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Near-infrared (NIR) spectroscopy for the non-destructive and fast determination of geographical origin of Angelicae gigantis Radix

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Abstract

Near-infrared (NIR) spectroscopy has been utilized to discriminate the Korean and Chinese Angelicae gigantis Radix. Decursin, one of the major ingredients in Korean Angelicae gigantis Radix, was preliminarily identified using TLC and HPLC. Decursin was then used as a unique marker component for successful discrimination between two geographical origins. Second derivative spectra were used to reduce baseline variations observed in original diffuse reflectance spectra as well as to enhance spectral features. The unique 1625 nm band of decursin in Korean samples provided clear spectral differences over Chinese samples. To develop a calibration model, soft independent modeling of class analogy (SIMCA) was used. With the use of the SIMCA model, independent sample datasets collected at two different periods were predicted. The Korean and Chinese samples were clearly identified with 100% accuracy.

Keywords: Angelicae gigantis Radix; SIMCA; Identification NIR spectroscopy

1. Introduction

Herbal medicines have played an important role in oriental medical history, especially in China, Japan, and Korea. Each country has developed its own characteristic remedies and used different origins of herbal plants to treat the same human diseases. As a result, standard analytical methods and specifications vary significantly from country to country even for the same herbal medicine. Some herbal medicines have the same pharmacopoeia name, but their botanical origins are different. On the contrary, some herbal medicines have the same botanical origin, but their pharmacopoeia names are different. With this kind of confusion, there are possibilities of misuse and overuse of herbal medicines when these are heavily traded among East Asian countries [1]. Therefore, it is of critical importance to establish standard specifications of herbal medicines and related analytical procedures among these countries.

The major goal of this paper is to investigate near-infrared (NIR) spectroscopy for the fast determination of geographical origins (China and Korea) of Angelicae gigantis Radix, one of most popular herbal medicines in East Asia. Three East Asian countries use Angelicae gigantis Radix with the same pharmacopoeia name; however, their botanical origins as well as major ingredients are different. Accordingly, the resulting efficacy and remedy of Angelicae gigantis Radix from each country is different. As such, it is necessary to identify geographical origin for proper medical treatment and prescription. Because the shape of Angelicae gigantis Radix is almost the same, a chemical method that involves an assessment of the major component, decursin, is necessary. While TLC and HPLC have been used to this end, they are destructive and require long analysis time. Since NIR spectroscopy is fast and non-destructive and does not require reagents, we have successfully employed NIRs for identification [2], dis-

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crimination [3–5], and determination of major ingredients [6,7] in herbal or oriental medicines. Therefore, this approach can be utilized for the fast identification of country origin of Angelicae gigantis Radix, as its chemical composition is sufficiently different according to where it has been cultivated. To establish a NIR spectroscopic method, Korean and Chinese Angelicae gigantis Radix samples were collected from various cultivation areas during a period of more than one year. Soft independent modeling of class analogy (SIMCA) was used to build a calibration model for classification. Fast identification is important for the protection of general public health as it is vital for proper use of herbal medicines (such as Angelicae gigantis Radix), along with the correct geographical information. Additionally, this is also necessary for fair evaluation and distribution of products.

2. Experimental

2.1. Sample preparation

One hundred and fifty-one Korean and eighty-one Chinese samples of Angelicae gigantis Radix were obtained from National Agricultural Products Quality Management Service (NAQS), Anyang, Korea. All samples were prepared over 14 months to include wider compositional variations such as seasonal change and cultivation area. The samples were cut and grinded into powder. The final powder samples were prepared by passing the ground powder through a 20-mesh sieve.

2.2. Thin layer chromatography (TLC) and high performance liquid chromatography (HPLC)

For the identification of major ingredients, TLC as well as HPLC were performed. Initially, 1 g of powder sample was dissolved in 5 mL of methanol and kept at 50 °C in a water bath during a period of 10 min for extraction. Then, the sample solution was cooled and filtered. The final filtrated solution was used. At the same time, a standard decursin/methanol solution (0.1 mg/mL) was prepared to identify the decursin reference peak. A TLC plate was made from silica gel GF_{254} and 2:1 acetic acid:ethyl acetate solution was used as an eluent. TLC peaks were identified using fluorescence with excitation wavelengths at 254 and 366 nm.

For a more detailed investigation, HPLC was performed (Waters, Milford, USA). Capcellpack C_{18} and acetonitrile: H_2O (52:8) were used as the column and mobile phase, respectively. For signal detection, a UV detector (absorption at 280 nm) was used.

2.3. NIR spectra and data processing

All NIR spectra (1100–1750 nm) were collected using a NearIRSTA HN1100 NIR spectrometer (Spectrontech Co. Ltd., Kwangju, Korea) equipped with a tungsten-halogen source and a diode-array detector. The detector temperature was controlled at 30 °C and the signal was integrated over 42 ms for one scan. Each spectrum corresponded to an accumulation of 30 scans. To collect spectra, a fiber optic reflectance probe was directly contacted with the powder samples and then corresponding diffuse reflectance spectra were collected.

