Study on chemical constituents of different species of *Dendrobium*

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**Abstract.** The objective of this study is to determine the erianin of 10 species of *Dendrobium* by high performance liquid chromatography (HPLC). Processing techniques were washed, dried and ground. The research method for determining the content of erianin was adopted from *Chinese Pharmacopoeia* 2015 Edition. Acetonitrile-0.05% phosphoric acid (37:63) was used as mobile phase. The samples were separated on Sharpsil-TC18 column(4.6*150mm;5μm) at a flow rate of 1.2mL/min and detected at 230nm, and the column temperature was kept at 30°C. The injection volume was 20μL. The number of theoretical plates was not less than 6000 according to the chromatographic peak of erianin. The result of the study showed erianin was detected only in *Dendrobium chrysotoxum* among the 10 species of *Dendrobium*, and the content was 0.098%. Conclusion: The content of erianin in *Dendrobium chrysotoxum* met the specification of no less than 0.03% in the 2015 edition of *Chinese Pharmacopoeia*.

**1 INTRODUCTION**

*Dendrobium* is one of the richest genus of Orchidaceae with more than 1100 species widely distributed in Asian, Europe and Australia. There are 74 species and 2 variations of *Dendrobium* found in China which are mainly distributed in Yunnan, Sichuan and Guangxi provinces [1]. *Dendrobium* is one of the most well-known orchids in global horticultural trade due to its beautiful flowers and ideal characteristics as houseplants. Moreover, *Dendrobium* has been used as traditional herbal in many Asian countries for a long time. Thus, wild *Dendrobium* resources have been over-excavated, making wild *Dendrobium* endangered in recent years.

As a traditional medicinal and ornamental plant in China, *Dendrobium* has a long history of application. The stems of *Dendrobium* have been used as medicine for a thousand years. In recent decades, more than 100 compounds have been found and identified from the medical parts of *Dendrobium*, which include alkaloids, polysaccharide, phenols, flavonoids, amino acids, tanning, etc [2]. Modern pharmacological studies show that *Dendrobium* has the functions of anti-tumor, immune regulation, anti-oxidation, anti-angiogenesis, anti-inflammatory, and relieving diabetes [3]. At present, the research on the chemical components of *Dendrobium* is mainly focused on the polysaccharides and alkaloids, but the research on other chemical components is relatively less. Erianin(2-Methoxy-5-[2-(3,4,5-trimethoxyphenyl) ethyl] benzoil) is a low molecular weight natural compound isolated from *Dendrobium chrysotoxum*, with a relative molecular weight of 318.36. As a bibenzyl compound, erianin has many pharmacological activities. Sun et al. have found that erianin can significantly inhibit the proliferation of human breast cancer T47D cells, activate immune escape signal pathway to induce apoptosis of T47D cells, inhibit the expression of cyclin dependent kinase (CDKs), and lead to cell cycle arrest, and erianin does not affect the proliferation of normal breast epithelial mcf10a cells [4]. Deng et al found that erianin can induce apoptosis of human lung cancer A549 cells in vitro [5]. Su et al. showed that erianin could block the cell cycle of human hepatoma cell line Huh7 at G2 / m, thus inhibiting its proliferation [6]. Erianin can inhibit cancer through multi-mechanism and multi-layer surface, it has great application prospect in clinical treatment [7]. In this study, the content of erianin in 22 species of *Dendrobium* was detected by high performance liquid chromatography in order to provide the relevant basis for the quality comparison of different species of *Dendrobium*, and provide the relevant reference value for the further study of *Dendrobium*.

**2 MATERIALS AND METHODS**

2.1 Instrumentation and reagents

The tools used in this study were analytical scales (analytical balance) with accuracy of 0.01 mg, electricity heat drum, beaker cups, pipette, Agilent 1100 high performance liquid chromatograph(diode-array detector), ultrasonic cleaner, conical flask with cover, pulverizer, standard sieve, amber laboratory bottle, label paper, Sharpsil-T C18 column(4.6*150mm; 5μm), 0.45μm pore size (Millipore) membrane filter, vacuum pump.

