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Content Determination of Chlorogenic Acid and Luteoloside in Flos Lonicerae

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Abstract The contents of chlorogenic acid and luteoloside in Flos Lonicerae from Binhai New Area and Jinnan District of Tianjin were determined to provide basis for the quality identification of this medicinal material. The content of chlorogenic acid was determined by HPLC. In Flos Lonicerae from Binhai New Area and in Flos Lonicerae harvested at different stages from Jinnan District, the contents of chlorogenic acid were 3.804%, 5.507% (three green stage), 4.855% (silver flower stage) and 4.220% (golden flower stage), respectively, and the contents of luteoloside were 5.53%, 12.405% (three green stage), 14.370% (silver flower stage) and 0.917% (golden flower stage), respectively. The contents of chlorogenic acid in Flos Lonicerae from Jinnan District were higher than that from Binhai New Area. Among different stages, the content of chlorogenic acid was highest in three green stage, followed by that in silver flower stage. As the flowers bloomed, the content of chlorogenic acid in the medicinal material showed a significant downward trend. In Flos Lonicerae from Jinnan District, the content of luteoloside was highest in silver flower stage and lowest in golden flower stage.

Key words Flos Lonicerae, Chlorogenic acid, Luteoloside, Content determination

1 Introduction

According to *Chinese Pharmacopoeia*, Flos Lonicerae refers to dried flower buds or early blossoms of *Lonicera japonica* Thunb., *Lonicera hypoglauca* Miq., *Lonicera confusa* DC. or *Lonicera dasystyla* Rehd^[1]. Flos Lonicerae is cold in nature and sweet in taste, with aroma. It can be used to clear heat without hurting the stomach and dispel evil. It is distributed to lung, stomach and large intestine channels, with functions of clearing heat, detoxifying and dispelling wind-heat. It is widely used clinically for sores and carbuncles, pyreticosis and dysentery caused by wind-heat and warm-heat, manifested by wind-heat cold, upper respiratory infection, acute tonsillitis, pharyngitis, dysentery and swelling, and it has a long history of application^[2]. In China, Flos Lonicerae is widely distributed, cultivars in Henan and Shandong and wild species in Sichuan, Guangxi, Jiangxi, Jiangsu, Shaanxi and Gansu^[3]. Flos Lonicerae contains a variety of organic acids, and its main active ingredients are chlorogenic acid compounds, such as chlorogenic acid, isochlorogenic acid, caffeic acid and 3, 5-dicaffeoylquinic acid^[4].

2 Medicinal material, reagents and instruments

2.1 Medicinal material

Flos Lonicerae was collected from the

test sites in Binhai New Area and Jinnan District of Tianjin, and it was identified as the dried flower buds or early blossoms of *L. japonica* Thunb. by the associate professor Pei Yi from College of Horticulture and Landscape Architecture, Tianjin Agricultural University. After dried in an oven at 50-60°C, Flos Lonicerae was pulverized using a multifunctional pulverizer, passed through a 60-mesh sieve, packed and sealed for future use.

2.2 Reagents and instruments The reagents used included methanol (Tianjin Fengchuan Chemical Reagent Technology Co., Ltd.), acetonitrile [chromatographically pure, Concord Technology (Tianjin) Co., Ltd.], chlorogenic acid standard (National Institutes for Food and Drug Control), phosphoric acid (Tianjin Guangxia Fine Chemical Research Institute), ethanol (Tianjin Fengchuan Chemical Reagent Technology Co., Ltd.), luteoloside standard (Chengdu Ruifensi Biotechnology Co., Ltd.) and distilled water.

The apparatus and instruments used included BM255 mixer (Guangdong Midea Fine Electric Appliance Manufacturing Co., Ltd.), 101-2AB electric heating air drying oven (Tianjin Taisite Instrument Co., Ltd.), KQ-300DA digital ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.), METTLER AB54 electronic balance [Mettler Toledo Instruments (Shanghai) Co., Ltd.] and Waters e 2695-2998 high-performance liquid chromatograph (Waters Corporation).

3 Trait observation

External characteristics of Flos Lonicerae randomly collected from Binhai New Area (Binhai No. 1) and Flos Lonicerae randomly collected from Jinnan District at the three green stage (Jinnan No. 2), silver flower stage (Jinnan No. 3) and golden flower stage (Jinnan

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No.4) were observed, and the results were recorded.

