Analysis of bioactive substances and antioxidant activity in leaves and flower buds of *Flos Lonicerae*

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Abstract. In this experiment, the main bioactive substances and antioxidant activities of flower buds and leaves in different developmental stages were measured with three *Flos Lonicerae* varieties (lines) 'Hong Lei Yi Hao', 'Yin Cui Lei' and "Cui Lei 109" as materials, and their correlation was analyzed. The results showed that the polyphenols and flavonoids gradually decreased and then increased with the growth and development of leaves, and they were the highest in young leaves, followed by old leaves. The Vitamin c (Vc) content of the three varieties (lines) was the highest in old leaves, followed by young leaves, and there was no significant difference among the varieties (lines). The polyphenol content and flavonoid content in *Flos Lonicerae* were positively correlated with DPPH free radical scavenging rate, while the correlation between Vc content and antioxidant activity was low or negatively correlated.

1. Introduction

*Flos Lonicerae* refers to Lonicera macranthoides Hand.-Mazz., Lonicera hypoglauca Miq., Lonicera confusa DC. or Lonicera fulvotomentosa Hsuet S.C. Cheng) dried flower buds or flowers with initial opening [1]. Lonicera has a long history as a commonly used Chinese medicinal material. The chemical components contained in this genus are complex, mainly including organic acids, flavonoids, triterpenoid saponins and volatile oils [2], with high medicinal value [3-4]. At present, the research on *Flos Lonicerae japonicae* is relatively early and comprehensive. From the research results on *Flos Lonicerae*, the efficacy ingredients are basically similar to *Flos Lonicerae japonicae*, and the content of multiple components is significantly higher than that of *Flos Lonicerae japonicae* [5-6], which pharmacological effect is not inferior to *Flos Lonicerae japonicae*. In summary, *Flos Lonicerae* has great potential for future medicinal and tea cultivation [7].

At present, polyphenols, flavonoids and Vitamin c (Vc) are generally considered to be the main bioactive substances in *Flos Lonicerae* [3-4], and the correlation between these components and the antioxidant activity of *Flos Lonicerae* has not been further studied. The experiment uses 'Hong Lei Yi Hao', 'Yin Cui Lei' and "Cui Lei 109" as materials to compare the main bioactive substances and antioxidant activities of leaves and flower buds of three varieties (lines). The experiment aims to provide basic data and reference basis for the evaluation of health function of the leaves and flower buds, breeding of new varieties and the efficient use of resources.

2. Materials and methods

2.1. Experiment materials
The experimental materials are 'Hong Lei Yi Hao', 'Yin Cui Lei' and "Cui Lei 109". The row spacing of the plants was 1.0m×1.6m, and 420 plants were planted in acre. Plant soil, fertilizer, water management and pest control are routinely managed.

2.2. Sample collection and Processing
In the flowering period of *Flos Lonicerae*, three trees of each variety (lines) with good growth and consistent growth were selected as a sampling plant, which were repeated 3 times. Buds (2.5 cm-3 cm long, swollen white on the top, greenish on the base), young leaves (1-2 nodes at the top of the original shoot tip), extended leaves (5-6 nodes at the middle of the original shoot tip), mature leaves (9-10 nodes at the bottom of the original shoot tip) and old leaves (5-6 nodes at the middle of the perennial shoot tip) were picked. Young leaves and flower buds of *Flos Lonicerae* are rich in phenolic substances, leading to the tendency of browning in normal temperature after harvest[8]. Therefore, samples were collected around 8:00 a.m. on a cloudy day, and were taken back to the laboratory immediately with an ice box. It is dried by microwave for 30s-90s, then dried in an oven at 60 °C to constant weight, pulverized, and passed through a 60 mesh sieve for determination of total flavonoids and polyphenol content. After fresh liquid nitrogen treatment, it was stored in a -80 °C ultra-low temperature freezer for analysis of sample Vc content and antioxidant enzyme activity.

