Arctii Fructus

Figure 1 A photograph of Arctii Fructus

1. NAMES

Official Name: Arctii Fructus

Chinese Name: 牛蒡子

Chinese Phonetic Name: Niubangzi

2. SOURCE

Arctii Fructus is the dried ripe fruit of *Arctium lappa* L. (Asteraceae). The infructescence is collected in autumn when fruit is ripe, and dried under the sun; the fruit is tapped out, foreign matter removed, then dried under the sun again to obtain Arctii Fructus.

3. DESCRIPTION

Long-obovoid, slightly flattened, somewhat curved, 5-7 mm long, 2-3 mm wide. Externally greyish-brown, purplish-black mottled, with several longitudinal ribs, usually the 1-2 middle ribs relatively distinct. Summit obtuse-rounded, slightly broad, with a circular ring at the top, and a pointed remain of style in the centre; base slightly narrowed, bearing surface pale in colour. Pericarp relatively hard, cotyledons 2, yellowish-white, oily. Odourless; taste bitter, followed by slight pungency and numbness (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Exocarp consists of 1 layer of cells, wall crooked, most of wall cracked. Mesocarp varies in number of layers, up to more than 10 layers, walls slightly thickened and lignified, with small collateral vascular bundles. Prism of calcium oxalate 3-9 μm in diameter, occurring abundantly in mesocarp near the boundary with the endocarp. Endocarp consists of 1 layer of palisade-aligned stone cells. Testa consists of several rows of decadent cells, the borders of cell indistinct. Endosperm consists of 1-2 layers of cells. Cotyledon cells filled with aleurone grains, oil droplets, some containing small crystals (Fig. 2).

Powder

Colour greyish-brown. Stone cells of endocarp slightly flattened, tapering-fusiform, long-elliptic to tapering-ovate, closely mosaic in surface view, 70-224 µm long, 13-70 µm wide, the walls up to 20 µm thick; polychromatic under the polarized microscope. Prisms of calcium oxalate 3-9 µm in diameter, occurring abundantly in parenchymatous cells of mesocarp; polychromatic under the polarized microscope. Reticulated cells of mesocarp elongated in longitudinal sectional view, their walls have fine and dense crisscross striations. Cells of cotyledon filled with aleurone grains, some containing small crystals and oil droplets. Exocarp consists of 1 layer of cells, wall crooked, most of wall cracked (Fig. 3).

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

Standard solution

Arctiin standard solution

Weigh 5.0 mg of arctiin CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of dichloromethane, methanol and water (40:8:1, v/v).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol. Sonicate (560 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL round-bottomed flask and evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of ethanol.

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 arctiin standard solution (5 µL) and the test solution (3 µL) 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).

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 arctiin.

