Food and Chemical Toxicology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Discrimination of three Siegesbeckiae Herba species using UPLC-QTOF/MS-based metabolomics approach

Hong-Xun Tao[a,1], Wei Xiong[a,1], Guan-Ding Zhao[a], Yu Peng[a], Zhang-Feng Zhong[a], Liang Xu[e], Ran Duan[d], Karl W.K. Tsim[d], Hua Yu[a,b,c,*], Yi-Tao Wang[a,**]

[a] Institute of Chinese Medical Sciences, State Key Laboratory of Quality Research in Chinese Medicine, University of Macau, Macao Special Administrative Region, China
[b] HKBU Shenzhen Research Center, Shenzhen, Guangdong, China
[c] School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong Special Administrative Region, China
[d] Division of Life Science, Center for Chinese Medicine, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong Special Administrative Region, China
[e] School of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian, China

ARTICLE INFO

Keywords:
Metabolomics
Traditional Chinese medicines
Multi-origin herbs
Siegesbeckiae Herba species
UPLC-QTOF/MS

ABSTRACT

The plant origin is one of the most important factors for the quality control of traditional Chinese medicines (TCMs) and highly affected on their safety and effectiveness in clinical applications. Multi-origin has been widely observed for many TCMs. Siegesbeckiae Herba (SH) is a traditional anti-rheumatic TCM which is originated from the plants of *Siegesbeckia pubescens* Makino (SP), *S. orientalis* L. (SO), and *S. glabrescens* Makino (SG). In the present study, an UPLC-QTOF/MS method were validated and successfully applied for the determination of the chemical profiles in the three SH species. The data were statistical analyzed with the OPLS-DA analysis and One-Way ANOVA *F*-test. Obvious differences in chemistry were observed in different SH species and 40 components were identified. Finally, 6 components were selected as potential chemical markers for the discrimination of SP, SO and SG based on the characteristic distribution in individual SH species.

1. Introduction

The property of multi-origin (multi-based source) is a historical issue in many traditional Chinese medicines (TCMs), which may lead to problems related to consumption safety, clinical efficacy and quality controllability. Systemic investigations of the chemical compositions of the Chinese medicinal herb with different origins are critical for the standardization and modernization of the TCMs. Siegesbeckiae herba (SH, also called *Xi-xian* in Chinese), originated from the plants of *Siegesbeckia pubescens* Makino (SP), *S. orientalis* L. (SO) and *S. glabrescens* Makino (SG) (Fig. 1), is one of the extensively used Chinese herbal medicines for treating chronic diseases such as arthritis and numbness of limbs (Yang, 2015). Among them, only SP has been traditionally used for hundreds of years (Ju et al., 2000). Owing to enrich medical resources, the other two species, SO and SG are also extensively used in recent decades. However, the chemical compositions and contents of these three species of SH showed significantly differences (Teng et al., 2015). Diterpenes, sesquiterpene and flavonoids are the commonly compositions reported in all three SH species. Diterpenes and their glycosides are considered to be the active components for SH.

So far, several methods such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) have been employed for the chemical analysis of SH herbs (Huo et al., 2012; Yao et al., 2015). In the current Chines Pharmacopeia (2015 edition), only the kirenol, a diterpenoid component found in all SH species, is used for the quality control of SH. However, using one chemical marker might be failed to meet the modern demands of qualitative identification and quantitation determination for the multi-originated SH herbal materials. Metabolomics, an emerging technology used in authentication of herbal medicines, has been applied to qualitatively and quantitatively monitor the variations in chemical profiles of abundant TCMs originated from different species (Xiang et al., 2011; Liu et al., 2016; Chen et al., 2016), as well as those subjected to different processing approaches (Park et al.,

