Journal of Food and Drug Analysis 25 (2017) 946–959

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Qualitative and quantitative characterization of secondary metabolites and carbohydrates in *Bai-Hu-Tang* using ultraperformance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry and ultraperformance liquid chromatography coupled with photodiode array detector

Wei-Fang Zhong [a,b,1], *Wing-Sum Tong* [a,1], *Shan-Shan Zhou* [a,c,1], *Ka-Man Yip* [a], *Song-Lin Li* [c], *Zhong-Zhen Zhao* [a], *Jun Xu* [a,*], *Hu-Biao Chen* [a,*]

[a] *School of Chinese Medicine, Hong Kong Baptist University, Hong Kong*
[b] *Ocean College, Agricultural University of Hebei, Qinhuangdao, China*
[c] *Department of Pharmaceutical Analysis and Metabolomics, Jiangsu Province Academy of Traditional Chinese Medicine and Jiangsu Branch of China Academy of Chinese Medical Sciences, Nanjing, China*

ARTICLE INFO

Article history:
Received 5 July 2016
Received in revised form
14 December 2016
Accepted 19 December 2016
Available online 14 February 2017

Keywords:
Bai-Hu-Tang
carbohydrate
secondary metabolite
ultraperformance liquid chromatography coupled with photodiode array detector
ultraperformance liquid

ABSTRACT

Bai-Hu-Tang (BHT), a classic traditional Chinese medicine (TCM) formula used for clearing heat and promoting body fluid, consists of four traditional Chinese medicines, i.e., Gypsum Fibrosum (*Shigao*), Anemarrhenae Rhizoma (*Zhimu*), Glycyrrhizae Radix et Rhizoma Praeparata cum Melle (*Zhigancao*), and nonglutinous rice (*Jingmi*). The chemical composition of BHT still remains largely elusive thus far. To qualitatively and quantitatively characterize secondary metabolites and carbohydrates in BHT, here a combination of analytical approaches using ultraperformance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry and ultraperformance liquid chromatography coupled with photodiode array detector was developed and validated. A total of 42 secondary metabolites in BHT were tentatively or definitely identified, of which 10 major chemicals were quantified by the extracting ion mode of quadrupole time-of-flight mass spectrometry. Meanwhile, polysaccharides, oligosaccharides, and monosaccharides in BHT were also characterized via sample pretreatment followed by sugar composition analysis. The quantitative results indicated that the determined chemicals accounted for 35.76% of the total extract of BHT, which demonstrated that the study could be instrumental in chemical dissection and quality control of BHT. The research deliverables not only laid the root for

* *Corresponding authors.* Jockey Club School of Chinese Medicine Building, 7 Baptist University Road, Kowloon Tong, Kowloon 999077, Hong Kong.
E-mail addresses: davidxujun@hkbu.edu.hk (J. Xu), hbchen@hkbu.edu.hk (H.-B. Chen).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jfda.2016.12.007

chromatography coupled with quadrupole time-of-flight mass spectrometry

further chemical and biological evaluation of BHT, but also provided a comprehensive analytical strategy for chemical characterization of secondary metabolites and carbohydrates in traditional Chinese medicine formulas.

1. Introduction

Bai-Hu-Tang (BHT, also known as white tiger decoction), originally documented in Treatise on Exogenous Febrile Diseases (*Shang-Han-Lun*, a renowned medicinal classic at the Eastern Han Dynasty), is a classic traditional Chinese medicine (TCM) formula used for clearing heat and promoting body fluid [1]. The potential of BHT for treating septicemia and hyperglycemia is also demonstrated by pharmacological experiments [2,3]. BHT is composed of one mineral drug, i.e., Gypsum Fibrosum (GF, *Shigao*), and three herbal drugs including Anemarrhenae Rhizoma (AR, *Zhimu*), Glycyrrhizae Radix et Rhizoma Praeparata cum Melle (GR, *Zhigancao*), and nonglutinous rice (NR, *Jingmi*) [1]. Bioactive ingredients of the four drugs have been individually explored. Specifically, GF is constituently explicit with no less than 95% hydrated calcium sulfate ($CaSO_4 \cdot 2H_2O$) [4]; secondary metabolites, mainly involving saponins (steroidal and triterpene) and flavonoids (xanthones, flavanonols, and chalcones), are believed to be largely responsible for pharmacological effects of AR [5] and GR [6]; NR mainly contains multiple primary metabolites (e.g., carbohydrates, amino acids, and vitamins) and microelements, of which polysaccharides, especially amylose and amylopectin, were much abundant occupying up to around 80% [7]. Nevertheless, the constituents of BHT still remain qualitatively and quantitatively unknown, especially those contributed by the three herbal drugs due to their complex chemical compositions. Whether and how the bioactive chemicals in the individual drugs occur in BHT are crucial to both control the quality and ascertain the effective substances of BHT, and therefore deserve further investigation.

