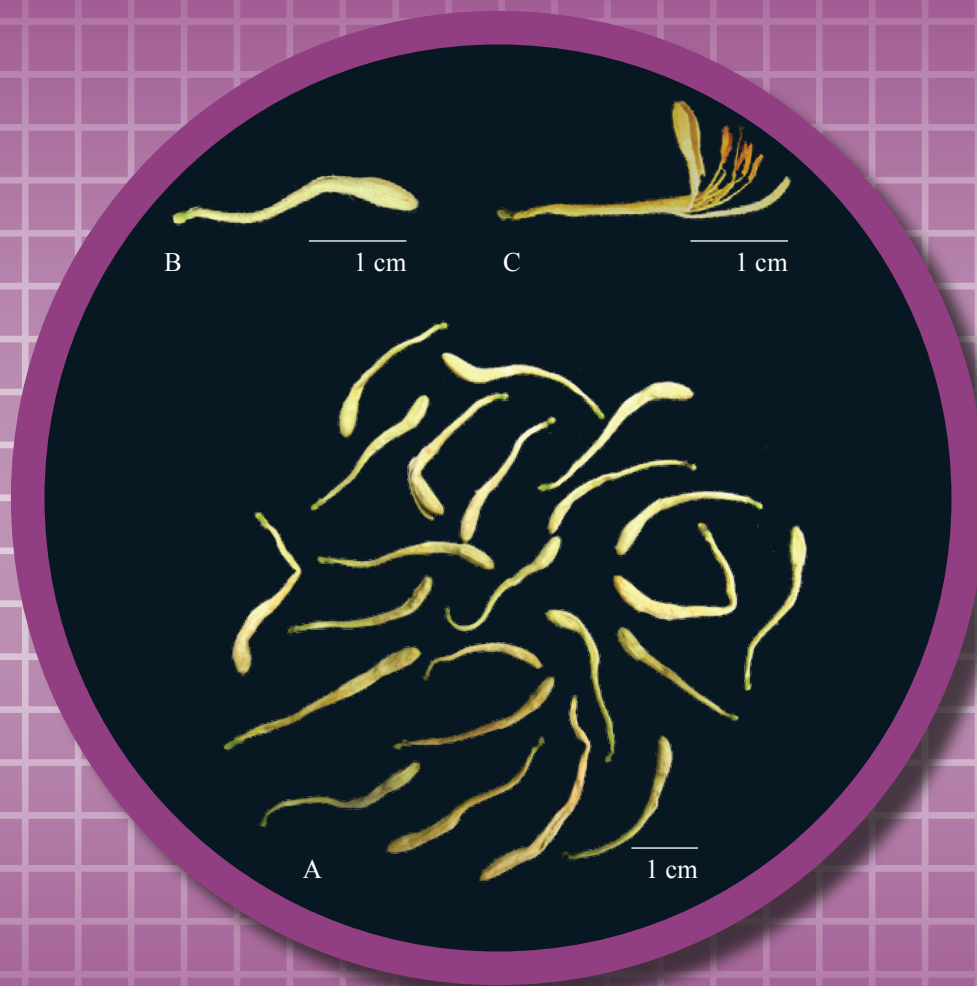


# Lonicerae Japonicae Flos



**Figure 1** A photograph of Lonicerae Japonicae Flos

A. Lonicerae Japonicae Flos    B. Magnified image of a flower bud  
C. Magnified image of flower

## 1. NAMES

Official Name: *Lonicerae Japonicae Flos*

Chinese Name: 金銀花

Chinese Phonetic Name: Jinyinhua

## 2. SOURCE

*Lonicerae Japonicae Flos* is the dried flower bud of *Lonicera japonica* Thunb. (Caprifoliaceae). The flower bud is collected in early summer before flowering, then dried under the sun to obtain *Lonicerae Japonicae Flos*.

## 3. DESCRIPTION

Clavate, stout in upper part and tapered downwards, slightly curved, 2-3 cm long, about 3 mm in diameter on upper part and 1.5 mm in diameter on lower part. Externally yellowish-white or greenish-white, gradually darken on keeping, densely covered with pubescence. Foliaceous bracts occasionally visible. Calyx green, 5-lobed at the apex, lobes pubescent, about 2 mm long. Corolla tubular when open, apex 2-lipped; stamens 5, epipetalous, yellow; pistil 1, ovary glabrous. Odour delicately fragrant; taste bland, slightly bitter and sweet (Fig. 1).

