Radix Platycodi

Figure 1 A photograph of Radix Platycodi

1. NAMES

Official Name: Radix Platycodi

Chinese Name: 桔梗

Chinese Phonetic Name: Jiegeng

2. SOURCE

Radix Platycodi is the dried root of *Platycodon grandiflorum* (Jacq.) A. DC. (Campanulaceae). The root is collected in the spring or autumn after removal of the rootlets, it is peeled or unpeeled, then dried thoroughly.

3. DESCRIPTION

Cylindrical, fusiform, or somewhat thin and long, slightly twisted, gradually tapering, often branched, main root 7-26 cm in length, 5-25 mm in diameter at the upper end. Sometimes the apex showing a shorter rhizome with round or crescent-shaped stem scars. Externally white or yellowish-brown, covered with coarse longitudinal wrinkles, lateral furrows and lenticel-like lateral lines. Texture fragile, fracture surface, even or uneven, cortex white or somewhat yellowish, xylem yellowish-white or pale brown, cambium ring brown, pale brown or not clear. Odourless or slightly scented of caramel smell. Tasteless at first, later bittersweet (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork cells occasionally remain, consisting of several layers of suberized cells. Cortex narrow, often with clefts. Groups of laticiferous tubes scattered in a radial fashion in the phloem, containing yellowish-brown granules. Cambium in a ring. Xylem vessels scattered singly or in groups, arranged radially (Fig. 2).

Powder

Colour light greyish-yellow to light greyish-brown. Cork cells occasionally observed, polygonal in shape. Fragments of groups of laticiferous tubes easily observed, containing yellowish or yellowish-brown granules in the cells. Inulin abundant, fan-shaped or irregular, with radial striations, scattered or contained within parenchyma cells. Vessels reticulate, scalariform, or bordered-pitted, 13-78 μm in diameter (Fig. 3).

4.2 Physicochemical Identification

Procedure

Weigh 1.0 g of the powdered sample and put into a 100-mL conical flask, then add 10 mL of dichloromethane. Sonicate (240 W) the mixture for 30 min. Centrifuge at about 2000 × *g* for 15 min. Filter and transfer 1 mL of the filtrate to a test tube. Cautiously add about 1 mL of sulphuric acid along the inner wall of the test tube. Allow to stand for about 20 min. An orange-brown ring is observed at the interface of the two solvent layers.

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

Standard solution

Platycoside E standard solution

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

Developing solvent system

Prepare a mixture of dichloromethane, methanol and acetic acid (5:8:1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add 30 mL of methanol. Sonicate (240 W) the mixture for 30 min. Centrifuge at about 2000 × *g* for 10 min. Transfer the supernatant to a 100-mL round-bottomed flask and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 3 mL of water. Load the water extract onto a Hydrophilic-Lipophilic Balance (HLB) extraction cartridge (3 mL, 60 mg). Wash the cartridge with 6 mL of water. Elute with 2 mL of methanol.

