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ORIGINAL ARTICLE

Separation, identification, and quantification of active constituents in *Fructus Psoraleae* by high-performance liquid chromatography with UV, ion trap mass spectrometry, and electrochemical detection

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Abstract The qualitative and quantitative analysis of active constituents in *Fructus Psoraleae* is presented by high-performance liquid chromatography (HPLC) coupled with different detections. Extracts of *Fructus Psoraleae* were examined by HPLC with ion trap mass spectrometry (IT-MS) and 18 major compounds of coumarins, benzofuran glycosides, flavonoids, and meroterpene were identified. The determination of four major constituents including bavachin, isobavachalcone, bavachinin, and bakuchiol was accomplished by HPLC with UV, MS, and electrochemical detection (ECD). These methods were evaluated for a number of validation characteristics (repeatability, LOD, calibration range, and recovery). ECD obtained a high sensitivity for analysis of the four components; MS provided a high selectivity and sensitivity for determination of bavachin, isobavachalcone, and bavachinin in negative-ion mode. After optimization of the methods, separation, identification. and quantification of the four components in *Fructus Psoraleae* were comprehensively tested by HPLC with UV, MS, and ECD.

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1. Introduction

Fructus Psoraleae (Buguzhi in Chinese), the dried ripe fruit of *Psoralea corylifolia* L. (Fabaceae), is a well-known traditional Chinese medicine (TCM) with many beneficial effects [1]. It is traditionally used to alleviate asthma and diarrhea, and to treat vitiligo and alopecia areata in East Asian countries. The major active constituents of this herb contain coumarins, benzofuran glycosides, flavonoids, and meroterpene, such as psoralenoside, isopsoralenoside, psoralen, isopsoralen, neobavaisoflavone, bavachin, psoralidin, isobavachalcone, corylifol

A, bavachinin, and bakuchiol, etc. [2–4]. Bavachin, isobavachalcone, and bavachinin are three typical flavonoid ingredients and bakuchiol is typical meroterpene in *Fructus Psoraleae*. Their chemical structures are shown in Fig. 1. They have functions in common such as inhibition to α-glucosidase activities [3] and anti-oxidation [5]. Clinical studies have shown that bavachin can stimulate bone formation [6] and that isobavachalcone exhibits a broad spectrum of biological activities, such as enhancing cardiac contractility, preventing cardiac fatigue due to lactic acid, and so on [7], bavachinin inhibits the accumulation of nitric oxide (NO) [8]. In addition, bakuchiol, a principal constituent, and its related compounds show a variety of bioactivities such as inhibition of monoamine transporters, immunosuppressive effect, caspase-3-depended apoptosis, hepatoprotective effect, antimicrobial activity, anti-inflammatory effect, inhibition of DNA polymerase, and topoisomerase II, cytotoxic effect, and anti-diabetic effects [9,10].

As we know, the active constituents in traditional Chinese medicine (TCM) are very complex so that the mechanism to cure disease is still unclear. In order to isolate and identify these active compounds in *Fructus Psoraleae*, many methods such as thin-layer chromatography (TLC) [11], high-performance liquid chromatography (HPLC) [12,13], liquid chromatography-mass spectrometry (LC/MS) [14,15], high-speed counter-current chromatography (HSCCC) [16], and micellar electrokinetic chromatography (MEKC) [17] were reported. Compared with CE, HPLC can afford better analytical precision and higher sample loading capacity. HPLC coupled to UV [12,13] and MS detection [14,15] is applied in the determination of the active compounds in *Fructus Psoraleae*. HPLC in combination with tandem mass spectrometry (MS) appears to be a more suitable technique for the identifying of the active compounds in plant samples in terms of sensitivity and selectivity.

In recent years analytical techniques have made great advances. LC-MS and LC-tandem MS (LC-MS/MS) such as quadrupole time of flight (Q-TOF) [18] and quadrupole-linear ion trap (Q-LIT) [19] have played a crucial role in plant analysis. The application of ion trap (IT) mass spectrometers in the structure elucidation and in the identification of unknowns in complex matrices has been well established [20]. In previous work, we developed an LC-IT-MS for identification and quantification of oleanolic acid and ursolic acid in nine Chinese herbs [21].

Electrochemical detection (ECD) is a powerful detection technique for HPLC separation. ECD has many advantages such as simple, rapid, inexpensive, and sensitive. HPLC coupled with ECD has been reported to analyze phenolic and flavonid compounds in natural products. Analysis of amino acid biomarkers and active constituents in Chinese medicine by HPLC-ECD has been reported in our group [22,23].

The aim of the present work was the identification of the main active compounds in *Fructus Psoraleae* by HPLC-ESI-IT-MS and development of HPLC-UV, HPLC-MS, and HPLC-ECD methods for simultaneous determination and quantification of four active components (bavachin, isobavachalcone, bavachinin, and bakuchiol). The analytical characteristics of HPLC coupled with different detections have been compared and discussed.

2. Experimental

2.1. Chemicals and materials

The ripe seed of *Psoralea corylifolia* L. was purchased from Liuyouyu Drugstore, Wuhan (Hubei, China). Bavachin (purity ≥ 99%), isobavachalcone (purity ≥ 99%), bavachinin (purity ≥ 99%), and bakuchiol (purity ≥ 99%) were obtained from Shanghai Shunbo Bio-engineering Technology (Shanghai, China). Methanol and acetonitrile of HPLC grade were purchased from Tedia (USA) and VBS (USA), respectively. Deionized water was purified using a Milli-Q system (Millipore, Bedford, MA, USA); Helium (purity, 99.999%), and liquid nitrogen were obtained from Wuhan Analytical

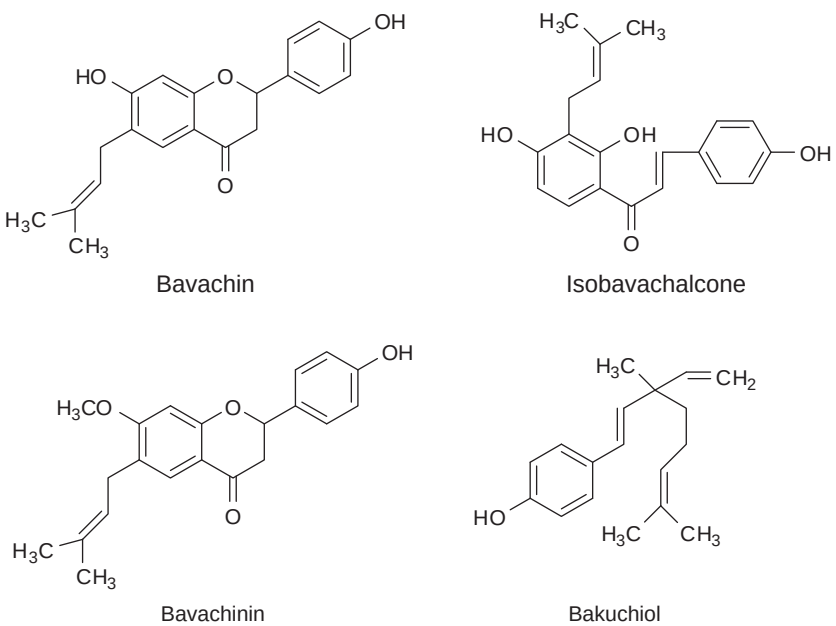

Figure 1 Chemical structures of bavachin, isobavachalcone, bavachinin and bakuchiol.

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