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Chemical identification and quality assessment of Radix Angelicae sinensis (Danggui roots)

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ABSTRACT

The objective of this study is to develop the chemical methods for the genuine identification of Danggui (DG, the roots of *Angelica sinensis* (Oliv.) Diels) and the assessment of its quality. Over fifty herbal samples including different forms of DG with its related herbs, namely Japanese Danggui (JDG, the roots of *Angelica acutiloba* Kitagawa and *A. acutiloba* Kitagawa var. *sugiyamae* Hikino), Chuanxiong (CX, the rhizomes of *Ligusticum chuanxiong* Hort.) and Japanese Chuanxiong (JCX, the rhizomes of *Cnidium officinale* Makino), were collected from different cultivation areas in China and Japan. These herbal samples were analyzed by high-performance liquid chromatography (HPLC), interfaced with diode array detection (DAD) and mass spectrometry (MS) techniques, and Fourier Transform Infrared Spectroscopy (FTIR).

The HPLC-fingerprints of DG was developed based on the analysis of fifty-three herbal samples containing forty DG samples, four JDG samples, six CX samples and three JCX samples by HPLC-DAD-atmospheric pressure chemical ionization (APCI)-MS techniques. Ferulic acid and *Z*-ligustilide were unequivocally determined whilst senkyunolide I, senkyunolide H, coniferyl ferulate, senkyunolide A, 3-butylphthalide, *E*-ligustilide, *E*-butylidenephthalide, *Z*-butylidenephthalide and levistolide A were tentatively identified in their chromatograms. A combination of mathematics and computer approaches was employed to study the similarity among the HPLC-chromatographic patterns. Principal component analysis (PCA) was also utilized to generate a visual three dimensional (3D) projection plots for qualitative evaluation on the resemblance and difference of these samples using the entire chromatographic profiles as input data. Besides, the relative retention time (RRT) and relative peak area (RPA) of each of the characteristic peak related to the reference peak were calculated for the quantitative expression of the chemical properties in the chromatographic pattern of these samples. The consistency of the chromatograms was observed among the intra-species samples whilst the chromatograms among the other species samples were obviously different. The different species samples can be distinguished by either their chromatogram features (entire chromatograph pattern, correlation coefficient or PCA plots) or their specific chemical compounds (senkyunolide A or conferyl ferulate). The mean chromatogram was therefore used as a representative standard fingerprint for a group of chromatograms.

Bioactive compounds, *Z*- and *E*-ligustilide were quantitatively analyzed in DG and its related herbs by the newly developed HPLC method. By choosing an appropriate UV absorption wavelength at 350 nm, *Z*- and *E*-ligustilide were baseline separated and their interference peaks avoided in the HPLC chromatograms. The identities of *Z*- and *E*-ligustilide in the samples were unambiguously determined by the respective quasi-molecular ions ($[M+H]^+$) in APCI mass spectrometry. Based on the stability data, acetonitrile was chosen for the preparation of the standard solutions in order to minimize the isomerization of *Z*-ligustilide. The overall analytical procedure is rapid and reproducible and therefore considered suitable for the quantitative analysis of large number of samples.

Altogether fifty-six herbal samples containing twenty-two DG whole roots, eight DG root heads, four DG rootlets, four DG root slices, five DG whole root samples divided into root head and rootlet, four JDG samples, six CX samples and three JCX were quantified for the contents of *Z*- and *E*-ligustilide. A relatively higher level of

ligustilide is found to be existing in the DG, CX and JCX samples despite belonging to different genus. Ligustilide is therefore not the unique characteristic compound used as the chemical identity for DG. However, the content of ligustililde in the JDG samples was one-tenth of that in the DG whole root, an obvious quantitative difference between them even though they belong to the same genus. The contents of ligustilide in the different DG samples were further compared. The content of ligustilide in the rootlet was higher than that in the root head. This variation in the DG samples is in part due to the processing methods, storage time and conditions contained different amount of ligustilide. Besides, analysis of an extract from a sample root of *Angelica gigas* Nakai using LC-MS could not detect the presence of ligustilide in this herb.

Activity of DG is often linked to ferulic acid but the stability of ferulic acid during extraction 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 weak organic acid. Based on the stability evaluation, two new terms, namely free ferulic acid and total ferulic acid, were suggested and defined. Meanwhile, the 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.

Moreover, the contents of the free and total ferulic acid were investigated in a total fifty-seven herbal samples containing twenty-four DG whole roots, eight DG root heads, five DG rootlets, four DG slices, six DG whole root samples divided into root head and rootlet, four JDG samples, four CX samples and two JCX samples. The amount of variation of free ferulic acid was larger than that of their counterparts, and the ratio of total ferulic acid to free ferulic acid was in the range of 1.1-9.7. It was recommended that the chemical assay of DG using total ferulic acid content would be a better choice to assess the herbal quality. An almost equal level of ferulic acid is observed in DG, CX and JCX, which indicates that ferulic acid is not a characteristic to represent the medicinal functions of DG. However, the content of total ferulic acid in JDG samples is one-tenth less than those in the DG (whole root), CX and JCX samples. The contents of ferulic acid in the different DG samples were also compared. The content of the total ferulic acid in the DG samples collected from Minxian, Gansu of China is not generally higher than those in other cultivating areas. The DG rootlet contains a higher content of ferulic acid than the root head. Besides, the difference in the processing methods, storage time and conditions for the DG samples also result in a varied level of ferulic acid.

FTIR spectroscopy combined with the secondary derivative technique and two-dimensional correlation IR spectroscopy (2D-IR) were employed to analyze the different DG samples, extracts and extracted material residue. Six DG samples processed by the various processing approaches, namely (a) dried by freezing, (b) fumigated by fumes from burning broad bean stems, (c) wheat stems, (d) wood, (e) dried in shade or (f) sunlight, respectively, show different general IR, secondary derivative and 2D-IR spectra. The processing methods (b) and (c) are considered as the most suitable to process DG. Moreover, six DG whole roots samples were divided into

the root head and the rootlet, and analyzed by IR spectroscopy. The difference of the chemical properties in the root head and the rootlet were found in both the IR and the 2D-IR spectra. Besides the different chemical information in the DG raw material, various extracts and extracted material residue appeared in the FTIR and secondary-derivative spectra. It was therefore suggested that the FTIR spectroscopy could be used to rapidly screen the quality of DG samples.

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