Secondary metabolites and carbohydrates are commonly deemed as the two major kinds of ingredients in most TCMs, in particular herbal TCMs [8,9]. However, nowadays chemical characterization of TCMs extensively focuses on secondary metabolites since they have been understood adequately [10], just as in the cases of AR and GR. By contrast, carbohydrates (polymeric and monomeric carbohydrates) are largely overlooked due to scientifically restricted definition. However, the situation is becoming increasingly debatable given the recently revealed chemical and bioactive roles of TCM carbohydrates. To be specific, first, natural carbohydrates are abundant in many TCMs and are easily extracted as the taken chemicals since TCMs are usually prepared by water extraction [11]. Second, accumulated *in vivo* and *in vitro* studies have demonstrated that TCM carbohydrates have various pharmacological actions—anticancer [12], immune regulation [13,14], hyperglycemic [15], and prebiotic-like effects [16], to name but a few. In addition to their direct bioactivity, we recently verified that carbohydrates could also exert indirect effects *in vivo* by synergy with co-occurring secondary metabolites in herbal medicines [17]. Therefore, carbohydrates should be taken into account for overall chemical characterization of TCMs as well as TCM formulas.

Thus, we seek to further explore chemical compositions of BHT in this study by characterizing both carbohydrates and secondary metabolites. First, BHT was accordingly prepared. Then, an ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS/MS) method was developed for qualitative and quantitative characterization of the secondary metabolites in BHT, for the latter of which the extracting ion mode of QTOF-MS was adopted. Meanwhile, carbohydrates in BHT, including polysaccharides, oligosaccharides, and monosaccharides, were also qualitatively and quantitatively determined by sample pretreatment and then sugar composition analysis using ultra-performance liquid chromatography coupled with photodiode array detector (UPLC-PDA).

2. Methods

2.1. Reagents, chemicals, and materials

Acetonitrile [high-performance liquid chromatography (HPLC) and MS grade], ammonium acetate (HPLC grade), ethanol (absolute), and formic acid (MS grade) were purchased from Merck (Darmstadt, Germany). Trifluoroacetic acid used for acid hydrolysis of polymeric carbohydrates was from Riedel-de Haën (Honeywell, Seelze, Germany). For monosaccharide derivatization, 3-methyl-1-phenyl-5-pyrazolone (PMP) was bought from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was produced by a Milli-Q water purification system (Merck Millipore, Milford, MA, USA).

Reference substances including neomangiferin, isomangiferin, mangiferin, isoliquiritin, liquiritin, neoliquiritin, liquiritin apioside, timosaponin BII, glycyrrhizic acid, and timosaponin AIII (Figure 1) were supplied from Chengdu Pufei De Biotech Co. Ltd (Szechwan, China). The purity of these reference standards was determined to be higher than 95% by HPLC–MS analysis (the same analytical condition as described in Section 2.3). Sugar reference substances, namely, D-mannose (Man), L-rhamnose monohydrate (Rha), D-glucuronic acid (GlcA), D-galacturonic acid monohydrate (GalA), D-glucose (Glc), D-galactose (Gal), L-arabinose (Ara), D-fucose (Fuc), sucrose, and maltotriose, were obtained from Sigma.

GF and decoction pieces of AR and GR were purchased from Hong Kong herbal market, and were authenticated by Professor H.B. Chen based on the morphological and histological features according to the standards of Chinese