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Assay of free ferulic acid and total ferulic acid for quality assessment of *Angelica sinensis*

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Abstract

Activity of Chinese Danggui (DG), the processed root of *Angelica sinensis* (Oliv.) Diels, is linked to the ferulic acid content but the stability of ferulic acid during extraction for medicinal use is not known. The stabilities of ferulic acid and coniferyl ferulate were evaluated in the extracts of DG using a variety of extraction solvents. These included various combinations and proportions of methanol, water, formic acid, 1 M aqueous hydrochloric acid and 2% sodium hydrogen carbonate ($NaHCO_3$) in water. Coniferyl ferulate was found liable to hydrolyze into ferulic acid in neutral, strongly acidic and basic solvents, where heat and water could facilitate this hydrolysis. However, the hydrolysis was relatively resisted in weakly organic acid. Based on the stability evaluation, two new terms, namely: free ferulic acid and total ferulic acid, were suggested and defined. Free ferulic acid refers to the natural content of ferulic acid in herbs. Total ferulic acid means the sum of free ferulic acid plus the amount of related hydrolyzed components. Meanwhile, the high-performance liquid chromatographic (HPLC) method was developed to assay free ferulic acid and total ferulic acid in DG using methanol–formic acid (95:5) and methanol–2% $NaHCO_3$ in water (95:5) as extraction solvents, respectively. Ten DG samples were investigated on their contents of free and total ferulic acid. The results indicated that the amount variety of free ferulic acid was larger than that of their counterparts, and the ratio of total ferulic acid to free ferulic acid was 4.07 ± 2.73 (mean $\pm$ SD, $n = 10$). The chemical assay of DG using total ferulic acid content would be a better choice to assess the herbal quality and was recommended.

Keywords: Ferulic acid; Coniferyl ferulate; *Angelica sinensis*; HPLC; Pharmaceutical analysis

1. Introduction

Chinese Danggui (Radix *Angelicae Sinensis*, DG) is the processed root of *A. sinensis* (Oliv.) Diels, which is one of the widely used traditional Chinese medicinal (TCM) materials to enrich blood, activate blood circulation, regulate menstruation, relieve pain and relax bowels, etc. There are over eighty composite formulae of TCM containing DG [1]. Furthermore, this herb is commonly used as a female tonic, dietary supplements and one of the cosmetic ingredients sold in China, Europe, USA and/or other countries [2–5]. Its medicinal value has been demonstrated by numerous clinical trials, pre-clinical studies and traditional or modern experiences [6–11].

Ferulic acid was isolated from DG and was also found in other plants [12–14]. Pharmacological studies showed that ferulic acid and/or sodium ferulate had been found to inhibit platelet aggregation, increase coronary blood flow, relax or stimulate smooth muscle, possess anti-arrhythmic affects, anti-oxidate, immunostimulate, anti-inflammatory effects, etc. [2,6,15–19]. Some of these bioactivities were related to the medicinal functions of DG. Therefore, ferulic acid was widely used as one of the marker compounds to assess the quality of DG and its products [20–26]. However, the reported content of ferulic acid in DG varies within the range of 0.211–1.43 mg/g, and which were quantified by a variety of methods (Table 1) [20–36]. Apart from the variation in natural abundance among the samples, the

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Table 1
The variation in contents of ferulic acid for *Angelica sinensis* (Oliv.) Diels analyzed by the different methods in literatures

Extraction solvent	Extraction method	Analytical method	Content (mg/g)	Reference
Methanol	Reflux	TLC	1.08	27
Methanol	Reflux	HPLC	0.418–1.20	28
Methanol	Sonication	HPLC	0.423–1.03	20, 21
Methanol	Sonication	CE	0.211–0.226	22
Methanol–formic acid (95:5)	Immersion	HPLC	0.425	29
Methanol–formic acid (95:5)	Immersion	TLC	0.529	30
Methanol–formic acid (95:5)	Soxhlet	TLC	0.673–1.17	23
Methanol–formic acid (95:5)	Sonication	HPLC	0.233–0.479	31
Aqueous methanol	Sonication	HPLC	0.35–1.43	24
70% methanol	Reflux	HPLC	0.915–1.37	32
Ethanol	Reflux	HPLC	0.468	33
70% Ethanol	Sonication	CE	0.486–1.02	34
50% Ethanol	Reflux	HPLC	0.582–0.606	35, 36
Diethyl ether–methanol (20:1)	Reflux	HPLC	0.271	25
Water	Reflux	CE	0.415	26

