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Analysis of main nutrient components in leaves and buds of three kinds of *Lonicera confusa* varieties (lines)

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**Abstract.** On *Lonicera macranthoides* varieties (lines) 'red bud 1', 'silver Cui Lei' and 'Cui Lei 109' flower buds and leaves of different maturity, the soluble sugar, protein, amino acid and Vc were analyzed. The results showed that the buds and leaves of 'red bud 1', 'silver Cui Lei' and 'Cui Lei 109' were rich in nutrients. The buds and leaves of 'Red bud 1' were rich in nutrients, and the contents of protein in the leaves of 'Cui Lei 109' and 'silver Cui Lei' were higher.

1. Introduction

Honeysuckle, also known as *Lonicera japonica* Thunb., is a semi-evergreen shrub or vine of *Lonicera japonica*. In PRC codex, it is mentioned that the *Lonicera confusa* is the dry flower bud or the initial flower of *Lonicera japonica*, including *Lonicera macranthoideoni* Hand.-Mazz., *Lonicera hypoglauca* Miq., *Lonicera confusa* DC.[1]. *L. confusa* has the functions of clearing heat and detoxifying, and can be used for treating fever, blood stasis and other symptoms. In addition, it has a strong inhibitory effect on thromboxane biosynthesis and hydroperoxide-induced endothelin fragile damage[2]. The main components of *L. confusa* are volatile oil, chlorogenic acid, luteolin and various amino acids[3-5], as well as soluble sugars. According to research, polysaccharides have antibacterial, antifungal, immune-enhancing, anti-tumor[6] and other biological activities[7-8]. The process of opening *L. confusa* can be divided into flower bud stage (green flower bud, length about 1 cm), Sanqing period (green flower bud, length 2.2~3.4 cm), two white stage (light white flower bud, length 3.0~3.9 cm), large white period (white flower buds, length 3.6~4.6 cm), silver flower stage (white flower buds that have just opened in the big white period, length 4.2~4.8 cm), golden flower stage (2 to 3 hours after silver flower opening, the color turns yellow into golden flower stage, long 4.0~4.5 cm).

'Red bud 1', also known as 'Lu lei No.1', is an ornamental, tea drink and medicinal *L. confusa* variety selected by Shandong. Its flower buds, leaves and stems are all red, very beautiful, and the ornamental value is extremely high. With its flower bud soaked in water, it has a sweet pink color and a rich aroma. 'Silver Cui Lei' is a medicinal and tea drink species selected from Hunan. The buds of the medicinal and tea-flavored *L. confusa* variety 'Yu lei No.1' selected by Chongqing are large, with high yield and good quality. 'Cui Lei 109' is an excellent line selected from the introduction of the variety “Yu lei No.1” in Muchuan County. The parental source of ‘red bud 1’, ‘silver Cui lei’ and 'Yu lei No.1' are the same *L. japonica* Thunb.. According to 'Lonicera confusa 2010 Chinese Pharmacopoeia Quality Standard', *L. macranthoides* belongs to *L. confusa*.

Most of the researches on *L. confusa* are based on their flower buds, and the studies on the chemical constituents of *L. confusa* mainly focus on chlorogenic acids, flavonoids, triterpenoid saponins and volatile oils[9-10]. There were few studies on the nutrients such as polysaccharides,
amino acids and proteins in leaves and flower buds in different periods. Therefore, this experiment intended to use the three L. confuse varieties ‘red bud 1’, ‘silver Cui lei’ introduced from Shandong and Hunan by Yizhichuncha Co., Ltd., and “Cui lei 109”, which was the mutant strain of ‘Yu lei No.1’ from Chongqing in Muchuan. The main nutrients such as Vc, soluble sugar, amino acid and protein in different flowering buds and different maturity leaves were analyzed, and provide basis for development and utilization of L. confuse.

2. Materials and methods

2.1. Materials
This experiment used the three L. confuse varieties ‘red bud 1’, ‘silver Cui lei’ introduced from Shandong and Hunan by Yizhichuncha Co., Ltd. and “Cui lei 109”, which was the mutant strain of ‘Yu lei No.1’ from Chongqing in Muchuan.

