Medulla Junci

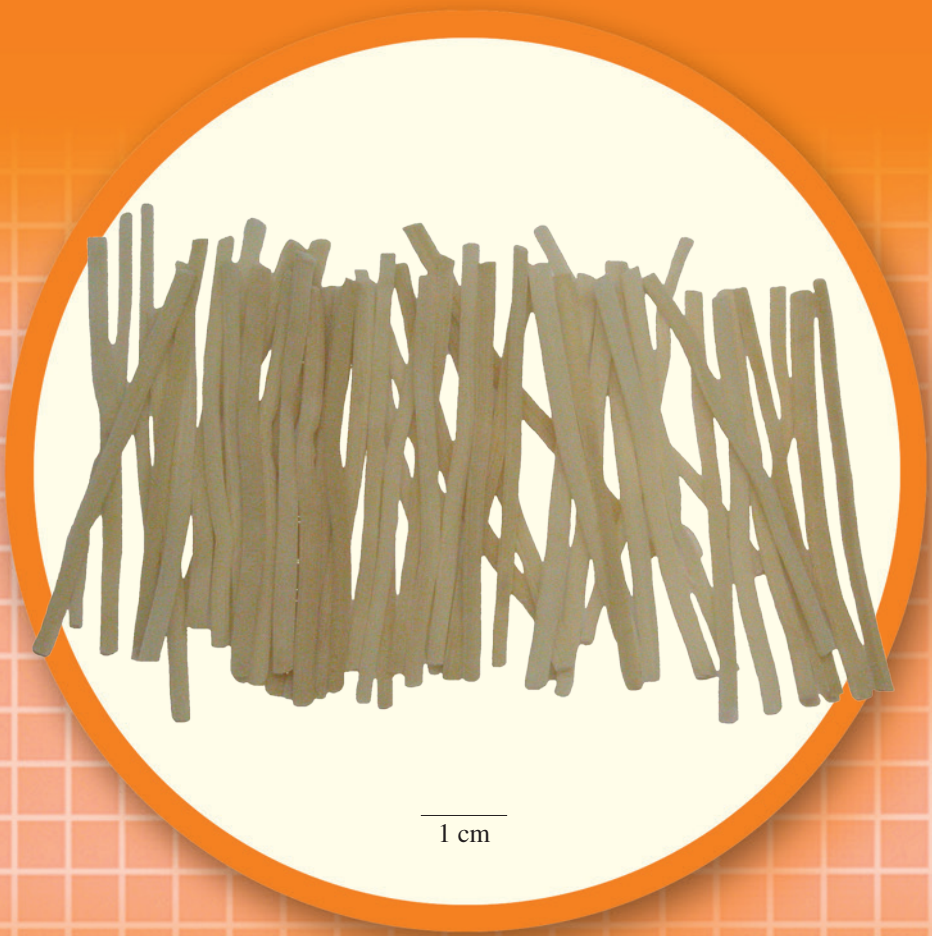


Figure 1 A photograph of Medulla Junci

1. NAMES

Official Name: Medulla Junci

Chinese Name: 燈心草

Chinese Phonetic Name: Dengxincao

2. SOURCE

Medulla Junci is the dried stem pith of *Juncus effusus* L.(Juncaceae). The stem is collected from late summer to autumn, dried under the sun, the outer part removed, the pith so obtained is then straightened out or ligated into a small bundle to obtain Medulla Junci.

3. DESCRIPTION

Long-slender and cylindrical, up to 90 cm long, 1-3 mm in diameter. Externally white or pale yellowish-white, with fine longitudinal striations. Texture light, soft, slightly elastic, when broken, the fracture is white. Odour slight; tasteless (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Medulla Junci is composed of ventilate parenchyma tissue. The parenchyma cells stellate in shape, branches 4-8, 5-51 μm long, 5-12 μm in diameter, each cell branch is connected with branches of other stellate cells to form air cavities, mostly in triangular shape, occasionally quadrilateral (Fig. 2).

Powder

Colour pale yellow. The parenchyma cells stellate in shape, branches 4-8, 5-51 μm long, 5-12 μm wide, connected with branches of other stellate cells to form air cavities mostly in triangular shape; fine pits occasionally observed, intercellular layer thin, sometimes beaded (Fig. 3).

