# Radix Aucklandiae


Figure 1 A photograph of Radix Aucklandiae

## 1. NAMES

Official Name: Radix Aucklandiae

Chinese Name: 木香

Chinese Phonetic Name: Muxiang

## 2. SOURCE

Radix Aucklandiae is the dried root of *Aucklandia lappa* Decne. (Asteraceae/Compositae). The root is collected in autumn and winter, after removal of rootlets and soil, it is cut into sections. The larger section is further cut into pieces longitudinally, dried, then the rough outer bark removed by dashing to obtain Radix Aucklandiae.

## 3. DESCRIPTION

Cylindrical or semicylindrical, 5-20 cm long, 5-50 mm in diameter. Externally yellowish-brown to greyish-brown, showing distinct wrinkles, longitudinal furrows, and lateral root scars. Texture hard, not easy to break; fracture greyish-brown to dark brown; the outer layer greyish-yellow or pale brownish-yellow; cambium ring brown, having radial lines and scattered brown dotted oil cavities. Odour aromatic and characteristic; taste slightly bitter (Fig. 1).

## 4. IDENTIFICATION

### 4.1 Microscopic Identification *(Appendix III)*

**Transverse section**

The cork consisting of several layers of cork cells. Phloem broad; phloem fibres in bundles, sparsely distributed or arranged in 1-3 interrupted whorls. Cambium in a ring. Xylem fibres mostly distributed near the cambium and the root center. Oil secretory cavities round or elliptic in shape, varying in size, up to 400 μm in diameter (Fig. 2).

**Powder**

Colour yellowish-brown to brown. Inulin fairly abundant, with radial striations. Reticulate vessels

very frequent, bordered-pitted vessels also present, 11-110 μm in diameter. Xylem fibres mostly in bundles, long-fusiform, 11-40 μm in diameter, pit apertures horizontal-porous, crisscross or V-shaped. Some parenchyma cells with very fine oblique crisscross striatons. Cork cells subsquare, subrectangular or polygonal in shape. Fragments of oil secretory cavity sometimes visible, containing yellow or brown secretion (Fig. 3).

## 4.2 Physicochemical Identification

**Procedure**

Weigh 0.2 g of the powdered sample and put into a 15-mL centrifugal tube, then add 10 mL of ethanol. Sonicate (560 W) the mixture for 5 min. Centrifuge at about 3000 × *g* for 5 min. Transfer 1 mL of the supernatant to a test tube, add 4 mL of ethanol and mix. Transfer 1 mL of the solution to another test tube. Cautiously add about 0.5 mL of sulphuric acid along the inner wall of the test tube. Allow to stand for 10 min. A reddish-purple to purple ring is observed at the interface of the two solvent layers.

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

**Standard solutions**

*Costunolide standard solution*

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

*Dehydrocostus lactone standard solution*

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

**Developing solvent system**

Prepare a mixture of dichloromethane and cyclohexane (5:1, v/v).

**Spray reagent**

Mix 1 mL of dilute sulphuric acid (50%, v/v) and 10 mL of *p*-hydroxybenzaldehyde in methanol (2%, w/v). Freshly prepare the reagent.

