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Analysis of bioactive constituents contents and their antioxidant activity in the flowers and leaves of two *Lonicera acuminata* Wall germplasm

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Abstract. "Cuilei 001", "Cuilei 003" two *Lonicera acuminata* Wall germplasm as test materials, the content of bioactive constituents and antioxidant activity of the flowers and leaves at different periods were discussed. The results showed that the content of polyphenol, flavonoid, and vitamin C (Vc) were the highest in the young leaves of the "Cuilei 001". The scavenging ability of the two germplasms to 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was similar and was highest in the flowers, but in the leaves, the scavenging ability to DPPH free radical gradually decreased with the development of the leaves. The flavonoid content, Vc content, peroxidase (POD) activity, superoxide dismutase (SOD) activity, and DPPH free radical scavenging rate of "Cuilei 001" and "Cuilei 003" were significantly positively correlated, and the polyphenol content and DPPH free radical scavenging rate were significantly negatively correlated.

1. Introduction

There are many kinds of honeysuckle and its sources are complicated, among which *Lonicera acuminata* Wall (*L. acuminata* Wall) is used as honeysuckle in parts of Sichuan and Xizang [1], and the chemical constituents of *L. acuminata* Wall are basically the same as those of authentic honeysuckle[2]. They are rich in vitamin C (Vc), polyphenol, flavonoid, peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), and other natural antioxidants, have the physiological activities of anti-oxidation, anti-aging and increasing the immunity of the body[3-6]. At present, the components and antioxidant activities of *Lonicera macranthodes* Hand-Mass, *Lonicera hypoglauca* Miq, *Lonicera confusa* DC, and *Lonicera fulvotomentosa* Hsu et S C Cheng in Chinese pharmacopoeia are often studied, but there are few reports on *Lonicera acuminata* Wall. It has been reported that the leaves of *Lonicera* are rich in many bioactive substances, but have not been used reasonably for a long time. Therefore, "Cuilei 001" and "Cuilei 003" were used as materials to determine the contents of main bioactive substances and to analyze the antioxidant activities of the flowers and leaves at different growth stages. In order to provide basic data and reference basis for evaluation of flower bud and leaf health function of *Lonicera japonica* germplasm in Muchuan County, we selection of new varieties and efficient utilization of resources.

2. Materials and methods

2.1. Experimental materials and sample treatment



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The experimental materials are “Cuilei 001” and “Cuilei 003”, which are collected from Muchuan County, Sichuan Province, China.

Samples were collected from 20 branches in different directions on each tree at the flowering stage of large white stage to two white stage, and the tree vigor was uniform. The flower buds which were uniform size, no disease and insect pests were selected, and the leaves at 4 different periods were also selected, which were the young leaves (5 - 10 d), the unrolled leaves (25 - 30 d), the mature leaves (> 40 d) and the old leaves (365 d), and repeated three times. Immediately after the harvest, used the ice box to bring the samples back to the laboratory. The dry sample was prepared by microwave drying method, microwave power 3 KW, drying time 30 ~ 90 s, temperature 60 °C, drying to constant weight, crushing, over 60 mesh sieves, used for the determination of flavonoid and polyphenol in the sample. Fresh samples were treated with liquid nitrogen and stored in the ultra-low temperature refrigerator at - 80 °C for the analysis of Vc content, antioxidant enzyme activity and antioxidant activity.

2.2. Index and measuring method

The content of polyphenol was measured by the method of Lu Xiaoting [7] et al. The content of flavonoid was measured by the method of Guan Bingfeng[8] et al. The content of Vc was measured by Fe^{3+} reduction method [9]. POD activity was measured by Guaiacol method [10], CAT activity was measured by Spectrophotometry [11], and SOD activity was measured by NBT Reductive method [12]. The preparation of sample extracts for the determination of DPPH free radical scavenging was described in the method of Liang [13] et al. The method of determination was referenced the method of Chen Jinming[14] et al. and Niki E[15] et al.

2.3. Statistical analyses

SPSS Statistics 17.0 software (IBM, Chicago, USA) were used for analysis of variance ($p < 0.05$).

