# **Ardisiae Crenatae Radix**



A. Ardisiae Crenatae Radix

B. Magnified image of transverse section of root

望江南 陳皮 Mielicopes Ptelefioliae Caulis 二义古 Rhi anthemi Indici Flos 仟節参 Smilacis Chinae Rhizoma 豆蔻 野菊花 Panacis Japonici Rhizoma Lycoridis Radiatae Bulbus Tinosporae I 季陵菜 乙誌 洋合花 Daturae Flog 人 巴 L

## Ardisiae Crenatae Radix

金花 Daturae Flo

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## 1. NAMES

Official Name: Ardisiae Crenatae Radix

Chinese Name: 朱砂根

Chinese Phonetic Name: Zhushagen

# 2. SOURCE

Ardisiae Crenatae Radix is the dried root of *Ardisia crenata* Sims (Myrsinaceae). The root is collected in autumn and winter, washed clean, then dried under the sun to obtain Ardisiae Crenatae Radix.

# 3. **DESCRIPTION**

Cylindrical, slightly curved, 5-30 cm long, 2-10 mm in diameter, clustered on the slightly swollen rhizomes. Externally greyish-brown to brown, longitudinal wrinkles visible, with transverse or annular cracked traces, bark easily separated from wood. Texture hard and fragile, easily broken, fracture uneven, bark thick, whitish or pink, scattered with purplish-red dots on the outside, known as "cinnabar dots", wood yellowish-white. Odour slight; taste slightly bitter, irritating to tongue (Fig. 1).

# 4. **IDENTIFICATION**

## 4.1 Microscopic Identification (Appendix III)

## **Transverse section**

Cork consists of 3-10 layers of subsquare or subrectangular cells, neatly arranged, some of cork cell inner walls thickened, appearing as stone cells. Cortex consists of 10 to more than 20 layers of subrounded parenchymatous cells, some containing reddish-brown masses, sometimes scattered with stone cells. Endodermis distinct. Pericyclic stone cells arranged in an interrupted ring. Phloem narrow. Cambium sometimes visible. Xylem vessels radially arranged into single row, some containing reddish-brown to brown masses. Parenchymatous cells filled with starch granules (Fig. 2).

L 豆根 Saururi Herba 三白草 Saussureae Involucratae Herba 天山雪蓮 Polygoni Perfoliati Herba Hantaginis Herba Fulgani Perfoliati Herba 天山雪蓮 Plumbaginis Zeylanicae Radix Henispermi Rhizoma Plumbaginis Zeylanicae Radix

#### Powder

Colour pale brown. Simple starch granules abundant, subrounded, helmet-shaped, elliptic or irregular in shape, 4-36  $\mu$ m in diameter, with dotted, cleft-like or V-shaped hilum, striations indistinct; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-4 units. Vessels mostly bordered-pitted, 10-45  $\mu$ m in diameter. Xylem fibres slender, 10-15  $\mu$ m in diameter. Cork cells subsquare, subrectangular or subpolygonal, walls slightly thick. Stone cells subsquare, subrounded, subrectangular or irregular in shape, 40-120  $\mu$ m in diameter, pit canals distinct, some with striations, lumens relatively large; bright blue-white or polychromatic under the polarized microscope. Reddish-brown to brown masses visible (Fig. 3).





Figure 2 Microscopic features of transverse section of Ardisiae Crenatae Radix

A. Sketch B. Section illustration C. Cork cells (thickened inner walls → )
 D. Section magnified

Cork 2. Stone cell in cortex 3. Reddish-brown masses (cinnabar dots) 4. Cortex
 Endodermis 6. Pericyclic stone cells 7. Phloem 8. Cambium 9. Xylem



Figure 3 Microscopic features of powder of Ardisiae Crenatae Radix

- 1. Starch granules 2. Bordered-pitted vessels 3. Fibres 4. Cork cells
- 5. Stone cells 6. Brown masses
- a. Features under the light microscope b. Features under the polarized microscope

## Ardisiae Crenatae Radix

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

#### **Standard solution**

Bergenin standard solution

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

#### **Developing solvent system**

Prepare a mixture of ethyl acetate, ethanol (95%) and formic acid (8:0.6:0.4, v/v).

