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Short communication

Development of gas chromatographic/mass spectrometry-pattern recognition method for the quality control of Korean Angelica

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Abstract

This paper describes gas chromatographic/mass spectrometry (GC/MS)-pattern recognition methods for the quality control of Korean Angelica. A total of 57 *Angelicae radix* samples, including *Angelica gigas* (Korean origin), *A. sinensis* (Chinese origin) and *A. acutiloba* (Japanese origin), were analyzed by GC/MS, with a principal component analysis (PCA) subsequently applied to 10 common peaks selected from each chromatogram. As a result, the samples were clustered according to their origins on the PC score plot. The loading plot revealed that decursin and decursinol angelate were the most contributive principles distinguishing Korean samples from Chinese and Japanese samples, In addition, a discriminant model was developed for classification of the *Angelicae radix*, using a discriminant analysis (DA), and validated with a training set (three from *A. gigas*, four from *A. sinensis*, and three from *A. acutiloba*). All samples tested were successfully classified according to their species origin.

Keywords: *Angelica radix*; GC/MS; Pattern recognition analysis; Fingerprint analysis

1. Introduction

Angelicae radix (root of Angelica; Danggui) is one of the most popular herbal medicines used in Asian countries, including Korea, China and Japan, which has been traditionally used for the treatment of gynecological diseases, such as menoxenia and anemia, via its hemogenic, analgesic and sedative activities in Korean herbal prescriptions [1]. There have been many reports about the pharmacologic activities of Korean *Angelicae radix* (*Angelica gigas* Nakai), including its antibacterial and antiamnestic effects, inhibitory effect on acetylcholinesterase, depression of cardiac contraction, activation of protein kinase C and antitumor activity. [2–5]. The principle active constituents of Korean *Angelicae radix* are coumarin derivatives, such as decursin and decursinol angelate, which are characteristic components of the root of *A. gigas* [6].

Different Angelica species, *A. sinensis* and *A. acutiloba*, have been described as *Angelicae radix* in Chinese and Japanese pharmacopeia. These Angelica species are considered to have different chemical compositions, pharmacological properties and efficacies from those of *A. gigas* [7]. Therefore, *Angelicae radix* requires classification, on a scientific basis, to prevent adulteration of the different Angelica species available in the herbal market. Several reports have described the chemical profiles of various Angelica species [8,9], but these used HPLC/UV system as the analytical tool, and the methods for detection of the major components of the root of *A. gigas*, such as decursin and decursinol angelate, have not been well validated for such a system. Decursin and decursinol angelate are principle bioactive components, as well as the distinctive components of the root of *A. gigas* and; thus, the detection of these coumarin derivatives is necessary for characterization of the root of *A. gigas*. Coumarin derivatives generally exhibit better responses in a gas chromatography (GC) system due to their non-polar property [10–14]. Therefore, a GC system was selected as the analytical tool, with a GC/MS-based pattern recognition analysis method developed for the quality control of Korean *Angelicae radix*.

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In this investigation, three different *Angelicae radix* samples were collected from Korea (18 samples as *A. gigas*), China (23 samples as *A. sinensis*) and Japan (16 samples as *A. acutiloba*) and analyzed by GC/MS. Subsequently, multivariate analytical methods, such as principal components analysis (PCA) and discriminant analysis (DA), were applied to the chromatographic data, with a classification model for three different *Angelicae radix* presented.

2. Materials and methods

2.1. Plant materials

Sixty-seven *Angelicae radix* samples were collected from oriental herb stores in 3 different countries, namely, 21, 27 and 19 samples from Korea, China and Japan, respectively. The collected samples were identified by Emeritus Prof. D. S. Han of Seoul National University, Korea, with voucher specimens (AR-K-001∼021, AR-C-001∼027, AR-J-001∼019) deposited at the Herbarium of College of Pharmacy, Seoul National University, Korea.