4 Methods^[5]

4.1 Content determination of chlorogenic acid in Flos Lonicerae

The chromatographic conditions were as follows: column, BDSHYPERSIL column (5 μ m, 4.6 mm \times 15 mm); mobile phase, acetonitrile (B) – 0.4% phosphoric acid solution (A) (13: 87); detection wavelength, 327 nm; column temperature, 30°C; flow rate, 1.0 mL/min; and injection volume, 10 μ L. The number of theoretical plates should be no less than 1 000 based on the chromatographic peak of chlorogenic acid.

4.1.1 Preparation of reference solution. An accurate amount of chlorogenic acid standard was placed in a brown volumetric flask and dissolved in 50% methanol to prepare into a reference solution of 40 μ g/mL. The reference solution prepared was stored below 10°C.

4.1.2 Preparation of test solution. An accurate amount (around 0.5 g) of the powder of Flos Lonicerae (passed through No. 4 sieve) was placed in a conical flask with a stopper, added with 50 mL of 50% methanol, weighed, sonicated (250 W, 35 Hz) for 30 min, cooled, and weighed again, and the lost weight was supplemented with 50% methanol. The solution was filtered. A certain volume (5 mL) of the subsequent filtrate was collected and diluted to 25 mL with 50% methanol. Thus, the test solution was prepared.

4.1.3 Precision test. The reference solution of chlorogenic acid was detected chromatographically under the conditions described above six times repeatedly at an injection volume of 10 μ L. The peak areas of chlorogenic acid were recorded. The *RSD* value of the peak areas of chlorogenic acid was 0.05%, indicating that the precision of the instrument is good.

4.1.4 Stability test. The test solution of Binhai No. 1 prepared in Section 4.1.2 was detected 0, 2, 4, 8, 12 and 24 h after its preparation, respectively at an injection volume of 10 μ L. The peak areas of chlorogenic acid were recorded. The *RSD* value of the peak areas of chlorogenic acid was 0.09%, indicating that the test solution was stable within 24 h.

4.1.5 Sample determination. The samples of Flos Lonicerae were prepared into test solutions according to the method described above, and the contents of chlorogenic acid were detected by HPLC at an injection volume of 10 μ L.

4.2 Content determination of luteoloside in Flos Lonicerae

The chromatographic conditions were as follows: column, Ultima-TeXB-Phenyl (5 μ m, 4.6 mm \times 250 mm); mobile phase, acetonitrile (A) – 0.5% glacial acetic acid solution (B); detection wavelength, 350 nm; column temperature, 30°C. The number of theoretical plates should be no less than 20 000 based on the chromatographic peak of luteoloside.

4.2.1 Preparation of reference solution. An accurate amount of luteoloside standard was dissolved in 70% ethanol to prepare into a reference solution of 40 μ g/mL.

4.2.2 Preparation of test solution. An accurate amount (around 2 g) of the powder of Flos Lonicerae (passed through No. 4 sieve) was placed in a conical flask with a stopper, and added with an

accurate volume of 70% ethanol, and the lost weight was supplemented. The solution was filtered. A certain volume (10 mL) of the subsequent filtrate was concentrated to dryness, and the residue was dissolved with 70% ethanol to 5 mL. Thus, the test solution was prepared.

4.2.3 Precision test. The reference solution of luteoloside prepared in Section 4.2.1 was detected chromatographically six times repeatedly at an injection volume of 10 μ L. The peak areas of luteoloside were recorded. The *RSD* value of the peak areas of luteoloside was 0.97%, indicating that the precision of the instrument is good.

4.2.4 Stability test. The test solution of Jinnan No. 4 prepared according to the method of Section 4.2.2 was detected 0, 2, 4, 8 and 12 h after its preparation, respectively at an injection volume of 10 μ L. The peak areas of luteoloside were recorded. The *RSD* value of the peak areas of luteoloside was 1.70%, indicating that the test solution was stable within 12 h.

4.2.5 Sample determination. The samples of Flos Lonicerae were prepared into test solutions according to the method described above, and the contents of luteoloside were detected by HPLC at an injection volume of 10 μ L.