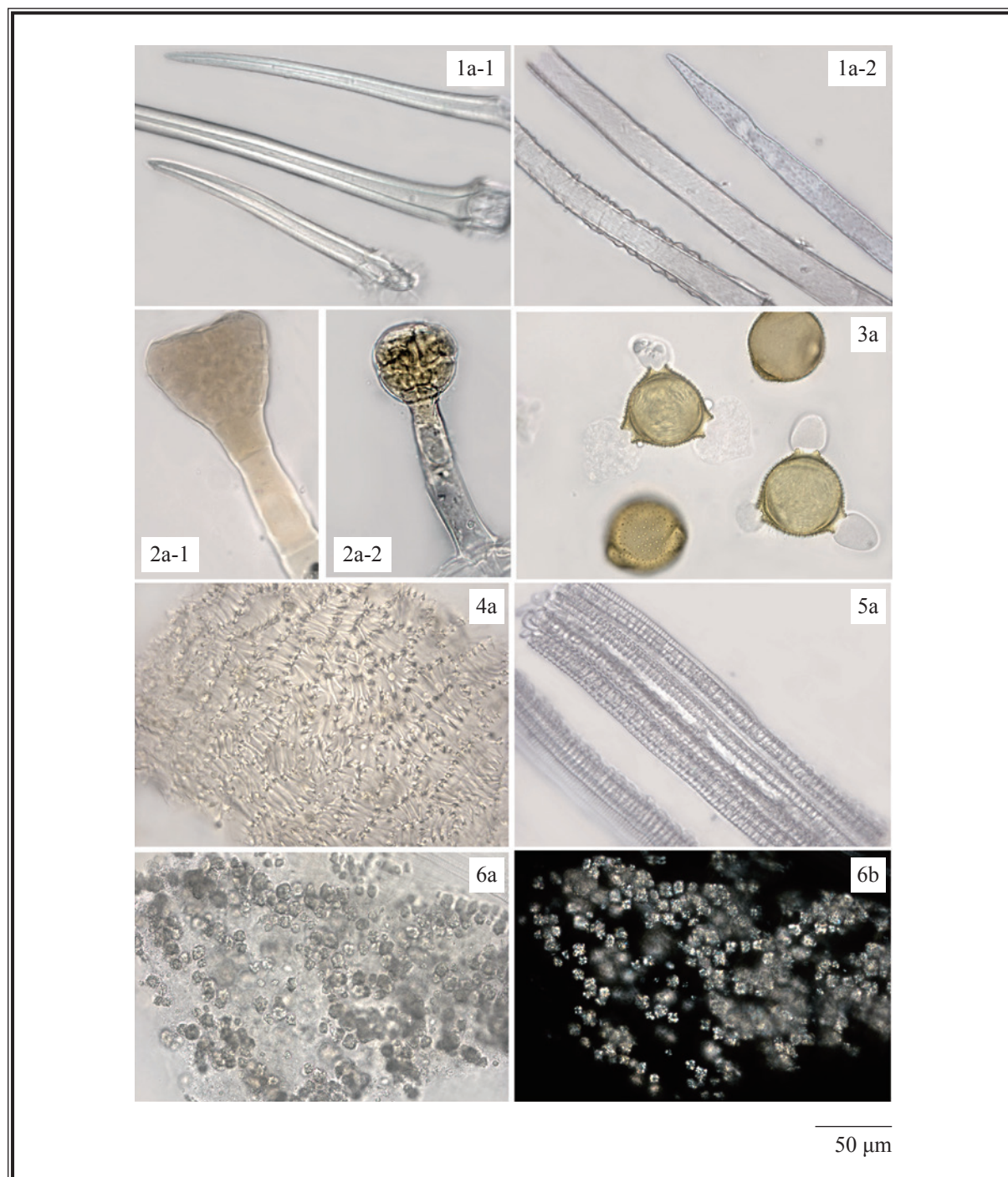
## 4. IDENTIFICATION

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

#### Powder

Colour pale green to pale yellowish-green. Non-glandular hairs numerous, occurring in two types: one type thick-walled non-glandular hairs, extremely numerous, unicellular, bicellular rare, straight or slightly curved, 45-900  $\mu\text{m}$  long, 14-37  $\mu\text{m}$  in diameter, walls 5-10  $\mu\text{m}$  thick; another type thin-walled non-glandular hairs, unicellular, extremely long, bent or wrinkled, 11-36  $\mu\text{m}$  in diameter, surface with fine warty protrusions. Glandular hairs numerous, occurring in two types: one type with an inverted conical head, apex flat, consisting of 10-33 cells, arranged in 2-4 layers in lateral view, 48-108  $\mu\text{m}$  in diameter, some cells contain pale yellow

contents; stalk 1- to 5-celled, 70-250  $\mu\text{m}$  long, 15-58  $\mu\text{m}$  in diameter, with thin walls and indistinct cuticle striations; another type with subrounded or slightly oblate head, consisting of 6-20 cells, 20-80  $\mu\text{m}$  in diameter; stalk 2- to 4-celled, 24-80  $\mu\text{m}$  long, 13-32  $\mu\text{m}$  in diameter. Pollen grains extremely numerous, yellow, subglobular or rounded-triangular, 60-92  $\mu\text{m}$  in diameter, exine with fine and dense short spiny and granular sculptures, 3 germinal