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

Standard solutions

Dehydroeffusol standard solution

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

Effusol standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, cyclohexane and glacial acetic acid (30:70:0.1, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 250-mL conical flask, then add 100 mL of methanol. Sonicate (220 W) the mixture for 1 h. Filter and evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol. Filter through a 0.45-μm PTFE filter.

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 dehydroeffusol standard solution, effusol standard solution and the test solution (6 μL each) to the plate. Develop over a path of about 15 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 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 dehydroeffusol and effusol.

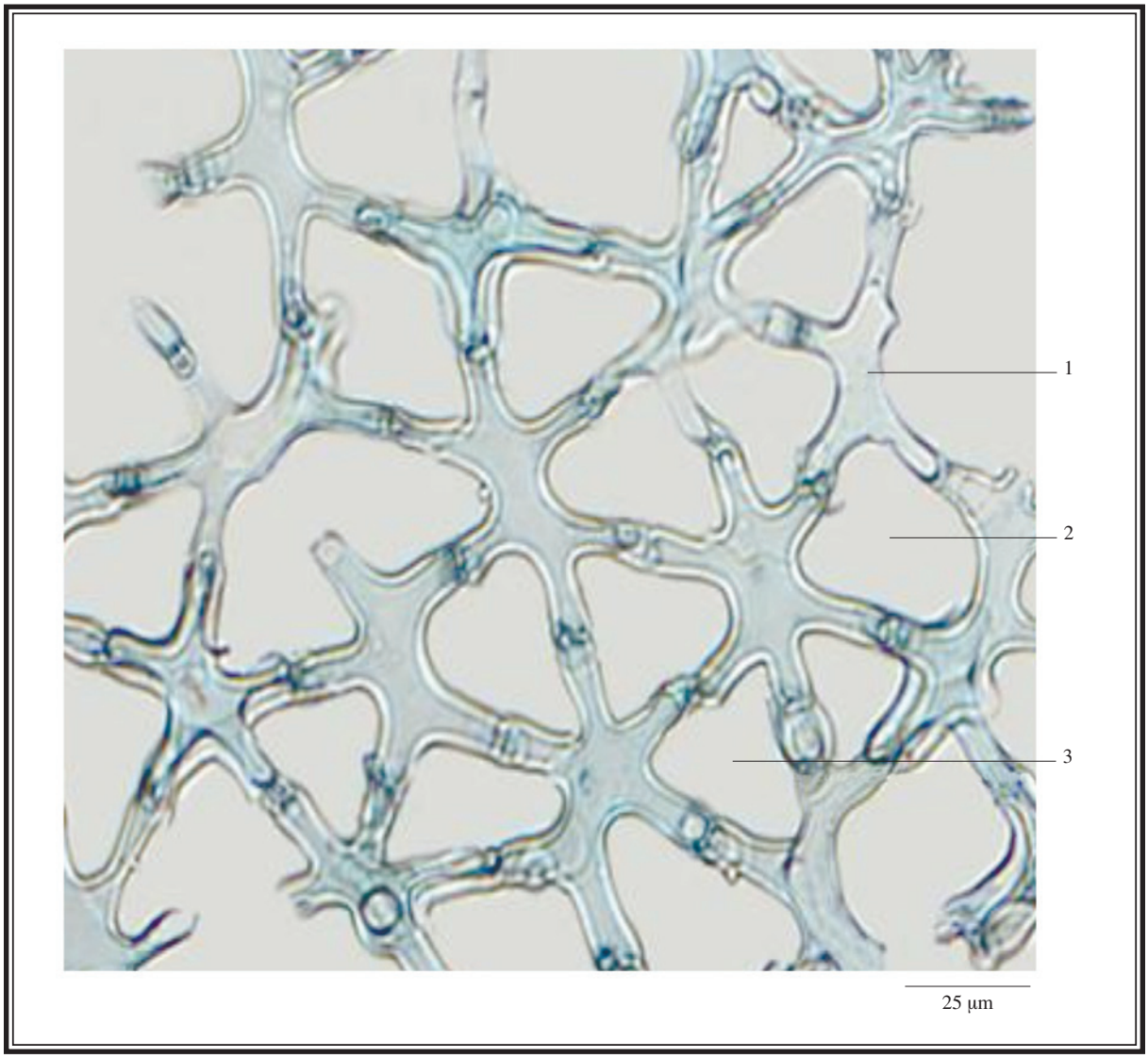


Figure 2 Microscopic features of transverse section of Medulla Junci

1. Stellate parenchyma cell 2. Quadrilateral cavity 3. Triangular cavity

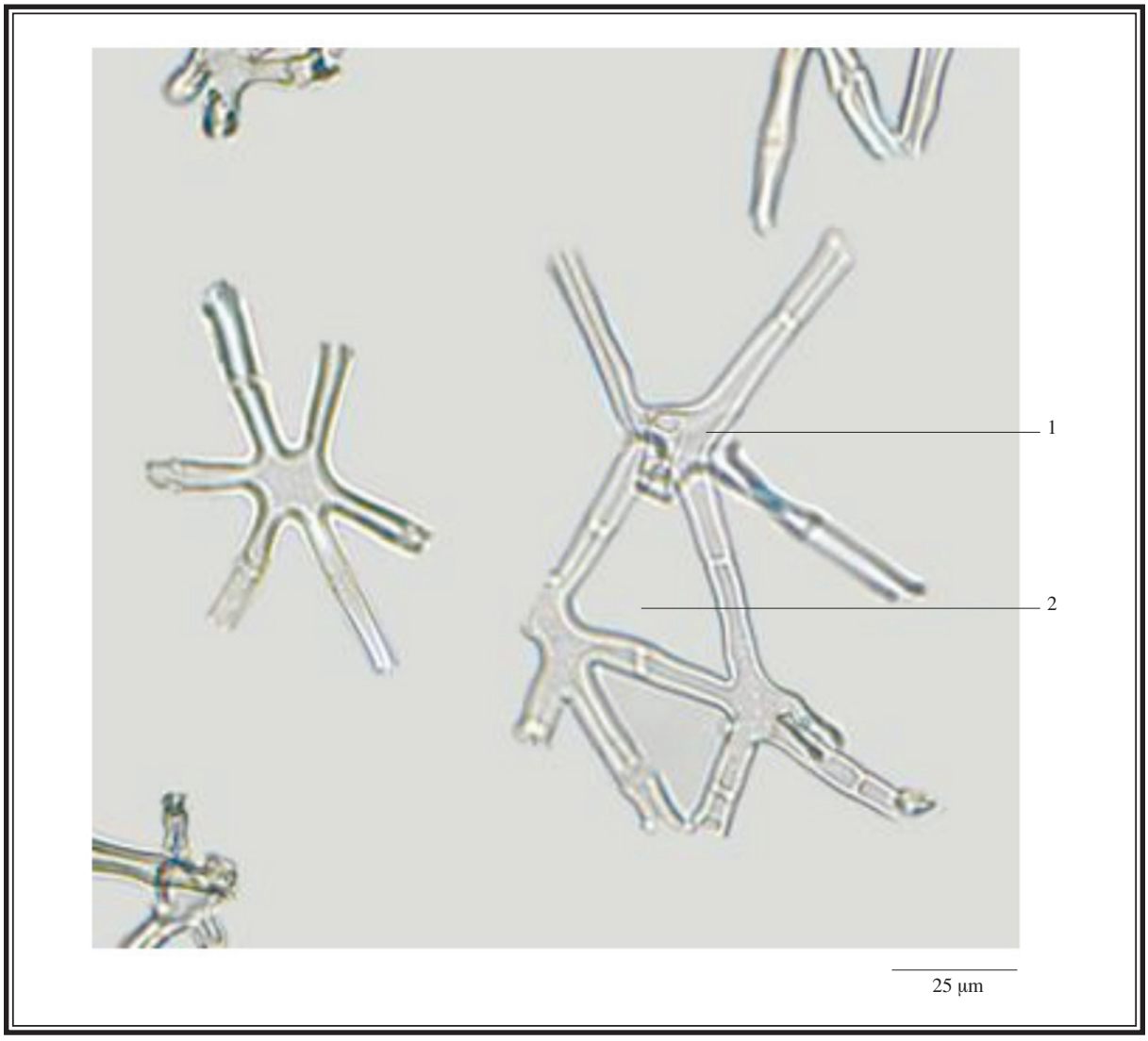
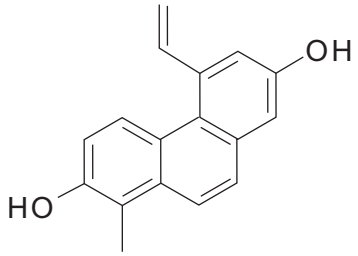