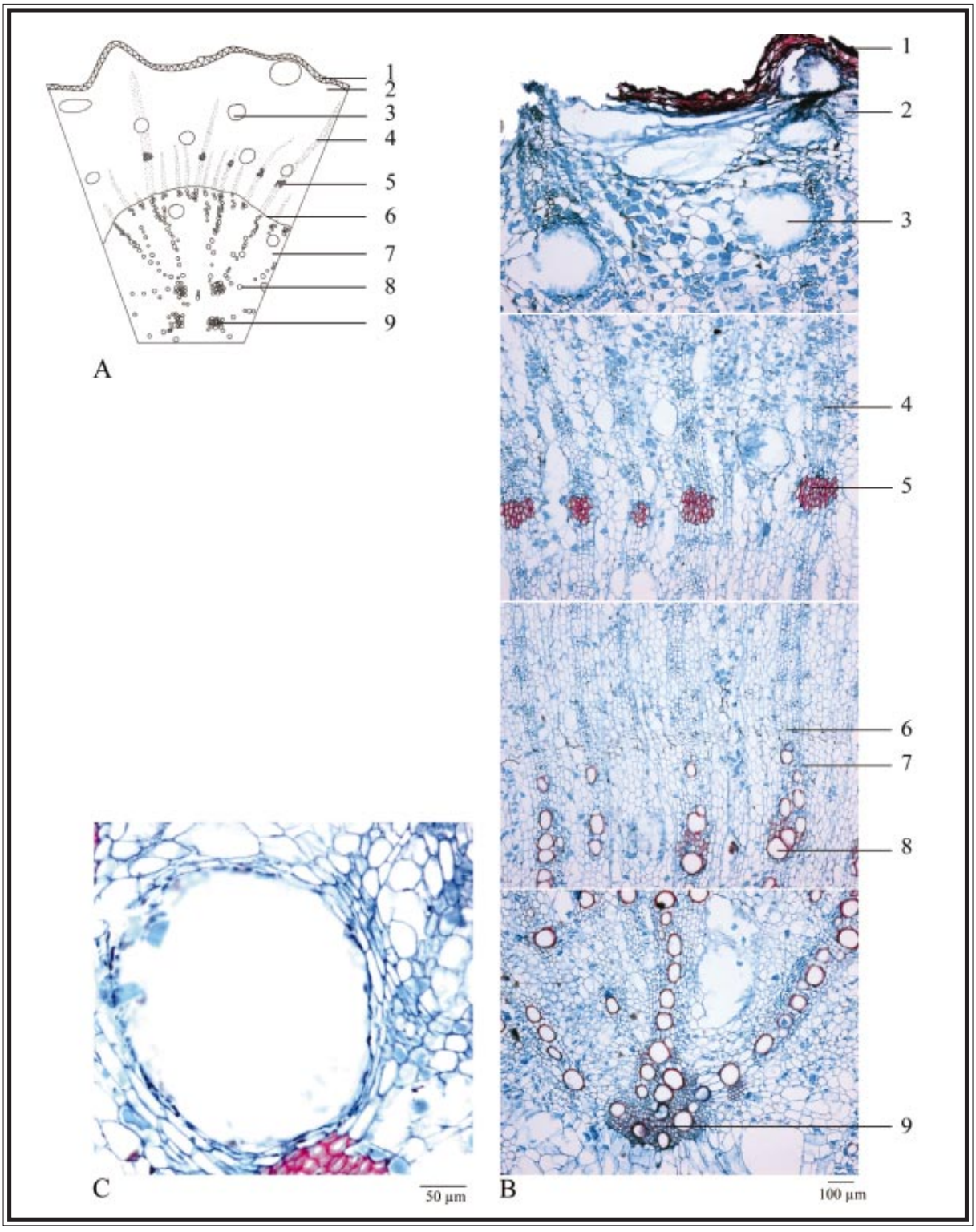



Figure 2 Microscopic features of transverse section of Radix Aucklandiae

A. Sketch B. Section illustration C. Oil secretory cavity

1. Cork 2.Cortex 3. Oil secretory cavity 4. Phloem 5. Phloem fibres 6.Cambium 7. Xylem 8. Vessels 9. Xylem fibres

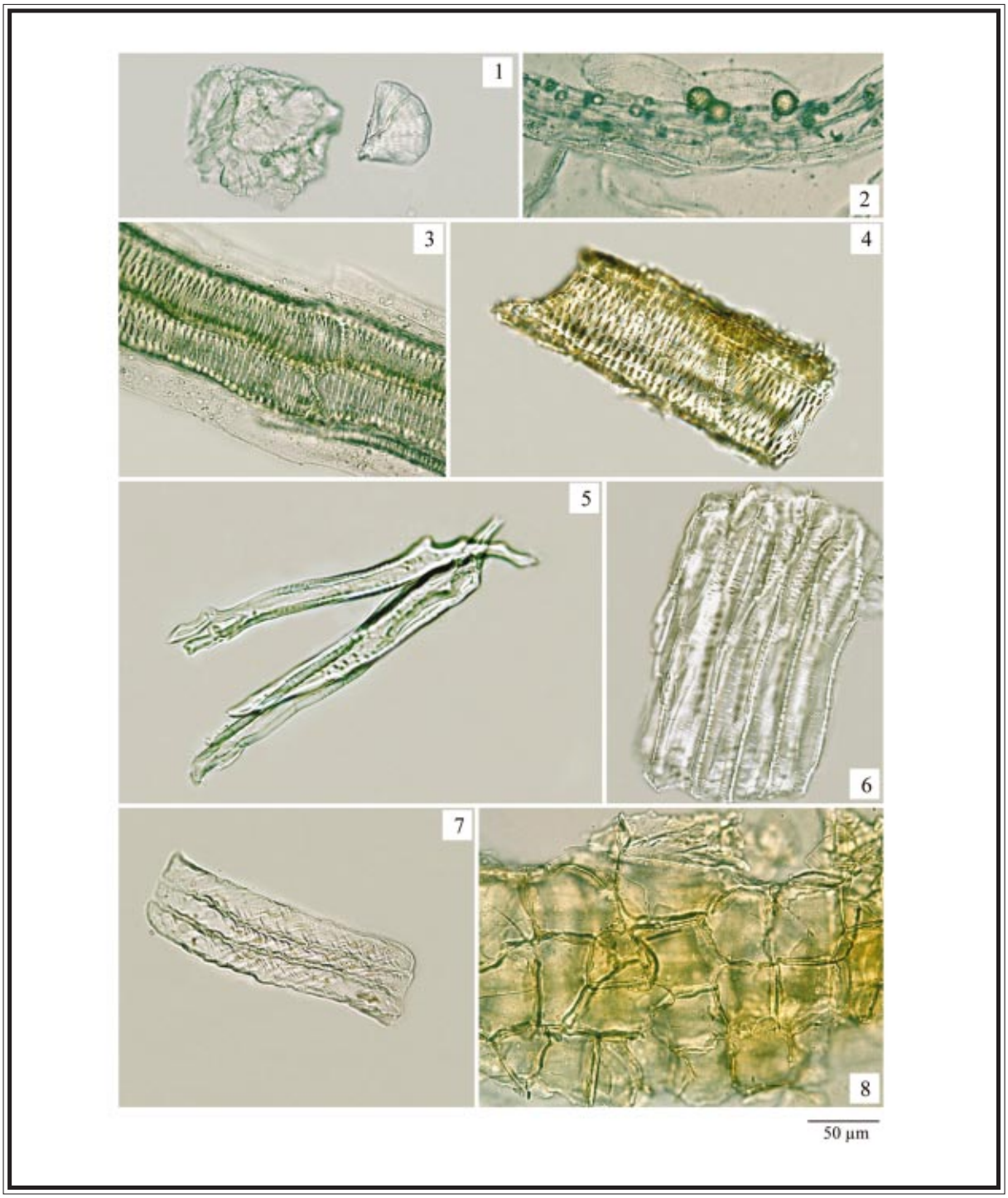



Figure 3 Microscopic features of powder of Radix Aucklandiae (under the light microscope)

1. Inulin 2. Fragment of oil secretory cavity 3. Reticulate vessels 4. Bordered-pitted vessel 5. Xylem fibres 6. Xylem fibres in a bundle 7. Parenchyma cells 8. Cork cells

**Test solution**

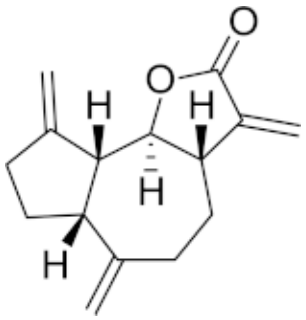
Weigh 1.0 g of the powdered sample and put into a 100-mL conical flask, then add 20 mL of dichloromethane. Sonicate (560 W) the mixture for 30 min. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethyl acetate.

**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 costunolide standard solution (2 µL), dehydrocostus lactone standard solution (2 µL) and the test solution (0.5 µL) to the plate. 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 and heat at about 100°C until the spots or bands become visible (about 10 min). Examine the plate under visible light. Calculate the $R_f$ values by using the equation as indicated in Appendix IV(A).

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the $R_f$ values, corresponding to those of costunolide and dehydrocostus lactone.

(i)




(ii)

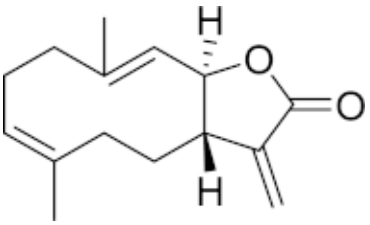



Figure 4 Chemical structures of (i) dehydrocostus lactone and (ii) costunolide

### 4.4 High-Performance Liquid Chromatographic Fingerprinting *(Appendix XII)*

**Standard solution**

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

Weigh 1.0 mg of costunolide CRS and dissolve in 10 mL of methanol.