pores. Anther cells subpolygonal or irregular in shape, walls beaded, with slightly curved or straight striations. Vessels mostly in groups, mainly spiral, relatively small, 10-40  $\mu\text{m}$  in diameter. Clusters of calcium oxalate scattered or occurred in parenchymatous cells, 6-45  $\mu\text{m}$  in diameter, with sharp angles; polychromatic under the polarized microscope (Fig. 2).



**Figure 2** Microscopic features of powder of *Lonicerae Japonicae Flos*

1. Non-glandular hairs (1-1 thick-walled non-glandular hairs, 1-2 thin-walled non-glandular hairs)
2. Glandular hair (2-1 inverted conical head, 2-2 subrounded or slightly oblate head)
3. Pollen grains
4. Anther cells
5. Vessels
6. Clusters of calcium oxalate

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

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

### Standard solutions

#### *Chlorogenic acid standard solution*

Weigh 1.0 mg of chlorogenic acid CRS (Fig. 3) and dissolve in 1 mL of ethanol (70%).

#### *Luteolin-7-O-glucoside standard solution*

Weigh 1.0 mg of luteolin-7-O-glucoside CRS (Fig. 3) and dissolve in 1 mL of ethanol (70%).

### Developing solvent system

Prepare a mixture of ethyl acetate, acetone, formic acid and water (20:3:1.5:1.5, v/v).

### Spray reagent

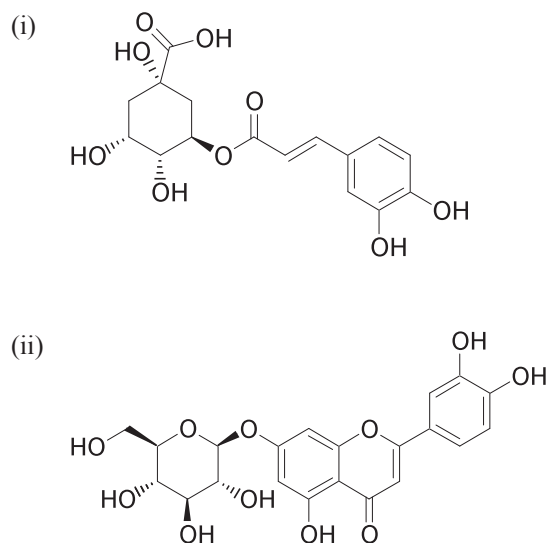
Weigh 2 g of ferric trichloride and dissolve in 100 mL of ethanol.