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The material that will be used in this research is 10 species of Dendrobium collected from Yunnan and Sichuan. All the Dendrobium stems are healthy and disease-free fresh. These 10 species of Dendrobium are Dendrobium thyrsiflorum, Dendrobium williamsonii, Dendrobium pendulum, Dendrobium nobile, Dendrobium linovianum, Dendrobium densiflorum, Dendrobium wardianum, Dendrobium chrysanthum, Dendrobium ellipsophyllum, Dendrobium chrysotoxum. The chemical used for extracting erianin is methanol (grade HPLC). Erianin (purity≥98.0% HPLC) was purchased from Shanghai yuanye Bio-Technology Company. The chemicals used for mobile phase are acetonitrile (grade HPLC), phosphoric acid (grade AR), and pure water which was prepared using purified water from a Mili-Q filtration system.

2.2 Method

2.2.1 Chromatographic condition

The mobile phase was acetonitrile-0.05% - phosphoric acid (37:63). The mobile phase was filtered through a 0.45μm pore size (Millipore) membrane filter. The separation was carried out on a Shapsil-T C18 column (4.6*150mm; 5μm). The temperature of the column was kept at 30℃ and the injection volume was 20μl. The total run time was set at 30 min. The flow rate was set at 1.2ml/min and the detection wavelength was set at 230nm. Under the above conditions, the number of theoretical plates is 6542 which is better than the regulation in Chinese Pharmacopoeia.

2.2.2 Preparation of standard solution

A standard stock solution of erianin was prepared by dissolving appropriate erianin in 50ml methanol to make a standard solution with a concentration of 15.3μg/ml.

2.2.3 Preparation of sample solution

Take the healthy and disease-free fresh stem segments of this 10 species of Dendrobium, and remove the leaves and leaf sheaths. Dry these stems in the electricity heat drum, after that, crush the dry stems, sift them through No.3 sieve. Accurately take 1g powders, put them in a conical flask with a stopper, add 50ml methanol in it. After soaking them for 20min, then exposed the solution to ultrasound treatment (250W, 40kHz) for 45min. Make up the weight loss with methanol, then filtered through a 0.45μm pore size membrane filter, take the subsequent filtrate as sample solution.

2.2.4 System suitability test

Accurately weigh 9.3mg (M1) of standard erianin, add appropriate methanol to prepare standard solution (std1) with a concentration of 13.95 μg / ml; precisely weigh 10.2mg (M2) of standard erianin, add appropriate methanol to prepare standard solution (std2) with a concentration of 15.3 μg / ml. Inject std1 6 times continuously, as shown in Table 1; inject std2 9 times continuously, as shown in Table 2. The recovery rate of standard solution = ((S2 / S1) * M1) / m2 * 100%, the average recovery rate of standard solution is 97.4%, which indicating that the system has good suitability.

Table1. STD1 continuous injection

| Number of injection | Peak area | Mean peak area(S1) | RSD |
|---------------------|-----------|--------------------|-----|
| 1                   | 323       |                    |     |
| 2                   | 318.2     |                    |     |
| 3                   | 327.7     |                    |     |
| 4                   | 327.3     | 325.8              | 1.5%|
| 5                   | 325.6     |                    |     |
| 6                   | 333.1     |                    |     |

Table2. STD2 continuous injection

| Number of injection | Peak area | Mean peak area(S2) | RSD |
|---------------------|-----------|--------------------|-----|
| 1                   | 347.6     |                    |     |
| 2                   | 348.2     |                    |     |
| 3                   | 345.4     |                    |     |
| 4                   | 333.1     |                    |     |
| 5                   | 352.7     | 348.2              | 2.0%|
| 6                   | 357       |                    |     |
| 7                   | 353.5     |                    |     |
| 8                   | 351.6     |                    |     |
| 9                   | 345       |                    |     |

2.2.5 Determination of samples

Inject 20μl standard solution and sample solution precisely and inject them into the high performance liquid chromatograph for determination, record the peak area. Calculate the content of erianin in the sample by external standard method.