2.2. Chemicals

Standard compounds; 4-ethylene-2-methoxyphenol, hexadecane, butylphthalide, butylidenephthalide, *Z*-ligustilide, *E*-ligustilide, palmitic acid, linoleic acids, decursin, and decursinol angelate, were provided from the Pharmaceutical analysis laboratory of College of Pharmacy, Seoul National University, Korea. HPLC grade solvents were purchased from Duksan (Kyungki-do, Korea) and *N*-eicosane ($C_{20}H_{42}$) from Alltech (Alltech Associates, Inc., USA).

2.3. Sample preparation

One gram of the pulverized *Angelicae radix* was added to 2 mL of an ethyl acetate solution, containing 400 μg of *N*-eicosane (I.S.), in a capped vial, with the mixture was sonicated at 50 °C for 30 min.

2.4. GC/MS analysis

An Agilent 6890 series GC system, with a Jeol JMS-600 mass spectrometer (JEOL, Tokyo, Japan) and HP-5 (30 m × 0.32 mm, 0.25 μm, Agilent Technologies, USA) column, was used for the GC/MS analysis. A 1 μL aliquot of each sample extract was injected onto the GC column. The oven temperature was programmed from 120 to 280 °C at a rate of 5 °C/min. The peaks in the resulting chromatograms were identified using the corresponding standard compounds. The relative peak areas were calculated from the peak area ratio of each analyte to that of the internal standard in the chromatograms. The peaks with peak area ratios greater than 0.001 (S/N > 5) were measured for the following pattern recognition analysis.

For validation of the GC/MS method, the reproducibility was determined by repeated analyses of three QC samples (AR-K-005, AR-C-011, and AR-J-016, $n = 5$) on three separate days. For the 10 common peaks, the peak area ratio values were calculated; the precision (as a percent relative standard deviation) did not exceed 10% for all the tested QC samples. Variance in the retention times was within ± 2% for all the detectable peaks.

2.5. Statistical analysis

The SAS 8.1 (SAS Institute Inc., Cary, NC, USA) software was used for statistical analyses of the data. Data processing for the pattern recognition analysis was as follows: the chromatographic data was obtained using the chromatographic data processing software, Chromate (Interface, Seoul, Korea), which were then imported into SAS, where the peak alignment and subsequent chemometric analyses were performed. Ten common peaks from the GC chromatograms were selected and used as variables. A principal component analysis (PCA) was carried out after normalization of the data using an auto-scaling method, i.e., the average was subtracted from each variable, with each variable divided by its standard deviation. The eigenvalues of ≥1.0 obtained by Kaiser and the cumulative proportion of eigenvalues of ≥80% were considered sufficiently salient for interpretation. Using random selection, 67 samples were divided into two sets: one as a training set (18 from Korea, 23 from China, and 16 from Japan) to develop a discriminant model, and the other as a validation set (3 from Korea, 4 from China, and 3 from Japan). A discriminant analysis (DA) was carried out to develop a classification model, with the model subsequently validated.

3. Results and discussion

Fig. 1 shows typical chromatograms of the *Angelica radix* samples from the three different countries. Different chromatographic patterns were observed according to the species origin. As expected, decursin and decursinol angelate were shown to be major constituents of the root of *A. gigas* from the GC/MS analysis. In the case of *A. sinensis*, *Z*-ligustilide was found to be the principle component, as previously reported [8,9]. *A. acutiloba* exhibited no notable distinctive peak in the GC/MS chromatogram. *A. gigas* was apparently differentiated from other samples by the high decursin and decursinol angelate contents. The decursin and decursinol angelate contents in *A. sinensis* and *A. acutiloba* were less than 1% of that found in *A. gigas* (Table 2). Ten common peaks were selected from the each chromatogram for a pattern recognition analysis. The retention time, mass spectrum profile and compound name corresponding to each peak are summarized in Table 1. The peak area ratios of the 10 common peaks for each Angelica species are summarized in Table 2.

