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Simultaneous quantification of eight major constituents in Herba *Siegesbeckiae* by liquid chromatography coupled with electrospray ionization time-of-flight tandem mass spectrometry

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ABSTRACT

A simple and reliable high-performance liquid chromatography coupled with electrospray ionization time-of-flight tandem mass spectrometry method was developed and validated for the determination of the major diterpenoids and flavonoids in the aerial parts of Herba *Siegesbeckiae*, including Kirenol, hythiemoside B, *ent*-16β,17,18-trihydroxy-kauran-19-oic acid, *ent*-17,18-dihydroxy-kauran-19-oic acid, *ent*-16β,17-dihydroxy-kauran-19-oic acid, 16α-hydro-*ent*-kauran-17,19-dioic acid, Rhamnetin, 3′,4′-dimethoxy quercetin. The separation of eight compounds was performed on a Waters Symmetry Shield TM RP18 column (250 mm × 4.6 mm i.d., 5 μm) with gradient elution using a mobile phase consisting of 0.1% aqueous formic acid and acetonitrile containing 0.1% formic acid in selected ion monitoring mode. All calibration curves showed good linearity ($r > 0.999$) within the test ranges. The precision was evaluated by intra- and inter-day tests, which revealed relative standard deviation (RSD) values less than 3.7%. The recoveries for the quantified compounds were between 97.4 and 101.2% with RSD values below 2.4%. According to the literatures, this study represents the first investigation of the simultaneous analysis of multiple components and the method can be applied to determine the amounts of the major compounds in Herba *Siegesbeckiae*.

1. Introduction

The plants of the genus *Siegesbeckiae* (Compositae) are annual herbs widely distributed in tropical, subtropical, and temperate parts of the world. Three species of *Siegesbeckiae* (*Siegesbeckiae orientalis* L., *Siegesbeckiae pubescens* Makino, *Siegesbeckiae glabrescens* Makino) grow in China, and their aerial parts have been used as a traditional Chinese medicine, "Xi-Xian", for the treatment of rheumatic arthritis, hypertension, malaria, neurasthenia, and hepatitis [1]. Extracts of Herba *Siegesbeckiae* (HS) have been reported to exhibit antioxidant [2], antiallergic [3] and antifertility effects [4]. In the past few decades, systematic chemical studies have been performed, and HS is known to contain a large number of compounds, including diterpenoids, flavonoids, steroids and fatty acids. Pharmacological studies have suggested that characteristic diterpenoids are the main bioactive constituents of HS, a series of *ent*-pimarane and *ent*-kaurane diterpenoids from the herbs have been reported to be responsible for the antifertility [4], anti-inflammatory [5] and PTP1B inhibitory activities [6]. Flavonoids were also the bioactive compositions, Kim et al. suggested that flavonoids in HS provided the activity of inhibiting the production of NO in the lipopolysaccharide (LPS)-induced microglia [7]. Therefore, the quality control of HS should be focused on the determination of the diterpenoids and flavonoids.

To our knowledge, only several papers employed high pressure liquid chromatography-ultraviolet (HPLC-UV) to determine Kirenol or Darutigenol in HS [8–10]. And very few literatures were referred to the quantitative assay of *ent*-pimarane and *ent*-kaurane diterpenoids. An intractable problem is that most diterpenoids found in HS are not detectable by HPLC-UV analysis for the lack of an UV chromophore. Consequently, it is particularly difficult to simultaneously determine these diterpenoids with HPLC-UV. By contrast, MS is a sensitive and selective technique that allows for detection of the constituents of no UV absorption, and TOF-MS allows the generation of mass information with greater accuracy and precision. Due to high sample throughput, HPLC–TOF-MS methods are suitable for the analysis of traditional Chinese medicine (TCM) especially for low-abundance and complex compounds, which are difficult to analyze by conventional isolation.

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In this paper, HPLC–ESI-TOF-MS method was proposed for the quantification of the major diterpenoids and flavonoids including Kirenol (1), hythiemoside B (2), *ent*-16β,17,18-trihydroxy-kauran-19-oic acid (3), *ent*-17,18-dihydroxy-kauran-19-oic acid (4), *ent*-16β,17-dihydroxy-kauran-19-oic acid (5), 16α-hydro-*ent*-kauran-17,19-dioic acid (6), Rhamnetin (7), 3′,4′-dimethoxy quercetin (8) in five different sources of HS. According to the literatures, this study represents the first investigation of the simultaneous analysis of multiple components in HS.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade acetonitrile was purchased from Concord Technology Co., Ltd. (TianJin, China). Distilled water was prepared from demineralized water in our laboratory. Analytical reagent grade formic acid was obtained from Kermel Chemical Reagent Co., Ltd. (TianJin, China). The aerial parts of HS from five sources, including *S. pubescens* Makino from Hebei (two batches, samples A and B purchased from Anguo Province Zhenyu Chinese Medicine Pieces Company Limited) and Henan (sample C gathered from Xin County Henan Province), *S. orientalis* L. from Hunan (sample D bought from Anhui Jioujioutong Pharmaceutical Company Limited) and *S. glabrescens* Makino from Yunnan (sample E bought from Ynnan Qiancaoyuan Pharmaceutical Company Limited), were authenticated by Qi-shi Sun, Professor of Pharmacognosy. Compounds 1–8 (Fig. 1) were isolated and purified from the extract of *S. pubescens* Makino (sample A) in our laboratory, and identified by ^{1}H NMR, ^{13}C NMR and comparison with those reported in the literatures among which compounds 7 and 8 were not yet reported from SH [11–17]. The purities of the above ingredients using as standards were above 98% by LC analysis.