* Corresponding author. Room 8008, Building N22, Institute of Chinese Medical Sciences, State Key Laboratory of Quality Research in Chinese Medicine, University of Macau, Avenida da Universidade, Taipa, Macao Special Administrative Region, China.
** Corresponding author. Room 1050, Building N22, Institute of Chinese Medical Sciences, State Key Laboratory of Quality Research in Chinese Medicine, University of Macau, Avenida da Universidade, Taipa, Macao Special Administrative Region, China.
E-mail addresses: bcalecyu@umac.mo (H. Yu), YTWang@umac.mo (Y.-T. Wang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.fct.2017.12.068
Received 4 December 2017; Received in revised form 25 December 2017; Accepted 30 December 2017

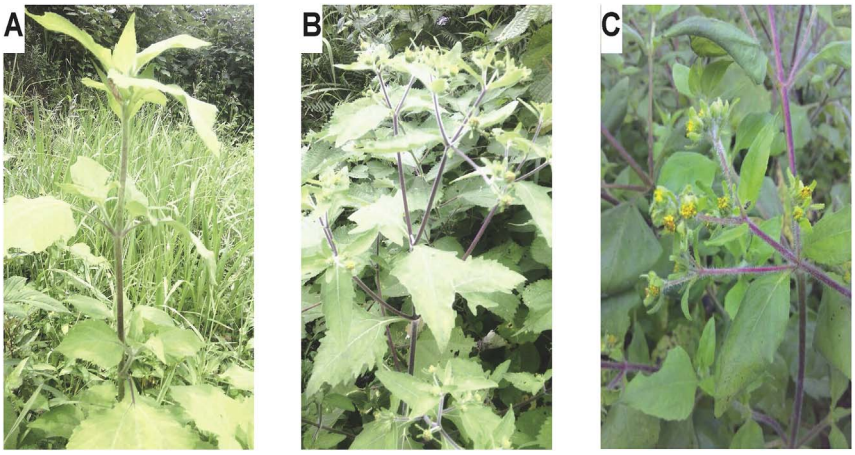

Fig. 1. The plant species of Siegesbeckiae herba. A: *Siegesbeckia pubescens* Makino (SP); B: *S. orientalis* L. (SO); C: *S. glabrescens* Makino (SG).

Table 1
Information of the collected Siegesbeckiae herbs.

Batch No.	Species	Location
SP-001	*S. pubescens* Makino	Muyang, Jiangsu
SP-002	*S. pubescens* Makino	Guiyang, Guizhou
SP-003	*S. pubescens* Makino	Laiwu, Shandong
SP-004	*S. pubescens* Makino	Gaoyou, Jiangsu
SP-005	*S. pubescens* Makino	Tianjin, Tianjin
SP-006	*S. pubescens* Makino	Anshan, Liaoning
SP-007	*S. pubescens* Makino	Dalian, Liaoning
SP-008	*S. pubescens* Makino	Shenyang, Liaoning
SP-009	*S. pubescens* Makino	Changchun, Jilin
SP-010	*S. pubescens* Makino	Tonghua, Jilin
SP-011	*S. pubescens* Makino	Chengde, Hebei
SP-012	*S. pubescens* Makino	Zunhua, Hebei
SP-013	*S. pubescens* Makino	Nanning, Guangxi
SP-014	*S. pubescens* Makino	Baise, Guangxi
SP-015	*S. pubescens* Makino	Leye, Guangxi
SP-016	*S. pubescens* Makino	Yulin, Guangxi
SP-017	*S. pubescens* Makino	Guangzhou, Guangdong
SO-001	*S. orientalis* L.	Meizhou, Guangdong
SO-002	*S. orientalis* L.	Guangzhou, Guangdong
SO-003	*S. orientalis* L.	Lishui, Zhejiang
SO-004	*S. orientalis* L.	Yulin, Guangxi
SO-005	*S. orientalis* L.	Ganzhou, Jiangxi
SO-006	*S. orientalis* L.	Guiyang, Guizhou
SO-007	*S. orientalis* L.	Nanling, Anhui
SO-008	*S. orientalis* L.	Anguo, Hebei
SO-009	*S. orientalis* L.	Changde, Hunan
SO-010	*S. orientalis* L.	Xiaogan, Hubei
SO-011	*S. orientalis* L.	Zhenjiang, Jiangsu
SO-012	*S. orientalis* L.	Zhaoyang, Hunan
SO-013	*S. orientalis* L.	Suzhou, Jiangsu
SG-001	*S. glabrescens* Makino	Yongkang, Zhejiang
SG-002	*S. glabrescens* Makino	Jinyun, Zhejiang
SG-003	*S. glabrescens* Makino	Dandong, Liaoning
SG-004	*S. glabrescens* Makino	Fengcheng, Liaoning
SG-005	*S. glabrescens* Makino	Jinhua, Zhejiang
SG-006	*S. glabrescens* Makino	Shaoxing, Zhejiang
SG-007	*S. glabrescens* Makino	Bozhou, Anhui
SG-008	*S. glabrescens* Makino	Suining, Sichuan
SG-009	*S. glabrescens* Makino	Ziyang, Sichuan
SG-010	*S. glabrescens* Makino	Luzhou, Sichuan