A principal component analysis (PCA) was carried out for classification of the Angelica samples. The dataset for the statistical analysis consisted of the 10 selected peaks and the peak area ratios for a total of 57 Angelicae samples. From the PCA results, the first two principal components, PC1 and PC2, were able to describe more than 90% of the total variability (data not shown). The scores plot of PC2 versus PC1 revealed a defined clustering of the *A. radix* samples according to their origin (Fig. 2). Samples originating from Korea were characterized by positive scores on PC1, while samples originating from China and Japan showed negative scores on PC1. Samples originating from China and

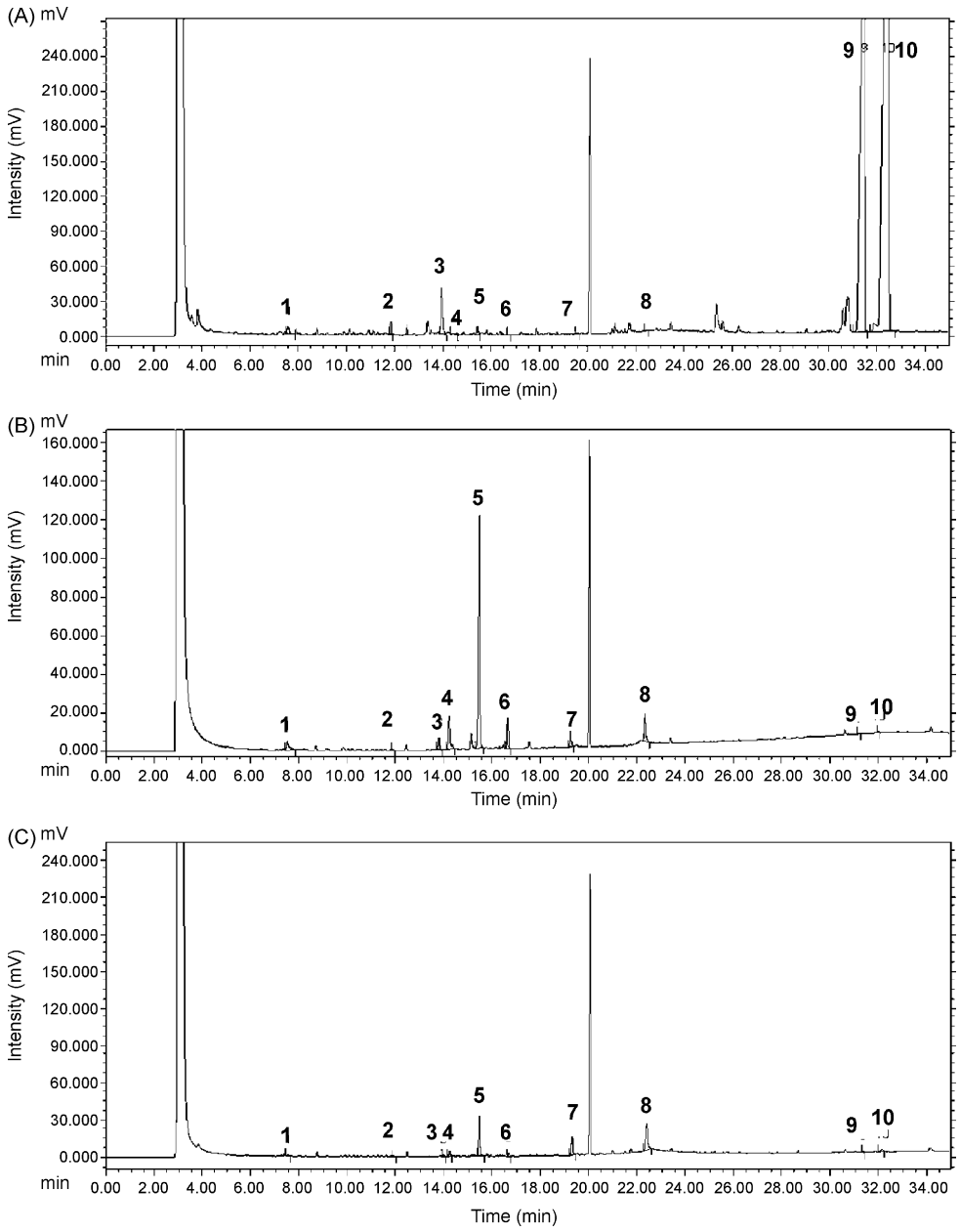

Fig. 1. Representative GC/MS chromatograms of (A) *A. gigas*, (B) *A. sinensis* and (C) *A. acutiloba*. The information for each peak is summarized in Table 1.

