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Comparative study of galuteolin and chlorogenic acid contents in different germplasms of *Lonicerae Japonicae* flos

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ABSTRACT

Traditional Chinese medicine plays important role in China's public health services. *Lonicerae Japonicae* Flos is one of the often used herbs. The differences of germplasms and planting geographic position of *Lonicerae Japonicae* Flos make its quality uneven. Galuteolin and chlorogenic acid are the major active ingredients of *Lonicerae Japonicae* Flos. In this research, galuteolin and chlorogenic acid contents of ten kinds of *Lonicerae Japonicae* Flos were firstly systematicly measured. The results showed that the contents of galuteolin from 1 to 10 samples were between 0.08% and 0.22%, which were higher than 0.050% of requirement by the pharmacopoeia of China, and the contents of chlorogenic acid from 1 to 10 samples were between 1.94% and 4.00%, higher than 0.05% of requirement by the pharmacopoeia of China. This research is important for quality control of *Lonicerae Japonicae* Flos medicine.

KEYWORDS

Lonicerae Japonicae Flos;
Galuteolin;
Chlorogenic acid;
Traditional chinese medicine;
Germplasms.

INTRODUCTION

The traditional Chinese medical system and the western medical system run parallel in China. There are about 9 hundred million outpatient services of traditional Chinese medical in one year. The annual production of Chinese medicinal herb is about 2.5 million tones. *Lonicerae Japonicae* Flos is the dried buds or nascent open flowers of *Lonicera Japonica* Thunb plants, and the often used traditional Chinese medicine, which has the curative effects of heat-clearing and detoxifying and dispelling wind[1]. The annual production of *Lonicerae Japonicae* Flos is about 10 thousands tones.

The flavonoid compounds is one kind of major active ingredients of *Lonicerae Japonicae* Flos, in which, galuteolin has the inhibition effects to influenza virus NA[2] and K562 cell proliferation[3], and protection effects on adriamycin (ADR) induced myocardial damage[4], and is used as a new index for its quality control. The galuteolin content in *Lonicerae Japonicae* Flos should be higher than 0.05% requested by the pharmacopoeia of China[1].

Chlorogenic acid is another major active ingredients of *Lonicerae Japonicae* Flos, has multiple pharmacological actions, like antioxidation, antibacteria, antivirus, antiinflammatory, anti-liver fibrosis, antitumor, liver protection, and antiendotoxin effects[5-11]. The chlorogenic acid content in *Lonicerae Japonicae* Flos

should be higher than 0.05% requested by the pharmacopoeia of China[1].

In reality, the contents of the major active ingredients of *Lonicerae Japonicae* Flos have large differences for different germplasms and geographic position and growing conditions of plantation. In this research, the major active ingredients of galuteolin and chlorogenic acid of 10 kinds of *Lonicerae Japonicae* Flos will be systematic investigated, which will be benefit for the choice of medicinal material sources, plantation, and seed breeding.

MATERIALS AND METHODS

The germplasms and plantation area of the tested samples of *Lonicerae Japonicae* Flos were listed in TABLE 1. The standards of galuteolin and chlorogenic acid were obtained from national institute for food and drug control of China.

Galuteolin and chlorogenic acid were measured by using Waters-600 HPLC with Waters-996 type detector (Waters Co., USA), and Diamonsil-C_{18} column (150 mm×4.6 mm,5μm) (DikmaTechnologies, China).

In galuteolin measurement, gradient elution was used with the mobile phase A of benzyl cyanide and mobile phase B of 0.5% glacial acetic acid. The elution gradients are as follows, 0-15 minÿA increased from 10 to 20%, B decreased from 90 to 80%; 15-30 min, A 20% and B 80%; 30-40 min, A increased from 20 to 30%, B decreased from 80 to 70%. The mobile phase flow speed of 1.0 mL/min, column temperature of 35ºC, detector wave length of 350 nm and the sample injection volume of 10 μL were used.

