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Analytical comparison of different parts of Radix Angelicae Sinensis by gas chromatography coupled with mass spectrometry

Shui-Yin Wei [a], Cheng-Jian Xu [a,*], Daniel Kam-Wah Mok [a], Hui Cao [b], Tsui-Yan Lau [a], Foo-Tim Chau [a,*]

[a] *State Key Laboratory of Chinese Medicine and Molecular Pharmacology, Chemometrics and Herbal Medicines Laboratory, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong, China*
[b] *National Engineering Research Center for Modernizing Traditional Chinese Medicine, Zhuhai, Guangdong, China*

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Abstract

The three parts of Radix Angelicae Sinensis (Danggui) were reported to have different therapeutic effects. In order to investigate their chemical compositions of different parts of Danggui, gas chromatography–mass spectrometry (GC–MS) combined with chemometrics data analysis was applied to provide a more detailed study. Subwindow factor analysis (SFA) and a modified augmented evolving window orthogonal projection (AEWOP) method were used to resolve the batch GC–MS data sets from the Danggui samples. Then, *t*-test, Wilcoxon rank sum test, and principal component analysis (PCA) were applied to carry out the comparison job. The results indicate which components are found to have significant differences among the three parts. These findings may be helpful for further research of the pharmacological activities of Danggui.

Keywords: Radix Angelicae Sinensis; Danggui; Chemometrics; GC–MS; Factor analysis

1. Introduction

Recent years have seen an increasing interest worldwide in the use of traditional Chinese medicines (TCMs) for the prevention and treatment of various illnesses [1–3]. Radix Angelicae Sinensis, named as Danggui in Chinese, is one of the most commonly used traditional Chinese medicines. Danggui was first recorded for its therapeutic effect in Shen Nong Ben Cao Jing around 100 B.C., and it has also been regarded as 'women ginseng' for over 2000 years. Danggui is the root of *Angelica sinensis* (Oliv.) Diels. Its main pharmacological effects are tonifying blood, promoting blood circulation, regulating menstruation, alleviating pain, and lubricating the bowels to relieve constipation [4]. It has been recorded that 98 prescriptions in China and 56 in Japan contain Danggui [4,5]. Besides the common usage in Asia, Danggui is also used as a health food product for women's care in Europe and America. Therefore, the analytical research of Danggui has been a hot topic, especially in chromatographic science [5–14]. Most of these studies [5–13] treat Danggui as a "whole part", while, according to Chinese Pharmacopoeia 2005 [4], Danggui can be further divided into three parts from its growing positions, which are head, body, and tail. Moreover, these three parts were reported to have different therapeutic activities. As shown in Fig. 1, the head or topmost part of Danggui, called Guitou in Chinese, is about 1.5–4 cm in diameter, annulated, apex obtuse and rounded, showing purple or yellowish-green remains of stems and leaf sheaths, and is used mainly to stop bleeding. The body or middle part of Danggui, named Guishen, which is lumpy on the surface, is good for regulating and nourishing blood. The tail or end part of the Danggui, named Guiwei, is about 0.3–1 cm in diameter, the upper portion thick and the lower portion thin, mostly twisted and exhibiting a few rootlet scars, and it is utilized primarily to promote blood circulation, remove blood stagnation and reduce pain. In Chinese medical treatment, Guitou is important in the case of traumatic injuries like fall and bleeding, or heavy flow of menstrual periods. Guishen nourishes blood circulation of pregnant women while one should avoid using Guiwei, Guiwei actively enhances blood circulation which should not be over-stimulated during

* Corresponding author. Tel.: +86 852 3400 8672; fax: +86 852 2364 9932.
E-mail addresses: xucj@fg702-6.abct.polyu.edu.hk (C.-J. Xu), bcftchau@polyu.edu.hk (F.-T. Chau).

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pregnancy. However, after childbirth, Guiwei is an ideal choice because it helps to remove the stagnant blood in the womb. The above expressions about pharmacological activities of different parts of Danggui, to great extent, are based on empirical observations of thousands of years [15,16], and are not substantial or convincing from modern scientific viewpoint yet. Therefore, it is important to reveal chemical knowledge background of different parts of Danggui at a molecular level to elucidate the pharmacological mechanism underlied.

The chemical constituents of Danggui are generally categorized into essential oils, phthalide dimers, organic acids and their esters, vitamins, and polyacetylenes [17]. Among them, the essential oils are usually attributed to the main pharmacological activities of Danggui [18]. In the present work, hyphenated gas chromatography–mass spectrometry (GC–MS) and chemometrics resolution methods (CRMs) have been employed to carry out qualitative and quantitative analyses of Danggui samples. The recent development of CRMs provides good tools to analyze complex herbal medicine systems. Such resolution methods, to name but a few, heuristic evolving latent projections (HELP) [19], subwindow factor analysis (SFA) [20], orthogonal projection approach-alternating least square (OPA-ALS) [21] and evolving window orthogonal projection (EWOP) [22], are especially efficient for co-elution systems. In this paper, SFA is applied to extract pure mass spectra of chemical constituents in Danggui samples, and a new modified augmented evolving window orthogonal projection (AEWOP) are manipulated to obtain pure chromatograms from batch data sets. Afterwards, the statistical tests and principal component analysis (PCA) are employed to reveal the difference and sameness among Danggui samples.