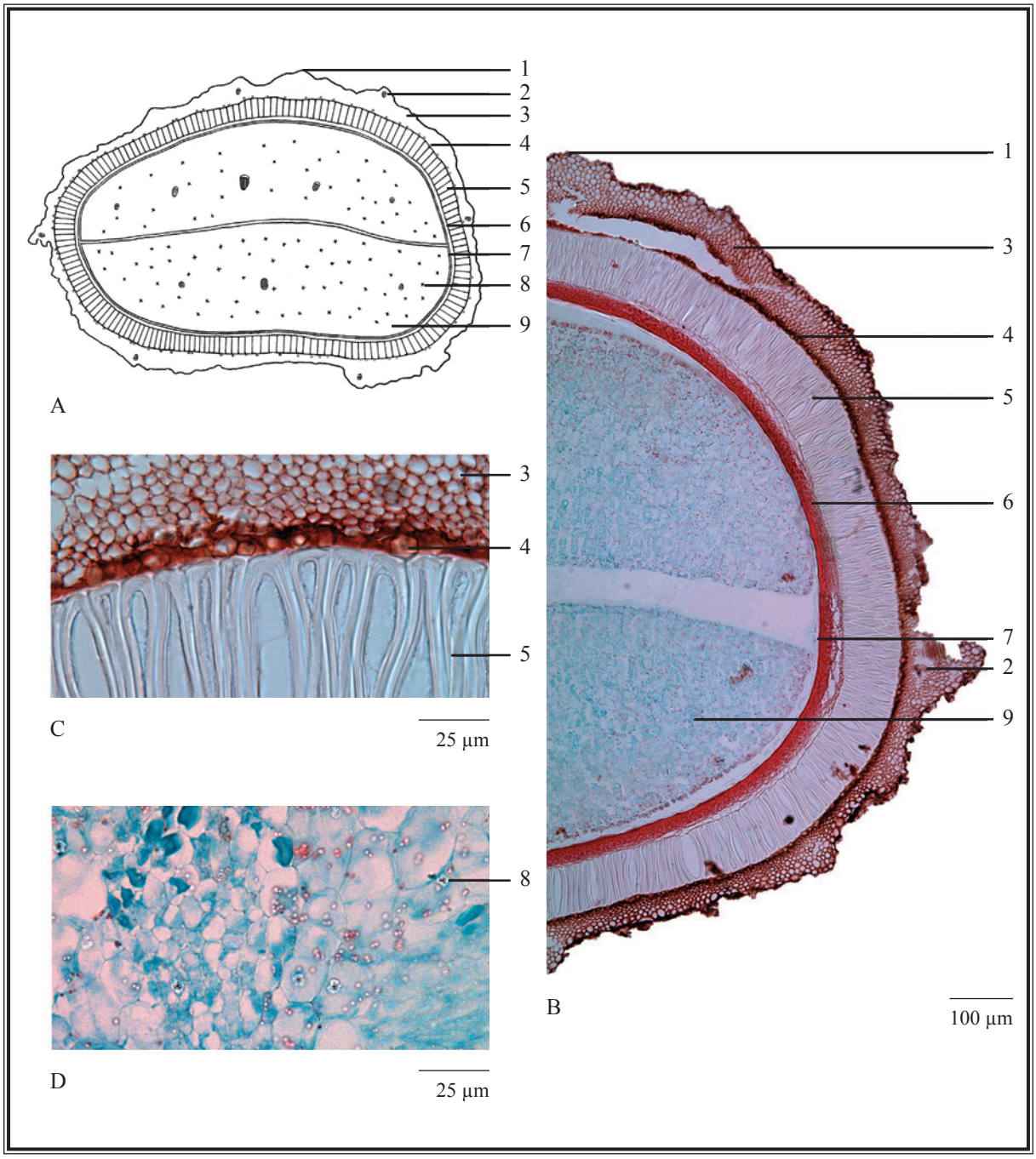


Figure 2 Microscopic features of transverse section of Arctii Fructus

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

1. Exocarp 2. Vascular bundle 3. Mesocarp 4. Prism of calcium oxalate
5. Endocarp 6. Testa 7. Endosperm 8. Crystal 9. Cotyledon

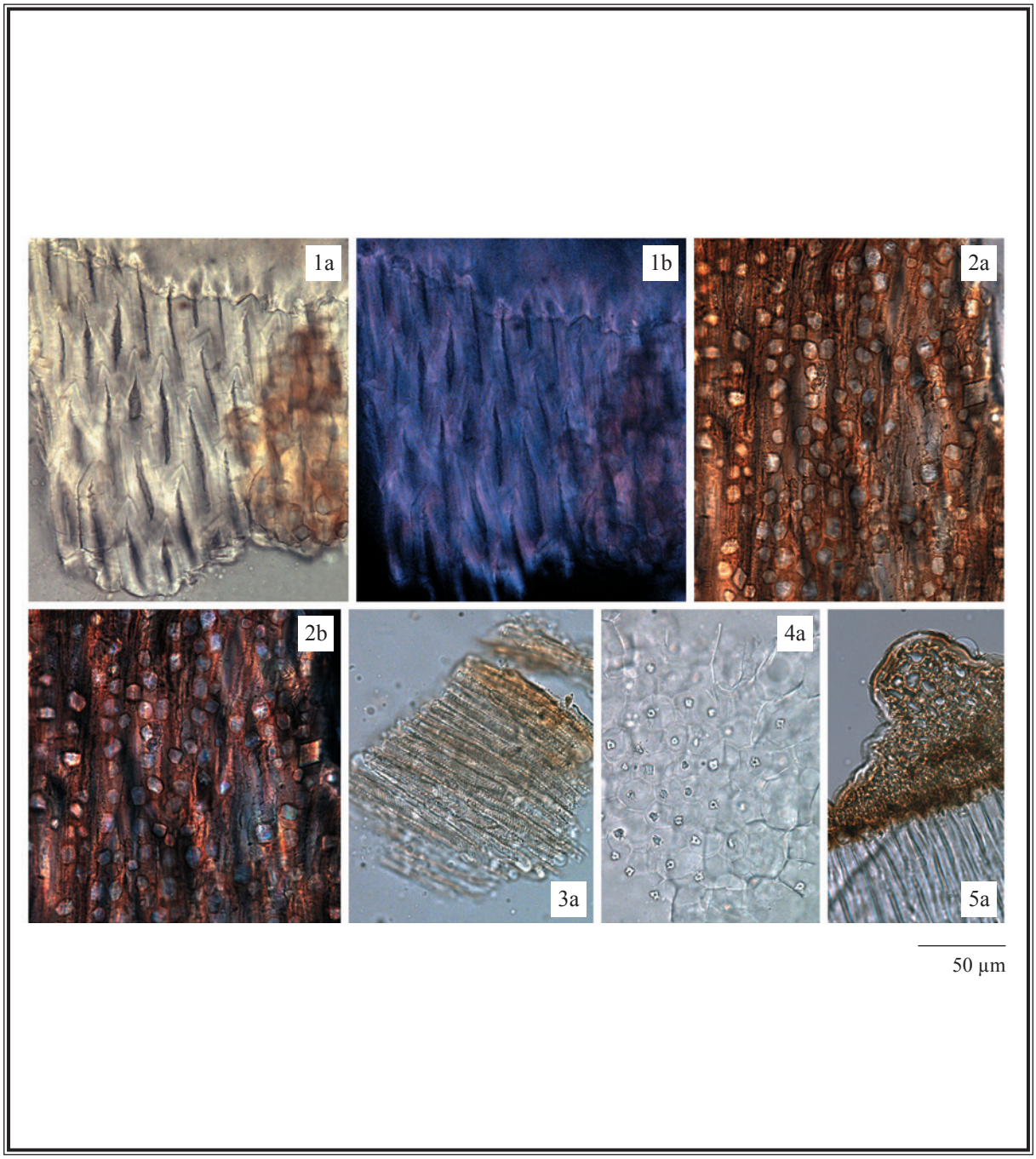
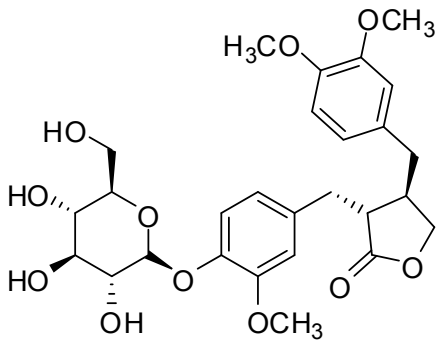