### Test solution

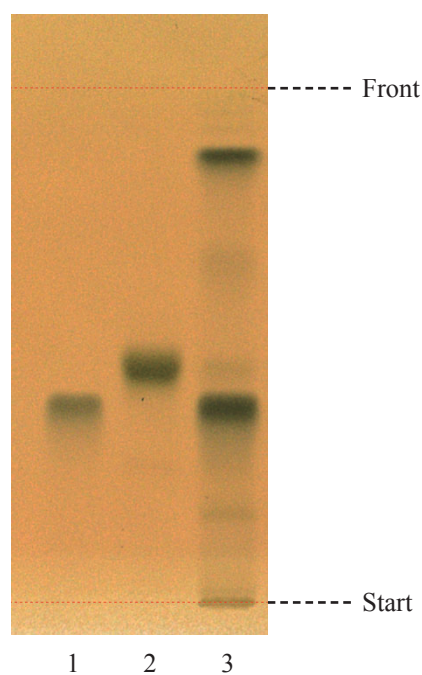
Weigh 1.0 g of the powdered sample and place it in a 25-mL conical flask, then add 5 mL of ethanol (70%). Sonicate (100 W) the mixture for 20 min. Filter the mixture.

### Procedure

Carry out the method by using a HPTLC silica gel F<sub>254</sub> plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately chlorogenic acid standard solution, luteolin-7-O-glucoside standard solution and the test solution (8 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 let equilibrate for about 15 min. 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 8 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 dry in air. Examine the plate under visible light. Calculate the  $R_f$  values by using the equation as indicated in Appendix IV (A).



**Figure 3** Chemical structures of (i) chlorogenic acid and (ii) luteolin-7-*O*-glucoside



**Figure 4** A reference HPTLC chromatogram of Lonicerae Japonicae Flos extract observed under visible light after staining

1. Chlorogenic acid standard solution
2. Luteolin-7-*O*-glucoside standard solution
3. Test solution

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 chlorogenic acid and luteolin-7-*O*-glucoside (Fig. 4).

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

#### Standard solutions

*Chlorogenic acid standard solution for fingerprinting, Std-FP (1000 mg/L)*

Weigh 5.0 mg of chlorogenic acid CRS and dissolve in 5 mL of methanol (50%).

*Luteolin-7-O-glucoside standard solution for fingerprinting, Std-FP (20 mg/L)*

Weigh 1.0 mg of luteolin-7-O-glucoside CRS and dissolve in 50 mL of methanol (50%).

#### Test solution

Weigh 2.0 g of the powdered sample and place it in a 100-mL conical flask, then add 50 mL of methanol (50%). Sonicate (100 W) the mixture for 30 min. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 3000 x g for 5 min. Transfer the supernatant to a 50-mL volumetric flask and make up to the mark with methanol (50%). Filter through a 0.45- $\mu$ m PTFE filter.

#### Chromatographic system

The liquid chromatograph is equipped with a DAD (350 nm) and a column (4.6  $\times$  250 mm) packed with phenyl modified ODS bonded silica gel (5  $\mu$ m 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)	0.2% Phosphoric acid (% v/v)	Acetonitrile (% v/v)	Elution
0 – 5	92	8	isocratic
5 – 15	92 $\rightarrow$ 80	8 $\rightarrow$ 20	linear gradient
15 – 35	80	20	isocratic
35 – 45	80 $\rightarrow$ 70	20 $\rightarrow$ 30	linear gradient
45 – 60	70 $\rightarrow$ 92	30 $\rightarrow$ 8	linear gradient

#### System suitability requirements

Perform at least five replicate injections, each using 10  $\mu$ L of chlorogenic acid Std-FP and luteolin-7-O-glucoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of chlorogenic acid and luteolin-7-O-glucoside should not be more than 5.0%; the RSD of the retention times of chlorogenic acid and luteolin-7-O-glucoside peaks should not be more than 2.0%; the column efficiencies determined from chlorogenic acid and luteolin-7-O-glucoside peaks should not be less than 30000 and 20000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and the *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