All the spectral processing including SIMCA and a second derivative algorithm were accomplished using ChemoHN 1100 software (Spectrontech Co. Ltd., Kwangju). All samples were divided into a training set and two prediction sets. A classification model was developed in a training set, which is a group of samples for modelling and the developed model was evaluated by two prediction sets including samples not used for modeling. A total of 232 spectra were put into 130 spectra (90 Korean and 40 Chinese) for the training set, 50 spectra (30 Korean and 20 Chinese) for prediction set I, and 52 spectra (31 Korean and 21 Chinese) for prediction set II. The difference between prediction sets I and II was the collection period of samples. Each period lasted approximately 3 months.

3. Results and discussion

3.1. Identification of decursin using TLC and HPLC

In the Korean Pharmacopoeia, Angelicae gigantis Radix is defined as the root of *Angelica gigas* Nakai [8]. In Japan, it is defined as the root of *Angelica acutiloba* Kitagawa or *Angelica acutiloba* Kitagawa var. It is termed *sugiyamae* Hikino (*Umbelliferae*), and *Angelica sinensis* Diels in China. One of the major active ingredients of Korean Angelicae gigantis Radix is decursin (pyranocoumarin class), and other components include angelate, umbelliferone, and nodakenin. The major components of Chinese Angelicae gigantis Radix are ligustilide, *n*-butyllidene phthalide, butylphthalide, and sedannoic acid lactone (phthalide class). Chinese Angelicae gigantis Radix is similar to Japanese, but has lower content of ligustilide than the Japanese root. Overall, Korean Angelicae gigantis Radix mainly contains pyranocoumarin class, while Chinese and Japanese Angelicae gigantis Radixs contain phthalide class as major ingredients.

To examine the compositional difference between Korean and Chinese Angelicae gigantis Radix, TLC was performed initially. The results are shown in Fig. 1, which indicates that the TLC patterns between the two groups are clearly different. One Korean and two Chinese samples were compared with the standard decursin (molecular structure is shown in Fig. 3), which is the major ingredient in Korean Angelicae gigantis Radix. Two fluorescence images by excitation using 254 and 366 nm are presented. The standard peak of decursin was positioned at R_f 0.5 (d). The Korean sample showed the same peak value as decursin. In the Chinese samples (b and c), the strongest peak was observed at R_f 0.9 and no characteristic peak was observed at R_f 0.5. This is evidence of the higher decursin content in Korean samples. For more

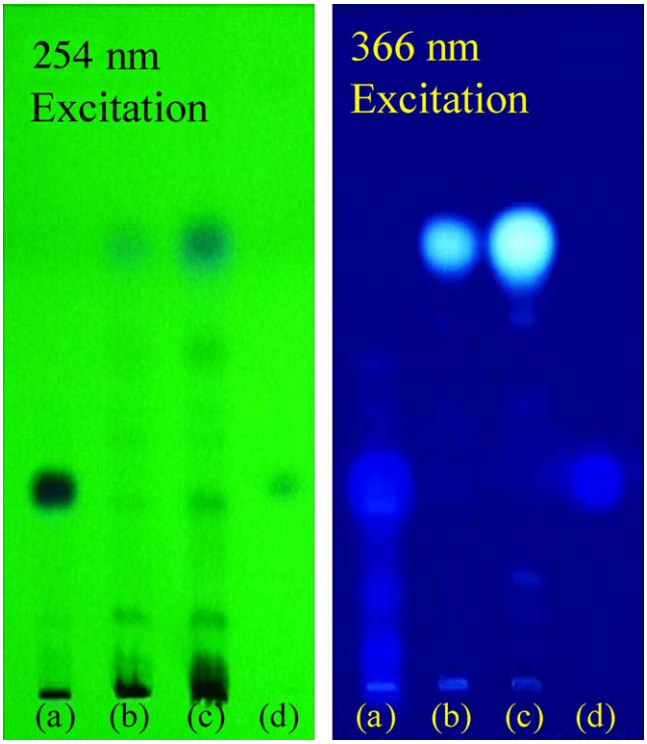

Fig. 1. TLC fluorescence chromatograms of one Korean (a), two Chinese samples (b and c) and decursin (d) by excitation using 254 and 366 nm.