Figure 3 Microscopic features of powder of Arctii Fructus

1. Endocarp stone cells 2. Prisms of calcium oxalate 3. Reticulated cells of mesocarp
4. Cotyledon cells 5. Exocarp cells

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

(i)



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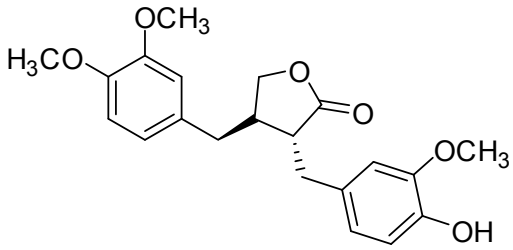


Figure 4 Chemical structures of (i) arctiin and (ii) arctigenin

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

Standard solution

Arctiin standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 2.0 mg of arctiin CRS and dissolve in 20 mL of methanol.

Test solution

Weigh 0.4 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (560 W) the mixture for 30 min. Filter through a 0.45-µm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 45	75 → 48	25 → 52	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of arctiin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of arctiin should not be more than 5.0%; the RSD of the retention time of arctiin peak should not be more than 2.0%; the column efficiency determined from arctiin peak should not be less than 20000 theoretical plates.

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

Procedure

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

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Arctii Fructus extract

Peak No.	RRT	Acceptable Range
1	0.81	± 0.03
2 (marker, arctiin)	1.00	-
3	1.11	± 0.03
4	1.17	± 0.03
5 (arctigenin)	2.19	± 0.06