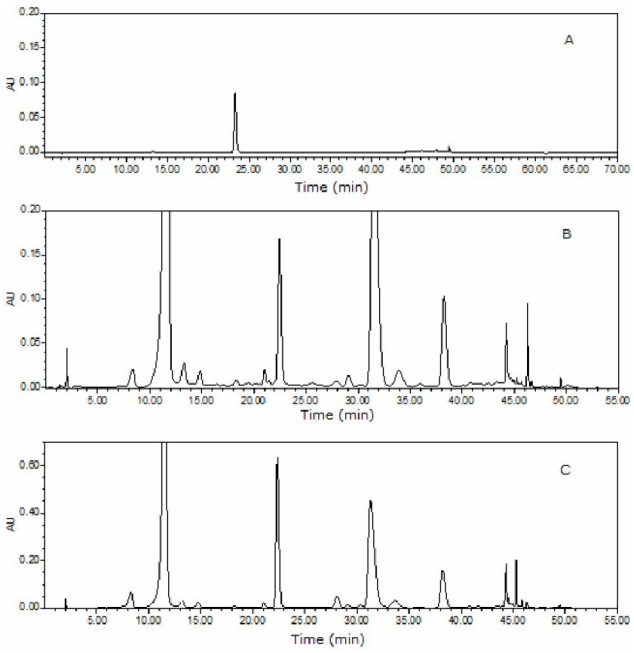
In chlorogenic acid measurement, the mobile phase of methanol- 3% formic acid (1:79), flow speed of 1.0 mL/min, column temperature of 30ºC, detector wave length of 327 nm and the sample injection volume of 10 μL were used.

The galuteolin sample solution was made as follows. *Lonicerae Japonicae* Flos powder of 2 g was accurately measured, droped into 50 mL taper bottle, filled in 50 mL of 70% ethanol. The taper bottle was capped, weighted, sonicated for 1 h, cooled to room temperature, and then, filled in with 70 % ethanol until the weight was the same as formerly weighted. The solution was mixed, filtered using 0.45 μm pore diameter filter and stored for measurements.

The chlorogenic acid sample solution was made as follows. *Lonicerae Japonicae* Flos powder of 0.5 g was accurately measured, droped into 50 mL taper bottle, filled in 50 mL of 50% ethanol. The taper bottle was capped, weighted, sonicated for 30 min, cooled to room temperature, and then, filled with 50% ethanol until the weight was the same as formerly weighted, mixed, filtered using 0.45 μm pore diameter filter. Above solution of 5 mL was taked into 25 mL brown colored volumetric flask, filled in with 5% methnol to 25 mL, mixed, filtered using 0.45 μm pore diameter filter and stored for measurements.

The measurement precision was confirmed as follows. Using the above galuteolin standard solution and measurement conditions, successively measured the galuteolin concentration for 6 times, and the relative standard deviation (RSD) (RSD= (standard deviation/ average)×100%) was calculated to be 2.09%. Using the same method as described above, the RSD for chlorogenic acid measurements was 1.60%.

The measurement constancy was confirmed as follows. The galuteolin or chlorogenic acid concentrations were measured using the same galuteolin or chlorogenic acid standard solution, at 0, 3, 6, 9, 12, 24 h, and calculated the RSD values, which were 1.13% and 0.71% for galuteolin and chlorogenic acid, respectively.