Figure 3 Microscopic features of powder of Medulla Junci (under the light microscope)

1. Stellate parenchyma cell 2. Triangular cavity

(i)



(ii)

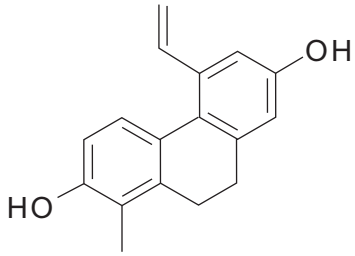


Figure 4 Chemical structures of (i) dehydroeffusol and (ii) effusol

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

Standard solution

Dehydroeffusol standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 1.0 mg of dehydroeffusol CRS and dissolve in 100 mL of methanol.

Test solution

Weigh 0.3 g of the powdered sample and place it in a 100-mL conical flask, then add 100 mL of methanol. Sonicate (220 W) the mixture for 30 min. Filter and evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-mL volumetric flask and make up to mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (282 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)	Water (%, v/v)	Methanol (%, v/v)	Elution
0–30	85→46	15→54	linear gradient
30–60	46	54	isocratic

System suitability requirements

Perform at least five replicate injections, each using 20 μL of dehydroeffusol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of dehydroeffusol should not be more than 5.0%; the RSD of the retention time of dehydroeffusol peak should not be more than 2.0%; the column efficiency determined from dehydroeffusol peak should not be less than 40000 theoretical plates.

The *R* value between peak 3 and peak 4 in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject dehydroeffusol Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of dehydroeffusol peak in the chromatogram of dehydroeffusol Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify dehydroeffusol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of dehydroeffusol Std-FP. The retention times of dehydroeffusol 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 Medulla Junci extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Medulla Junci extract

Peak No.	RRT	Acceptable Range
1	0.41	±0.03
2	0.71	±0.03
3 (effusol)	0.97	±0.03
4 (marker, dehydroeffusol)	1.00	-
5 (juncusol)	1.12	±0.04

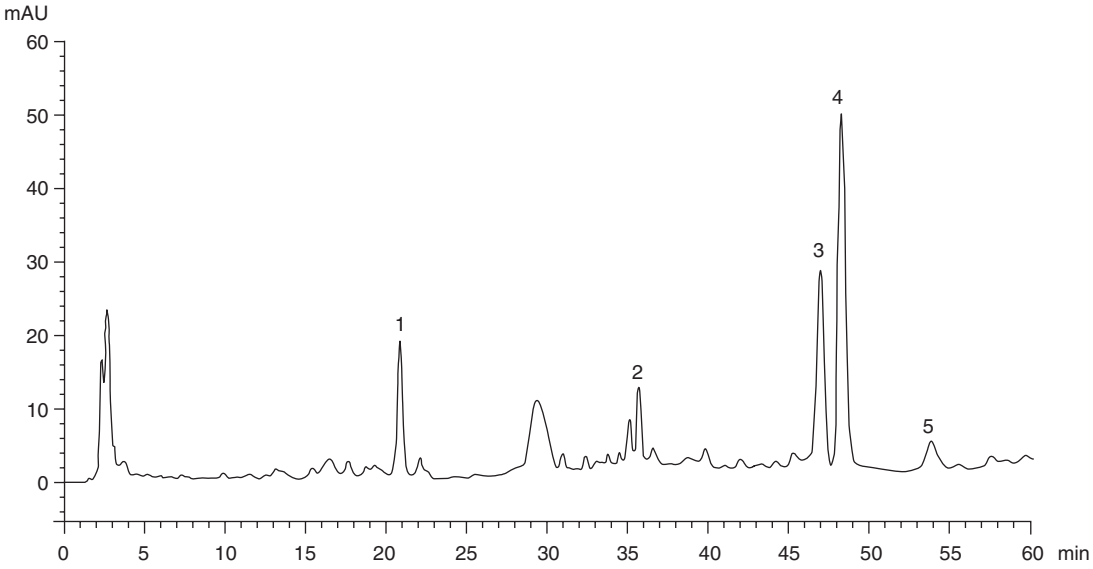


Figure 5 A reference fingerprint chromatogram of Medulla Junci extract

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 XV)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 4.0%.