2.3. Experiment methods
Polyphenols was determined by the optimized ethanol extraction method and content determination method such as Zhang [9]. Flavonoids was extracted and measured by Song [10]. Vc was determined by the 2, 6-dichlorophene indophenol method [11]. The determination of DPPH free radical scavenging capacity refered to the method of Yang [12]. Determination of antioxidant enzyme activity [13]: The determination of superoxide dismutase (SOD) activity was performed using the reduction method of NBT. The activity of peroxidase (POD) was determined by guaiacol method. The activity of catalase (CAT) was determined by ultraviolet absorption.

2.4. Statistical Analyses
Data statistics and correlation tests were performed with Microsoft Excel 2010, and variance analysis and significance tests were performed with SPSS 22.0. The differences between the varieties (lines) was marked with different lowercase letters \((p < 0.05)\), and the data in the table were represented by mean ± standard deviation.

3. Results

3.1. Polyphenol content of leaves
The polyphenol content in the leaves of three varieties (lines) showed a trend of gradually decreased and then increased with the growth and development (table1). The content of polyphenols in the three varieties (lines) was highest in young leaves, followed by old leaves, and lowest in mature leaves. The content of 'Yin Cui Lei' polyphenols in young leaves was the highest, which was 12.73% higher than that of 'Hong Lei Yi Hao' \((p < 0.05)\). The polyphenols content of "Cui Lei 109" in old leaves was the highest, 11.63% \((p < 0.05)\) and 16.52% \((p < 0.05)\), respectively, than 'Hong Lei Yi Hao' and 'Yin Cui Lei'.

3.2. Flavonoids content of leaves
The variation of flavonoids content in leaves of *Flos Lonicerae* was consistent with the change of polyphenol content (table 2). With the growth and development of leaves, the flavonoids content in leaves gradually decreased and then increased. The flavonoid content of "Cui Lei 109" in young leaves was higher than that of 'Yin Cui Lei' and 'Hong Lei Yi Hao', respectively, which was 63.86% \((p < 0.05)\) and 87.44% \((p < 0.05)\). The flavonoids content of "Cui Lei 109" in the mature leaves and the old
leaves was the highest, and the content of flavonoids in the old leaves was significantly higher than that of 'Yin Cui Lei' and 'Hong Lei Yi Hao'.

### Table 1. Polyphenol content of leaves.

| Varieties (lines) | Young leaves (mg/g) | Extended leaves (mg/g) | Mature leaves (mg/g) | Old leaves (mg/g) |
|-------------------|---------------------|------------------------|----------------------|-------------------|
| "Cui Lei 109"     | 220.75 ± 3.12 a     | 153.35 ± 3.60 a        | 124.59 ± 1.42 b      | 196.03 ± 4.79 a   |
| 'Yin Cui Lei'     | 228.21 ± 1.56 a     | 156.59 ± 4.01 a        | 146.32 ± 1.47 a      | 168.23 ± 2.43 b   |
| 'Hong Lei Yi Hao' | 195.82 ± 1.98 b     | 159.71 ± 2.45 a        | 145.43 ± 3.15 a      | 175.60 ± 2.95 b   |

### Table 2. Flavonoids content of leaves.

| Varieties (lines) | Young leaves (mg/g) | Extended leaves (mg/g) | Mature leaves (mg/g) | Old leaves (mg/g) |
|-------------------|---------------------|------------------------|----------------------|-------------------|
| "Cui Lei 109"     | 50.29 ± 0.96 a      | 24.38 ± 0.98 a         | 20.08 ± 0.76 a       | 31.71 ± 1.08 a    |
| 'Yin Cui Lei'     | 30.69 ± 1.02 b      | 24.35 ± 0.64 a         | 19.85 ± 0.35 a       | 20.60 ± 0.94 b    |
| 'Hong Lei Yi Hao' | 26.83 ± 0.54 c      | 24.60 ± 0.56 a         | 19.37 ± 0.12 a       | 19.78 ± 0.37 b    |

3.3. Vc content of leaves

The variation of Vc content in the leaves of the three varieties was consistent with that of polyphenol content and flavonoid content (table 3). The Vc content in the leaves gradually decreased with the growth and development, and increased, and the content in the old leaves reached the maximum. Compared with "Cui Lei 109" and 'Hong Lei Yi Hao', the content of Vc in the young leaves, extended leaves and old leaves of 'Yin Cui Lei' were the highest. Compared with "Cui Lei 109" and 'Yin Cui Lei', the content of Vc in the mature leaves of 'Hong Lei Yi Hao' was the highest at 1848.45 mg/100g, but the difference did not reach a significant level.

