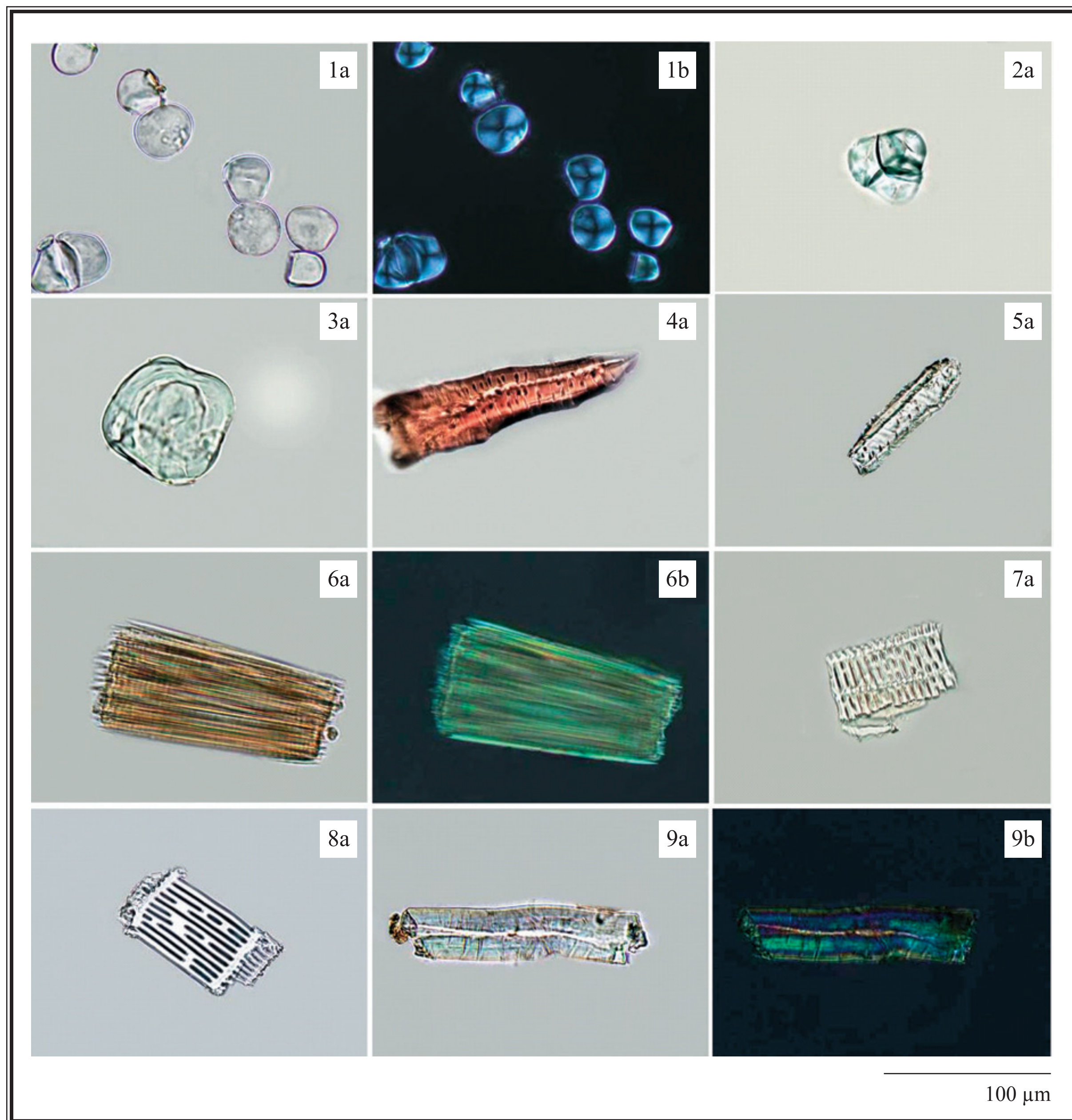

Figure 3 Microscopic features of powder of Smilacis Glabrae Rhizoma

1. Starch granules 2. Compound starch granules 3. Subsquare stone cell 4. Dark brown stone cell 5. Subellipitic stone cell 6. Raphides of calcium oxalate bundles 7. Bordered-pitted vessel 8. Reticulate vessel 9. Fibre

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

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 astilbin.

Figure 4 Chemical structure of astilbin

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

Standard solution

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

Weigh 5.0 mg of astilbin CRS and dissolve in 10 mL of ethanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (70%). Sonicate (90 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Filter through a 0.45-µm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (320 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 – 15	82 → 78	18 → 22	linear gradient
15 – 40	78 → 60	22 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of astilbin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of astilbin should

not be more than 5.0%; the RSD of the retention time of astilbin peak should not be more than 2.0%; the column efficiency determined from astilbin peak should not be less than 15000 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. 5).

Procedure

Separately inject astilbin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of astilbin peak in the chromatogram of astilbin Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify astilbin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of astilbin Std-FP. The retention times of astilbin 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 Smilacis Glabrae Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Smilacis Glabrae Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.53	± 0.03
2 (marker, astilbin)	1.00	-
3	1.16	± 0.03
4	1.77	± 0.07

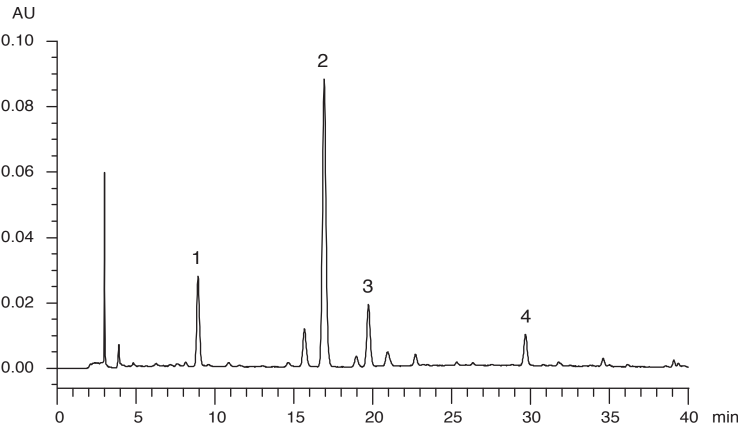

Figure 5 A reference fingerprint chromatogram of Smilacis Glabrae Rhizoma extract

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. 5).

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 XVIII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 2.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

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

7. ASSAY

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

Standard solution
Astilbin standard stock solution, Std-Stock (500 mg/L)
Weigh accurately 2.5 mg of astilbin CRS and dissolve in 5 mL of ethanol (70%).
Astilbin standard solution for assay, Std-AS
Measure accurately the volume of the astilbin Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 5, 40, 80, 120, 180 mg/L for astilbin.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 10 mL of ethanol (70%). Reflux the mixture for 15 min. Cool down to room temperature. Transfer the solution to a 10-mL volumetric flask. Make up to the mark with ethanol (70%). Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (290 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 acetonitrile and 0.1% phosphoric acid (22:78, v/v). The elution time is about 20 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of astilbin Std-AS (80 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of astilbin should not be more than 5.0%; the RSD of the retention time of astilbin peak should not be more than 2.0%; the column efficiency determined from astilbin peak should not be less than 5000 theoretical plates.

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

Limits

The sample contains not less than 0.45% of astilbin ($C_{21}H_{22}O_{11}$), calculated with reference to the dried substance.