

ORIGINAL ARTICLE

Analysis of the bioactive components from different growth stages of *Fritillaria taipaiensis* P. Y. Li

Rui Peng*, Peng Ma, Rangyu Mo, Nianxi Sun

Chongqing Academy of Chinese Materia Medica, Chongqing 400065, China

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Alkaloid;
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Nucleobase;
Quality control

Abstract High-performance liquid chromatography (HPLC) coupled with an evaporative light scattering detector (ELSD) or a diode array detector (DAD) were utilized for the quantitative analysis of 4 alkaloids (peimisine, sipeimine, peimine and peiminine) and 9 nucleosides and nucleobases (uracil, uridine, adenosine, adenine, inosine, thymine, cytidine, guanosine and thymidine) from *Fritillaria taipaiensis* P. Y. Li that had been cultivated in the same field for 2–6 years. The content of peimisine, sipeimine, peimine, peiminine, uracil, thymine, adenine and inosine in plants cultivated for 2–4 years was significantly higher than that of plants cultivated for 5–6 years, while the content of cytidine, uridine, guanosine, thymidine and adenosine did not change over this period. This is the first evaluation of variation in the bioactive compounds in *F. taipaiensis* over its life cycle.

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Abbreviations: DAD, diode array detector; ELSD, evaporative light scattering detection; HPLC, high pressure liquid chromatography; RSD, relative standard deviation

*Corresponding author. Tel.: +86 23 89029186; fax: +86 23 89029008.

E-mail address: rpeng5572@yahoo.com.cn (Rui Peng).

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1. Introduction

Taibaibeimu refers to the bulbs of *Fritillaria taipaiensis* P. Y. Li from the family Liliaceae, and it has long been used as an antitussive and anti-asthmatic herb in China. It is documented as Chuanbeimu in the China Pharmacopoeia¹. Numerous pharmacological studies have shown that the alkaloid content of this medicinal plant is responsible for its antitussive and expectorant activity²⁻⁴. Additionally, many nucleosides and nucleobases present in the water extract of *Fritillaria* bulbs act as antihypertensives, platelet aggregation inhibitor and smooth muscle relaxation promoter⁵⁻⁷.

The original plants used for the traditional Chinese medicine Chuanbeimu, such as *Fritillaria cirrhosa* D. Don, *Fritillaria unibracteata* Hsiao et K. C. Hsia, *Fritillaria przewalskii* Maxim. ex Batal, *Fritillaria delavayi* Franch., *F. taipaiensis* P. Y. Li and *Fritillaria wabuensis* S. Y. Tang et S. C. Yueh, are perennial herbs. In traditional Chinese medicine, the smaller the bulb size of Chuanbeimu the better the therapeutic effect. Thus, the tradition in application of Chuanbeimu is that 50 g Songbei should contain no less than 240 bulbs and 50 g Qingbei should contain 190 bulbs. It is thought that the content and composition of the drugs during the early growth stage is more effective than that during the late growth stage. However, there is as yet no evidence that the content of alkaloids, nucleosides and nucleobases in *F. taipaiensis* P. Y. Li changes over the course of growth and maturation.

In this study, an HPLC coupled with diode array detector (DAD) and an HPLC coupled with evaporative light scattering detector (ELSD) were used to quantify 9 nucleosides and nucleobases including uracil, uridine, adenosine, adenine, inosine, thymine, cytidine, guanosine and thymidine, and 4 alkaloids including peimisine, sipeimine, peimine and peiminine in *F. taipaiensis* in a single analysis. The content of these components were compared at different growth stages of this plant to ascertain the optimal harvesting time and regulate the yield and quality of *F. taipaiensis*.

2. Materials and methods

2.1. Plant materials

Five samples of *F. taipaiensis* were collected after 2–6 years of cultivation in Lanying, Wuxi county, Chongqing municipality, China. The plants were harvested in July 2011. Plant material was authenticated at the Chongqing Academy of Chinese Materia Medica (Chongqing, China).

2.2. Chemicals and reagents

Alkaloid standards, including peimisine (MUST-11042112), sipeimine (MUST-10031103), peimine (MUST-10072201) and peiminine (MUST-11012001), were purchased from Chengmust Biotech Co., Ltd. (Chengdu, China). Nucleosides and nucleobases, such as uracil (Batch No. 100469-200702), uridine (Batch No. 887-200202), adenosine (Batch No. 110879-200202), adenine (Batch No. 886-200002), inosine (Batch No. 140669-200702), thymine (Batch No. T0376-5G), cytidine (Batch No. C4651-1G), guanosine (Batch No. G6752-1G) and thymidine (Batch No. T9250-1G), were purchased from Sigma (Shanghai, China). The purities of these compounds were determined to be more than 99.0% by HPLC. Their structures are shown in Fig. 1. Methanol for liquid chromatography was purchased from Honeywell (Burdick & Jackson, USA). Other reagents were of HPLC grade or the highest grade commercially available.

2.3. Solution preparation

2.3.1. Preparation of standard solutions

The stock solution of alkaloid standards peimisine (920 µg/mL), sipeimine (442 µg/mL), peimine (440 µg/mL) and peiminine (392 µg/mL) were prepared and diluted with methanol to obtain

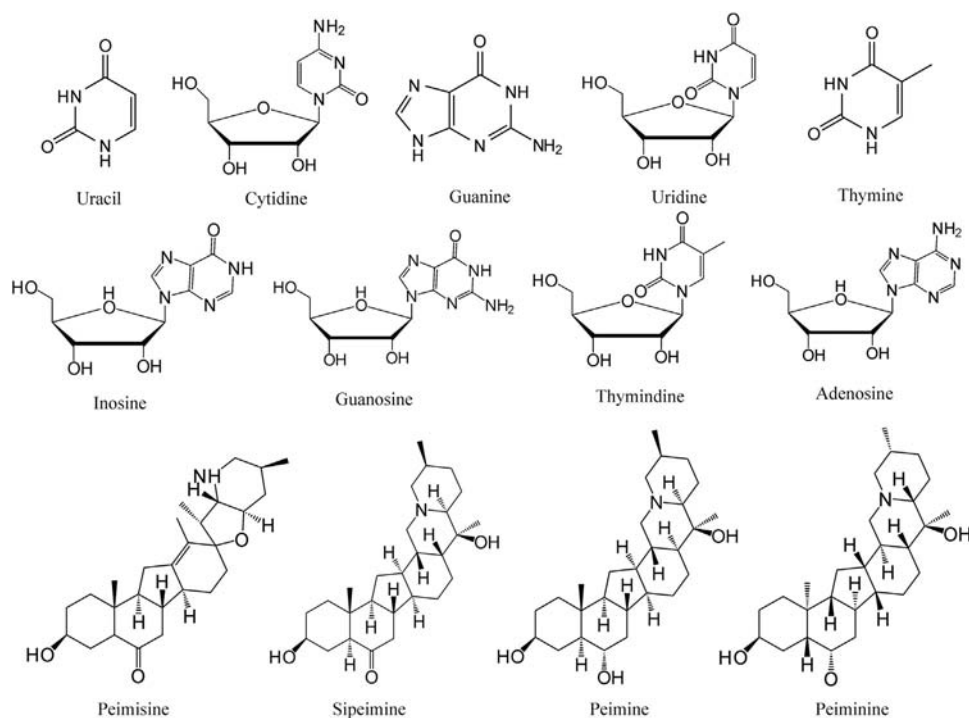


Figure 1 Chemical structures of nucleosides, nucleobases and alkaloids in *Fritillaria taipaiensis* P. Y. Li.

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