#### **Test solution**

Weigh 0.2 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Filter and transfer the filtrate to an evaporating dish. Evaporate the solvent to dryness on a water bath. Dissolve the residue in 1 mL of ethanol (70%).

#### 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 bergenin standard solution and the test solution (2 µL each) to the plate. Develop over a path of about 4 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). Calculate the  $R_f$  value by using the equation as indicated in Appendix IV (A).



Figure 4 Chemical structure of bergenin





- Figure 5 A reference HPTLC chromatogram of Ardisiae Crenatae Radix extract observed under UV light (254 nm)
- 1. Bergenin 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 bergenin (Fig. 5).

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

## **Standard solution**

*Bergenin standard solution for fingerprinting, Std-FP (200 mg/L)* Weigh 2.0 mg of bergenin CRS and dissolve in 10 mL of methanol.

#### **Test solution**

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about  $5000 \times g$  for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol (70%). Filter through a 0.45-µm RC filter.

#### Chromatographic system

The liquid chromatograph is equipped with a DAD (272 nm) and a column ( $4.6 \times 250$  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	Acetonitrile	Water	Elution
(min)	(%, v/v)	(%, v/v)	
0-35	$5 \rightarrow 40$	$95 \rightarrow 60$	linear gradient

#### System suitability requirements

Perform at least five replicate injections, each using 10  $\mu$ L of bergenin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of bergenin should not be more than 5.0%; the RSD of the retention time of bergenin peak should not be more than 2.0%; the column efficiency determined from bergenin peak should not be less than 25000 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. 6).

#### Procedure

Separately inject bergenin Std-FP and the test solution (10  $\mu$ L each) into the HPLC system and record the chromatograms. Measure the retention time of bergenin peak in the chromatogram of bergenin Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify bergenin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of bergenin Std-FP. The retention times of bergenin 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 Ardisiae Crenatae Radix extract are listed in Table 2.



Table 2	The RRTs and acceptable ranges of the four characteristic peaks of Ardisiae Crenatae Radix
	extract

Peak No.	RRT	Acceptable Range
1	0.69	$\pm 0.03$
2 (marker, bergenin)	1.00	-
3	1.63	± 0.03
4	1.94	± 0.05



Figure 6 A reference fingerprint chromatogram of Ardisiae Crenatae Radix 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. 6).

# 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 4.0%. Acid-insoluble ash: not more than 1.0%.

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 14.0%. Ethanol-soluble extractives (cold extraction method): not less than 16.0%.

# 7. ASSAY

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

#### **Standard solution**

Bergenin standard stock solution, Std-Stock (1000 mg/L)
Weigh accurately 5.0 mg of bergenin CRS and dissolve in 5 mL of methanol.
Bergenin standard solution for assay, Std-AS
Measure accurately the volume of the bergenin Std-Stock, dilute with methanol to produce a series of solutions of 5, 100, 200, 300, 400 mg/L for bergenin.

#### **Test solution**

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about  $5000 \times g$  for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol (70%). Filter through a 0.45-µm RC filter.

#### Chromatographic system

The liquid chromatograph is equipped with a DAD (272 nm) and a column ( $4.6 \times 250$  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 3) –

	Ardisiae	e Crenatae R	adix

Time	Acetonitrile	Water	Elution	
(min)	(%, v/v)	(%, v/v)		
0-35	$5 \rightarrow 40$	$95 \rightarrow 60$	linear gradient	

#### Table 3 Chromatographic system conditions

#### System suitability requirements

Perform at least five replicate injections, each using 10  $\mu$ L of bergenin Std-AS (200 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of bergenin should not be more than 5.0%; the RSD of the retention time of bergenin peak should not be more than 2.0%; the column efficiency determined from bergenin peak should not be less than 25000 theoretical plates.

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

## Procedure

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

#### Limits

The sample contains not less than 2.0% of bergenin ( $C_{14}H_{16}O_9$ ), calculated with reference to the dried substance.





Figure 1 A reference assay chromatogram of Ardisiae Crenatae Radix extract