The flower bud materials were taken from the four flowering stages of Sanqing period, large two white stage, silver flower stage and golden flower stage. The leaves were sampled into the old leaves on the 2-year-old branches, and the functional mature leaves and young leaves in the same year. Each sample was taken from the upper, middle and lower parts of 20 branches of 3-5 trees of the same variety (lines), and the flower buds and leaves of uniform size were picked and brought back to the laboratory with an ice box. The test samples were dried at 60°C. To the constant quality, it is naturally dried after being taken out, and then crushed, passed through a 60 mesh sieve, and used. Fresh samples should be stored in a refrigerator at -20°C.

2.2. Measuring indicators and methods
The soluble sugar content was determined by the phenol-sulfuric acid method[11]. The content of protein in flower buds and leaves was determined by Coomassie Brilliant Blue method[11]. The amino acid content was determined using the ninhydrin colorimetric method[12]. The Vc content was determined by the 2,6-dichlorophenol indophenol method[13].

3. Results and analysis

3.1. Soluble sugar content in flower buds and leaves
It can be seen from Table 1 that with the development of flower buds, the content of soluble sugar in flower buds increased first and then decreased, but the variation rules among varieties (lines) were inconsistent. ‘Silver CuiLei’ and “CuiLei 109” both had the highest total sugar during the silver flower period, while the ‘red bud 1’ flower bud was the highest in the large two white period. Among them, the 'Silver Cui Lei’ flower bud had the highest total sugar content, and the 'red bud 1’ had the lowest.

| Variety(line) | Sanqing period | Large two white period | Silver flower stage | Golden flower stage |
|---------------|----------------|------------------------|---------------------|---------------------|
| Red bud 1     | 13.60          | 15.08                  | 14.09               | 14.38               |
| Cui lei 109   | 13.11          | 14.05                  | 15.78               | 14.83               |
| Silver cui lei| 12.82          | 15.52                  | 15.99               | 15.09               |

It can be seen from Fig. 1 that the soluble sugar content of 'Red bud 1' leaves was the highest and stable, while the "Cui lei 109" leaves had the lowest soluble sugar content, but the soluble sugar content of the two leaves had the same trend old leaves > mature leaves> young leaves, the soluble sugar content of 'silver cui lei' leaves was middle, but the difference was large. The highest soluble sugar content was young leaves, followed by old leaves and the lowest was mature leaves. Therefore, the soluble sugar-rich variety was 'red bud 1', and the suitable picking period of soluble sugar in the leaves of the honeysuckle was old leaves.

2
Flower buds and leaf protein content

It can be seen from Table 2 that the protein of “Cui lei 109” was the highest and stable in the whole flowering period, but the overall trend was downward, while the ‘silver Cui lei’ was generally on the rise, only the large two white period was bigger than silver flower stage. The protein of ‘red bud 1’ varied greatly throughout the flowering period, and the protein content of the large two white period was the highest among the four periods of the three L. confusa varieties, but the protein content of the silver flower stage was the lowest. Therefore, “Cui lei 109” was the best protein content of the three varieties, and the suitable flowering period of the protein in L. confusa was the large two white period.

### Table 2. Protein content of flower buds at different developmental stages. (unit: %)

| Variety (line) | Sanqing period | Large two white period | Silver flower stage | Golden flower stage |
|---------------|----------------|-------------------------|---------------------|---------------------|
| Red bud 1     | 12.45          | 15.81                   | 8.66                | 11.62               |

**Figure 1. Leaf soluble sugar content**

**Figure 2. Leaf protein content**
It can be seen from Fig. 2 that in 'red bud 1', the protein content of the young leaves was the highest in the three leaf stages, but in 'Cuilei 109' and 'silver Cuilei' it was the lowest. The mature leaf protein content of bud 'cuilei 109' and 'silver cuilei' was the highest in the three leaf stages, but in 'red bud 1' mature leaves it was the lowest, the old leaves of the three varieties were all centered. Therefore, according to the overall situation, the protein content of 'red bud 1' was the highest among the three varieties of L. confusa, and the picking period of the leaves of L. confusa was in the old leaves.

