Smilacis Chinae Rhizoma



C. Magnified image of longitudinal slices of rhizome

Smilacis Chinae Rhizoma

NAMES 1.

Official Name: Smilacis Chinae Rhizoma

Chinese Name: 菝葜

Chinese Phonetic Name: Bagia

2. SOURCE

Smilacis Chinae Rhizoma is the dried rhizome of Smilax china L. (Liliaceae). The rhizome is collected from late autumn to next spring, rootlets removed, washed clean and then dried under the sun; or cut into slices when fresh, then dried under the sun to obtain Smilacis Chinae Rhizoma.

DESCRIPTION 3.

Irregular masses or flat-cylindrical, slightly curved, 1.7-14.7 cm long, 4-51 mm in diameter. Externally yellowish-brown to purplish-brown, uneven, with conical protuberance scars of stem on the swollen nodes, and remnants of hard spinous rootlets or rootlets on the surface. Heavy in weight, texture very hard, uneasily broken, cut surface brownish-yellow to reddish brown, fibrous, with vein spots on transverse section and vein striations on longitudinal section. Odour slight; taste slightly bitter and astringent (Fig. 1).

IDENTIFICATION 4.

4.1 Microscopic Identification (Appendix III)

Transverse Section

Hypodermis consists of several layers of lignified parenchymatous cells and 2 layers of stone cells, the cells outside the first stone cells layer often fallen off. Parenchymatous cells contain starch granules, slightly lignified, some filled with secretion. Mucilage cells scattered at the outer side of parenchyma, containing raphides of calcium oxalate. Vascular bundles collateral, densely scattered at the inner side of parenchyma, surrounded by fibres (Fig. 2).

山豆根 Saururi Herba 三白草 壮荆葉 車前 Plantagir 天山雪蓮 白花丹 和板歸 Menispermi Rhizoma 山銀花 Shumbaginis Zeylanicae Radix 和板歸 Menispermi Rhizoma 山銀花 Smilacis Chinae

Bruceae Fructus 鴉.腑

Smilacis Chinae Rhizoma

Powder

Colour brown. Starch granules numerous, subrounded or ellipsoid, 4-36 µm in diameter; compound starch granules composed of 2-9 units; hilum dotted or cleft-like, striations indistinct; black and cruciate-shaped under the polarized microscope. Raphides of calcium oxalate in bundles or singly scattered, sometimes in mucilage cells, 24-140 µm long; polychromatic or yellowish-white under the polarized microscope. Stone cells pale yellow or reddish-brown, singly scattered or in groups; subsquare, long-ellipsoid or irregular in shape, 31-445 µm long, 17-164 µm in diameter, with distinct branched pit canals, walls thick, lumens relatively small, cell walls 4-60 µm thick, pits distinct; bright reddish-brown or slightly polychromatic under the polarized microscope. Fibres singly scattered or in bundles, pale yellow or reddish-brown, 9-50 µm in diameter; yellowish-white under the polarized microscope. Vessels mainly scalariform. Lignified parenchymatous cells abundant, always full of starch granules, some filled with yellowish-brown substances; yellowish-white under the polarized microscope (Fig. 3).





Figure 2 Microscopic features of transverse section of Smilacis Chinae Rhizoma

- A. Sketch B. Section illustration
- C. Magnified image of hypodermis
- D. Magnified image of raphides of calcium oxalate and starch granules
- 1. Stone cells 2. Lignified parenchymatous cells 3. Hypodermis 4. Mucilage cell
- 5. Raphides of calcium oxalate 6. Collateral vascular bundle
- 7. Fibres 8. Phloem 9. Xylem 10. Starch granules

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壮荆葉

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Smilacis Chinae Rhizoma



Figure 3 Microscopic features of powder of Smilacis Chinae Rhizoma

- 1. Starch granules 2. Raphides of calcium oxalate 3. Stone cell
- 4. Fibre 5. Scalariform vessel 6. Lignified parenchymatous cell
- a. Features under the light microscope b. Features under the polarized microscope

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

Standard solutions

Chlorogenic acid standard solution

Weigh 1.0 mg of chlorogenic acid CRS (Fig. 4) and dissolve in 1 mL of methanol (70%). *Resveratrol standard solution*

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

Developing solvent system

Prepare a mixture of *n*-butyl acetate, methanol, formic acid and water (16:3:1:1, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about $2800 \times g$ for 10 min. Transfer the supernatant to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 10 mL of water. Transfer the solution to a 50-mL centrifuge tube. Add 10 mL of *n*-butanol. Centrifuge at about $2800 \times g$ for 10 min. Transfer the upper layer to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 4 mL of methanol (70%). Filter through a 0.45-µm nylon filter.

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 chlorogenic acid standard solution (3 µL), resveratrol standard solution (1 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 cm. After the development, remove the plate from the chamber, mark the solvent front 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).

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Figure 4 Chemical structures of (i) chlorogenic acid (ii) oxyresveratrol (iii) polydatin and (iv) resveratrol







1. Chlorogenic acid standard solution 2. Resveratrol standard solution

3. Test solution

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the $R_{\rm f}$ values, corresponding to those of chlorogenic acid and resveratrol (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solutions

Chlorogenic acid standard solution for fingerprinting, Std-FP (50 mg/L)
Weigh 0.5 mg of chlorogenic acid CRS and dissolve in 10 mL of methanol (70%).
Oxyresveratrol standard solution for fingerprinting, Std-FP (50 mg/L)
Weigh 0.5 mg of oxyresveratrol CRS (Fig. 4) and dissolve in 10 mL of methanol (70%).
Polydatin standard solution for fingerprinting, Std-FP (50 mg/L)
Weigh 0.5 mg of polydatin CRS (Fig. 4) and dissolve in 10 mL of methanol (70%).
Resveratrol standard solution for fingerprinting, Std-FP (50 mg/L)
Weigh 0.5 mg of polydatin CRS (Fig. 4) and dissolve in 10 mL of methanol (70%).
Resveratrol standard solution for fingerprinting, Std-FP (50 mg/L)
Weigh 0.5 mg of resveratrol CRS and dissolve in 10 mL of methanol (70%).

