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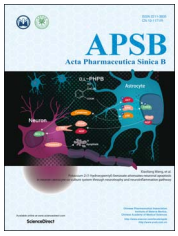

ORIGINAL ARTICLE

Application of ^{1}H NMR-based metabolomics for discrimination of different parts and development of a new processing workflow for *Cistanche deserticola*

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Tricarboxylic acid cycle metabolites;
Echinacoside;
Acteoside

Abstract *Cistanche deserticola* (CD) is one of the two authoritative source plants of Cistanches Herba, a well-known medicinal plant. Herein, ^{1}H NMR spectroscopy was employed to characterize the chemical profile and to distinguish the different parts, as well as to propose a new processing workflow for CD. Signal assignment was achieved by multiple one and two dimensional NMR spectroscopic techniques in combination with available databases and authentic compounds. The upper parts of the plant were distinguished from the lower parts by combining ^{1}H NMR spectroscopic dataset with multivariate statistical analysis. A new processing method that hyphenated steaming with freeze-drying, was demonstrated to be superior to either steaming coupled with oven-drying or direct freeze-drying *via* holistic ^{1}H NMR-based metabolomic characterization. Phenylethanoid glycosides, mainly echinacoside and acteoside, were screened out and confirmed as the chemical markers responsible for exhibiting the superiority of the new processing workflow, whereas serial primary metabolites, especially carbohydrates and tricarboxylic acid cycle metabolites, were found as the primary molecules governing the discrimination between the upper and lower parts of the plant. Collectively, ^{1}H NMR spectroscopy was demonstrated as a versatile analytical tool to characterize the chemical profile and to guide the in-depth exploitation of CD by providing comprehensive qualitative and quantitative information.

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1. Introduction

Cistanches Herba (CH, Chinese name: Roucongrong), initially archived in *Shen Nong's Chinese Materia Medica*, has been extensively regarded as one of the most well-known edible tonic and medicinal plants, and honored as "Ginseng of the deserts"[1,2]. As one of the two official source plants of CH, *Cistanche deserticola* (CD, Orobanchaceae) is a holoparasitic plant and mainly distributed in the north and northwest of China[2,3]. It has been widely utilized for the treatments of kidney deficiency characterized by impotence, pain in the loins and knees, female sterility, and constipation in traditional Chinese medicinal practices for centuries[3–5]. However, the wild sources of CD are on the edge of extinction in recent years due to over-harvesting, and it has been listed as one of the class II plants needing protection in China[1]. Moreover, CD offers important contribution for desert control. Therefore, it is critical but challenging to use this herbal material more efficiently.

Scientific studies on *Cistanche* plants initiated in the 1980s[6]. Phytochemical investigations revealed the existence of diverse chemical types, *e.g.*, phenylethanoid glycosides (PhGs), iridoids, lignans, fatty acids, alditols, and carbohydrates, within CD[7]. Among them, PhGs are most frequently mentioned owing to their broad spectrum of biological activities, including anti-oxidation, anti-aging, anti-fatigue, anti-inflammation, enhancing body immunity, improving the learning and memory of Alzheimer's disease mice, etc[1]. Recently, PhGs are attracting increasing attention as potential new drug candidates for treating neurodegenerative disorders. In particular, an amalgamation of the total PhGs found in CH has been developed as a new drug, registered as total *Cistanche* glycoside capsule (Memoregain®) for the treatment of vascular dementia[8]. Echinacoside, the most abundant and effective constituent of the total PhGs exhibits anti-apoptotic effects on SHSY5Y neuronal cells following TNFα-induced apoptosis, and reverses deficits in Parkinson's disease mice[9]. Moreover, another primary active compound, acteoside (also known as verbascoside) is able to antagonize the apoptosis in neurons[10] to defend against neurotoxicity in PC12 cells induced by 1-methyl-4-phenylpyridium or glutamate[11], and to improve scopolamine-induced memory deficits[2].

Similar to *Cordyceps* and *Ginseng*, CD has been extensively consumed and valued as health food. The actual and perceived benefits of CD are likely to play the determinant roles for the price of the crude drug. Given the large body of the crude drugs of CD, the slices are more popular in the market. In general, enzymatic inactivation and water deprivation are the two key steps during the medicinal slice processing. The conventional drying methods of CD include insulation, oven-drying, salting, and cellar storage[12–14]. However, products from these processes usually suffer from ill-looking appearance and low content of PhGs, thus hindering the wide application and consumption of CD. In 2007, our group proposed a new processing technique for CD which dramatically preserved the contents of echinacoside and acteoside in slices[14]. However, since the products from this process still suffer from an unpleasant appearance, we presently describe a processing methodology to improve the appearance and to further perserve the contents of PhGs in the processed materials.

Serial analytical tools have hitherto been applied for the chemical analysis of CD, including thin-layer chromatography, high performance liquid chromatography (HPLC) coupled with various detectors, such as diode array detector (DAD), evaporative light scattering detector (ELSD), electron capture detector (ECD), and tandem mass spectrometer (MS/MS). PhGs, in particular echinacoside and acteoside, have been most frequently adopted as the quality markers[1]. The fingerprint of this herbal drug has also been developed using HPLC–DAD and HPLC–DAD–MS/MS[15,16]. Nonetheless, it remains a challenge to assess the quality of CD because of its extremely complex chemical profile. Generally speaking, approaches targeting several analytes are not able to offer a holistic chemical view for the crude extracts, although abundant qualitative and quantitative information can be obtained from LC–MS/MS. Moreover, HPLC-related analytical strategies can be limited by large amounts of solvents, tedious sample preparation, and/or time-consuming procedures. Therefore, a new fit-for-purpose analytical tool being capable of yielding comprehensive information of the compound pool is required for optimal chemical analysis. Fortunately, ^{1}H NMR spectroscopy has been exactly demonstrated as an attractive "all in one" tool being capable of offering not only qualitative dataset but also quantitative information for a wide range of both primary and secondary metabolites with simple sample preparation and rapid acquisition[17–20]. Until now, wide applications of ^{1}H NMR spectroscopy have been launched for simultaneous determination, chemical profiling, and metabolomics of complex matrices.

Although several investigations have been carried out for this precious herbal medicine, its global chemical profile is largely unknown. Because CD is a parasitic plant, the lower parts should be responsible for transmitting nutrient substances, mainly primary metabolites, from the host towards the upper parts, whereas vigorous energy metabolism, such as blossom, usually occurs at the upper parts. Therefore, it is reasonable to assume that differences occur for the metabolome of different parts. Therefore, in current study, we aim: 1) to comprehensively characterize the chemical profile of CD using ^{1}H NMR spectroscopy coupled with diverse two dimensional (2D) NMR measurements; 2) to clarify the differences between the upper and the lower parts; and 3) to propose a new processing workflow through ^{1}H NMR-based metabolomic study. The findings obtained are expected to provide solid guidelines for the further exploitation of this medicinal herb in a better way, in particular for the employment of different parts and processing techniques.

2. Experimental

2.1. Plant materials

Twelve batches of fresh materials (CD1–CD12, Table S1, Supplemental information) were collected from Inner Mongolia, Xinjiang, and Ningxia autonomous regions in China. The botanical origins of all crude materials were authenticated as *C.*

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