Determination of effective medicinal components in *Lonicera macranthoides* flower buds

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Abstract. This study is to determination of active medicinal components in *Lonicera macranthoides*. The results of High-performance liquid chromatography showed that the contents of chlorogenic acid were as follows: Baiyun: 6.297%, jincuilei: 5.293%, yincuilei: 5.288%, common variety: 7.455%, common variety (after flowering) : 4.140% . The contents of Luteolin in different *Lonicera macranthoides* varieties were as follows: Baiyun: 0.0264% ; Jincuilei: 0.0573% ; Yincuilei: 0.0347% ; common variety: 0.0394% ; common variety (after flowering) : 0.0197% .

1 Introduction

In the 2005 edition of the Chinese Pharmacopoeia, one of the bases for distinguishing flos *Lonicerae* from Flos *Lonicerae japonicae* was "analysis of chemical constituents, the active constituents of Flos *Lonicerae* are mainly Luteolin glycosides, but the other varieties contain little chlorogenic acid, there have also been a number of reports in recent years of differences in the contents of *Lonicera Japonica* and honeysuckle, in order to further determine the market value of *Lonicera macranthoides*, in this experiment, the contents of Luteolin and chlorogenic acid in the flower buds of *Lonicera macranthoides* were determined[1-2]. Chlorogenic acid, alias: Chlorogenic acid, CAFFEIC tannic acid, 3-caffeoylquinic acid, formula:C16H18O9.

Luteolide (English name: Luteolide, alias name: luteolin-7-O-glucoside); Cynaroside, molecular formula: C₂₁H₂₀O₁₁[3].

2 Materials and methods

2.1 Experimental materials and apparatus

2.1.1 Experimental materials

This experimental material is *Lonicera macranthoides* dry flower bud, from Hunan Longhui honeysuckle science and technology development center. The four common varieties are Jincuilei, Yincuilei, Baiyun and Longhui (before and after flowering) . The control samples, chlorogenic acid (batch number: 110753-200413) and Luteolin (batch number: 111720-201106; 99.3%) were all from China Institute of Pharmaceutical and biological products.

2.1.2 Equipment and chromatographic conditions

P230IIhigh-pressure constant-current pump; UV230Iultraviolet-visible detector; Zwi Column Incubator; EC2006 Data Chromatographic Workstation; Diamonsil C18 column. Octadecyl Silane Bonded Silica Gel was used as Filler, acetonitrile (a)-0.1% phosphoric acid solution (b) was used as mobile phase gradient elution:

Table1. Gradient elution program

Time(min)	Mobile phase A (%)	Mobile phase B(%)
0→5	14	86
5→15	14→28	86→72
15→27	28→25	72→75
27→30	25→14	75→86

The flow rate of the mobile phase was 1.0 ml/min, the column temperature was 30 °c, the detection wavelength was 327 nm (0-12 Min) , 350 nm (12-30 Min) and the injection volume was 10μl.

2.2 Assay

2.2.1 Preparation of Reference Solution

This experiment take 19.10mg of chlorogenic acid reference substance, put it in a brown measuring flask, add 50% methanol to make 0.382mg/ml, 0.0382mg/ml solution, that is (stored below 10°C). Take 10.58mg of luteolin reference substance, add 50% methanol to make 0.01mg/ml, 0.001mg/ml solution, that is (stored below 4°C).

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2.2.2 Preparation of Test Solution

This experiment take about 0.5g of this product powder (passed through the No. 4 sieve), accurately weigh it, place it in a stoppered conical flask, add 50% methanol 50ml accurately, weigh it, and ultrasonically treat it (power 250W, frequency 30kHz) for 30 minutes. Let it cool and weigh it again. Use 50% methanol to make up the lost weight. Shake well and filter. Accurately measure 5ml of the filtrate and place it in a 25ml brown measuring flask. Add 50% methanol to the mark and shake well to get it.

2.2.3 Investigation of linear relationship

In this experiment, precisely aspirate 0.0382 mg/ml chlorogenic acid reference substance 1 μ l, 3 μ l, 0.382mg/ml chlorogenic acid reference substance 2 μ l, 5 μ l, 8 μ l, 13 μ l, 18 μ l, 26 μ l, 28 μ l, respectively according to the chromatographic conditions, and use the reference substance to inject samples The amount (μ g) is the abscissa and the corresponding peak area is the ordinate to obtain the standard curve of chlorogenic acid.

In the meantime, Precisely draw 0.001050594 mg/ml luteolin reference substance 1 μ l, 8 μ l, 12 μ l, 16 μ l, 0.01050594 mg/ml luteolin reference substance 4 μ l, 5 μ l, 8 μ l, 10 μ l, 12 μ l, 16 μ l, 20 μ l, 25 μ l, 30 μ l, respectively according to the chromatographic conditions For sample injection, take the injection amount of the reference substance (μ g) as the abscissa and the corresponding peak area as the ordinate to obtain the standard curve of luteolin.