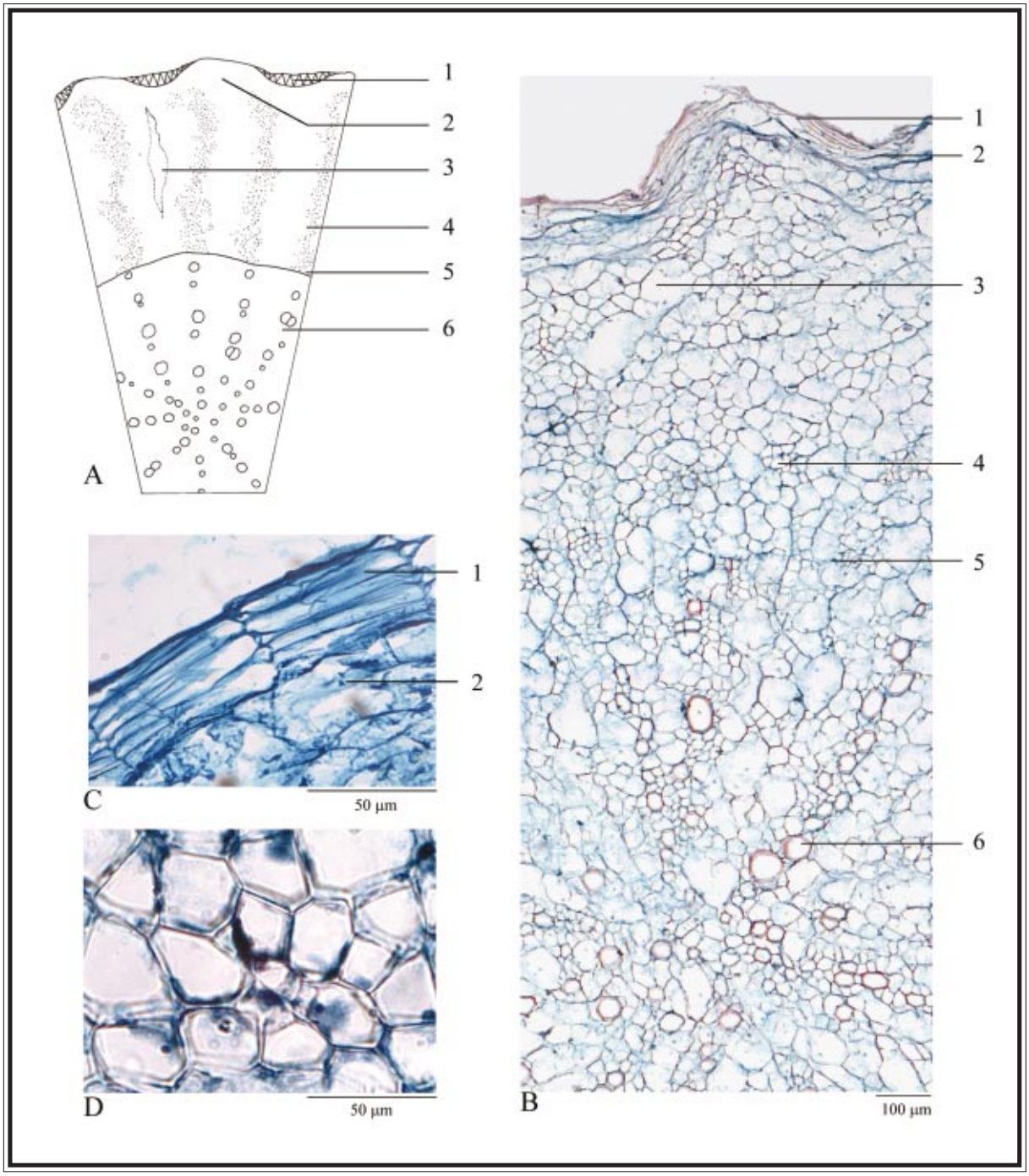

Figure 2 Microscopic features of transverse section of Radix Platycodi

A. Sketch B. Section illustration C. Cork cell D. Laticiferous tube group

1. Cork 2. Cortex 3. Cleft 4. Phloem 5. Cambium 6. Xylem

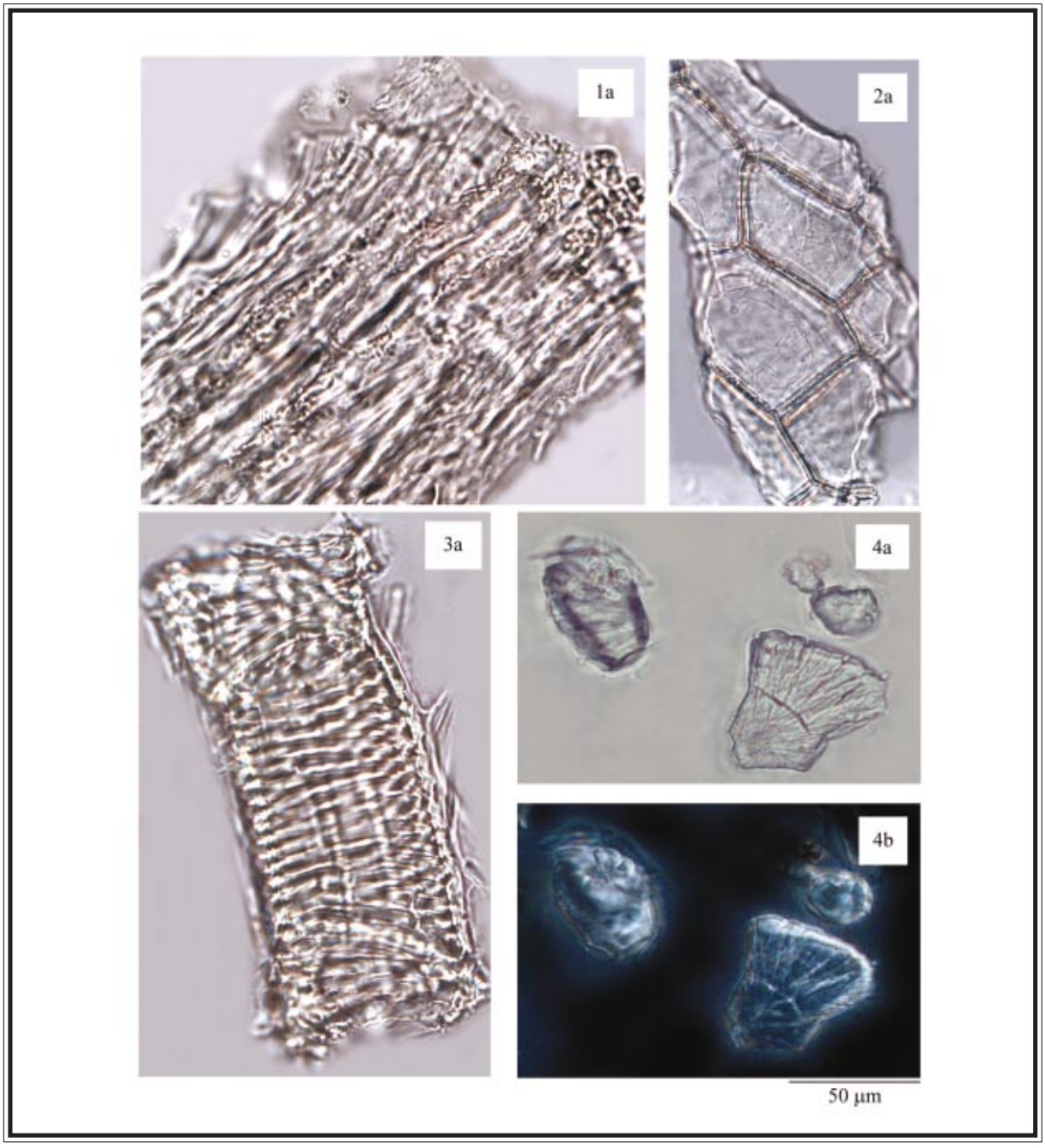

Figure 3 Microscopic features of powder of Radix Platycodi

1. Laticiferous tube 2. Cork cells 3. Scalariform vessel 4. Inulin

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

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 platycoside E standard solution and the test solution (5 µL each) 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 leave for about 30 min for equilibrium. 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 7.5 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, then heat at about 105°C until the spots or bands become visible (about 5–10 min). Examine the plate under visible light. 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 platycoside E.

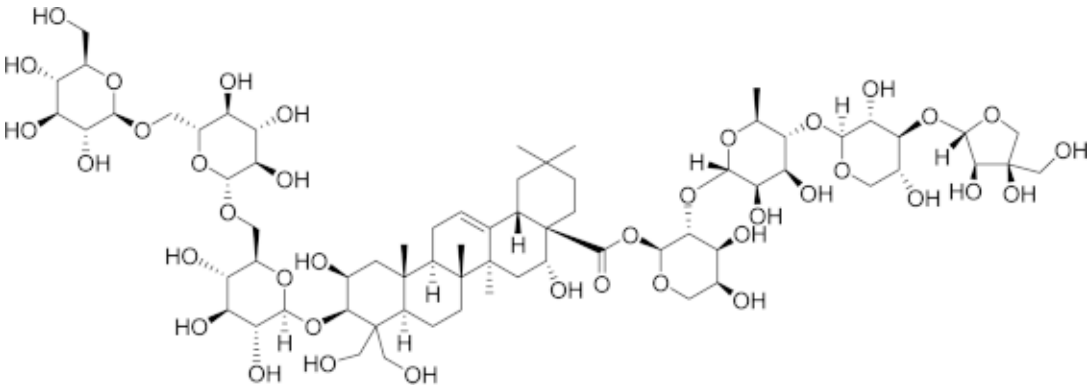

Figure 4 Chemical structure of platycoside E

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

Standard solution

Platycoside E standard stock solution, Std-Stock (1000 mg/L)

Weigh 1.0 mg of platycoside E CRS and dissolve in 1 mL of methanol.

Platycoside E standard solution for fingerprinting, Std-FP (500 mg/L)

Pipette 1 mL of platycoside E Std-Stock into a 2-mL volumetric flask and make up to the mark with methanol.

Test solution

Weigh 1.0 g of the powdered sample and put into a 250-mL conical flask, then add 100 mL of methanol. Sonicate (240 W) the mixture for 50 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction once. Combine the extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 10 mL of methanol. Add 50 mL of diethyl ether and shake for 1 min. Allow to stand for 12 h. Collect the precipitate and dissolve in 3 mL of methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (201 nm) and a column (3.9 × 300 mm) packed with ODS bonded silica gel (4 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 – 60	90 → 70	10 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 μL of platycoside E Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of platycoside E should not be more than 5.0%; the RSD of the retention time of platycoside E peak should not be more than 2.0%; the column efficiency determined from platycoside E peak should not be less than 5000 theoretical plates.