### Table 3. Vc content of leaves.

| Varieties (lines) | Young leaves (mg/100g) | Extended leaves (mg/100g) | Mature leaves (mg/100g) | Old leaves (mg/100g) |
|-------------------|------------------------|--------------------------|------------------------|---------------------|
| "Cui Lei 109"     | 1726.68 ± 8.76 a       | 1621.28 ± 8.65 a         | 1393.24 ± 6.91 a       | 1815.54 ± 5.94 a    |
| 'Yin Cui Lei'     | 1801.35 ± 5.98 a       | 1693.91 ± 9.04 a         | 1405.21 ± 13.42 a      | 2001.35 ± 10.71 a   |
| 'Hong Lei Yi Hao' | 1650.42 ± 6.04 a       | 1479.7 ± 8.34 b          | 1484.45 ± 8.75 a       | 1889.86 ± 9.47 a    |

### Table 4. SOD activity of leaves.

| Varieties (lines) | Young leaves (U/g. min) | Extended leaves (U/g. min) | Mature leaves (U/g. min) | Old leaves (U/g. min) |
|-------------------|-------------------------|---------------------------|--------------------------|----------------------|
| "Cui Lei 109"     | 242.77 ± 1.07 a         | 259.24 ± 2.59 c           | 163.81 ± 2.01 c          | 271.39 ± 1.37 b      |
| 'Yin Cui Lei'     | 195.71 ± 1.25 c         | 472.22 ± 3.06 a           | 341.79 ± 1.93 a          | 350.72 ± 3.06 a      |
| 'Hong Lei Yi Hao' | 225.89 ± 1.63 b         | 299.90 ± 2.43 b           | 188.32 ± 0.97 b          | 262.54 ± 0.93 b      |

3.4. SOD activity of leaves

The activity of SOD in the leaves of three varieties (lines) showed a trend of increasing first, then decreasing and then rising, and reached the highest value in the unfolded leaves (table 4). The SOD activity of the young leaves of "Cui Lei 109" was significantly higher than that of 'Yin Cui Lei' and 'Hong Lei Yi Hao', which were 24.05% \((p < 0.05)\) and 7.47% \((p < 0.05)\), respectively. The highest levels of SOD in extended leaves, mature leaves and old leaves were 'Yin Cui Lei', respectively, higher than 'Cui Lei 109' \((p < 0.05)\), 108.65% \((p < 0.05)\) and 29.23% \((p < 0.05)\), higher than 'Hong Lei Yi Hao' 57.46% \((p < 0.05)\), 82.16% \((p < 0.05)\), 1.49% \((p < 0.05)\) and 33.59% \((p < 0.05)\).

3.5. POD activity of leaves

The changes of POD activity in the leaves of "Cui Lei 109" and 'Yin Cui Lei' were both the first to increase the thickness and then increase, and reached the maximum in the "Cui Lei 109" old leaves and
the 'Yin Cui Lei' leaves (table 5). The trend of POD activity of 'Hong Lei Yi Hao' first increased and then decreased, and reached the maximum in mature leaves. The POD activity of the young leaves was the lowest in all four developmental stages. Compared with POD activities of "Cui Lei 109" and 'Hong Lei Yi Hao', 'Yin Cui Lei' was the lowest in all four developmental stages, and the differences reached a significant level.