3. Results and analysis

3.1. Comparison of bioactive substances of “Cuilei 001”

Among the leaves at different developmental stages, the content of polyphenol, flavonoid and Vc were the highest in the young leaves (Table 1). With the development of the leaves, the contents of polyphenol and flavonoid decreased significantly; the change trend of Vc content was first decreased, then increased, and decreased again. The content of polyphenol in the flowers was higher, only less than that in the young leaves; the content of flavonoid in the flowers was significantly lower than that of the young leaves and unrolled leaves, the content of Vc in the flowers was significantly lower than that of the leaves at all developmental stages, compared with the young leaves, flavonoid content and Vc content decreased by 54.53% ($p < 0.05$) and 59.09% ($p < 0.05$), respectively.

Table 1. Comparison of bioactive substances of “Cuilei 001”^a.

materials	Polyphenol (mg/g·DW)	Flavonoid (mg/g·DW)	Vc (mg/100g·FW)
flowers	272.86±0.63a	48.60±0.29c	616.22±0.75e
young leaves	288.51±0.19a	106.88±1.02a	1506.42±1.21a
unrolled leaves	245.21±0.53b	72.33±0.75b	666.89±1.01d
mature leaves	233.22±0.39b	46.67±0.52c	1207.43±0.33b
old leaves	231.39±0.21b	47.77±0.36c	1046.96±0.15c

^aThe data are mean ± standard error. The lowercase letters in the same column of data indicate that the differences between different treatments have significant differences ($p < 0.05$). Same as below.

3.2. Comparison of antioxidant enzyme activity and antioxidant ability of “Cuilei 001”

In the leaves, POD activity was highest in the unfolded leaves and lowest in the young leaves; CAT activity was highest in the young leaves and lowest in the old leaves, and SOD activity was highest in mature leaves (Table 2). With the development of the leaves, the ability to scavenge DPPH free

radicals gradually decreased, and the scavenging rate of old leaves decreased by 9.27% ($p < 0.05$), compared with the young leaves. The antioxidant enzyme activity and antioxidant ability in flowers were roughly at a higher level.

Table 2. Comparison of antioxidant enzyme activity and antioxidant ability of "Cuilei 001".

materials	POD activity (u/g·min·FW)	CAT activity (u/g·min·FW)	SOD activity (u/g·FW)	DPPH·scavenging rate (%)
flowers	889.17±0.64b	486.00±0.32b	394.91±0.17a	76.49±0.12a
young leaves	463.33±0.13e	805.80±0.06a	304.37±0.14b	73.12±0.11b
unrolled leaves	893.33±0.19a	387.60±0.24c	304.70±0.25b	69.43±0.19c
mature leaves	500.00±0.23d	274.80±0.17d	177.92±0.19c	67.81±0.11d
old leaves	871.67±0.22c	257.00±0.21e	301.46±0.39b	66.34±0.27e

3.3. Comparison of bioactive substances of "Cuilei 003"

With the development of the leaves, the content of polyphenol decreased gradually, and the content of polyphenol in the old leaves was the lowest, which decreased by 27.85% ($p < 0.05$), compared with the young leaves (Table 3). The content of flavonoid increased first and then decreased, the content of flavonoid in unrolled leaves was the highest (70.31 mg/g·DW), followed by young leaves, but there was no significant difference between mature leaves and old leaves. The content of Vc decreased first and then increased, then decreased again. Compared with the leaves, the contents of polyphenol, flavonoid and Vc in flowers showed a moderate level.

Table 3. Comparison of bioactive substances of "Cuilei 003".

materials	Polyphenol (mg/g·DW)	Flavonoid (mg/g·DW)	Vc (mg/100g·FW)
flowers	256.71±0.23b	66.30±0.16b	1101.01±0.21c
young leaves	283.13±0.19a	51.83±1.04c	1168.58±0.11b
unrolled leaves	271.39±0.25a	70.31±0.34a	741.22±0.17e
mature leaves	245.58±0.06b	31.65±0.12d	1470.95±0.18a
old leaves	204.48±0.31c	30.33±0.26d	1065.54±0.13d

3.4. Comparison of antioxidant enzyme activity and antioxidant ability of "Cuilei 003"

The activities of POD and CAT in the unrolled leaves were significantly higher than those in the leaves of other stages (Table 4). The SOD activity and DPPH scavenging rate were highest in the young leaves, and the activity of SOD in the unrolled leaves was the lowest. The scavenging rate of DPPH decreased at first and then increased slightly with development of leaves. Except that the SOD activity was only lower than that of the young leaves, the POD activity, CAT activity, and DPPH scavenging rate in the flowers were significantly higher than those in the leaves.