2. Theory

The GC–MS instrument yields a bilinear data matrix $\mathbf{X}$ ($m \times n$) where m rows correspond to spectra taken at regular time intervals and the n columns represent chromatograms measured at different mass to charge ratios. The bilinear data matrix

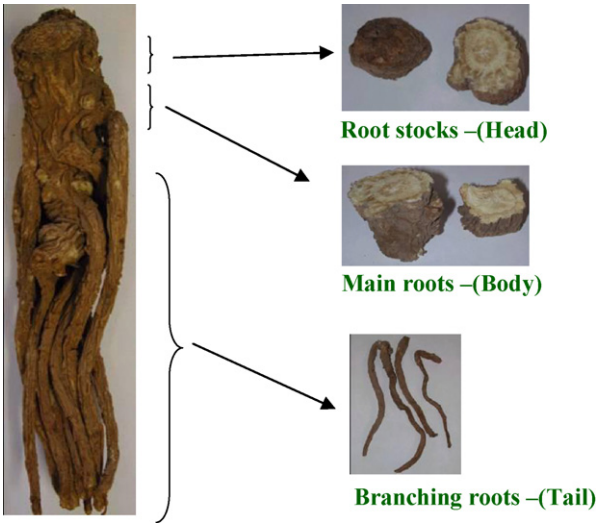

Fig. 1. A picture of different parts of Danggui.

$\mathbf{X}$ can be decomposed into the products of two matrices, one containing the concentration profiles, $\mathbf{C}$ ($m \times k$) and the second the individual mass spectra, $\mathbf{S}$ ($n \times k$) where k is the number of components.

$$\mathbf{X} = \mathbf{C}\mathbf{S}^{\mathrm{T}} \tag{1}$$

Here, we apply CRMs to obtain the pure contribution of each component. A detailed mathematical explanation of the methods used has been given extensively elsewhere [19–23]. Therefore, only a brief description will be given here.

2.1. Subwindow factor analysis (SFA)

SFA is method directly extracting component spectra from overlapping structures obtained from hyphenated chromatography without first resolving chromatographic profiles. This is of advantage when a complete resolution cannot be obtained or is of less interest in the analytical situation. SFA is based on that, if there are two subwindows in the concentration direction containing one and only one common component, and then the pure spectra, $\mathbf{s}_{\mathrm{p}}$, of the common component can be resolved uniquely. Suppose $\mathbf{E}$ and $\mathbf{D}$ are the orthonormal base sets of two subwindows (these base sets were obtained by Singular Value Decomposition (SVD) of the submatrices of $\mathbf{X}$ in these two subwindows), then the pure spectral variables can be obtained by minimizing the squared norm.

$$\mathbf{N} = ||\mathbf{E}\boldsymbol{\alpha} - \mathbf{D}\boldsymbol{\beta}||^2 \tag{2}$$

Here $\boldsymbol{\alpha}$, $\boldsymbol{\beta}$ are the weight vectors, and also are the first left and the first right singular vectors when N is minimized respectively, of matrix $\mathbf{E}^{\mathrm{T}}\mathbf{D}$. Then pure spectra $\mathbf{s}_t$ can be calculated by $\mathbf{E}\boldsymbol{\alpha}$ or $\mathbf{D}\boldsymbol{\beta}$.

2.2. Augmented evolving window orthogonal projection (AEWOP)

After the spectrum of a certain component is calculated by SFA, the next step is to obtain the chromatographic profiles of that component. Here evolving window orthogonal projection (EWOP) is utilized to obtain pure chromatographic profiles.

By using zero-concentration graph with projecting the extracted pure spectrum onto a series of moving windows, the EWOP method can resolve the concentration profiles of the component more conveniently and accurately. Suppose $\mathbf{s}_t$ is the normalized pure spectrum of the component, and Span ($\mathbf{X}_i$) represents hyperplane spanned by the column vectors in the moving window matrix $\mathbf{X}_i$ ($i = 1, 2, \ldots, m - w + 1$, with w being the size of the moving window). If the component belongs to the subsystem in the moving window matrix $\mathbf{X}_i$, the column vector $\mathbf{s}_t$ will exist in Span ($\mathbf{X}_i$) and the length of the residual vector will be nearly equal to zero. Thus, the length of the residual vector is plotted against time to evaluate the presence of the certain components.

$$\mathbf{re}_i = ||(\mathbf{I} - \mathbf{X}_i^{\mathrm{T}}(\mathbf{X}_i^{\mathrm{T}})^{+})\mathbf{s}_t||_2^2 \quad i = 1, 2, \ldots, m - w + 1 \tag{3}$$