5 Results and analysis

The contents of chlorogenic acid and luteoloside in Flos Lonicerae from Binhai New Area and Jinnan District were determined. The results show that the contents of chlorogenic acid in the Flos Lonicerae samples were all higher than 1.5%. The content of chlorogenic acid in Binhai No. 1 was lower than those in the Flos Lonicerae samples from Jinnan District. Jinnan No. 2 had the highest chlorogenic acid content, followed by Jinnan No. 3. The contents of luteoloside in the Flos Lonicerae samples were all above 0.05%. Jinnan No. 3 had the highest luteoloside content, and Jinnan No. 4 had the lowest luteoloside content (Table 1). The contents of chlorogenic acid and luteoloside in the Flos Lonicerae samples all met the standards stipulated by *Chinese Pharmacopoeia*.

Table 1 Contents of chlorogenic acid and luteoloside in the Flos Lonicerae samples

Sample	Chlorogenic acid	Luteoloside	%
Binhai No. 1	3.804	5.530	
Jinnan No. 2	5.507	12.405	
Jinnan No. 3	4.855	14.370	
Jinnan No. 4	4.220	0.917	

6 Discussion

In this experiment, the quality of Flos Lonicerae from Binhai New Area and Flos Lonicerae at only three stages from Jinnan District was evaluated, and the exploration of the production area and the optimal harvest time may have some limitations. In the future, Flos Lonicerae at more stages and more cultivation sites can be collected to conduct a more comprehensive investigation, so as to enhance the scientific and comprehensive nature of the research

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Table 3 Sensory evaluation of wines from different glutinous rice varieties

Variety	Grain morphology	Color	Aroma	Taste	Sensory evaluation score
Jiahua No. 1	Broken and irregular	Milky white	Rice aroma ++, wine aroma +++	Strong	85
Bancangnuo	More complete	Milky white	Wine aroma ++	Strong	86
Zhongshannuo	Complete	Milky white	Wine aroma ++	Strong	83
Hongnuo	More complete	Light yellow ++, clear	Wine aroma +++	Stronger	91
Hexiangnuo	Broken, flocculent precipitation, clear upper layer	Light pink, clear	Wine aroma ++	Strong	82
Haibainuo No. 1	More complete, flocculent precipitation	Light yellow +	Wine aroma ++	Strong	85
Jingnuo No. 6	Broken, flocculent precipitation, agglomerated	Milky white	Wine aroma +++	Strong	82
Nuo S687	Broken, turbid	Light yellow +, clear	Wine aroma ++	Strong	80

3.4 Correlation analysis between chemical composition of glutinous rice and quality of wine brewed The correlations between the main chemical constituents of glutinous rice and the quality of wine are shown in Table 4. The starch content of glutinous rice was negatively correlated with the total acidity, alcohol content and sensory quality and positively correlated with the total residual sugar of wine brewed ($P > 0.05$); and there were positive correlations between protein content of glutinous rice and various indices of wine (total acidity, $P < 0.05$; others, $P > 0.05$). It indicates that the higher the protein content in glutinous rice, the higher the total acidity, which is not conducive to the flavor and stability of rice wine; and the fat content of glutinous rice has a significant negative correlation with alcohol content, and a significant positive correlation with sensory quality of rice wine.

Table 4 Correlation analysis between main chemical constituents of glutinous rice and quality of wine

Main chemical constituents	Total acidity	Alcohol content	Total residual sugar	Sensory score
Starch	-0.376 2	-0.148 3	0.078 5	-0.181 4
Protein	0.664 7*	0.033 0	0.178 3	0.089 0
Fat	0.110 8	-0.694 5*	0.044 8	0.712 7*

4 Conclusions

The main chemical constituents of 8 special glutinous rice varieties, and the main physical and chemical properties and sensory quality of the wines from these glutinous rice varieties were studied, and the correlations between the main chemical constituents of glutinous rice and the quality of wine were analyzed. There were little differences in the contents of main chemical constituents among the special glutinous rice varieties. In the glutinous rice, starch had the highest content, followed by protein, and the con-

tent of fat was extremely low. From week 4 to week 8, the total acidity decreased with time, with an average decrease of 28.60%; the total residual sugar significantly reduced, with an average reduction of 67.33%; and the alcohol content increased significantly, by an average of 15.13%. The correlations between starch content of glutinous rice and physical and chemical indices and sensory quality of wine were small; the protein content of glutinous rice was positively correlated with the physical and chemical indices and sensory quality of wine (total acidity, $P < 0.05$); and the fat content of glutinous rice was significantly negatively correlated with alcohol content, and significantly positively correlated with sensory quality of wine.

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results, provide a reliable basis for the exploration of the optimal harvest time of *Flos Lonicerae* and provide a reference for the reasonable promotion of *Flos Lonicerae* in Tianjin and even China.

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