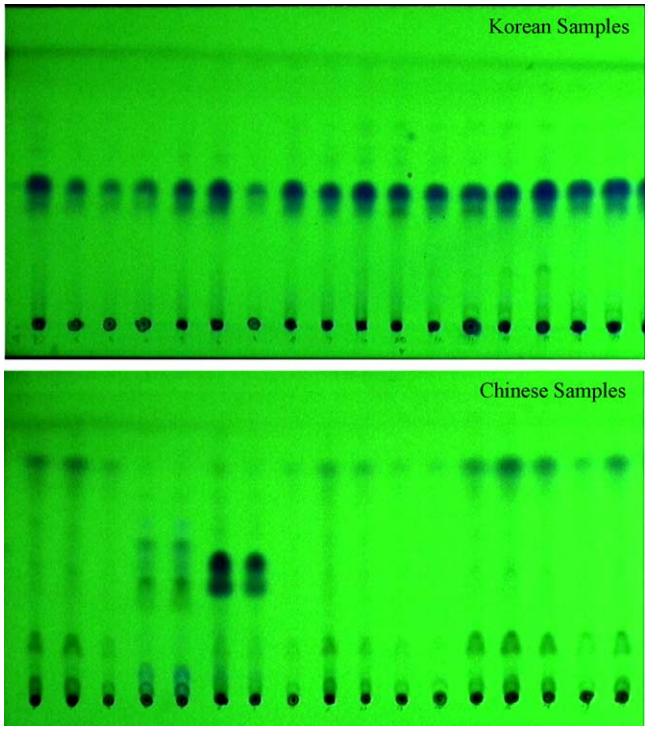

Fig. 2. TLC fluorescence chromatograms of 17 randomly selected samples from Korea and China by 254 nm excitation.

confirmation, TLC was further performed for 17 samples (randomly selected) from Korea and China. Fig. 2 shows the corresponding TLC fluorescence images at 254 nm excitation. For all Korean samples, the decursin peak is identified and their TLC patterns are similar. TLC patterns for the Chinese samples vary without strong features of decursin

Additionally, HPLC was employed to investigate the compositional difference between the Korean and Chinese samples. Fig. 3 shows chromatograms of decursin standard (a), one Korean (b), and two Chinese samples (c and d). The decursin peak is clearly identified for the Korean sample at the same retention time as that of the standard. This is further clear evidence of the presence of decursin in the Korean samples. Moreover, a peak of the isomer (decursinol angelate) is also observed immediately after the decursin peak. On the contrary, two chromatograms of typical Chinese samples are significantly different with higher compositional complexity, as indicated by TLC. There is no decursin or decursinol angelate peaks from the Chinese samples and the peak patterns are apparently different from each other. Overall, the HPLC results are consistent with those from TLC.

3.2. SIMCA analysis

In order to classify the Korean and Chinese samples, soft independent modeling of class analogy, one of the most powerful classification algorithms, has been utilized in this study [9]. Before performing SIMCA, it is important to examine the spectral bands that are useful for classification. Since all Korean samples contain larger amounts of decursin, in contrast with the Chinese samples in particular, the distinct peaks of decursin should help to discriminate two different geographical origins.

For spectral comparison, the second derivative spectra of 25 randomly selected Korean and Chinese samples were examined. The baselines of near-infrared spectra collected from powder samples vary widely due to the difference in particle size as well as packing density. Therefore, a second derivative spectrum was used to reduce baseline variations as well as to enhance spectral features. The corresponding NIR spectra (1540–1680 nm range) are shown in Fig. 4. The spectra of the Chinese samples were offset for better visual comparison. The most significant difference is observed at 1625 nm. This peak can be assigned as the characteristic band of decursin. Generally, it is known that the 1625 nm band corresponds to the overtone C–H bands of double bonds or aromatic rings (Ex: = C–H at 3070 cm^{-1} in IR region; 3070 cm^{-1} × 2 = 6140 cm^{-1} = 1629 nm) [10]. It is clearly noticeable that the 1625 nm band is identified for Korean samples, while no significant feature is observed at 1625 nm for the Chinese samples. Consequently, decursin is the important marker component in terms of discrimination and its NIR band can be successfully utilized in this regard.

A model was developed using the 1500–1700 nm range, which encompasses the decursin band at 1625 nm as well as other spectral features. Before performing SIMCA, all spectra were pre-processed using the second derivative algorithm, as noted. With the use of only two principal components (first

