Available online at SciVarse ScienceDirect
Chinese Herbal Medicines (CHM)
ISSN 1674-6384
Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Original article

Comprehensive Evaluation of Powdered Chinese Herbal Medicines-An Exemplification of *Isatidis Radix*

Xue Han[1, 2†], Ming-quan Wu[1, 2†], Zhi-rui Yang[1, 2], Ding-kun Zhang[1, 2], Yi Mao[3], Jin Han[3], Yin-qiu Huang[1, 2], Yong-feng Zhou[2], Yan-ling Zhao[3*], Jia-bo Wang[2*], Xiao-he Xiao[4]

1. College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 610075, China.
2. China Military Institute of Chinese Medicine, 302 Military Hospital, Beijing 100039, China.
3. Department of Pharmacy, 302 Military Hospital, Beijing 100039, China.
4. Department of Integrative Medical Center, 302 Military Hospital, Beijing 100039, China.

ARTICLE INFO

Article history
Received: November 11, 2015
Revised: February 3, 2016
Accepted: April 1, 2016
Available online:
July 1, 2016

DOI:
10.1016/S1674-6384(16)60052-7

ABSTRACT

Objective Currently, powdered Chinese herbal medicines (CHMs) were mainly evaluated through physical property, chemical dissolution, and bioactivity independently. It could not reflect the quality comprehensively. This paper was to explore and establish a comprehensive evaluation method for powdered CHMs. **Methods** *Isatidis Radix* was chosen as an exemple. Firstly, powdered *Isatidis Radix* in different particle size was prepared. Then, their physical properties were characterized. The dissolution of index component epigoitrin was determined, and their antiviral activities were evaluated by neuraminidase-based bioassay. **Results** As the particle size decreased, powder distribution tended to be uniform, and the dissolution of epigoitrin increased, antiviral activity enhanced. According to cluster analysis of above results, the sequence of evaluation consequence was ultrafine powder S2 (D_{90}: 32.80 ± 0.29) > ultrafine powder S1 (D_{90}: 52.08 ± 0.53) > fine powder S0 (D_{90}: 118.16 ± 0.76) (from the superior to the inferior). **Conclusion** Overall, the comprehensive evaluation for powdered CHMs based on the physical characterization, chemical dissolution, and bioassay could not only be used to evaluate powdered herbs, but also guide the screening and optimization of the particle size of powder.

Key words
chemical dissolution; comprehensive evaluation; *Isatidis Radix*; neuraminidase-based bioassay; physical characterization; powdered Chinese herbal medicine

1. Introduction

With the increasing consumption of Chinese herbal medicines (CHMs) and the deterioration of ecotope, the resource supply of CHMs is facing a greater challenge. One of the effective solutions is to improve its utilization level. Powdered herb as an effective way has been widely applied in clinic and manufacture with the advantages of being high in

*Correspondence authors: **Zhao YL** Tel/Fax: +86-10-6693 3320 E-mail: zhaoyl2855@yeah.net
Wang JB Tel/Fax: +86-10-6693 3325 E-mail: pharm_sci@126.com
†These authors contributed equally to this work.

Fund: National Natural Science Foundation of China (81473316).

use ratio and good in uniformity (Cai et al, 2013; Liu et al, 2010; Feng et al, 2011).

However, lots of practical issues remain unsolved. For instance, how the crush degree is determined, as powders' particle size making a great influence on the physical, chemical, and biological properties (Prescott et al, 1970; Fincher et al, 1968). In addition, the stable efficacy is mainly insured by the uniformity of powder, and its uniformity mostly depends on the particle size and particle size distribution of powder. Besides (Komblum et al, 1970), the existing assessments are executed in a single aspect from the physical characterization, chemical dissolution, and bioactivity. However, there was a lack of connection interrelating them to evaluate powder, which is unable to provide a guideline for practical and clinic application. So a comprehensive evaluation including physical characterization, chemical dissolution, and bioactivity is essential.