The *R* value between peak 1 and peak 2 in the chromatogram of the test solution should not be less than 1.0 and the *R* value between peak 4 and peak 5 in the chromatogram of the test solution should not be less than 0.7 (Fig. 5).

Procedure

Separately inject platycoside E Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of platycoside E peak in the chromatogram of platycoside E Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Under the same HPLC conditions, identify platycoside E peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of platycoside E Std-FP. The retention times of platycoside E 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 Radix Platycodi extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the five characteristic peaks of Radix Platycodi extract

Peak No.	RRT	Acceptable Range
1	0.95	±0.03
2 (marker, platycoside E)	1.00	-
3	1.19	±0.03
4	1.56	±0.08
5	1.59	±0.08

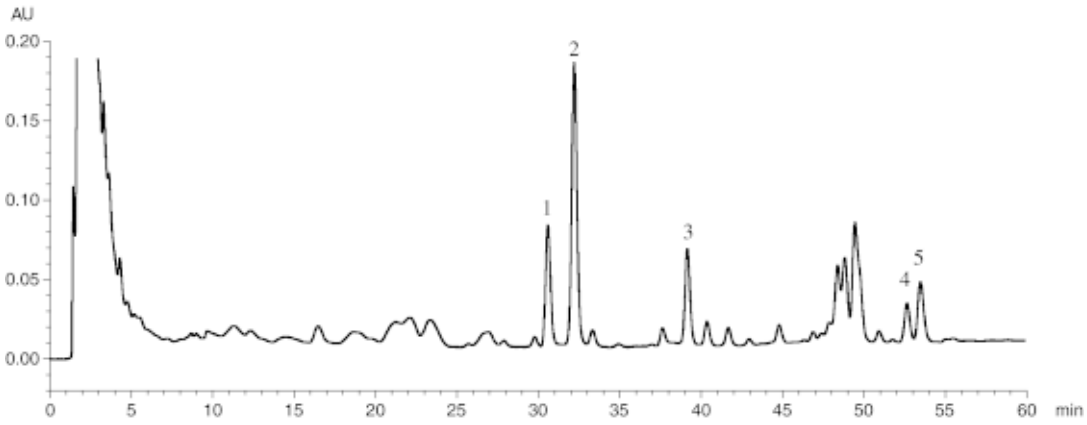

Figure 5 A reference fingerprint chromatogram of Radix Platycodi extract

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 XVII)*: 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)*: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 49.0%.

Ethanol-soluble extractives (cold extraction method): not less than 25.0%.

7. ASSAY

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

Standard solution

Platycoside E standard stock solution, Std-Stock (2000 mg/L)

Weigh accurately 10.0 mg of platycoside E CRS and dissolve in 5 mL of methanol.

Platycoside E standard solution for assay, Std-AS

Measure accurately the volume of the platycoside E Std-Stock, dilute with methanol to produce a series of solutions of 300, 500, 1000, 1500, 2000 mg/L for platycoside E.

Test solution

Weigh accurately 1.0 g of the powdered sample and put into a 50-mL conical flask, then add 50 mL of methanol. Sonicate (240 W) the mixture for 50 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Repeat the extraction once. Combine the extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 2-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD and a column (3.9 × 300 mm) packed with ODS bonded silica gel (4 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	82 → 78	18 → 22	linear gradient
10 – 20	78	22	isocratic
20 – 30	78 → 65	22 → 35	linear gradient
30 – 40	65 → 0	35 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 μL of platycoside E Std-AS (500 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of platycoside E should not be more than 5.0%; the RSD of the retention time of platycoside E peak should not be more than 2.0%; the column efficiency determined from platycoside E peak should not be less than 4000 theoretical plates.

The *R* value between platycoside E 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 platycoside E Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of platycoside E against the natural logarithm of the corresponding concentrations of platycoside E Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 μL of the test solution into the HPLC system and record the chromatogram. Identify platycoside E peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of platycoside E Std-AS. The retention times of platycoside E peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of platycoside E in the test solution by using the following equation –

$$\text{Concentration of platycoside E in the test solution} = e^{[\text{Ln (A)}-I]/m}$$

Where A = the peak area of platycoside E in the test solution,

I = the y-intercept of the 5-point calibration curve,

m = the slope of the 5-point calibration curve.

Calculate the percentage content of platycoside E in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.10% of platycoside E ($C_{69}H_{112}O_{38}$), calculated with reference to the dried substance.

Lane	Sample	Results
1	Standard (Platycoside E)	Platycoside E positive
2	Blank (Methanol)	Negative
3	Spiked sample (Sample plus platycoside E)	Platycoside E positive
4	Sample (Radix Platycodi)	Platycoside E positive
5	Sample duplicate (Radix Platycodi)	Platycoside E positive

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