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Original Article

Comprehensive quality evaluation and comparison of *Angelica sinensis* radix and *Angelica acutiloba* radix by integrated metabolomics and glycomics

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ABSTRACT

Angelica radix (*Danggui* in Chinese) used in China and Japan is derived from two species of *Angelica*, namely *Angelica sinensis* and *Angelica acutiloba*, respectively. The differences in quality between *A. sinensis* radix (ASR) and *A. acutiloba* radix (AAR) should be therefore investigated to guide the medicinal and dietary applications of these two species. Secondary metabolites and carbohydrates have been demonstrated to be the two major kinds of bioactive components of *Danggui*. However, previously, quality comparison between ASR and AAR intensively concerned secondary metabolites but largely overlooked carbohydrates, thus failing to include or take into consideration an important aspect of the holistic quality of *Danggui*. In this study, untargeted/targeted metabolomics and glycomics were integrated by multiple chromatography-based analytical techniques for qualitative and quantitative characterization of secondary metabolites and carbohydrates in *Danggui* so as to comprehensively evaluate and compare the quality of ASR and AAR. The results revealed that not only secondary metabolites but also carbohydrates in ASR and AAR were different in type and amount, which should collectively contribute to their quality difference. By providing more comprehensive chemical information, the research results highlighted the need to assess characteristics of both carbohydrates and secondary metabolites for overall quality evaluation and comparison of ASR and AAR.

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1. Introduction

Angelica radix (*Danggui* in Chinese) is one of the most common traditional herbal medicines used in Asian countries, especially in China and Japan. According to classical document records, 70 herbal formulae in China and 56 herbal formulae in Japan contain *Danggui*. *Danggui* has been traditionally prescribed in the treatment of gynecological diseases due to its ability to "replenish and invigorate the blood", in traditional Chinese medical terms [1]. Besides its medicinal usage, *Danggui* is also used by women worldwide as a health food supplement. *Danggui* used in China and Japan is derived from two species of the genus *Angelica*, namely *Angelica sinensis* and *Angelica acutiloba*, respectively. If and how *A. sinensis* radix (ASR) and *A. acutiloba* radix (AAR) differ in quality is a topic of intense interest. The answers would be helpful to guide their medicinal and dietary applications, such as whether they can be used alternatively or they should be employed for treating different diseases. To achieve this, systematic chemical comparison between ASR and AAR is essential.

Thus far, many studies have been performed comparing the chemical components of ASR and AAR in order to characterize their quality differences [2–4]. However, these studies focused on secondary metabolites and largely overlooked carbohydrates. It is well-recognized that secondary metabolites and carbohydrates are the two major kinds of bioactive chemicals in medicinal/dietary herbs, and *Danggui* is no exception [5–9]. Accumulated phytochemical and biological experiments have demonstrated that, in addition to such secondary metabolites as phthalides and organic acids [10,11], *Danggui* also contains abundant carbohydrates (poly-/oligo-/mono-saccharides) with multifaceted bioactivities including anti-oxidant, anti-tumor, and immune-regulatory effects [12–14]. Thus, in order to characterize and compare the holistic quality of ASR and ARR, carbohydrates should be adequately taken into account for overall chemical profiling.

Metabolomics aims to collectively and dynamically characterize a set of small biomolecules (metabolome) in an organism, and is being widely employed for the "overall" chemical characterization of herbal medicines [15,16]. Hyphenated liquid chromatography and mass spectrometry (LC-MS)-based metabolomics is a particularly powerful method to provide global profiles of complex (up to hundreds of) secondary metabolites by determining their presence, amount and sometimes their structures [17,18]. In contrast to secondary metabolites, the chemical characterization of carbohydrates is a challenge due to their different chemical properties, for example, macromolecular mass and intricate multidimensional structures of polysaccharides [19]. The recently-coined glycomics seeks to explore the qualitative and quantitative information of a certain glycome (the entire carbohydrate components), for which the combined deployment of various analytical approaches to determine multiple chemical parameters of different carbohydrates is essential [20,21]. For example, chemical modifications such as hydrolysis and/or derivative formation are always needed prior to chromatographic or mass spectrometric analysis of carbohydrates to decompose their advanced structures and thereby improve their analytical adaptability. Similar with metabolomics, glycomics is a promising approach for delineating carbohydrate components in herbal medicines from a holistic perspective.

In this study, metabolomics and glycomics were integrated to comprehensively characterize the chemical components of ASR and AAR so as to holistically compare the quality of the two *Angelica* cultivars. The experimental procedure was designed as follows. First, batches of ASR and AAR samples were collected. Since ASR is mainly grown in China but AAR is grown in Japan, Taiwan and China, ASR samples were collected only from China while AAR samples were collected from the three regions to additionally investigate the effects of cultivation regions on AAR quality. Then, untargeted/targeted metabolomics approaches by ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS/MS) and ultra-performance liquid chromatography-triple quadrupole mass spectrometry (UPLC-TQ-MS/MS) were developed to qualitatively and quantitatively determine secondary metabolites in the samples. Meanwhile, targeted glycomics that combined analytical techniques including UPLC-TQ-MS/MS, high performance gel permeation chromatography coupled with evaporative light scattering detector (HPGPC-ELSD) and high performance liquid chromatography coupled with evaporative light scattering detector (HPLC-ELSD) were applied to characterize polysaccharides, oligosaccharides and monosaccharides in the samples. Finally, the obtained data were integrated and processed by multivariable statistical analysis for holistic quality comparison of all ASR and AAR samples.

2. Materials and methods

2.1. Chemicals and materials

MS-grade acetonitrile, formic acid, ammonium acetate and methanol were purchased from Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA) used for acid hydrolysis of polysaccharides was from Riedel-de Haën (Seelze, Germany). 1-Phenyl-3-methyl-5-pyrazolone (PMP) for monosaccharide and oligosaccharide derivatization was bought from Sigma (St. Louis, USA). Ultra-pure water was produced by a Milli-Q water purification system (Millipore, Bedford, USA).

The reference substances of dextrans with known molecular sizes (1–670 kDa), together with D-galacturonic acid monohydrate (GalA), D-glucuronic acid (GlcA), L-arabinose (Ara), D-mannose (Man), D-galactose (Gal), D-glucose (Glc), L-rhamnose monohydrate (Rha), D-fucose (Fuc), D-fructose (Fru), maltose (Mal), maltotriose (Tri), maltopentaose (Pen), maltohexaose (Hex), maltoheptaose (Hep), cellobiose (Cel), melibiose (Meli) and sucrose (Suc) were purchased from Sigma. The reference substances of coniferyl ferulate (CF), senkyunolide H (SH) and senkyunolide I (SI) were provided by Phytomarker Ltd (Tianjin, China); ferulic acid (FA), senkyunolide A (SA), Z-ligustilide (Z-lig), butylphthalide (BP) and levistolide A (LA) were obtained from Chengdu Must Bio-Technology Co., Ltd (Chengdu, China); Z-butylidenephthalide (BDP) was bought from Sigma. The purity of these references was higher than 95.0% as indicated by HPLC analysis.

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