Isatidis Radix (Banlangen in Chinese), a kind of CHMs, is the dry roots of *Isatis indigotica* Fort., which belongs to the family Brassicaceae. It has been used for over 2000 years and for the treatment of viral infection in recent decades. It was reported that *Isatidis Radix* showed the antiviral effects on the influenza virus (Bo et al, 2007), mumps, HCMV (Jian et al, 2011), HSV-1 (Fang et al, 2005), HSV-2 (He et al, 2013), coxsackie virus B3 (Lian et al, 1999), HBsAg, HBeAg, HBcAg, and HBV-DNA (Wei et al, 2013). During SARS, H1N1, and other influenza outbreaks, *Isatidis Radix* was frequently used as an effective anti-flu drug, so it was chosen as an exemplification in this paper.

It has been reported that the antiviral activity of common powdered *Isatidis Radix* was lower than that of micropowder (Liu et al, 2013). But to the best of our knowledge, there is no previous report about any comprehensive evaluation of powdered CHMs. So the physical and chemical properties and bioactivity were combined to evaluate the quality of powder in the study. At first, physical characterization was measured by classical methods. Then, the influence of particle size on dissolution of epigoitrin was determined by HPLC. As previous studies showed that the antiviral effective components of *Isatidis Radix* were alkaloids, and the monomer component epigoitrin has been proved to be effective and could be isolated (Huang et al, 1982; Ma et al, 2014). After that, the antiviral activity was determined using neuraminidase-based bioassay which was optimized by our research group before (Li et al, 2009a; 2009b). This method was mature, simple, high through put, and suitable to evaluate various samples of antiviral effect (Fairchok et al, 2015; Müller et al, 2015; Gubareva et al, 2004; 2002). Then cluster analysis was taken to analyze and compare the results. A comprehensive evaluation method for powdered CHMs was established preliminarily.

2. Materials and methods

2.1 Materials and reagents

Isatidis Radix was purchased from Beijing Lvye Medicine Company Co., Ltd. (13112201, China) and was identified by Prof. Xiao-he Xiao (PLA Institute of Chinese Materia Medica, 302 Hospital of People's Liberation Army, Beijing, China). Standard reference of epigoitrin (111753-201103) was obtained from The National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purity was more than 98%. Neuraminidase Inhibitors Screen Kit was purchased from Beyotime Co., Ltd. (1104281411, China).

Methanol of HPLC grade was purchased from Fisher Chemicals (Pittsburg, USA). Water was purified using a Milli-Q Water Purification System (Millipore, USA). Glycine was purchased from Amresco Co. (20140103480, USA). Phosphoric acid and absolute ethyl alcohol were purchased from Beijing Chemical Factory (20140304, 20140409, China). Sodium hydroxide was purchased from Xilong Chemical Co., Ltd. (120828, China). All other chemicals used were of analytical grade and available locally.

2.2 Preparation of samples

Preparation of *Isatidis Radix* powders with different size: *Isatidis Radix* (200 g) was grinded into fine powder S0. Ultrafine powders S1 and S2 were achieved by ultrafine mill to grind about 6 and 8 min, respectively.

2.3 Physical property characterizations

The particle size distribution was determined by the MS2000 Laser Particle Size Analyzer (Malvern Instruments Ltd., UK). Based on the light scattering theory, measurements were finished by using dry dispersion with air as medium. The D_{10}, D_{50}, and D_{90} values were calculated to represent the maximal particle size diameters including10%, 50%, and 90% of the particles, respectively. For example, the D_{90} value means that 90% of particles are smaller than this particle diameter, whereas the remaining 10% of the particles have larger diameters. Each sample was tested for three times, and the average value was figured out. The span represents the width of particles distribution. The more uniform the powder is, the smaller the span will be.

The microscopic morphology of *Isatidis Radix* powders was observed by JSM−7500F Scanning Electron Microscope (Japanese Electronics Co., Japan). Right amount of powders was pasted upon the conductive plastic and gold was sprayed to make samples.

2.4 HPLC analysis on epigoitrin dissolution

2.4.1 Establishing methodologies

Isatidis Radix powders (1 g) was taken into a 100 mL round-bottom flask after a precise weighing, completely dissolved by 50 mL of water. The flask was weighed, decocted for 2 h, and cooled down. Reference solution of epigoitrin (10 mg/L) was prepared by dissolving with methanol. All samples were filtered through a millipore membrane filter with an average pore diameter of 0.22 μm, and 10 μL of filtrate was injected into the HPLC system for analysis.