### Procedure

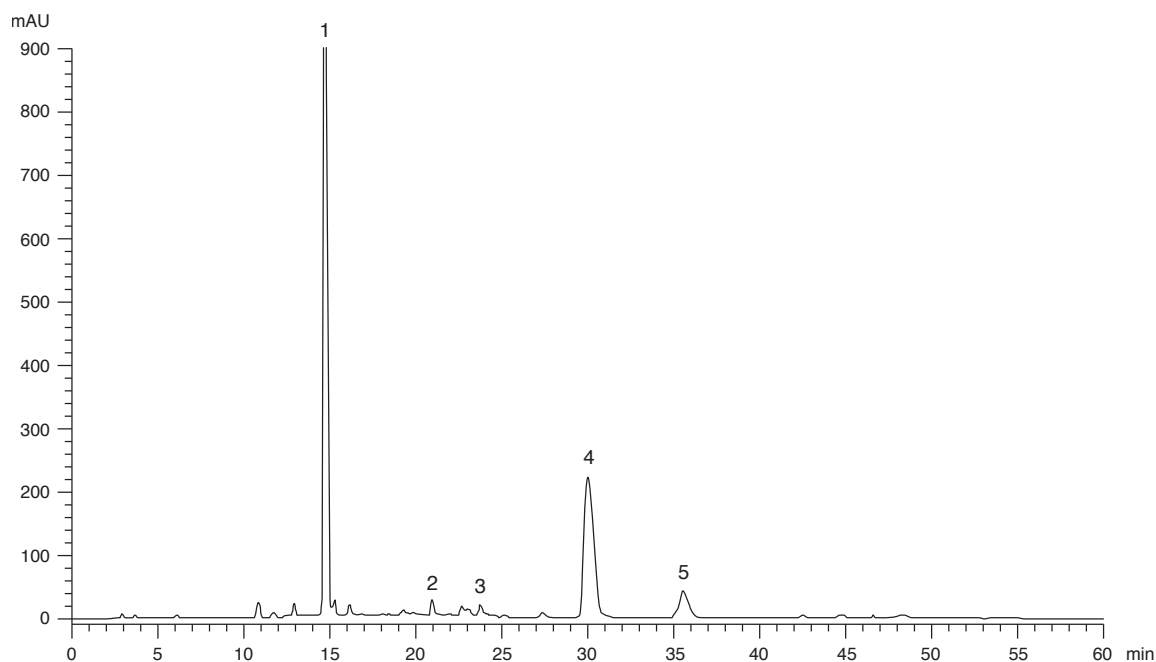
Separately inject chlorogenic acid Std-FP, luteolin-7-*O*-glucoside Std-FP and the test solution (10  $\mu$ L each) into the HPLC system and record the chromatograms. Measure the retention times of chlorogenic acid and luteolin-7-*O*-glucoside peaks in the chromatograms of chlorogenic acid Std-FP, luteolin-7-*O*-glucoside Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify chlorogenic acid and luteolin-7-*O*-glucoside peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of chlorogenic acid Std-FP and luteolin-7-*O*-glucoside Std-FP. The retention times of chlorogenic acid and luteolin-7-*O*-glucoside peaks in the chromatograms of the test solution and the corresponding Std-FP 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 Lonicerae Japonicae Flos extract are listed in Table 2.

**Table 2** The RRTs and acceptable ranges of the five characteristic peaks of Lonicerae Japonicae Flos extract

Peak No.	RRT	Acceptable Range
1 (chlorogenic acid)	0.62	$\pm 0.03$
2	0.88	$\pm 0.03$
3 (marker, luteolin-7- <i>O</i> -glucoside)	1.00	-
4	1.27	$\pm 0.03$
5	1.49	$\pm 0.05$





**Figure 5** A reference fingerprint chromatogram of *Lonicerae Japonicae Flos* 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** (*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 7.5%.

Acid-insoluble ash: not more than 1.5%.

**5.7 Water Content** (*Appendix X*)

Oven dried method: not more than 12.0%.

## 6. EXTRACTIVES (Appendix XI)

Water-soluble extractives (hot extraction method): not less than 37.0%.

Ethanol-soluble extractives (hot extraction method): not less than 32.0%.

## 7. ASSAY

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

### 7.1 Assay of Chlorogenic acid

#### Standard solution

*Chlorogenic acid standard stock solution, Std-Stock (1000 mg/L)*

Weigh accurately 2.0 mg of chlorogenic acid CRS and dissolve in 2 mL of methanol (50%).

