Analysis of Anthocyanins in Different Parts of *Michelia* with High Performance Liquid Chromatography

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Abstract. In this paper, high-performance liquid chromatography was used to analyze the composition and content changes of anthocyanins in various parts of the ground in Michelia, such as flowers, leaves and stems. The results showed that its flower contained cyanidin and Petunidin, while the stem contained Peonidin, and no anthocyanins were detected in the leaves. The types of single anthocyanin components contained in different parts are different. It is intended to provide a reference for the cultivation and evaluation of new varieties of ornamental plants of Michelia.

1. Introduction
Michelia is a Michelia plant of Magnoliaceae, which is mainly distributed in southwest to east of China. There are more than 70 kinds of Michelia plants, which are evergreen shrubs or trees, with beautiful shape and big fragrant flowers. They are widely used in landscaping. Previous studies have shown that Michelia figo, M. maudia and M. platypetala are commonly used in gardens, which have better cold resistance (Li et al., 2016; Li et al., 2017), but their flower colors are all white and single. The red flower Michelia is a rare red plant, which is very popular in the application of landscape.

The color of flowers directly determines the prospect of the development and utilization of ornamental resources of Michelia, and anthocyanins are important indicators of the color of petals. The color of petals is mainly related to the type, amount and distribution of anthocyanins (Liu et al., 2016; Zhang et al., 2002; Gao et al., 1995; Liao et al., 2015; Qiu et al., 2017). Therefore, this paper intends to use high performance liquid chromatography to analyze the composition and content changes of anthocyanins in flowers, leaves, stems and other parts of Michelia, in order to clarify the content and distribution characteristics of anthocyanins, which aims to provide reference for the evaluation and utilization of Michelia resources and the breeding of new varieties.
2. Materials and Methods

2.1 Materials and Equipment

2.1.1 Materials and Reagents
The experiment was conducted in the Botanical Garden of South Subtropical Crop Research Institute (Zhanjiang, Guangdong), Chinese Academy of Tropical Agricultural Sciences. A total of 5 single plants were selected in the experiment. Each single plant took 5 flowers, 2 stems, and 10 leaves distributed at different locations. After picking, they were taken back to the laboratory immediately, crushed and mixed under the condition of liquid nitrogen, and stored at -20 ℃ for standby.

Absolute ethanol, acetonitrile, formic acid, methanol (Chromatographic level), salt Acid (super pure), ultrapure water (deionized water). Standard sample of Delphinidin, Cyanidin, Pelargonidin, Petunidin, Peonidin, Malvidin.

Extraction solution: absolute ethanol + water + hydrochloric acid = 2 + 1 + 1 (V + V + V)

2.1.2 Instruments and Equipment
High Performance Liquid Chromatograph (LC-20A, equipped with UV detector), Shimadzu Corporation, Japan; Water bath (accuracy ± 0.2 °C), balance (accuracy 0.01mg), grinder, ultrasonic cleaner.

2.2 Method

2.2.1 Pretreatment method
Refer to the method of sample extraction and hydrolysis of NY / T 2640-2014 (Hu et al., 2014), and adjust it according to the actual situation.

Anthocyanin extraction: accurately weigh 1.0 g of powder into a 10 ml stoppered colorimetric tube, add the extraction solution to the scale, shake well for 1 min, and then ultrasonically extract for 30 min.

Hydrolysis of anthocyanin to anthocyanin: after ultrasonic extraction, hydrolyze it in boiling water bath for 1 h, take it out and cool down, use the extraction solution to volume again, then leave it to stand and take the supernatant, and filter it with a 0.22 μm organic phase filter membrane to be tested. After sample preparation, the storage time shall not exceed 3 days at 4 ℃.

The whole sample preparation process needs to be performed under dark conditions.

2.2.2 Chromatographic Methods
The Column was InertSustain C18 (250 mm × 4.6 mm × 5 μm), the column temperature was 30 ℃, the flow rate was 1 ml / min, the detector wavelength was 535 nm, and the injection volume was 10 μL. Mobile phase A was methanol: acetonitrile: water: formic acid = 22.5:22.5:40:10; mobile phase B was 10% formic acid water, formic acid: water = 1:9 (V / V) mixed and prepared (the above reagents need to be filtered by 0.45 μ M filter membrane and degassed by ultrasonic for 5 min before they can be used on the machine).

The gradient elution method was: 0-2 min: 7-40% B; 2.0 min-11 min: 40-67% B; 11-12 min: 67-100% B; 12-14 min: 100% B; 14-15 min: 100-7% B; 15-20 min: 7% B, the total operation time was 20 min.

2.2.3 Data processing method
Linear regression analysis and mapping were completed with Office 2010 (Microsoft Inc) software.

3. Results and analysis

3.1 Standard curve of anthocyanin
Linear regression analysis was performed with the six anthocyanin standards, and the standard curves were drawn which taken the mass concentration as the abscissa and the peak area as the ordinate. The
correlation coefficient R2 of each standard curve is 0.99, which shows that the linear correlation between the concentration of anthocyanin and the peak area is good. The name, peak time, standard curve equation, correlation coefficient and linear range of anthocyanin standards are shown in Table 1.

| Standard name | Retention time /min | Regression equation | Correlation coefficient R2 | Linear range / (μg/mL) |
|---------------|---------------------|---------------------|---------------------------|------------------------|
| Delphinidin   | 8.447               | y = 20100x + 31482  | 0.997                     | 1～50                   |
| Cyanidin      | 9.645               | y = 22911x + 44149  | 0.993                     | 1～50                   |
| Pelargonidin  | 10.056              | y = 24153x + 38851  | 0.996                     | 1～50                   |
| Petunidin     | 11.212              | y = 19138x + 33515  | 0.995                     | 1～50                   |
| Peonidin      | 11.839              | y = 28584x + 39871  | 0.997                     | 1～50                   |
| Malvidin      | 12.109              | y = 22975x + 14796  | 0.999                     | 1～50                   |

Note: X. Molar concentration (mg·L⁻¹); Y. Peak area.

According to the HPLC analysis method described in this paper, the six anthocyanins are well separated in liquid chromatography (see Figure 1).

3.2 Analysis of anthocyanin species and content in different parts of Michelia

It can be seen from Figure 2 that there are Cyanidin and Petunidin in its flowers, Pelargonidin and Peonidin in stems, and no anthocyanin is detected in the leaves. Different parts contain different kinds of single anthocyanin components. The other pigments were not detected, because of the small content and the interference of the baseline, which could not be determined accurately. It needs to be further determined by pre-treatment concentration or mass spectrometry and other instruments.

The main pigment of Michelia flower is Petunidin, whose content is more than 25 times that of Cyanidin. It can be seen that Petunidin is the main color substance of Michelia, and red character is a rare variety in Michelia, which can cultivate new varieties with high ornamental value. It is of great significance to improve the ornamental value and garden application value of Michelia.
4. Conclusion
In this paper, high-performance liquid chromatography was used to analyze the composition and content changes of anthocyanins in various parts of the ground in Michelia, such as flowers, leaves and stems. The results showed that its flower contained cyanidin and geranin, while the stem contained petunidin and paeoniflorin, and no anthocyanins were detected in the leaves. The types of single anthocyanin components contained in different parts are different, which need to be further confirmed. Petunidin is the main color substance of Michelia, and red character is the rare quality, which can cultivate new varieties with high ornamental value. It is of great significance to improve the ornamental value and garden application value of Michelia.

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