2014; Zou et al., 2017), cultivated duration (Kim et al., 2011; Shin et al., 2016) and original regions (Song et al., 2014; Hoffmann et al., 2017). Of all profiling techniques in metabolomics, various analytical methods were applied widely to differentiate plant species based on their chemical component profiles (Schripsema, 2010; Allwood and Goodacre, 2010). Unfortunately, to the best of our knowledge, metabolomics has never been established for discriminational investigation for SH species.

In this study, consequently, an UPLC-QTOF/MS-based metabolomics method was first developed to discriminate the chemical profiles of SH species. The orthogonal partial least-squares discriminate analysis (OPLS-DA) and One-Way ANOVA *F*-test were applied to data analysis. Moreover, the potential chemical markers for discrimination of the SH species were selected from the identified components.

2. Materials and methods

2.1. Materials

Total 40 batches of SH samples (including 17 batches of SP, 13 batches of SO and 10 batch of SG) were collected from different location of China (Table 1). All samples were authenticated by Dr. Tingxia DONG from the Hong Kong University of Science & Technology, and the corresponding author. The voucher specimens were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao, China.

Formic acid, acetonitrile and Leucine-enkephalin were purchased from Sigma-Aldrich (St. Louis, MO, USA) of LC-MS grade. Methanol was purchased form RCI Labscan Limited (Thailand) of HPLC grade. All other reagents and chemicals were of analytical grade. Milli-Q water was prepared using a Milli-Q system (Millipore, MA, USA).

2.2. Sample preparation

All herbal materials were powdered and passed through 30 mesh sieve. One gram of the powdered herb was weighted accurately and mixed with 50 mL methanol in a 100 mL glass flask. The total weight was recorded. Subsequently, the herb was extracted under reflux for 5 h, cooled to room temperature and weighed again. Brought to original mass with methanol and mixed well, the solution was filtered through a syringe filter (0.22 μm, Millipore) and stored at 4 °C until analysis.

2.3. UPLC-QTOF/MS analysis

The sample analysis was carried out on a Waters ACQUITY-UPLC CLASS system (Waters Corp., Milford, USA) coupled with an ACQUITY UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μm) maintained at 45 °C. Elution was performed with a mobile phase of A (0.1% FA in water) and B (0.1% FA in ACN) under a gradient program: 0–3 min, 1%–30% B; 3–9 min, 30–60% B; 9–11 min, 60–80% B; 11–15 min, 80–100% B. The flow rate was 0.45 mL/min, and the injection volume was 5 μL.

Mass spectrometric detection was carried out on a quadrupole time-of-flight (QTOF) SYNAPT G2Si High Definition Mass Spectrometer (Waters Corp., Milford, USA) with an electrospray ionization (ESI)