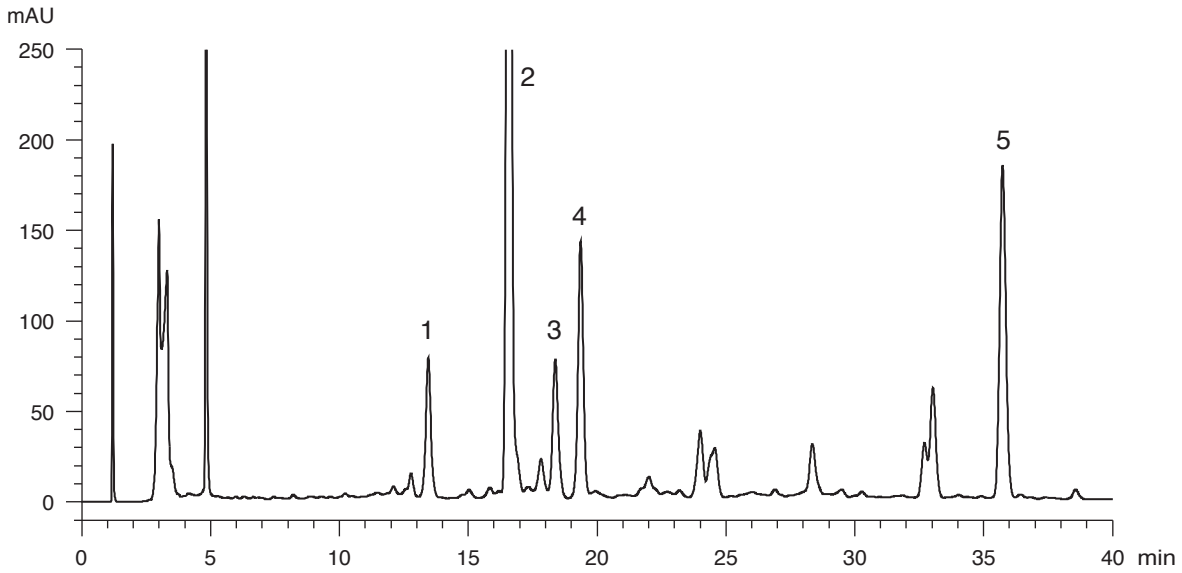


Figure 5 A reference fingerprint chromatogram of Arctii Fructus extract

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. 5).

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 XVIII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII):* not more than 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.5%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 9.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 11.0%.
Ethanol-soluble extractives (hot extraction method): not less than 18.0%.

7. ASSAY

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

Standard solution

Mixed arctiin and arctigenin standard stock solution, Std-Stock (500 mg/L for arctiin and 50 mg/L for arctigenin)

Weigh accurately 5.0 mg of arctiin CRS and 0.5 mg of arctigenin CRS (Fig. 4), and dissolve in 10 mL of ethanol.

Mixed arctiin and arctigenin standard solution for assay,Std-AS

Measure accurately the volume of the mixed arctiin and arctigenin Std-Stock, dilute with ethanol to produce a series of solutions of 50, 100, 150, 200, 400 mg/L for arctiin and 5, 10, 15, 20, 40 mg/L for arctigenin.

Test solution

Weigh accurately 0.4 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol. Sonicate (560 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	75 → 30	25 → 70	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed arctiin and arctigenin Std-AS (150 mg/L for arctiin and 15 mg/L for arctigenin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of arctiin and arctigenin should not be more than 5.0%; the RSD of the retention times of arctiin and arctigenin peaks should not be more than 2.0%; the column efficiencies determined from arctiin and arctigenin peaks should not be less than 10000 theoretical plates.

The *R* value between arctiin peak and the closest peak; and the *R* value between arctigenin 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 arctiin and arctigenin Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of arctiin and arctigenin against the corresponding concentrations of the mixed arctiin and arctigenin Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

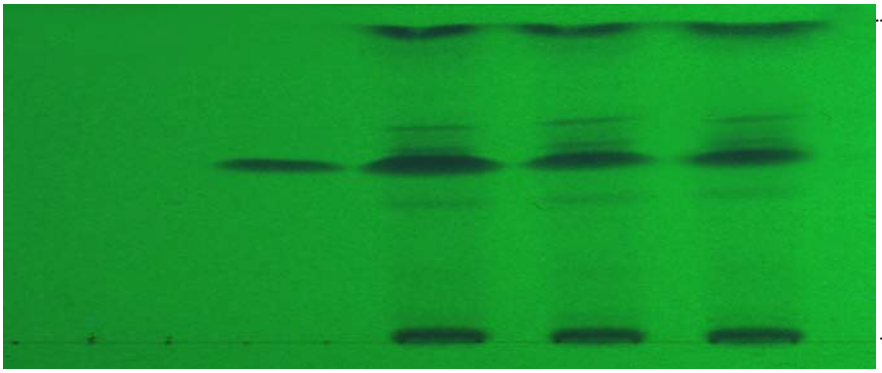
Procedure

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify arctiin and arctigenin peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed arctiin and arctigenin Std-AS. The retention times of arctiin and arctigenin 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 arctiin and arctigenin in the test solution, and calculate the percentage contents of arctiin and arctigenin in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 5.0% of the total content of arctiin ($C_{27}H_{34}O_{11}$) and arctigenin ($C_{21}H_{24}O_6$), calculated with reference to the dried substance.

Arctii Fructus　(牛蒡子)



Lane	Sample	Results
1	Blank (Ethanol)	Negative
2	Standard (Arctiin)	Arctiin positive
3	Spiked sample (Sample plus arctiin)	Arctiin positive
4	Sample (Arctii Fructus)	Arctiin positive
5	Sample duplicate (Arctii Fructus)	Arctiin positive

Figure 1　TLC results of Arctii Fructus extract observed under UV light (254 nm)