(A), the standard, (B), flower sample (C) leaf sample

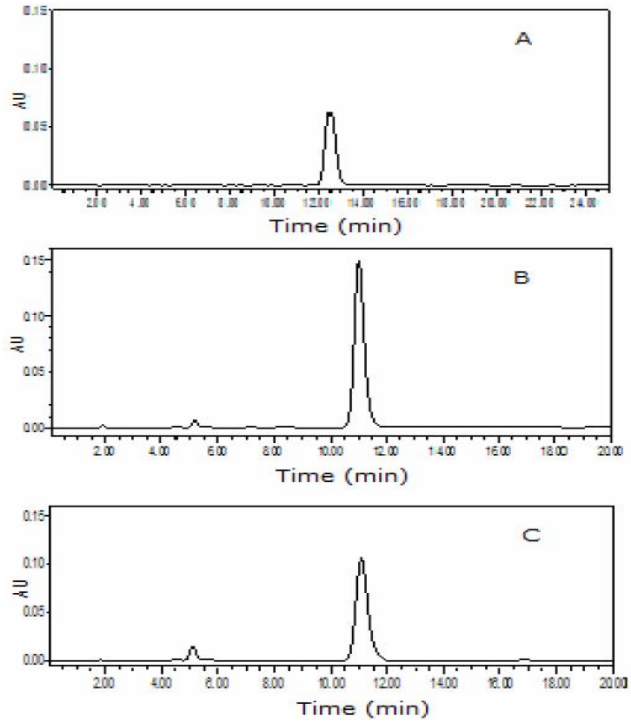
Figure 1 : HPLC measurements of galuteolin.

FULL PAPER

The measurement reproducibility was confirmed as follows. Six samples of galuteolin or chlorogenic acid sample solutions were made, measured, and the RSD values were calculated, which were 1.36% and 0.74% for galuteolin or chlorogenic acid, respectively.

The sample recovery of the measurements was confirmed as follows. Six samples of the galuteolin or chlorogenic acid sample solutions were made, added with some amount of galuteolin or chlorogenic acid standard solution, measured the galuteolin or chlorogenic acid concentrations and calculated the recovery and RSD values. The recovery and RSD values were 99.11% and 2.97% for galuteolin, and 99.11% and 2.97% for chlorogenic acid, respectively.

The measurements of galuteolin and chlorogenic acid were shown in Figure 1 and 2, respectively.



(A), the standard, (B), flower sample (C) leaf sample.

Figure 2 : HPLC measurements of chlorogenic acid

RESULTS AND DISCUSSION

The galuteolin contents of different *Lonicerae Japonicae* Flos germplasms

The results of galuteolin measurements were shown in TABLE 1. The galuteolin contents of 1~10 samples were between 0.08~0.22%, higher than the 0.05% level required by the pharmacopoeia of China[1]. The 9th sample (Italy germplasm) had the highest galuteolin content of 0.22% among the tested *Lonicerae Japonicae* Flos germplasm samples. The 3rd, 5th, 8th had the contents of 0.15, 0.13, and 0.12%, respectively. The lowest was the 4th sample of 0.08%.

The galuteolin contents in the leaf of two germplasm samples (11th and 12th samples) were measured, which were 0.75 and 0.76%, respectively, much higher than that in the flowers.

The top two of the 9th (Italy germplasm) and the 3rd samples were considered the high quality sources for medicine, plantation, and seed breeding uses.

The chlorogenic acid contents of different *Lonicerae Japonicae* Flos germplasms

The results were shown in TABLE 1. The chlorogenic acid contents of 1~10 samples were between 1.94~4%, higher than the 1.5% level required by the pharmacopoeia of China[1]. The top 4 samples of chlorogenic acid content were the 9th (Italy germplasm), 6th, 1st, and 3rd samples, had the chlorogenic acid contents of 4%, 3.74%, 3.67%, and 3.32%, respectively, more than doubled than the pharmacopoeia standard level. The lowest was the 10th sample of 1.94%.

The chlorogenic acid contents of two leaf samples (the 11th and 12th samples) were measured, which were 3.17% and 1.21%, respectively, lower than that in the flowers. The low limit of the pharmacopoeia is 1.5%.

