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Qualitative and quantitative determination of polyacetylenes in different *Bupleurum* species by high performance liquid chromatography with diode array detector and mass spectrometry

Hai-Qiang Huang[a], Juan Su[a,**], Xi Zhang[a], Lei Shan[a], Wei-Dong Zhang[a,b,*]

[a] Department of Natural Medicinal Chemistry, School of Pharmacy, Second Military Medical University, Shanghai 200433, China
[b] School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, China

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ABSTRACT

Polyacetylenes are main toxic ingredients in *Bupleurum longiradiatum*, a poisonous plant that has ever been misused as substitutes for *Chaihu* (Bupleuri Radix). For the first time, a high-performance liquid chromatography method coupled with diode array detector and mass spectrometry (HPLC-DAD–MS) was developed for qualitative and quantitative analysis of nine polyacetylenes in *Bupleurum* species. All references, including two new polyacetylenes, were isolated from *B. longiradiatum* and purified using a semi-preparation HPLC chromatography. The analysis was performed on a TSKgel ODS-100 V C18 column (3 μm, 150 mm × 4.6 mm i.d.) using a gradient system of acetonitrile and water, with diode array detection (254 nm). The method was validated for linearity, precision, accuracy, limit of detection and quantification. A total of 27 *Bupleurum* samples were examined with this method, which showed a great variety in the distribution and contents of the polyacetylenes. It was found that polyacetylenes (**1**–**8**) were the main ingredients in *B. longiradiatum*, while a few kinds of polyacetylenes (**5**–**8**) were also identified in *B. smithii*, *B. smithii* var. *parvifolium*, *B. bicaule* and *B. angustissimum*. However, no polyacetylenes (**1**–**9**) were detected in the authentic *Chaihu* samples and the other *Bupleurum* species. The results indicated that the toxic *B. longiradiatum* could readily be distinguished from other medicinal *Bupleurum* species based on the polyacetylene profiles, and the guidelines and quality control of polyacetylenes for *Chaihu* are useful. The acute toxicity of the ethanol extract of *B. longiradiatum* and its fractions was also investigated.

1. Introduction

Traditional Chinese medicine (TCM) herbs and their manufactured products have been used for thousands of years for prevention and treatment of disease in China. When prescribed according to Chinese medical philosophy and practice, those medications and crude materials recorded in the Chinese Pharmacopoeia have been approved for safety and efficacy [1]. However, increasing concerns and fears have been expressed to the efficacy, toxicity and quality of TCM herbs, because many popular and expensive TCM herbs are in short supply and inferior substitutes or fake crude herbs have been found in the market [2–4]. Moreover, misuse and confusion of names of certain herbs enhances difficulty in identifying the correct herbs [3,4]. This can be dangerous to consumers because some substitution involve poisonous material. Examples of the substitution of indicated material for poisonous alternatives are described throughout the literature [3–5]. A famous case was the substitutions of *Stephania tetrandra* with poisonous *Aristolochia fangchi* that resulted in cases of rapidly progressive renal fibrosis found in young Belgian women [6–8]. Despite the growing interest in TCM herbs, there are still insufficient scientific data on the safety and efficacy of some herbs, and sometimes the toxicity of a particular plant and its constituents have not been well characterized. Therefore, reliable methods are in great demand for the efficient detection and rapid characterization of specific active or toxic components, and to ensure that the TCM herbs are safe and their labeling is truthful and not misleading.

Bupleuri Radix, with a Chinese name *Chaihu*, is recorded as the roots of *Bupleurum chinense* and *B. scorzonerifolium* (Apiaceae) in the Chinese Pharmacopoeia, which has been widely practiced to treat influenza, fever, inflammation, malaria and menstrual disorders [9,10]. In previous chemical studies on *Bupleurum* plants, saponins, flavonoids, coumarins, fatty acids, steroids, polysaccharides and polyacetylenes were identified [11,12]. Among them,

* Corresponding author. Tel.: +86 21 81871244; fax: +86 21 81871244.
** Corresponding author.
E-mail addresses: juansu_2008@126.com (J. Su), wdzhangy@hotmail.com (W.-D. Zhang).

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saponins were known to be the major bioactive compounds, which were commonly used as chemical standards for quality evaluation of *Chaihu* in the current Chinese Pharmacopoeia and recent publications [9,13–17]. However, a systematic study on the authentication and assessment on related *Bupleurum* species was not well defined. There are at least ten species of the genus *Bupleurum* in the family Apiaceae that are also used under the name of *Chaihu* locally [11]. Even worse, *B. longiradiatum*, widely distributed in northeastern mainland China, is a poisonous plant but sometimes has been found in herb markets [9,11]. The misuse of *B. longiradiatum* as *Chaihu* had caused several cases of human poisoning, and at least three people died after administration of this plant, which showed symptoms such as serious nausea, vomiting, twitching, opisthotonus, and so on [11,18]. Following investigation on the acute toxicity of extracts of *B. longiradiatum* also found its strong toxicity against mice, which was attributed to its high content of hydrophobic compounds [11,19]. Six polyacetylenes were then isolated from the roots of *B. longiradiatum*, among which bupleurotoxin and acetylbupleurotoxin were toxic, with LD_{50} values of 3.03 mg/kg and 3.13 mg/kg, respectively [18]. Another study also found that bupuleurotoxin showed strong toxicity to mice at high doses [28].

