



ORIGINAL ARTICLE

# Chemical profiling and histochemical analysis of *Bupleurum marginatum* roots from different growing areas of Hubei province

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## KEY WORDS

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Germlasm resources

**Abstract** Bupleuri Radix has been widely used in traditional Chinese medicine. In the current herbal market, the species *Bupleurum marginatum* Wall. ex DC. is the main source of Bupleuri Radix. Although Bupleuri Radix from the roots of *B. marginatum* grown wild in the North West of Hubei province has higher quality compared with those from other regions according to the previous investigations, the exhaustive exploitation driven by increasing demand has drastically reduced the wild resource. As a result, germplasm evaluation and quality resource exploration are important for the sustainable utilization and cultivation of *B. marginatum*. A preliminary study indicated differences in the tissue structure of *B. marginatum* grown in different areas of North Western Hubei province. In the current study, various tissues of the roots of *B. marginatum* grown in different areas of North Western Hubei were subjected to laser microdissection and analyzed by microscopy and ultra-high performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC–Q–TOF–MS). The results show that wild plants

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from Maqiao Town, Baokang County contain the most saikosaponins distributed mainly in cork, cortex and phloem. This study provides key chemical information for evaluating the quality of *B. marginatum* roots.

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## 1. Introduction

*Bupleuri Radix* (Chinese thorowax root, known as ‘Chaihu’ in Chinese) is commonly used in traditional Chinese medicine (TCM) for the treatment of fevers and colds, malaria, cholecystitis, hepatitis, pancreatitis and menstrual disorders. It is often found in clinical prescriptions and patent medicines including Xiao–Chaihu–Tang, Xiao–Yao–Wan, Jia–Wei–Xiao–Yao–Wan and Chai–Ling–Tang. Modern studies have indicated that the roots of *Bupleurum marginatum* contain large amounts of saikosaponins a, c and d<sup>1</sup> of which a and d are the main active components to which the clinical efficacy of *Bupleuri Radix* is attributed<sup>2</sup>.

In the herbal market, the species *Bupleurum chinense* DC., *Bupleurum scorzonerifolium* Willd. and *B. marginatum* Wall. ex DC. are the main sources of commercial *Bupleuri Radix*<sup>3</sup>. Although the former two are recorded as sources of *Bupleuri Radix* in Chinese Pharmacopoeia and are widely cultivated, the widespread demand for the herb has tended to far outstrip the supply<sup>4</sup>. This is particularly the case for *B. marginatum* which usually grows wild on hillside meadows in Hubei, Yunnan, Sichuan, Guizhou and Guangxi provinces. As with other important medicinal herbs, the conservation, germplasm evaluation, quality resource exploration and large-scale cultivation of wild resources have become critical for their sustainable utilization.

It is well known that the *Bupleuri Radix* produced in the North West of Hubei province is of higher quality than products from other regions<sup>5,6</sup>. It is assumed that this is due to favorable features of the geography and climate of the area. To investigate the distribution and usage of *Bupleurum* species in North Western Hubei, a systematic field survey was carried out<sup>5</sup>. The results indicate that *B. marginatum* is the main species of *Bupleurum* in the region and that its roots are the actual commodity sold and used as *Bupleuri Radix* in the region. In a preliminary study, we found that the microscopic features of transverse sections of *B. marginatum* from different growing areas were different, a fact that could be important in selecting sources for cultivation of this important medicinal plant.

A medicinal plant usually contains a complex mixture of chemical components, the production and distribution of which

is directly related to the species and the environmental conditions under which it is grown. In recent years, the technique of liquid chromatography–mass spectrometry (LC–MS) has been widely applied to profile the chemical composition of herbal medicines<sup>7,8</sup>. Additionally, laser microdissection (LMD) has been used to facilitate tissue- and cell-specific metabolite profiling of plants<sup>9,10</sup>. Recently, we applied this combination of techniques to analyze tissue-specific metabolites in the stems of *Sinomenium acutum* (Thunb.) Rehd. et Wils.<sup>11</sup>. The objective of the present study was to analyze and compare the chemical profiles of roots of *B. marginatum* from North Western Hubei in order to enhance the quality evaluation of *B. marginatum*.

## 2. Materials and methods

### 2.1. Materials

Four batches of dried roots (Samples 1–4) and one batch of fresh roots (Sample 5) of *B. marginatum* grown in North Western Hubei province (Table 1) were collected. Samples 1, 2, 3 and 5 were grown in different areas but samples 3 and 4 were cultivated and wild plants respectively from the same area. The diameter of dried roots selected for study was about 0.7 cm and the batch of fresh roots was separated into roots of three approximate sizes *viz* 0.3, 0.6 and 1.0 cm. All samples were authenticated by Dr. Guangyi Yang and deposited in the Bank of China (Hong Kong) Chinese Medicines Centre of Hong Kong Baptist University.

### 2.2. Chemicals and reagents

Pure samples (>98% by HPLC) of saikosaponins a, c and d were isolated in our laboratory<sup>1</sup>. HPLC grade acetonitrile and methanol were from E. Merck (Darmstadt, Germany). HPLC grade formic acid (purity 96%) was purchased from Tedia (USA). Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

**Table 1** Sources of the samples of *Bupleurum marginatum* Wall. ex DC. in North Western Hubei Province.

No.	Source	Growing condition	Collection time
1	Shangjin town, Yunxi county	Wild, altitude 500–1000 m, hillside meadow	Mar 16, 2011
2	Guandu town, Zhushan county	Wild, altitude 350–1100 m, under forest or hillside meadow	Aug 13, 2011
3	Maqiao town, Baokang county	Cultivation, flat ground	Sep 14, 2011
4	Maqiao town, Baokang county	Wild, altitude 500–800 m, hillside meadow	Aug 23, 2011
5	Qingfeng town, Fang county	Wild, altitude 800–1000 m, hillside meadow	Oct 26, 2011

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