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Analysis of antioxidant compounds and antioxidant activities from three materials of Lonicera

Guojing Cai¹, Qunxian Deng¹*, Dongmei Li¹, Lamei Wang¹ and Yaqian Sun¹

¹ College of Horticulture, Sichuan Agricultural University, Chengdu, Sichuan, 610000, China

*Corresponding author’s e-mail: 1324856299@qq.com

Abstract. The buds and leaves of 3 varieties (lines) of Lonicera were use d as the materials to measure the contents of total phenols, flavone, anthocyanin, luteoloside and chlorogenic acid, at the same time, to analysis and compare the capacity of flower buds of scavenging DPPH, O₂⁻ and ·OH to the leaves. The results showed that the contents of total phenolics in the leaves of 3 varieties (lines) were the highest, and the content of anthocyanins was the lowest. The content of total phenols, flavone, anthocyanin, luteoloside in leaves were higher than that of in flower buds; The capacity of flower buds and leaves of scavenging DPPH and O₂⁻ was higher than the capacity of scavenging ·OH. "Cuilei 109" buds and leaves had the highest antioxidant compounds and antioxidant activities.

1. Introduction

Honeysuckle is also known as Lonicera japonica (Lonicera japonica Thunb. ), half-evergreen shrub or vine of the genus Lonicera [1]. In the description of the Pharmacopoeia, honeysuckle is the blooming flower or dried flower bud of Lonicera japonica, including of Lonicera macranthoides Hand. -Mazz., Lonicera hypoglauca Miq., Lonicera confuse DC. and Lonicera fulvotomentosa HsuetS.C.CCheng[1]. It has the efficacy of anti-oxidation, anti-virus, heat-clearing and detoxification, etc, whose active components are mainly polyphenols, including of chlorogenic acid, galuteolin and flavonoid substance[2]. According to research, chlorogenic acid and Luteolin has the efficacy of anti-aging, anti-cancer and improving the health by scavenging O₂⁻, ·OH and H₂O₂[3]. Therefore, flavonoids are also a kind of natural organic antioxidants[4]. At present, there are many literatures on the extraction of chlorogenic acid and luteolin from the flower buds of Lonicera japonica, few studies on antioxidant substances and antioxidant activities except chlorogenic acid and luteolin in leaves.

According to the study by Zhang Yan et al.[5], the contents of antioxidants such as chlorogenic acid and luteolin in Lonicera lonicerae were the highest during the period from big white stage to two white stage. The flower buds collected during the period of big white stage or two white stage and the leaves at the end of the flower were used as materials to carry out flower buds. Determination of main antioxidant active substances in leaves and analysis of scavenging ability of O₂⁻ and ·OH, DPPH in order to provide basis for further.

2. Materials and Methods

2.1. Materials
The tested material was the medicinal and ornamental variety 'Honglei No.1', which had great potential in health care scented tea and landscaping ornamental flowers, 'Yincuilei' introduced from a fine variety of Lonicera japonica, which was bred and "Cuilei 109", an excellent variety of Lonicera japonica L. 'Yulei 1'. Plant in Muchuan County, Yizhichuncha Co., Ltd. Company Mountain Yinhuabase. The materials of flower buds were collected from two white stage to big white stage, and the leaves were taken from the end of flower. The buds and leaves with uniform size were collected from the top, middle and lower three parts of 20 branches of the same variety (line) 35 trees each time. The buds and leaves were brought back to the laboratory with ice box, and the dry samples were dried to constant mass at 60 °C. The buds and leaves were naturally dried and crushed after being taken out. Over 60 mesh sieve for the determination of chlorogenic acid, luteolin, flavonoids and anthocyanins, and the storage of fresh samples in -20 °C refrigerator for determination of total phenol.

2.2. Methods

2.2.1. Preparation of extract from Lonicerae japonica. The flower buds and leaves were crushed by 60 mesh sieve and extracted with 60% ethanol. The ratio of liquid to material was 1: 15 and 60 °C for 3 times, then rotated and evaporated at 45 °C. The concentrated liquid was centrifuged at 10 000 r/min 4 °C for 30 min, and the supernatant was collected.

2.2.2. Determination of antioxidants content. Detect the content of total phenol[6], chlorogenic acid, galuteolin, lavone, anthocyanin separately.

- The content of anthocyanin (mg \cdot g^{-1}FW)=\Delta A\times 3\times 0.05 \times 1000 \times 445.2/(30200 \times 1), \Delta A=(A_{510}-A_{700})\times (A_{510}-A_{700})^4.5

2.2.3. Determination of antioxidant activity. Detect the scavenging ability of DPPH [7] radica, O_{2^{-}} [8] and \cdot OH [9] separately.

- The cleatance of DPPH radical (%) = (1-\frac{A_{C}}{A_{I}}) \times 100
- The cleatance of O_{2^{-}}(\%)=(AC-AS)/AC \times 100%
- The cleatance of \cdot OH (%) = (1-\frac{A_{C}}{A_{I}}) \times 100

2.2.4. Data handling. The correlation analysis was carried out with SPSS software, and the average value was calculated by Excel.