3 RESULTS AND DISSCUSSION

Among the 10 species of Dendrobium detected, erianin was detected only in Dendrobium chrysotoxum. The HPLC chromatograms have been shown in Fig1 and Fig2.
Fig 2. It is shown in Fig 1 that retention time of erianin peak is about 18.5 min. The content of erianin in these 10 species of Dendrobium is shown in Table 3. Content of erianin in Dendrobium chrysotoxum is 0.098 % on the dried basis, which met the specification of no less than 0.03 % in the 2015 edition of Chinese Pharmacopoeia.

**Table 3. Content of erianin in 10 species of Dendrobium**

| Variety                | Sample weight (g) | Methanol volume (ml) | Sample peak area | Standard peak area | Standard concentration (μg/ml) | Content (%) |
|------------------------|-------------------|----------------------|------------------|-------------------|-------------------------------|------------|
| D. thrysiflorum        | 1.0075            | 50                   | 0                | 348.2             | 15.3                          | 0.00       |
| D. williamsonii        | 1.0031            | 50                   | 0                | 348.2             | 15.3                          | 0.00       |
| D. pendulum            | 1.0047            | 50                   | 0                | 348.2             | 15.3                          | 0.00       |
| D. nobile              | 1.0002            | 50                   | 0                | 348.2             | 15.3                          | 0.00       |
| D. linavianum          | 1.0030            | 50                   | 0                | 348.2             | 15.3                          | 0.00       |
| D. densiflorum         | 1.0067            | 50                   | 0                | 348.2             | 15.3                          | 0.00       |
| D. wardianum           | 1.0015            | 50                   | 0                | 348.2             | 15.3                          | 0.00       |
| D. chrysanthum         | 1.0034            | 50                   | 0                | 348.2             | 15.3                          | 0.00       |
| D. ellipsophyllum      | 0.9249            | 50                   | 0                | 348.2             | 15.3                          | 0.00       |
| D. chrysotoxum         | 1.0035            | 50                   | 447.2            | 348.2             | 15.3                          | 0.098      |

In this study, no erianin was found in the Dendrobium nobile, which was different from the study of Xu [8]. And the content of Dendrobium chrysotoxum in this study is significantly different from the study of Xia [9]. What caused these situations may have many explanations. Such as the different growth age, cultivation ways and origin [10,11]. Generally speaking, the content of erianin in Dendrobium is significantly affected by many factors. Thus it can be seen that the content of erianin in Dendrobium is controlled by the interaction between genes and environment.

**4 CONCLUSION**

Over the years, many scholars at home and abroad have studied the chemical constituents of Dendrobium, from which alkaloids, polysaccharides, phenanthraquinones, benzenes, amino acid, fluorenones, coumarins, steroids, triterpenoids glycosides and volatile oil have been isolated and identified, and their pharmacological activities have been widely studied. Xu et al found that only 11 species of Dendrobium are involved in the anti-tumour study, and there is little research on the anti-tumour of benzenes compounds extracted from Dendrobium, meanwhile there is no systematic and in-depth study [12]. As a natural product isolated from Dendrobium chrysotoxum, erianin has great potential in clinical application of anti-tumour therapy. A large number of studies have shown that erianin can inhibit the occurrence and development of tumour through multiple mechanisms and levels [13].

The suggestions for further research work are as follows. First, more Dendrobium species should be detected and compared. Second, the study of antitumor components in Dendrobium should not only be limited to the erianin. Third, as one of the main components in Dendrobium, bibenzyl may has similar pharmacological effect with erianin. Liquid chromatograph mass spectrometer (LC-MS) method can be used for structural analysis and identification before further research. Fourthly, in order to get high quality Dendrobium, cultivation ways of Dendrobium should be improved.

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