2.2. Sample preparation

The dried powders (1 g) of HS were extracted by refluxing with 10 ml of methanol for 1 h, subsequently the extraction was repeated two additional times. The combined extract was filtered, and the filtrate was concentrated to a final volume of 10 ml using a rotary evaporator at 45 °C. The sample was passed through a 0.45 μm filter.

2.3. HPLC conditions

HPLC-MWD analysis was carried out on an Agilent 1200 series HPLC system (Agilent Technologies, USA), consisting of a G1376A Capillary Pump, a G1316A Thermostat Column Compartment and a G1367B autosampler. A Symmetry Shield TM RP18 column (250 mm × 4.6 mm i.d., 5 μm, Waters) was used for chromatographic separation, the column temperature was set at 30 °C. A linear gradient system consisted of mobile phase A (0.1% formic acid aqueous solution) and mobile phase B (acetonitrile containing 0.1% formic acid). The gradient elution profile was as follows: 0–10 min, 70% A; 10–12 min, 70–62% A; 12–22 min, 62% A; 22–25 min, 62–50% A; 25–28 min, 50–38% A; 28–40 min, 38% A. The flow rate was kept at 1.0 ml/min. The injection volume was 2 μl.

2.4. MS conditions

The HPLC system was coupled to an Daltonic micrOTOF-Q mass spectrometer equipped with electrospray ionization (ESI) interface (Bruker, Germany) in negative ion mode. The MS operating conditions were optimized as follows: the dry gas temperature was set at 240 °C, the flow rate was 6.0 l/min, the nebulizer pressure was set at 1.2 bar, the capillary voltage at −4.5 kV and collision energy at −10.0 eV. Quantification was obtained by using selected ion

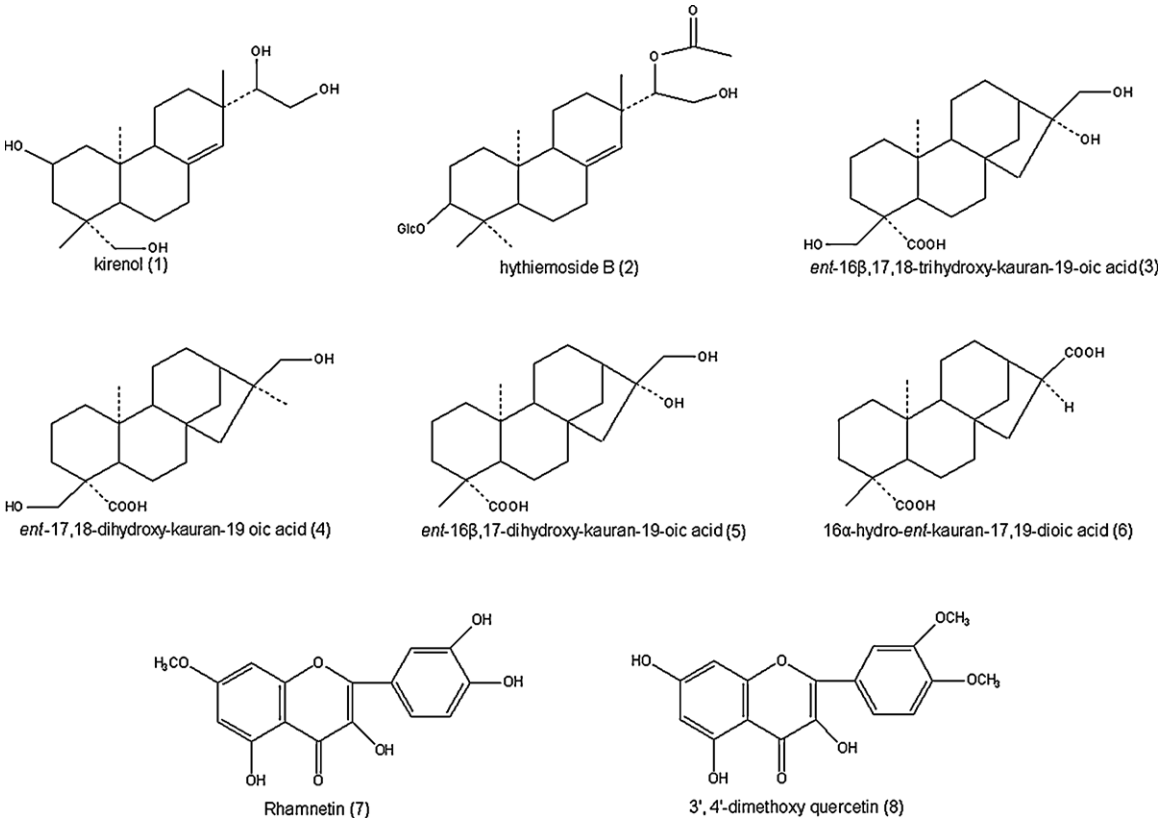

Fig. 1. Structures of compounds 1–8.