The best *Lonicerae Japonicae* Flos germplasm in view of chlorogenic acid contents was the 9th sample (Italy germplasm). In general, the *Lonicerae Japonicae* Flos germplasms from Pingyi city had higher chlorogenic acid contents (the 1st, 2nd, and 3rd samples in TABLE 1). The chlorogenic acid content of the 9th (Italy germplasm) sample had a chlorogenic acid content as high as 4%.

In addition to germplasm, the plantation geographic position and growing environments also affected the chlorogenic acid contents greatly. For the same germplasm planted in Linyi city had the chlorogenic acid content of 2.56% (7th sample), which was much higher than that of 1.98% planted in Lanzhou city (8th sample) (TABLE 1). The differences in soil, light intensity and duration, temperature, and humidity *et. al.*. It is reasonable to stress the place noted for the medicine, which

TABLE 1 : The contents of galuteolin and chlorogenic acid in *Lonicerae Japonicae* samples measured by HPLC

Samp Index	Serial Number	Germplasms	galuteolin contents(%)	chlorogenic acid contents(%)	Appearance of samples	Place of origin
1	S101201	flower of Lonicera japonica Thunb.	0.12	3.67	yellow white buds	Linyi city
2	S201202	flower of Lonicera japonica Thunb.	0.10	2.84	dull yellow white buds	Shandong province
3	S201203	flower of Lonicera japonica Thunb. cv.Jfyh	0.15	3.32	green young buds	Linyi city
4	S201204	flower of Lonicera japonica Thunb.	0.08	2.85	light green young buds	Fengqiu city
5	S201205	flower of Lonicera japonica Thunb.	0.13	2.28	green young buds	Henan province
6	S201206	flower of Lonicera japonica Thunb. cv.Yateliben	0.11	3.74	yellow white buds	Beijing
7	S201207	flower of Lonicera japonica Thunb. cv.Yate	0.11	2.56	yellow white buds	Rizhao city
8	S201208	flower of Lonicera japonica Thunb. cv.Yate	0.12	1.98	green young buds	Lanzhou city
9	S201209	flower of Lonicera japonica Thunb. f.Ytl	0.22	4.00	light yellow buds	Rizhao city
10	S201210	flower of Lonicera japonica var.chinensis	0.11	1.94	purple-lilac flowers	Rizhao city
11	S201211	leaf of Lonicera japonica Thunb. f.Ytl	0.75	3.17	green leaf	Rizhao city
12	S201212	leaf of Lonicera japonica Thunb. cv.Yate.	0.76	1.21	green leaf	Rizhao city

is called the genuine regional medicine.

The 11th sample was the diploid natural mutant, had the advantages of higher adaptivity, drought, waterlogging and cold tolerances, and multiple uses in medicine, ornamental, and water and soil conservation[12]. It had the galuteolin content of 0.11%, conformed to the pharmacopoeia of China[1], was the best choice for the muticle purposes of its uses.

In addition, *Lonicerae Japonicae* Folium was not yet the medicinal parts even if it had higher content of active ingredients. The total flavone content in the leaf was reported much higher than that in the flower[13], and many kinds of active compounds were contained in the leaf[14]. The leaf extracts had the antibacterial activity to *Escherichia coli*, *Bacillus subtilis*, *Bacillus breslaviensis*, *Penicillium*, *Aspergillus niger*, *Aspergillus flavus*, and *Saccharomyces cerevisiae*[15]. The flavones components of the leaf had 4 and 2 times higher antibacterial activity to *Staphylococcus aureus* and *E. coli*, respectively, compared with chlorogenic acid[16]. The leaf of *Lonicerae Japonicae* Flos needs to be further researched for medicinal part uses in order to make full use of *Lonicerae Japonicae* Flos resources and increase the herbal medicine sources.

In conclusion, galuteolin and chlorogenic acid contents of 10 kinds of *Lonicerae Japonicae* Flos germplasms were analyzed. The optimzal germplasms were determined. In addition, *Lonicerae Japonicae* Folium was regarded potential being used as the medicinal part.

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FULL PAPER

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