### Table 5. POD activity of leaves.

| Varieties (lines) | Young leaves (U/g. min) | Extended leaves (U/g. min) | Mature leaves (U/g. min) | Old leaves (U/g. min) |
|-------------------|-------------------------|---------------------------|--------------------------|----------------------|
| "Cui Lei 109"     | 570.00 ± 3.59 a         | 1380.00 ± 5.48 a          | 903.33 ± 2.01 a          | 1515.02 ± 5.09 a     |
| 'Yin Cui Lei'     | 135.00 ± 2.06 c         | 153.34 ± 1.03 c           | 136.25 ± 1.03 b          | 140.00 ± 1.37 c      |
| 'Hong Lei Yi Hao' | 183.33 ± 1.02 b         | 446.67 ± 2.95 b           | 1016.67 ± 4.08 a         | 653.33 ± 2.04 b      |

### Table 6. CAT activity of leaves.

| Varieties (lines) | Young leaves (U/g. min) | Extended leaves (U/g. min) | Mature leaves (U/g. min) | Old leaves (U/g. min) |
|-------------------|-------------------------|---------------------------|--------------------------|----------------------|
| "Cui Lei 109"     | 1272.01 ± 4.51 a        | 1603.82 ± 9.54 a          | 1159.26 ± 4.86 a         | 1404.01 ± 6.43 a     |
| 'Yin Cui Lei'     | 561.62 ± 1.34 b         | 142.86 ± 0.46 c           | 841.38 ± 2.17 b          | 947.43 ± 1.37 b      |
| 'Hong Lei Yi Hao' | 174.04 ± 1.02 c         | 547.59 ± 3.01 b           | 104.40 ± 0.76 c          | 360.01 ± 1.21 c      |

3.6. CAT activity of leaves

The CAT activity of the leaves of "Cui Lei 109" and 'Hong Lei Yi Hao' increased first, then decreased, and then increased, and they both reached the maximum in the extended leaves (table 6). 'Yin Cui Lei' was the trend that first declined and then rose, and reached the maximum in the old leaves. Compared with the CAT activities of 'Yin Cui Lei' and 'Hong Lei Yi Hao' leaves, the CAT activity of the four developmental stages of "Cui Lei 109" was the largest, and the difference reached a significant level.

3.7. The contents of polyphenols, flavonoids and Vc and the activities of POD, SOD and CAT in Flower buds

Among the buds of *Flos Lonicerae*, the highest content of flavonoids and Vc was 'Yin Cui Lei', while the activity of SOD and POD in antioxidant enzymes in 'Yin Cui Lei' was also the strongest, and the difference with the other two varieties reached a significant level (table 7). The polyphenol content of "Cui Lei 109" was the highest among the three varieties, which was higher than "Cui Lei 109" 17.83% ($p < 0.05$) and 'Hong Lei Yi Hao' 6.84% ($p < 0.05$). For CAT activity, the flower bud of 'Hong Lei Yi Hao' was higher than "Cui Lei 109": 17.29% ($p < 0.05$) and 2.45% ($p < 0.05$).

### Table 7. The contents of polyphenols, flavonoids and Vc and the activities of POD, SOD and CAT in flower buds.

| Varieties (lines) | Polyphenol content (mg/g) | Flavonoid Content (mg/g.) | Vc content (mg/100g) | SOD activity (U/g. min) | POD activity (U/g. min) | CAT activity (U/g. min) |
|-------------------|--------------------------|---------------------------|----------------------|-------------------------|------------------------|------------------------|
| "Cui Lei 109"     | 173.41±2.03 a            | 22.94±1.01 c              | 237.46±2.01 b        | 434.17±2.07 b          | 142.8±1.54 b            |
| 'Yin Cui Lei'     | 147.17±2.50 c            | 60.46±2.05 a              | 2437.16±4.81 a       | 329.55±1.25 a          | 608.33±1.09 a           | 48.6±0.99c             |
| 'Hong Lei Yi Hao' | 162.31±3.62 b            | 41.43±1.97 b              | 650.02±1.06 c        | 216.34±1.58 b          | 87.96±2.00 b            | 167.49±0.93 a          |