Table 4. Comparison of antioxidant enzyme activity and antioxidant ability of "Cuilei 003".

materials	POD activity (u/g·min·FW)	CAT activity (u/g·min·FW)	SOD activity (u/g·FW)	DPPH·scavenging rate (%)
flowers	876.67±0.12a	678.90±0.30a	354.89±0.18b	79.68±0.12a
young leaves	547.75±0.19c	346.80±0.34c	388.32±0.23a	75.01±0.08b
unrolled leaves	635.00±0.48b	421.20±1.09b	97.47±0.55e	71.97±0.18c
mature leaves	453.33±0.31d	357.00±0.16c	238.34±0.23d	68.31±0.09d
old leaves	240.50±0.13e	324.00±0.14d	303.72±0.56c	68.45±0.14e

3.5. Correlation Analysis between Bioactive substance content and Antioxidant activity of "Cuilei 001" and "Cuilei 003"

The flavonoid content, Vc content, POD activity, SOD activity and DPPH scavenging rate of "Cuilei 001" and "Cuilei 003" were significantly positively correlated (Table 5 and Table 6), the polyphenol content and DPPH scavenging rate were significantly negatively correlated.

Table 5. Correlation analysis between bioactive substance content and antioxidant activity of "Cuilei 001".

Index	Vc	Flavonoid	Polyphenol	POD activity	CAT activity	SOD activity	DPPH scavenging rate
Vc	1						
Flavonoid	0.6989*	1					
Polyphenol	0.2054	0.5260*	1				
POD activity	-0.2699	-0.4700	-0.8799	1			
CAT activity	-0.2602	-0.4700	0.4614	-0.7370	1		
SOD activity	0.5627	0.1074	-0.5265	0.6268	-0.8412	1	
DPPH scavenging rate	0.8658*	0.2906*	-0.2224*	0.0311*	-0.2572	0.7039*	1

Table 6. Correlation analysis between bioactive substance content and antioxidant activity of "Cuilei 003".

Index	Vc	Flavonoid	Polyphenol	POD activity	CAT activity	SOD activity	DPPH scavenging rate
Vc	1						
Flavonoid	0.6962*	1					
Polyphenol	-0.1766	-0.6795*	1				
POD activity	-0.2534	0.2500	-0.0962	1			
CAT activity	-0.9215	-0.5096	-0.0845	0.4148	1		
SOD activity	-0.0481	-0.2307	0.4744	0.5465	0.1773	1	
DPPH scavenging rate	0.5526*	0.7438*	-0.2108*	0.6661*	-0.3509*	0.4480*	1

4. Discussion and summary

At present, most of the antioxidants people can use are chemically synthesized and have certain toxic and carcinogenic effects, therefore, the evaluation and screening of plant materials containing natural strong antioxidants has become a new trend in medicine, biology and food science [16]. In this experiment, the contents of polyphenol, flavonoid and Vc of "Cuilei 001" and "Cuilei 003" were measured. The results showed that the content of polyphenol, flavonoid and Vc in the young leaves of "Cuilei 001" was the highest, which were 106.88 mg/g·DW, 288.51 mg/g·DW, 1506.42 mg/100g·FW, respectively. The results of DPPH assay showed that the extracts of flowers and leaves of "Cuilei 003" and "Cuilei 001" have good antioxidant capacity and can be used as a natural source of antioxidants. The results of this experiment indicated that the DPPH free radical scavenging rates of "Cuilei 001" and "Cuilei 003" flower and leaf extracts were correlated with polyphenol, flavonoid and Vc, and with POD, CAT and SOD activities, it is significantly correlated, which is consistent with Liu Hao[17] et al. and Lanhua[18] et al.

Therefore, in terms of nutrition, "Cuilei 001" and "Cuilei 003", as raw materials of natural antioxidants, should be harvested in flowers with high biological yield and antioxidant activity, but from the perspective of resources. A large number of abandoned branches and leaves can be used to a certain extent, especially young leaves.

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