nature of extraction solvents and methods were likely to be a critical cause. In the reported literatures, DG sample was commonly extracted using a variety of solvents, namely: methanol, methanol–formic acid (95:5), ethanol, diethyl ether–methanol (20:1) and/or water under reflux, sonication, immersion or soxhlet extraction (Table 1) [20–36]. However, coniferyl ferulate, the ester of ferulic acid, was also found in DG sample [37–39]. Kobayashi et al. reported that coniferyl ferulate was liable to hydrolyze into ferulic acid and coniferyl alcohol even if the pulverized sample of *Cnidium officinale* Makino was heated in water for 1 h (Fig. 1) [40]. According to this reported result, coniferyl ferulate is likely to be hydrolyzed in a variable extent in different extraction solvents and therefore resulting in a variety of level of ferulic acid determined in herbs. It is worth noting that TCM prescription is usually prepared and then decocted in boiling water. In this regard, coniferyl ferulate in TCM prescription materials are being easily converted into ferulic acid during extraction with boiling water. Therefore, ferulic acid remained as the major chemical constituent in this aqueous extract. If this is true, ferulic acid should be the principal functional compound instead of coniferyl ferulate in DG material according to the TCM practice. Another concern is about the reported levels of ferulic acid in literatures which might not represent the natural content in herbs or the actual amount for medicinal functions. Therefore, it is of top importance to examine the stability of ferulic acid in the different extraction conditions and to develop an accurate method for ferulic acid in DG sample for its quality assessment and evaluation of the therapeutic effect of DG.

This paper focuses mainly on studying the stability and relationship of ferulic acid with coniferyl ferulate, and developing a quantitative analysis method for the assay of ferulic acid in DG sample. Firstly, ferulic acid and coniferyl ferulate were identified in the HPLC chromatograms of DG extracts based on the on-line HPLC-atmospheric pressure chemical ionization (APCI)-MS and UV techniques. Then, the stabilities of ferulic acid and coniferyl ferulate were examined in extracts of DG samples by comparing their amounts using a variety of solvent/solvent combination and extraction methods. These solvents include methanol, methanol–formic acid, methanol–formic acid–water, methanol–hydrochloric acid (HCl) in water, methanol–water, water and methanol–sodium hydrogen carbonate ($NaHCO_3$) in water (Table 2). The results showed that coniferyl ferulate was liable to hydrolyze into ferulic acid in neutral, strongly acidic or basic media including methanol, methanol–water, water, methanol–1 M HCl in water and methanol–2% of $NaHCO_3$ in water resulting in a variety amount of ferulic acid being determined when the DG samples were extracted by different methods. However, a relatively stable amount of ferulic acid could be obtained in herb extracted with methanol–formic acid or methanol–2% $NaHCO_3$ in water.

Based on the observation, two new terms, ‘free ferulic acid’ and ‘total ferulic acid’, were suggested and defined. Free ferulic acid means the freely available ferulic acid and represents the natural content of ferulic acid in herb. Total ferulic acid is the sum of free plus the ferulic acid obtained from hydrolysis of conjugated ferulate, which represents the amount of ferulic acid in medicinal function. Through a

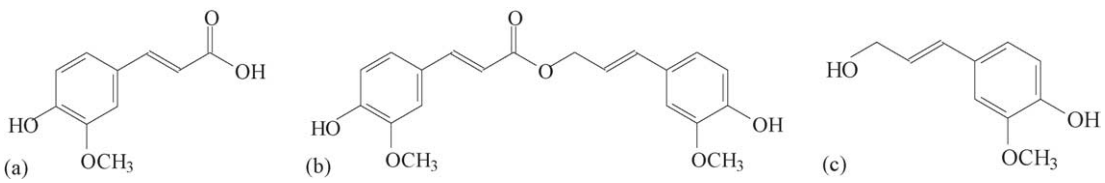

Fig. 1. Chemical structure of (a) ferulic acid, (b) coniferyl ferulate and (c) coniferyl alcohol.