**Test solution**

Weigh 0.2 g of the powdered sample and put into a 50-mL test tube, 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 detector (254 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 –

| Time (min) | 0.1% Trifluoroacetic acid (%, v/v) | Acetonitrile (%, v/v) | Elution |
|---|---|---|---|
| 0 – 20 | 100 → 70 | 0 → 30 | linear gradient |
| 20 – 28 | 70 | 30 | isocratic |
| 28 – 45 | 70 → 0 | 30 → 100 | linear gradient |
| 45 – 60 | 0 | 100 | isocratic |

**System suitability requirements**

Perform at least five replicate injections each with 10 μL of costunolide Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of costunolide should not be more than 5.0%; the RSD of the retention time of costunolide peak should not be more than 2.0%; the column efficiency determined from costunolide peak should not be less than 600000 theoretical plates.

The *R* value between peak 4 and peak 5 in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

**Procedure**

Separately inject costunolide Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of costunolide peak in the chromatogram of costunolide Std-FP and the retention times of the seven characteristic peaks (Fig. 5) in the chromatogram of the test solution. Under the same HPLC conditions, identify costunolide peak in

the chromatogram of the test solution by comparing its retention time with that in the chromatogram of costunolide Std-FP. The retention times of costunolide 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 seven characteristic peaks of Radix Aucklandiae extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the seven characteristic peaks of Radix Aucklandiae extract

| Peak No. | RRT | Acceptable Range |
|---|---|---|
| 1 | 0.37 | ±0.03 |
| 2 | 0.56 | ±0.03 |
| 3 | 0.58 | ±0.03 |
| 4 (marker, costunolide) | 1.00 | - |
| 5 (dehydrocostus lactone) | 1.01 | ±0.03 |
| 6 | 1.14 | ±0.03 |
| 7 | 1.15 | ±0.03 |

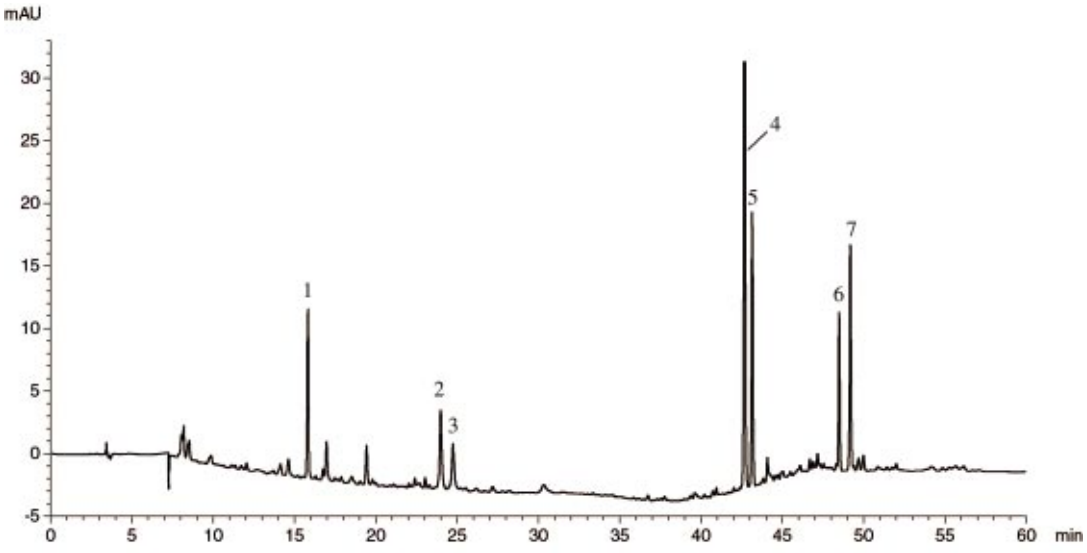



Figure 5 A reference fingerprint chromatogram of Radix Aucklandiae extract

For positive identification, the sample must give the above seven 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 XVII)*: meet the requirements.

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

**5.6** **Ash** *(Appendix IX)*

Total ash: not more than 4.5%.
Acid-insoluble ash: not more than 1.0%.

**5.7** **Water Content** *(Appendix X)*: not more than 14.0%.

## 6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 65.0%.
Ethanol-soluble extractives (cold extraction method): not less than 15.0%.

## 7. ASSAY

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

**Standard solution**

*Mixed costunolide and dehydrocostus lactone standard stock solution, Std-Stock (1000 mg/L each)*
Weigh accurately 10.0 mg of costunolide CRS and 10.0 mg of dehydrocostus lactone CRS and dissolve in 10 mL of methanol.
*Mixed costunolide and dehydrocostus lactone standard solution for assay, Std-AS*
Measure accurately the volume of the mixed costunolide and dehydrocostus lactone Std-Stock, dilute with methanol to produce a series of solutions of 2.5, 25, 100, 250, 500 mg/L for both costunolide and dehydrocostus lactone.