Acid-insoluble ash: not more than 2.5%.

5.7 Water Content *(Appendix X)*: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 4.0%.

Ethanol-soluble extractives (hot extraction method): not less than 5.0%.

7. ASSAY

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

Standard solutions

Dehydroeffusol standard stock solution, Std-Stock (100 mg/L)

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

Effusol standard stock solution, Std-Stock (100 mg/L)

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

Mixed dehydroeffusol and effusol standard solution for assay, Std-AS

Measure accurately the volume of the dehydroeffusol Std-Stock and effusol Std-Stock, dilute with methanol to produce a series of solutions of 0.2, 0.5, 1, 2, 4 mg/L for both dehydroeffusol and effusol.

Test solution

Weigh accurately 0.3 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 30 mL of methanol. Sonciate (220 W) the mixture for 30 min. Centrifuge at about 3200 × *g* for 10 min. Filter and transfer the solution to a 100-mL volumetric flask. Repeat the extraction for two more times. Combine the filtrate. Make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (270 nm for dehydroeffusol and 282 nm for effusol) 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 methanol and water (52:48, v/v). The elution time is about 40 min.

System suitability requirements

Perform at least five replicate injections, each using 20 μL of the mixed dehydroeffusol and effusol Std-AS (1 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of dehydroeffusol and effusol should not be more than 5.0%; the RSD of the retention times of dehydroeffusol peak and effusol peak should not be more than 5.0%; the column efficiencies determined from dehydroeffusol peak and effusol peak should not be less than 7500 and 7000 theoretical plates respectively.

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

Calibration curves

Inject a series of the mixed dehydroeffusol and effusol Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of dehydroeffusol and effusol against the corresponding concentrations of the mixed dehydroeffusol and effusol Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

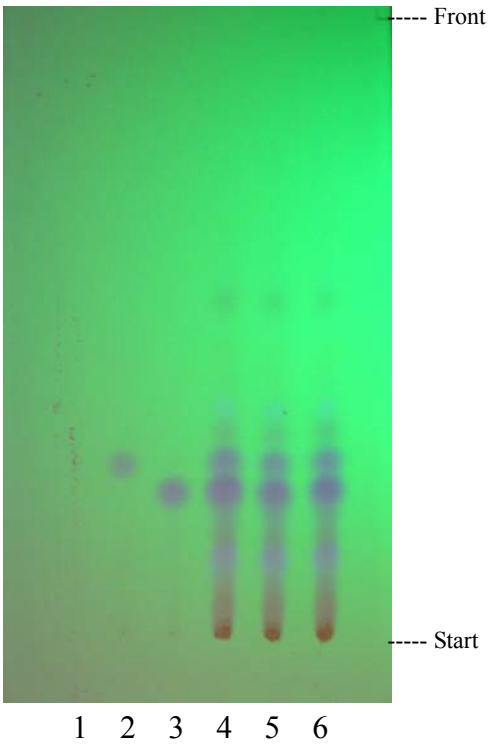
Procedure

Inject 20 μL of the test solution into the HPLC system and record the chromatogram. Identify dehydroeffusol and effusol peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed dehydroeffusol and effusol Std-AS. The retention times of dehydroeffusol and effusol peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of dehydroeffusol and effusol in the test solution, and calculate the percentage contents of dehydroeffusol and effusol in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.078% of the total content of dehydroeffusol ($C_{17}H_{14}O_2$) and effusol ($C_{17}H_{16}O_2$), calculated with reference to the dried substance.

Medulla Junci (燈心草)



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Standard (Effusol)	Effusol positive
3	Standard (Dehydroeffusol)	Dehydroeffusol positive
4	Spiked sample (Sample plus effusol and dehydroeffusol)	Effusol and dehydroeffusol positive
5	Sample (Medulla Junci)	Effusol and dehydroeffusol positive
6	Sample duplicate (Medulla Junci)	Effusol and dehydroeffusol positive

Figure 1 TLC results of Medulla Junci extract observed under UV light (254 nm)