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Original Article

Chemical material basis study of Xuefu Zhuyu decoction by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry

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ABSTRACT

Xuefu Zhuyu decoction, a classic prescription in traditional Chinese medicine, has been widely used in the clinical treatment of cardiovascular and cerebrovascular diseases. In order to profile the chemical material basis of this formula, an ultra-performance liquid chromatography (UPLC) coupled with quadrupole time-of-flight mass spectrometry (Q/TOF MS) method has been established for rapid separation and structural characterization of compounds in the decoction. As a result, 103 compounds including phenolic acids, spermidines, C-glycosyl quinochalcones, terpenoids, flavonoids, saponins, and others were detected; 35 of them were unambiguously identified, and 68 were tentatively characterized by comparing the retention time, MS data, characteristic MS fragmentation pattern and retrieving the literature. In conclusion, the UPLC coupled with quadrupole time-of-flight mass spectrometry method developed in this work is an efficient approach to perform chemical material basis studies of traditional Chinese medicine formulae.

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1. Introduction

Cardiovascular and cerebrovascular diseases are common diseases of the elderly that have seriously threatened human health in recent years. Even when the most advanced and comprehensive treatment was applied, more than 50% of the survivors from cardiovascular and cerebrovascular incidents were still unable to provide for themselves completely. Every year around the world, as many as 15 million people die of cardiovascular and cerebrovascular diseases. They have become one of the primary causes of human death.

The traditional Chinese medicine formula Xuefu Zhuyu decoction (XFZYD) was first recorded in Yilin Gaicuo (*Correction of Medical Errors*, 1850) by Qingren Wang (1768–1831) [1]. The herbal combination is regarded as a modification of two famous classic prescriptions, Taohong Siwu decoction (Peach Seed and Safflower Decoction of Four Ingredients) and Sinisan (Powder for Regulating Liver and Spleen) [2], which comprises 11 herbs: *Semen prunus* (Taoren) 12 g, Radix *Angelicae sinensis* (Danggui) 9 g, Rhizoma chuanxiong (Chuanxiong) 4.5 g, *Flos carthami* (Honghua) 9 g, Radix *Paeoniae rubra* (Chishao) 6 g, Radix rehmanniae (Dihuang) 9 g, *Fructus aurantii* (Zhiqiao) 6 g, Radix Bupleuri (Chaihu) 3 g, Radix platycodonis (Jiegeng) 4.5 g, Radix *Achyranthis bidentatae* (Niuxi) 9 g, and Radix and Rhizoma glycyrrhizae (Gancao) 6 g [3,4]. XFZYD has been demonstrated to show definite protection in the cardiovascular and cerebrovascular system, and modern pharmacological studies have elucidated the protective mechanisms [5,6]. XYZFD could induce endothelial progenitor cell angiogenesis, hasten tube formation [7], and regulate blood lipid [8,9]. Satisfactory clinical efficiency has been achieved for cardiovascular and cerebrovascular diseases [10] such as atherosclerosis, hypertension, hyperlipidemia, thromboembolism, and angina pectoris.

It is well known that the therapeutic effects of herbal medicine are due to the synergistic contribution of multiple constituents [11]. Since XFZYD has centuries of clinical use and reliable curative efficacy, developing a feasible and rapid analytical method for characterizing the constituents in the decoction is valuable and vital to ensuring its reliability and safety in clinical therapy. Many researchers have made significant contributions to the studies of substance foundation in XFZYD. Zhang et al [12] and Liu et al [13] used high-performance liquid chromatography–mass spectrometry (HPLC-MS) methods to identify antiatherogenic constituents of the decoction. Gao et al [14] introduced an HPLC–evaporative light scattering detector method to quantify chemical constituents in the XFZY capsule. In our previous study, an ultra-performance liquid chromatography (UPLC) coupled with diode array detector tandem MS method was undertaken to perform quantitative and qualitative analysis of the constituents in XFZYD products [15].

In order to deeply unveil the chemical compositions of XFZYD, a UPLC coupled with quadrupole time-of-flight (Q/TOF) MS method was introduced and established in this work. A total of 103 constituents were unambiguously identified or tentatively characterized. This also provides a valuable reference for further research and development of this formula and its related medicinal products.

2. Methods

2.1. Reagents and materials

HPLC grade acetonitrile and methanol were purchased from Merck (Merck, Darmstadt, Germany) and Sigma (Sigma–Aldrich, St Louis, MO, USA), respectively. Formic acid and dimethyl sulfoxide were obtained from Meridian Medical Technologies (Columbia, MD, USA). Water used in the experiment was purified by a Milli-Q water purification system (Millipore, Billerica, MI, USA).

Reference compounds (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, hydroxysafflor yellow A, amygdalin, albiflorin, paeoniflorin, *p*-hydroxycinnamic acid, ferulic acid, schaftoside, 6-hydroxy kaempferol-3-O-glucoside, liquiritin, rutin, isoquercitrin, verbascoside, astragalin, narirutin, β-ecdysterone, naringin, rhoifolin, hesperidin, neohesperidin, liquiritigenin, naringenin, kaempferol, platycodin D, isoliquiritigenin, formononetin, ginsenoside-Ro, 18β-glycyrrhizic acid, chikusetsu saponin Iva, nobiletin, and saikosaponin A) were obtained from the National Institute for Food and Drug Control (Beijing, China), Tianjin ZhongXin Pharmaceutical Group Co., Ltd. (Tianjin, China), and Top High Bio Technology Co., Ltd. (Nanjing, China). The purities of standards were >98%.

2.2. Preparation of standard solutions

Reference compounds were accurately weighed and directly prepared in methanol and dimethyl sulfoxide as individual standard stock solutions; following this mixed standard stock solutions containing all 35 standards were prepared. A working standard solution was prepared by diluting the mixed stock solution with water (v/v, 1:3) to obtain a suitable concentration.

2.3. Plant material and sample preparation

The plant materials (Taoren, Danggui, Chuanxiong, Honghua, Chishao, Dihuang, Zhiqiao, Chaihu, Jiegeng, Niuxi, and Gancao) were purchased from Anguo (Hebei, China) and identified by Professor Tianxiang Li. All herbs were deposited in Tianjin State Key Laboratory of Modern Chinese Medicine.

According to the traditional formula, 11 plant materials (total weight of 78 g) were mixed and immersed in 600 mL deionized water for 1 hour at room temperature, and then refluxed for 2 hours twice. After filtration and concentration, aqueous extract was dried at 45°C in an oven under vacuum to give 30 g original extract powder. The yield of preparation was 38.5%. A 10 mL aliquot of methanol was added to 0.4 g of extract powder and sonicated for 30 min at room temperature. The solution was diluted with deionized water (v/v, 1:1) and then centrifuged at 17,968 g for 10 min. Finally, the supernatant was transferred to autosampler vials for UPLC-Q/TOF MS analysis.