3. Results and Discussion

3.1. Contents of main antioxidants in flower buds

The contents of main antioxidant active substances in flower buds from two white stage to big white stage (Table 1) showed that all the three samples contain higher total phenols, and the total phenol content of "Cuilei 109" was 417mg/g, which was 35.7% higher than the others. The content of 'Yincuilei' was higher than that of the other two materials. The contents of meliloside and total anthocyanin in three samples were very low.

| Variety (line) | Total phenol (mg/g \cdot FW) | Flavone (mg/g \cdot DW) | chlorogenic acid (mg/g \cdot DW) | Galuteolin (mg/g \cdot DW) | Anthocyanin (mg/g \cdot DW) |
|---------------|----------------------------|------------------------|-------------------------------|---------------------------|---------------------------|
| HongleiNo.1   | 266.40 ± 0.32b              | 10.51 ± 0.14b          | 9.11 ± 0.34ab                | 0.30 ± 0.14a              | 0.20 ± 0.18ab             |
3.2. Contents of main antioxidants in leaves

The contents of main antioxidant active substances in the leaves of the three samples (Table 2) showed that the leaves of the three samples were rich in phenolic substances, in which the total phenol content was 406–654 mg/g, and there was the highest total phenol content in 'Yincuilei'. The content of total flavonoids was the second most in the leaves, and the content of chlorogenic acid was the third, in which the content of 'Honglei No.1' was higher than that of the other two materials. The content of total anthocyanin in flower buds of three samples was very low.

| Variety (line) | Total phenol (mg/g·FW) | Flavone (mg/g·DW) | chlorogenic acid (mg/g·DW) | Galuteolin (mg/g·DW) | Anthocyanin (mg/g·DW) |
|---------------|------------------------|-------------------|---------------------------|---------------------|---------------------|
| Honglei No.1  | 406.18 ± 0.23c         | 29.58 ± 0.27a     | 8.24 ± 0.18a              | 2.63 ± 0.21a        | 0.31 ± 0.16a        |
| Yincuilei     | 654.53 ± 0.16a         | 21.24 ± 0.19b     | 7.83 ± 0.19a              | 3.71 ± 0.21b        | 0.26 ± 0.17b        |
| Cuilei 109    | 524.08 ± 0.31b         | 27.14 ± 0.15c     | 4.73 ± 0.26c              | 1.58 ± 0.34c        | 0.24 ± 0.18bc       |

3.3. Analysis of Antioxidant activity of Flower Bud extract

The DPPH, ·OH and O₂⁻ scavenging rates of flower buds ethanol extract (Table 3) can be seen from the table that the removal rates of DPPH and O₂⁻ in the ethanol extracts of flower buds were similar, which were significantly higher than those for ·OH. The highest removal rates of DPPH, ·OH and O₂⁻ were obtained from bud extract of "Cuilei 109". 'Honglei No.1' and 'Yincuilei' had similar clearance rates to DPPH, ·OH and O₂⁻.

| Variety (line) | DPPH clearance (%) | ·OH clearance (%) | O₂⁻ clearance (%) |
|----------------|--------------------|-------------------|-------------------|
| Honglei No.1   | 72.06 ± 0.21a      | 41.38 ± 0.32c     | 71.24 ± 0.28b     |
| Yincuilei      | 70.71 ± 0.27c      | 42.44 ± 0.26b     | 73.24 ± 0.19b     |
| Cuilei 109     | 78.20 ± 0.18b      | 50.40 ± 0.17a     | 75.35 ± 0.23a     |

3.4. Analysis of antioxidant activity of leaf extract

The DPPH, ·OH and O₂⁻ scavenging rates of ethanol extracts from leaves (Table 4) can be seen that, like flower buds, ethanol extracts from leaves had the lowest clearance to ·OH. The removal rates of DPPH, ·OH and O₂⁻ were similar in the ethanol extracts of 'Honglei No.1' and 'Yincuilei' leaves, showing DPPH clearance > O₂⁻ clearance > ·OH clearance, but the removal rates of DPPH were much higher than those of ·OH and O₂⁻. The order of free radical scavenging ability of "Cuilei 109" leaf extract was O₂⁻ scavenging rate > DPPH scavenging rate > ·OH scavenging rate.

Comparing the free radical scavenging ability of flower bud and leaf ethanol extract, it was found that 'Honglei No.1' and 'Yincuilei' showed the same regularity. The ability of leaf to remove DPPH was higher than that of flower buds, but the ability of removing O₂⁻ and ·OH to flower buds was lower than that of flower buds. The ·OH scavenging ability of "Cuilei 109" leaf extract was higher than that of flower buds, but the scavenging ability to DPPH and O₂⁻ was lower than that of flower buds.

| Variety (line) | DPPH clearance (%) | ·OH clearance (%) | O₂⁻ clearance (%) |
|----------------|--------------------|-------------------|-------------------|
| Honglei No.1   | 81.70 ± 0.23a      | 39.26 ± 0.16c     | 58.09 ± 0.18b     |
| Yincuilei      | 85.08 ± 0.25b      | 46.68 ± 0.18b     | 50.69 ± 0.31c     |
4. Summary
The results showed that the buds and leaves of 'Honglei No. 1' and 'Cuilei 109' had high contents of total phenols and flavonoids, except for the physiological active substances chlorogenic acid acid and meliloside. The contents of total phenols, flavonoids and melilosides in leaves were significantly higher than those in flower buds, and had higher scavenging ability of DPPH, \( \cdot \)OH and \( \cdot \)O\(_2\)\. Therefore, the leaves of Lonicera had wide potential in extracting physiological active substances.

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