3.8. DPPH free radical scavenging rate of leaves and flower buds

The order of antioxidant activity of the three varieties (lines) of *Flos Lonicerae* flower buds was: "Cui Lei 109" > 'Hong Lei Yi Hao'> 'Yin Cui Lei', but the difference was not significant (table 8). The antioxidant activity of the leaves of *Flos Lonicerae* decreased gradually with the growth and development of leaves. Among the three varieties (lines) of the leaves of *Flos Lonicerae*, "Cui Lei 109" had higher antioxidant activity than four periods of 'Yin Cui Lei' and 'Hong Lei Yi Hao'.
### Table 8. DPPH free radical scavenging rate of leaves and flower buds.

| Varieties (lines) | Flower buds (%) | Leaves |       |       |       |
|-------------------|------------------|--------|-------|-------|-------|
|                   |                  | Young leaves (%) | Extended leaves (%) | Mature leaves (%) | Old leaves (%) |
| "Cui Lei 109"     | 74.41 ± 1.06a    | 78.89 ± 1.03a   | 75.02 ± 1.35a   | 72.30 ± 0.64a    | 71.29 ± 1.01a |
| ’ Yin Cui Lei ‘    | 68.15 ± 0.69a    | 77.04 ± 0.54a   | 72.13 ± 1.07a   | 70.51 ± 0.68a    | 69.78 ± 0.93a |
| ’ Hong Lei Yi Hao ‘| 72.01 ± 0.94a    | 71.14 ± 0.69b   | 66.54 ± 0.49b   | 64.57 ± 0.55b    | 63.95 ± 0.48b |

### Table 9. Correlation between bioactive substance content and antioxidant activity in leaves and flower buds.

| Index                                   | DPPH free radical scavenging rate | Polyphenol content | Flavonoid content | Vc content |
|-----------------------------------------|-----------------------------------|--------------------|------------------|------------|
| DPPH free radical scavenging rate       | 1                                 | 0.2556             | -0.1962          | 0.0785     |
| Polyphenol content                      |                                   |                    |                  |            |
| Flavonoid content                       | 0.4778                            | 0.7796             |                  |            |
| Vc content                              | -0.0432                           | 0.0785             |                  |            |

3.9. Correlation between bioactive substance content and antioxidant activity in leaves and flower buds

Polyphenols and flavonoids were positively correlated with DPPH free radical scavenging rate (table 9). Vc content was negatively correlated with DPPH free radical scavenging rate.

4. Discussion

This study founded that there were some differences in the changes in the content of bioactive substances in the leaves of different varieties (lines). Polyphenols and flavonoids gradually decreased and then increased with the growth and development of leaves, and were the highest in young leaves, followed by old leaves. The content of Vc in the three varieties (lines) was the highest in the old leaves, followed by the young leaves. The study founded that the bioactive substances and antioxidant activities in young leaves and old leaves of Flos Lonicerae were higher than flower buds.

During the young leaf period, the plants grow vigorously, the activity of related enzymes is higher, and the synthesis of polyphenols and flavonoids is promoted [14]. Then, as the plant tissues mature and senescence, the bioactive substances are decomposed or transferred, and the antioxidant activity is correspondingly reduced. The results showed that the polyphenol content, flavonoid content and DPPH free radical scavenging rate were positively correlated, while the correlation between Vc content and antioxidant activity was small or negatively correlated due to its buds and leaves. The main role of the anti-oxidation activity is chlorogenic acid [1].

5. Conclusion

In summary, the content of bioactive substances in the leaves was higher than the content of bioactive substances in the flower buds in a certain growth period. The medicinal function and health value of the leaves of Flos Lonicerae were not inferior to the buds. The antioxidant activity of the flower buds and leaves of Flos Lonicerae were affected by the content of bioactive substances, but polyphenols, flavonoids and Vc were not bioactive substances mainly related to antioxidant activity. The specific substances related to antioxidant activity of Flos Lonicerae and their correlation still need further study.

Acknowledgments
This work financially supported by the National Innovation Training Program 'Analysis of Active Matter Content and Antioxidant Activity of Honeysuckle in Muchuan County'. And thanks to Sichuan Yizhichuncha Co., Ltd. for providing experimental materials.

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