Our preliminary surveys on *B. longiradiatum* demonstrated that polyacetylenes were of particular abundance in root of this plant and proved to be responsible for the toxicity of *B. longiradiatum* [12]. Naturally occurring polyacetylenes were previously reported to be cytotoxic and neurotoxic at high concentrations, and they also to be potent skin sensitizers [20–22]. The well known polyacetylene toxin is oenanthotoxin, isolated from *Oenanthe fistulosa*, a toxic plant in the family Apiaceae [23,24]. As major toxic ingredients of *B. longiradiatum*, however, the contents of polyacetylenes in *B. longiradiatum* have not been studied yet. Moreover, their distributions in other related *Bupleurum* species are still unknown.

In general, the differences in constituents in TCM herbs will affect the efficacy and safety of pharmaceutical products and the standardization of this herbal medicine. Therefore, it is important to quantify the content of active constituents in herbal medicines, to ensure its efficacy. As to the clinic security, it is crucial to determine the toxic compounds, and to confirm the absence or presence of toxic ingredients in any drug products. Up until now, the quality control of *Chaihu* and related *Bupleurum* species mainly emphasized on the determination of bioactive saponin compounds [13–17], but not considered the scrutiny of various toxic ingredients in *Bupleurum*. To the best of our knowledge, no method for the determination of polyacetylenes in *Bupleurum* species has been reported so for.

Recently, HPLC-DAD–MS has shown its wide application in TCM research because the combination of DAD and MS can provide online UV and MS information at the same time for each individual peak in a chromatogram, while HPLC–UV is a convenient and effective method to control the quality of TCM for its rapid separation and quantitation [25].

This work focuses on developing a simple, effective and reliable method to analyze polyacetylenes in *B. longiradiatum* and related *Bupleurum* species. A high-performance liquid chromatography (HPLC) method coupled with diode array detection (DAD) and mass spectrometry (MS) was established to the qualitative and quantitative determination of nine polyacetylenes (**1**–**9**, Fig. 1). The structure of the constituents isolated was determined on the basis of UV, IR, NMR and MS data and the complete structural elucidation of compounds **1** and **3** is reported herein for the first time. The acute

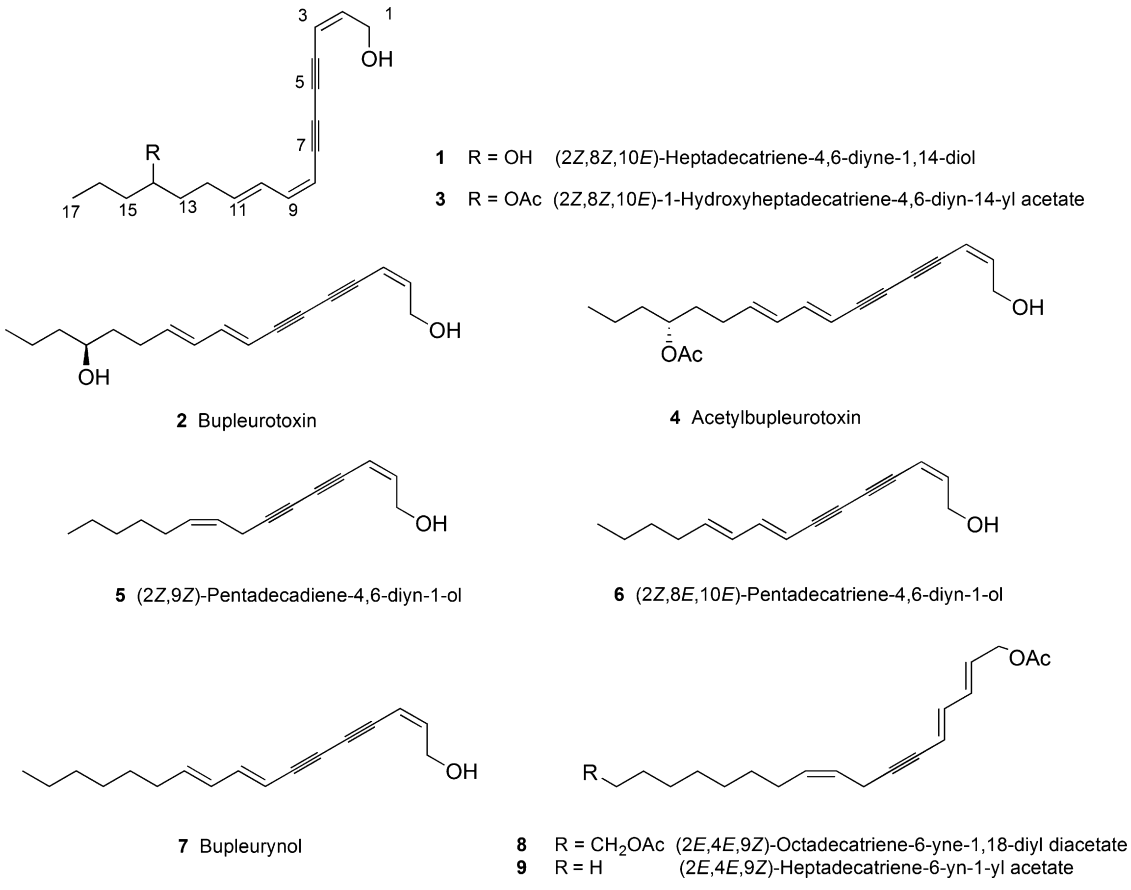

Fig. 1. Chemical structures of the nine standard compounds in the study.