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Holistic evaluation of San-Huang Tablets using a combination of multi-wavelength quantitative fingerprinting and radical-scavenging assays

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[ABSTRACT] The present study was designed to establish a multi-wavelength quantitative fingerprinting method for San-Huang Tablets (SHT), a widely used and commercially available herbal preparation, where high performance liquid chromatography (HPLC) with a diode array detector (DAD) was employed to obtain the fingerprint profiles. A simple linear quantitative fingerprint method (SLQFM) coupled with multi-ingredient simultaneous determination was developed to evaluate the quality consistency of the tested samples qualitatively and quantitatively. Additionally, the component–activity relationship between chromatographic fingerprints and total radical-scavenging capacity *in vitro* (as assessed using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay) was investigated by partial least squares regression (PLSR) analysis to predict the antioxidant capacity of new samples from the chromatographic fingerprints and identify the main active constituents that can be used as the target markers for the quality control of SHT. In conclusion, the strategy developed in the present study was effective and reliable, which can be employed for holistic evaluation and accurate discrimination for the quality consistency of SHT preparations and other traditional Chinese medicine (TCM) and herbal preparations as well.

[KEY WORDS] San-Huang Tablets; Radical-scavenging activity; Simple linear quantitative fingerprint method; Quality evaluation

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Introduction

Traditional Chinese medicine (TCM) and herbal preparations are widely used to treat various diseases and medical conditions [1], but the quality of TCM and herbal preparations is not always sufficiently evaluated. The chemical composition of TCM and herbal preparations is very complex and also subjected to the influence of a wide range of factors (e.g., climate, geographical location, and harvest time). The therapeutic efficacy of TCM is believed to achieve through synergistic effects of multiple components in herbal preparations [2]. The published methods have been focused on the analysis of either single or a limited number of chemical compounds; however, this approach has encountered inherent shortcomings and is incapable of ensuring quality consistency of herbal preparations. Therefore, developing suitable analytical methods is critical to the safe use of TCM and herbal medicines. Chromatographic fingerprinting techniques have been recognized by World Health Organization (WHO), USA Food and Drug Administration (FDA), and China Food and Drug Administration (CFDA), for authentication, identification and/or quality assessment of TCM and herbal preparations [3-11]. Most chromatographic fingerprinting methods employ HPLC coupled with UV or diode array detector (DAD) at a fixed wavelength; however, detection at a single wavelength may not be able to profile all chemical components with widely differing physicochemical properties. Thus, diverse strategies, such as enhanced fingerprinting at

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multi-wavelength [12] and two-dimensional (2D) fingerprinting [13-14], have been introduced to better address the chemical complexity of TCM and herbal preparations.

It has also been reported that increasing free radical generation is implicated in tissue damage during inflammation. The chemical components with antioxidant activities have been found to have anti-inflammatory effects [15]. This has led us to assess the antioxidant activity of the herbal preparations by quantifying the free radical scavenging capacity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. The antioxidant activities provide a second dimension to the quality assessment of TCM and herbal medicines in addition to chromatographic fingerprinting. Furthermore, developing a rapid and reliable fingerprint-efficacy relationship model would allow fast investigation of herbal medicines because fingerprinting is not directly related to efficacy and the separation processes are laborious and time-consuming [1].

San-Huang Tablets (SHT), one of the most popular herbal preparations used extensively in clinical practice, has various pharmacological activities, including anti-inflammatory, antimicrobial, antioxidant and antineoplastic activities. It may also have various effects of clearing heat, detoxification, and purging pathogenic fire, according to TCM theory [16]. It is composed of *Rhei* Radix et *Rhizoma*, *Scutellaria baicalensis* Georgi extractum, and berberine hydrochloride in the ratio of 300 : 21 : 5 (*W/W/W*). The major components in SHT include anthraquinones from *Rhei* Radix et *Rhizoma* (e.g., emodin, aloe-emodin, rhein, chrysophanol, and physcion), flavonoids from *Scutellaria baicalensis* Georgi extractum (e.g., baicalin and wogonoside), and berberine hydrochloride, which is an alkaloid. The structures of the compounds mentioned above are shown in Fig. 1. Only microscopic identification and thin-layer chromatography examination are officially recorded, and the number of ingredients quantified is less than four in the current Chinese Pharmacopeia, implying that it is inadequate to reflect the characteristics of the total chemical compositions of the complex preparation. There is relatively limited literature reporting studies for the holistic quality monitoring. Most previous reports are in reference to content determination of less than six components using HPLC [17-18] and qualitative analysis of fingerprint by HPLC [19-20], which lack overall quantitative assessment. Our previous study has established a capillary electrophoresis (CE) fingerprint for analysis of the quality homogeneity of SHT [21]; however, fingerprinting by CE has identified fewer peaks than chromatographic fingerprinting, due to its lower sensitivity. In addition, most related studies have only evaluated the similarity between sample fingerprint (SFP) and reference fingerprint (RFP) qualitatively [19-20]; and but quantitative fingerprint evaluation can provide a more comprehensive view, based on not only the similarity of peak distribution, but also quantitative content of the peaks. Based on the aforementioned considerations, the present study was designed to continue our exploration on the quality evaluation of thirty batches of SHT samples from various vendors, but from a different aspect and using novel experimental and analytical strategies. We developed the multi-wavelength fingerprinting method combined with multi-ingredient quantitative determination. An information entropy weight method (IEWM) was employed to integrate the multi-wavelength fingerprint information, and the relationship between the fingerprint components and the total antioxidant activity (obtained by DPPH assay) was established using partial least squares

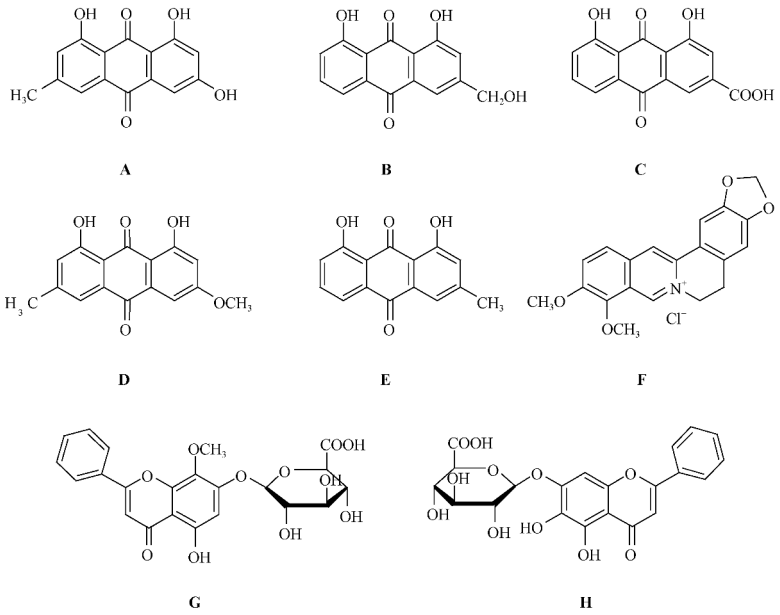

Fig. 1 The structures of main chemical compositions in the SHT. (A) emodin; (B) aloe-emodin; (C) rhein; (D) physcion; (E) chrysophanol; (F) berberine hydrochloride; (G) wogonoside ; and (H) baicalin