L) 豆根 aussureae Involucratae Herba 天山雪蓮 Polygoni Perfoliati Herba Polygoni Perfoliati Herba 王山雪蓮 Plumbaginis Zeylanicae Radix Di Lonicerae Flos Plumbaginis Zeylanicae Radix Di Lonicerae Flos Plumbaginis Zeylanicae Radix

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about $2800 \times g$ for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Make up to the mark with methanol (70%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (327 nm) and a column (4.6×250 mm) packed with ODS bonded silica gel (5 μ m particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Time (min)	0.4% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0-6	75	25	isocratic
6 - 30	$75 \rightarrow 65$	$25 \rightarrow 35$	linear gradient
30 - 45	$65 \rightarrow 60$	$35 \rightarrow 40$	linear gradient
45 - 60	$60 \rightarrow 40$	$40 \rightarrow 60$	linear gradient

 Table 1
 Chromatographic system conditions

System suitability requirements

Perform at least five replicate injections, each using 10 μ L of chlorogenic acid Std-FP, oxyresveratrol Std-FP, polydatin Std-FP and resveratrol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of chlorogenic acid, oxyresveratrol, polydatin and resveratrol should not be more than 5.0%; the RSD of the retention times of chlorogenic acid, oxyresveratrol, polydatin and resveratrol peaks should not be more than 2.0%; the column efficiencies determined from chlorogenic acid, oxyresveratrol, polydatin and resveratrol peaks should not be less than 10000, 10000, 10000 and 50000 theoretical plates respectively.

The *R* value between peak 2 and the closest peak; the *R* value between peak 3 and the closest peak; the *R* value between peak 4 and the closest peak; and the *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 6).

Procedure

Separately inject chlorogenic acid Std-FP, oxyresveratrol Std-FP, polydatin Std-FP, resveratrol Std-FP and the test solution (10 μ L each) into the HPLC system and record the chromatograms. Measure the retention times of chlorogenic acid, oxyresveratrol, polydatin and resveratrol peaks in the chromatograms of chlorogenic acid Std-FP, oxyresveratrol Std-FP, polydatin Std-FP, resveratrol Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of

the test solution. Identify chlorogenic acid, oxyresveratrol, polydatin and resveratrol peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of chlorogenic acid Std-FP, oxyresveratrol Std-FP, polydatin Std-FP and resveratrol Std-FP. The retention times of chlorogenic acid, oxyresveratrol, polydatin and resveratrol 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 five characteristic peaks of Smilacis Chinae Rhizoma extract are listed in Table 2.

 Table 2
 The RRTs and acceptable ranges of the five characteristic peaks of Smilacis Chinae Rhizoma extract

Peak No. RRT		Acceptable Range
1	0.82 (vs peak 2)	± 0.03
2 (marker 1, chlorogenic acid)	1.00	-
3 (polydatin)	2.19 (vs peak 2)	± 0.03
4 (oxyresveratrol)	2.67 (vs peak 2)	± 0.03
5 (marker 2, resveratrol)	1.00	-



Figure 6 A reference fingerprint chromatogram of Smilacis Chinae Rhizoma 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. 6).

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Smilacis Chinae Rhizoma

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

5.7 Water Content (Appendix X)

Oven dried method: not more than 12.0%.

6. EXTRACTIVES (Appendix XI)

Water-soluble extractives (cold extraction method): not less than 13.0%. Ethanol-soluble extractives (cold extraction method): not less than 11.0%.

7. ASSAY

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

Standard solution

Chlorogenic acid standard stock solution, Std-Stock (400 mg/L) Weigh accurately 4.0 mg of chlorogenic acid CRS and dissolve in 10 mL of methanol (70%). *Chlorogenic acid standard solution for assay, Std-AS* Measure accurately the volume of the chlorogenic acid Std-Stock, dilute with methanol (70%) to produce a series of solutions of 6, 12, 24, 50, 100 mg/L for chlorogenic acid.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about $2800 \times g$ for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the

residue with methanol (70%). Centrifuge at about $2800 \times g$ for 10 min. Combine the supernatants and make up to the mark with methanol (70%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (327 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 0.4% phosphoric acid and methanol (73:27, v/v). The elution time is about 25 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μ L of chlorogenic acid Std-AS (24 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of chlorogenic acid should not be more than 5.0%; the RSD of the retention time of chlorogenic acid peak should not be more than 2.0%; the column efficiency determined from chlorogenic acid peak should not be less than 3000 theoretical plates.

The R value between chlorogenic acid 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 chlorogenic acid Std-AS (10 μ L each) into the HPLC system and record the chromatograms. Plot the peak areas of chlorogenic acid against the corresponding concentrations of chlorogenic acid Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 10 μ L of the test solution into the HPLC system and record the chromatogram. Identify chlorogenic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of chlorogenic acid Std-AS. The retention times of chlorogenic acid 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 chlorogenic acid in the test solution, and calculate the percentage content of chlorogenic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.14% of chlorogenic acid ($C_{16}H_{18}O_9$), calculated with reference to the dried substance.

Smilacis Chinae Rhizoma (菝葜)



Figure 1 A reference assay chromatogram of Smilacis Chinae Rhizoma extract