**Test solution**

Weigh accurately 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate (560 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Filter the supernatant through a 0.45-μm RC filter. Repeat the extraction twice each with 5 mL of methanol. Combine the filtrate. Transfer the solution to a 25-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 detector (225 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 –

| Time (min) | Water (%, v/v) | Acetonitrile (%, v/v) | Elution |
|---|---|---|---|
| 0 – 10 | 40 → 20 | 60 → 80 | linear gradient |
| 10 – 20 | 20 | 80 | isocratic |

**System suitability requirements**

Perform at least five replicate injections each with 10 μL of the mixed costunolide and dehydrocostus lactone Std-AS (250 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of costunolide and dehydrocostus lactone should not be more than 5.0%; the RSD of the retention times of costunolide peak and dehydrocostus lactone peak should not be more than 2.0%; the column efficiencies determined from costunolide peak and dehydrocostus lactone peak should not be less than 20000 theoretical plates.

The *R* value between costunolide peak and dehydrocostus lactone peak in the chromatogram of the test solution should not be less than 1.5.

**Calibration curves**

Inject a series of the mixed costunolide and dehydrocostus lactone Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of costunolide and dehydrocostus lactone against the corresponding concentrations of the mixed costunolide and dehydrocostus lactone Std-AS. Obtain the slopes, y-intercepts and the $r^2$ values from the corresponding 5-point calibration curves.

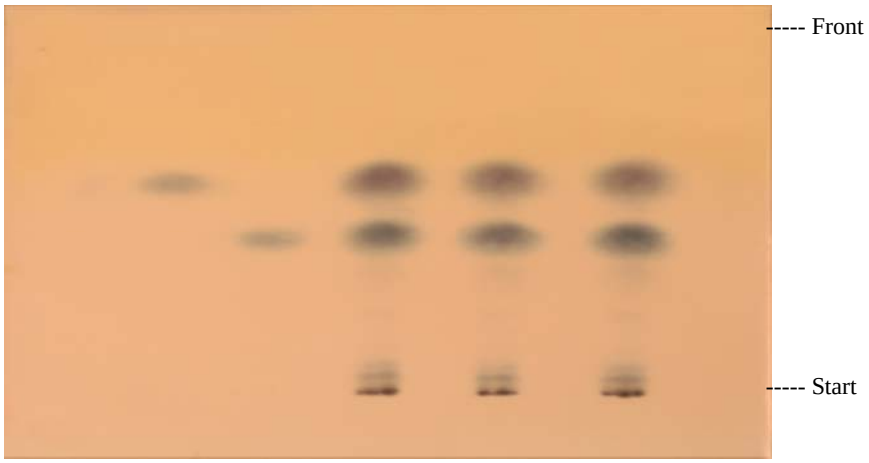
**Procedure**

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify costunolide peak and dehydrocostus lactone peak in the chromatogram of the test solution by comparing their retention

times with those in the chromatogram of the mixed costunolide and dehydrocostus lactone Std-AS. The retention times of costunolide peaks and dehydrocostus lactone peaks from the two chromatograms should not differ from their counterparts by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of costunolide and dehydrocostus lactone in the test solution, and calculate the percentage contents of costunolide and dehydrocostus lactone in the sample by using the equations indicated in Appendix IV(B).

**Limits**

The sample contains not less than 2.2% of the total content of costunolide ($C_{15}H_{20}O_2$) and dehydrocostus lactone ($C_{15}H_{18}O_2$), calculated with reference to the dried substance.




| Lane | Sample | Results |
|---|---|---|
| 1 | Blank (Ethyl acetate) | Negative |
| 2 | Standard (Dehydrocostus lactone) | Dehydrocostus lactone positive |
| 3 | Standard (Costunolide) | Costunolide positive |
| 4 | Spiked sample (Sample plus dehydrocostus lactone and costunolide) | Dehydrocostus lactone and costunolide positive |
| 5 | Sample (Radix Aucklandiae) | Dehydrocostus lactone and costunolide positive |
| 6 | Sample duplicate (Radix Aucklandiae) | Dehydrocostus lactone and costunolide positive |

Figure 1 TLC results of Radix Aucklandiae extract observed under visible light after staining