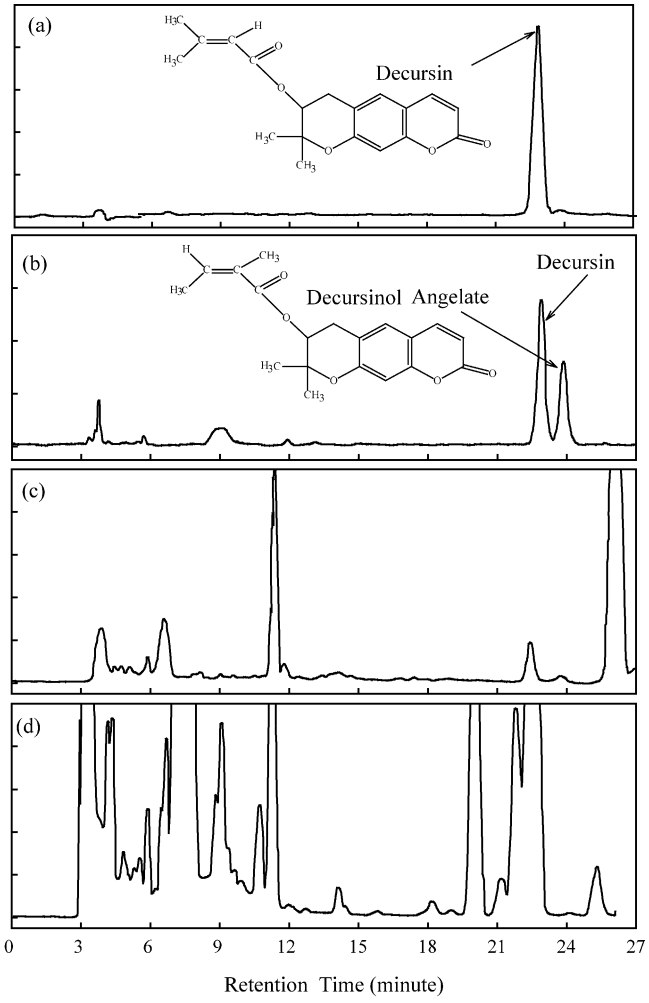

Fig. 3. HPLC chromatograms of decursin standard (a), one Korean (b). and two Chinese samples (c and d).

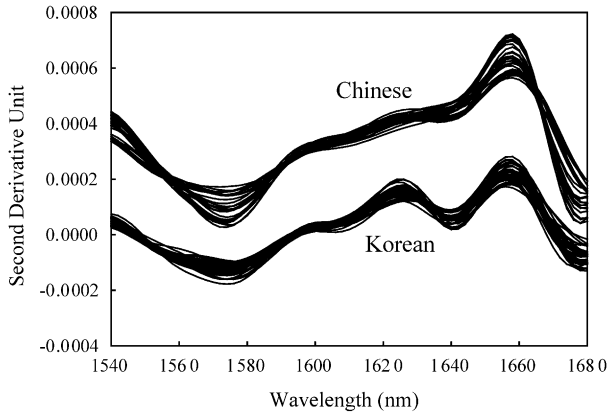

Fig. 4. Second derivative spectra of randomly selected 25 Korean and Chinese samples in the 1540–1680 nm range. The spectra of Chinese samples were offset for the better visual comparison.

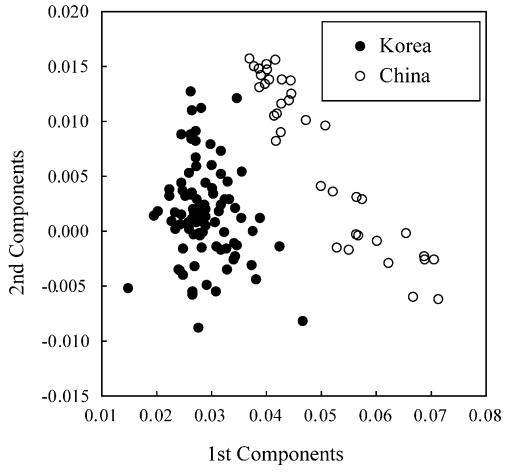

Fig. 5. The score scatter plot showing classification between Korean and Chinese samples.

Table 1
Prediction results using SIMCA model

Prediction set	Origin	Number of samples	Number of samples identified as Korean	Number of samples identified as Chinese
I	Korea	30	30	0
	China	20	0	20
II	Korea	31	31	0
	China	21	0	21

and second), the two groups were successfully segregated since there are clear spectral differences between the Korean and Chinese samples. The corresponding score scatter plot is shown in Fig. 5. As shown, two groups are clearly identified without overlapping.

With the use of the developed calibration model by SIMCA, the samples in prediction sets I and II are predicted; the results are summarized in Table 1. As shown, identification accuracy is 100% for both data sets. Even though the sample collection period is different (prediction sets I and II), reliable prediction results are achieved. Using NIR spectroscopy combined with SIMCA, the geographical origins of Angelicae gigantis Radix were effectively discriminated without failure.

4. Conclusions

The overall results strongly present the expandability of NIR spectroscopy to other herbal medicines for identification of geographical origins. To establish NIR spectroscopy as a more reliable and formal method, detailed understanding of major ingredients and marker components should be combined using conventional analytical methods such as HPLC. Through this approach, it is possible to

rationalize the relationship between NIR spectral features and classification results. A similar research strategy will be directed to study other important herbal medicines and Raman spectroscopy will also be evaluated for the same purpose.

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