2.2.4 Precision test

In this test, Precisely draw 10 μ l of the same test solution and repeat the injection 6 times to obtain the RSD of the peak area of chlorogenic acid and luteolin.

2.2.5 Stability Test

In this test, at 0 h, 3 h, 6 h, 9 h, 12 h, 15 h and 1 d, 2 d, 3 d, 4 d, and 5 d after the preparation of the test product, 10 μ l of the test product solution was precisely drawn into

the sample. Calculate the RSD of the peak area of chlorogenic acid and luteolin.

2.2.6 Repeatability Test

In this test, take the same sample, accurately weigh 6 parts, prepare the sample solution according to the method, inject the sample according to the chromatographic conditions, determine the content of chlorogenic acid and luteolin, and obtain the RSD of the peak area of chlorogenic acid and luteolin.

2.2.7 Sample recovery test

In this test, take 0.5g of the same sample powder of *Lonicera japonica*, 6 parts, accurately weighed, and accurately add 1ml of reference substance chlorogenic acid (1.0mg/ml) and 2ml of luteolin (0.1mg/ml) respectively, and prepare according to the method. The test solution was tested for content, and the average recovery rate of chlorogenic acid and luteolin was calculated.

2.2.8 Determination of sample content

The prepared test solution was subjected to gradient elution according to the gradient elution procedure in Table 1, and then the content of the sample was calculated according to the linear regression equation of the peak area of the sample and the corresponding reference substance.

3 Results and analysis

3.1 Investigation results of linear relationship

Chlorogenic acid-linear relationship investigation: According to the system adaptability test method, enter the chlorogenic acid reference substance of different concentrations, and then calculate the linear correlation number of chlorogenic acid. The regression equation is: $y = 2482.2219x + 164.7491$; $r = 0.9999$, and the linear range is 0.0382~10.696 μ g.

Table2. Analysis of linear relationship on chlorogenic acid and luteolin glycosides

Chlorogenic-acid injection volume (μ l)	Injection volume	Peak area	Luteolin injection volume (μ l)	Injection volume	Peak area
0.1	0.0382	104.11	0.1	0.001050594	2.67
0.3	0.1146	307.69	0.8	0.008404752	20.56
2	0.764	2041.21	1.2	0.012607128	30.62
5	1.91	4977.65	1.6	0.016809504	41.71
8	3.056	7945.76	4.0	0.042023760	106.28
13	4.966	12609.52	5.0	0.052529700	132.38
18	6.876	17424.46	8.0	0.084047520	212.21
26	9.932	24581.39	10	0.105059400	262.70
28	10.696	26691.11	12	0.126071280	312.44
			16	0.168095040	433.58
			20	0.210118800	535.19
			25	0.262648500	675.17
			30	0.315178200	806.19

Investigation of luteolin-linear relationship: According to the system adaptability test method, different concentrations of luteolin reference substance were added, and then the linear correlation number of luteolin was calculated. The regression equation is: $y=2565.4213x-2.4465$; $r=0.9999$, and the linear range is $0.001050594\sim0.3151782\mu\text{g}$.

The linear relationship investigation results show that the linear relationship between chlorogenic acid and luteolin is good.

3.2 Precision test results

Precisely draw 10uL of the same test solution and repeat the injection 6 times. The results are shown in Table 3. The RSD of the peak areas of chlorogenic acid and luteolin were 0.3% and 2.1%, respectively. It shows that the precision of the detection method in this experiment is good.

Table3. Precision results

	Chlorogenic acid peak area	Luteolin peak area
	3242.12	15.08
	3249.38	14.36
	3265.84	14.25
	3261.25	14.52
	3262.95	14.85
	3265.68	14.55
RSD	0.00301339	0.021260992

3.3 Stability test results

At 0, 3, 6, 9, 12, 15 h and 1, 2, 3, 4, and 5 days after the preparation of the test product, 10 uL of the test product solution was precisely drawn and injected. The results are

shown in Table 4. The intraday RSDs of the peak areas of chlorogenic acid and luteolin were 0.3% and 2.0%, respectively; the RSDs of the peak areas of chlorogenic acid and luteolin during the day were 0.4% and 2.1%, respectively. It shows that the stability of the test product during the test is good.

Table4. Stability Test Results

Time	Chlorogenic acid peak area	Luteolin peak area	Time	Chlorogenic acid peak area	Luteolin peak area
0 h	3265.68	15.09	1 d	3245.68	14.95
3 h	3249.38	14.76	2 d	3248.26	14.56
6 h	3265.84	14.56	3 d	3255.84	14.25
9 h	3261.25	14.52	4 d	3251.25	14.31
12 h	3262.95	14.5	5 d	3220.95	14.25
15 h	3240.06	14.22	RSD	0.00420428	0.020761881
RSD	0.003220575	0.020020526			

3.4 Repeatability test results

Take the same sample, accurately weigh 6 parts, and prepare the sample solution. The results are shown in Table 4-5: The RSD of chlorogenic acid and luteolin were determined to be 1.4% and 3.8%, respectively, indicating that the experimental method has good repeatability.