*Chlorogenic acid standard solution for assay, Std-AS*

Measure accurately the volume of the chlorogenic acid Std-Stock, dilute with methanol (50%) to produce a series of solutions of 20, 40, 60, 200, 400 mg/L for chlorogenic acid.

#### Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 100-mL centrifuge tube, then add 50 mL of methanol (50%). Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3500 x g for 10 min. Transfer the supernatant to a 100-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with methanol (50%). Pipette 10 mL of the solution to a 25-mL volumetric flask and make up to the mark with methanol (50%). Filter through a 0.45-µm PTFE filter.

#### Chromatographic system

The liquid chromatograph is equipped with a DAD (327 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 3) –

**Table 3** Chromatographic system conditions

Time (min)	0.4% Phosphoric acid (% v/v)	Acetonitrile (% v/v)	Elution
0 – 5	95	5	isocratic
5 – 10	95 → 87	5 → 13	linear gradient
10 – 30	87	13	isocratic

**System suitability requirements**

Perform at least five replicate injections, each using 10 µL of chlorogenic acid Std-AS (60 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of chlorogenic acid should not be more than 5.0%; the RSD of the retention time of chlorogenic acid peak should not be more than 2.0%; the column efficiency determined from chlorogenic acid peak should not be less than 40000 theoretical plates.

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

**Procedure**

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

**Limits**

The sample contains not less than 3.5% of chlorogenic acid ( $C_{16}H_{18}O_9$ ), calculated with reference to the dried substance.

**7.2 Assay of Luteolin-7-*O*-glucoside****Standard solution**

*Luteolin-7-*O*-glucoside standard stock solution, Std-Stock (500 mg/L)*

Weigh accurately 1.0 mg of luteolin-7-*O*-glucoside CRS and dissolve in 2 mL of ethanol (70%).

*Luteolin-7-*O*-glucoside standard solution for assay, Std-AS*

Measure accurately the volume of the luteolin-7-*O*-glucoside Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 3, 6.25, 12.5, 25, 50 mg/L for luteolin-7-*O*-glucoside.

### Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 50 mL of ethanol (70%). Reflux the mixture for 3 h. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL volumetric flask and make up to the mark with ethanol (70%). Filter through a 0.45- $\mu$ m PTFE filter.

### Chromatographic system

The liquid chromatograph is equipped with a DAD (350 nm) and a column (4.6  $\times$  250 mm) packed with ODS bonded silica gel (5  $\mu$ m particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 4) –

**Table 4** Chromatographic system conditions

Time (min)	0.5% Acetic acid (% v/v)	Acetonitrile (% v/v)	Elution
0 – 5	92	8	isocratic
5 – 15	92 $\rightarrow$ 80	8 $\rightarrow$ 20	linear gradient
15 – 35	80	20	isocratic
35 – 45	80 $\rightarrow$ 70	20 $\rightarrow$ 30	linear gradient

### System suitability requirements

Perform at least five replicate injections, each using 20  $\mu$ L of luteolin-7-*O*-glucoside Std-AS (12.5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of luteolin-7-*O*-glucoside should not be more than 5.0%; the RSD of the retention time of luteolin-7-*O*-glucoside peak should not be more than 2.0%; the column efficiency determined from luteolin-7-*O*-glucoside peak should not be less than 25000 theoretical plates.

The *R* value between luteolin-7-*O*-glucoside 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 luteolin-7-*O*-glucoside Std-AS (20  $\mu$ L each) into the HPLC system and record the chromatograms. Plot the peak areas of luteolin-7-*O*-glucoside against the corresponding concentrations of luteolin-7-*O*-glucoside Std-AS. Obtain the slope, y-intercept and the *r*<sup>2</sup> value from the 5-point calibration curve.

**Procedure**

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify luteolin-7-*O*-glucoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of luteolin-7-*O*-glucoside Std-AS. The retention times of luteolin-7-*O*-glucoside 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 luteolin-7-*O*-glucoside in the test solution, and calculate the percentage content of luteolin-7-*O*-glucoside in the sample by using the equations as indicated in Appendix IV (B).

**Limits**

The sample contains not less than 0.059% of luteolin-7-*O*-glucoside (C<sub>21</sub>H<sub>20</sub>O<sub>11</sub>), calculated with reference to the dried substance.