3.3. Flower buds and leaf amino acids

It can be seen from Table 3, the amino acid content of red bud 1' had a small change in the whole flowering period, which was relatively stable, and the overall amino acid content was high. The overall amino acid content of "Cuilei 109" was low, the amino acid content of 'silver cuilei' bud's varies greatly throughout the flowering period, and its value was unstable, but its amino acid content in the sanqing period and silver flower stages was high. In summary, the best variety of the overall amino acid content was 'silver Cuilei', and the suitable harvesting period of amino acids in the mountain silver flower was two flowering periods of Sanqing period and silver flower period.

| Variety(line) | Sanqing period | Large two white period | Silver flower stage | Golden flower stage |
|--------------|----------------|------------------------|---------------------|---------------------|
| Red bud 1    | 15.81          | 16.06                  | 15.59               | 14.74               |
| Cuilei 109   | 12.62          | 11.39                  | 13.45               | 14.02               |
| Silver cuilei| 16.96          | 9.96                   | 16.73               | 14.74               |

It can be seen from Fig. 3 that the amino acid contents of the three leaf cultivars (lines) varied greatly, but the overall amino acid content of 'silver Cuilei' was the highest. 'Red bud 1' and 'silver cuilei' had the highest content of mature leaves and the lowest content of young leaves, and the content of old leaves was middle. With the growth of leaves, the lower the amino acid content of "Cuilei 109", that was, young leaves>mature leaves>old leaves. Therefore, the variety of amino acids in the leaves was 'silver Cui Lei', and the picking leaf stage of the leaves of L. confusa was the mature leaf stage.

3.4. Flower buds and leaf Vc content

The determination of Vc was carried out by using the large two white period to measure its content. The leaves were selected from mixed leaves (old leaves, mature leaves and young leaves) to
comprehensively measure the content, and the *L. confusa* varieties with rich Vc content were analyzed. The results were shown in Table 4. In the average Vc content of the large two white period, "Cuilei 109" had the highest Vc content, followed by 'red bud 1', while 'silver Cuilei' was the lowest; in the mixed leaf Vc among the average content, “Cuilei 109” had the highest Vc content, 'red bud 1' was in the middle, and the lowest was 'silver Cuilei'. In summary, the overall content of the leaves of “Cuilei 109” was the highest, and the Vc content of the large two white period was also the highest, that was, 'Cuilei 109' was the most abundant variety of Vc in the three varieties of *L. confusa* (lines).

Table 4. Amino acid content of flower buds at different developmental stages. (unit: mg/g)

| Variety(line) | Large two white period | Mixed leaf |
|--------------|------------------------|-----------|
| Red bud 1    | 0.25                   | 0.29      |
| Cui lei 109  | 0.35                   | 0.39      |
| Silver cui lei | 0.15                   | 0.28      |

4. Conclusion

Studies have shown that the content of active constituents of honeysuckle and *L. confusa* in different flowering periods was quite different. In the past, it was reported in the literature that the difference in chlorogenic acid and flavonoids was used as an indicator to determine the optimal harvesting period [14-15]. The results of this experiment showed that the 'Red bud 1', 'silver Cuilei' and 'Cuilei 109' flower buds and leaves were rich in various nutrients, and the physiologically active substance chlorogenic acid should be combined with the main nutrients such as soluble sugar, protein, amino acid and Vc to determine the best harvesting period. The best harvesting period of flower buds was Sanqing period and large two white period, was consistent with the description of '2010 Chinese Pharmacopoeia on *L. confusa*' and literature analysis and the harvesting time in actual production. For leaves used as tea, the functional leaves that were mature in the current year should be selected. 'Red bud 1' flower buds and leaves were rich in nutrients. The flower buds and leaves of "Cuilei 109" contained higher protein, and the amino acid content of 'silver Cuilei' flower buds and leaves was higher.

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