Table5. Repeatability Test Results

Sample weight	Luteolin peak area	Luteolin peak area
0.5067	3487.07	15.58
0.4917	3384.82	14.36
0.4964	3416.94	14.25
0.5049	3475.02	14.52

0.4760	3276.91	14.85
0.4696	3232.17	14.55
0.4835	3327.76	15.08

3.5 Sample recovery rate

Take 0.5 g of the same gray felt honeysuckle sample powder, 6 parts, accurately weighed, and accurately add 5 mL of the reference substance chlorogenic acid (0.382mg/mL) respectively, and prepare the test solution according to the method under the item, and carry out the content determination and calculation. The average recovery rate of chlorogenic acid was 98.3%, and the RSD was 0.37%.

Table6. Sample recovery Test Results

Sample weight	Luteolin peak area	Recoveries (%)
0.5014	5330.34	100.12
0.4987	5301.30	99.78
0.4998	5312.88	98.98
0.5087	5407.60	100.09
0.4887	5195.01	101.12
0.5167	5492.64	99.99

3.6 Results of sample content determination

Prepare the test solution of each sample according to the method below, inject the sample according to the chromatographic conditions below, measure each sample 3 times, record the chromatogram and calculate the peak area according to the external standard method. The content of chlorogenic acid and luteolin in 5 different origins of *Lonicera japonica* is shown in Tables 7 and 8. The HPLC diagrams of the reference substance and the sample solution are shown in the figure below. The results of high performance liquid chromatography showed that the chlorogenic acid content of each *Lonicera japonica* varieties were as follows: Baiyun: 6.297%, RSD0.0137; Jin Cui Lei: 5.293%, RSD 0.0060; Yin Cui Lei: 5.288%, RSD 0.0029; common species : 7.455%, RSD0.0065; common species (after flowering): 4.410%, RSD0.0151. The content of luteolin in each *Lonicera japonica* variety is as follows, Baiyun: 0.0264%, RSD 0.0079; Jin Cui Lei: 0.0573%, RSD 0.0053; Yin Cui Lei: 0.0347%, RSD 0.0010; Common species: 0.0394%, RSD 0.0073; common varieties (after flowering): 0.0197%, RSD0.0031.

Table7. Content of chlorogenic acid in different species

Species	Chlorogenic acid (%)	RSD
Baiyun	6.297	0.0137
Jin Cuilei	5.293	0.0060
Silver Cuilei	5.288	0.0029
Common species	7.455	0.0065
Common species (after flowering)	4.410	0.0151

Table8. Content of Luteoloside in different species

Species	Luteoloside (%)	RSD
Baiyun	0.0264	0.0079
Jin Cuilei	0.0573	0.0053
Silver Cuilei	0.0347	0.0010
Common species	0.0394	0.0073
Common species (after flowering)	0.0197	0.0031

4 Conclusion and discussion

The results of this experiment show that the chlorogenic acid content of the flower buds of *Lonicera japonica* is far higher than that required for genuine honeysuckle. However, the content of luteolin is not as good as that of genuine honeysuckle. However, chlorogenic acid and luteolin have their own different pharmaceutical values[4-5].

The dry processing technology of honeysuckle flower buds directly affects the content of chlorogenic acid and luteolin in the flower buds. At present, the drying processing techniques used for the flower buds of the gray felt hair honeysuckle include sun drying, drying, shade drying and fumigation[6]. Comparing the effects of sun drying, shade drying, steaming and sulfur fumigation on the content of chlorogenic acid in *Lonicera japonica*, the results show that the content of chlorogenic acid in the shade drying is the highest. Compared with the other three methods, there are extremely significant differences. The sulfur fumigation method Second, but it is also significantly better than steaming and direct drying. The content of chlorogenic acid in honeysuckle dried by sun drying is the lowest. The sun-drying is divided into steaming, fumigating, frying, and raw drying. Studies have shown that steaming and fumigation are better, while the content of chlorogenic acid and luteolin is lower in raw sun-dried products. Studies have shown that honeysuckle produced by sulfur fumigation and drying methods not only has good appearance quality, but also has high chlorogenic acid content.

The samples in this experiment are divided into two drying methods, one is drying and the other is drying. The results showed that the raw sun exposure method greatly reduced the content of the effective ingredients in the flower buds of *Lonicera japonica*. Moreover, the dried flower buds are obviously darker, which also affects the appearance quality. The dried flower buds are green in appearance and good in quality.

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