Table 1
The GC/MS profile and identification for the common peaks selected for pattern recognition analysis

Peak	t_R (min)	M^+ (*m*/*z*)	Other ions (*m*/*z*)	Identification
p1	7.5	150	135, 107, 77	4-Ethenyl-2-methoxyphenol
p2	12.4	226	113, 85, 71, 57	Hexadecane
p3	13.8	190	133, 105, 77	Butylphthalide
p4	14.2	188	159, 146, 131, 103	Butylidenephthalide
p5	15.5	190	161, 148, 106	*Z*-ligustilide
p6	16.7	190	161, 148, 105	*E*-ligustilide
p7	19.3	256	213, 129, 97, 73, 57	Palmitic acid
p8	22.4	280	123, 109, 95, 81, 67	Linoleic acids
p9	30.6	328	228, 213, 83	Decursin
p10	32.1	328	228, 213, 83	Decursinol angelate

Table 2
Peak area ratio of 10 common peaks in extracts of *A. gigas*, *A. sinensis*, and *A. acutiloba*

Peak	Compound	*A. gigas*	*A. sinensis*	*A. acutiloba*
p1	4-Ethylene-2-methoxyphenol	0.068 ± 0.014	0.083 ± 0.018	0.022 ± 0.004
p2	Hexadecane	0.029 ± 0.004	0.011 ± 0.002	0.013 ± 0.003
p3	Butylphthalide	0.228 ± 0.039	0.041 ± 0.022	0.007 ± 0.002
p4	Butylidenephthalide	0.020 ± 0.006	0.105 ± 0.029	0.037 ± 0.009
p5	*Z*-ligustilide	0.030 ± 0.006	0.720 ± 0.196	0.145 ± 0.052
p6	*E*-ligustilide	0.001 ± 0.0004	0.131 ± 0.029	0.014 ± 0.005
p7	Palmitic acid	0.006 ± 0.003	0.037 ± 0.022	0.058 ± 0.032
p8	Linoleic acids	0.026 ± 0.014	0.139 ± 0.062	0.262 ± 0.098
p9	Decursin	5.613 ± 0.814	0.030 ± 0.008	0.013 ± 0.003
p10	Decursinol angelate	8.679 ± 1.003	0.043 ± 0.015	0.023 ± 0.009

The data is expressed as mean ± S.D. ($n = 18$ for *A. gigas*, $n = 23$ for *A. sinensis*, and $n = 16$ for *A. acutiloba*).

Japan were clearly separated on PC2, where the Chinese samples had positive scores on PC2, while the Japanese samples had negative scores.

The loading values of the variables associated with the first two PCs, PC1 and PC2, are displayed in Fig. 3. The loading plot revealed that decursin and decursinol angelate (p9 and p10) were the most contributive principles distinguishing the Korean samples from the Chinese and Japanese samples, which was also found in the chromatographic results. Butylidene phthalide, *Z*-ligustilide and *E*-ligustilide (p4, p5 and p6) for the Chinese Angelica samples, and palmitic acid and linoleic acid (p7 and p8) for the Japanese samples, were considered to be the distinctive components for discriminating the Chinese from the Japanese samples in the principle component analysis. Butylidene phthalide, *Z*-ligustilide, *E*-ligustilide, palmitic acid and linoleic acid were found in all the Angelica samples tested, as well as in other species [14–18]. Therefore, these compounds cannot be characteristic marker compounds of *A. sinensis* or *A. acutiloba*. However, a GC/MS analysis, adopting a pattern recognition analysis, successfully classified the *A. radix* samples according to their origin, which also reflected the overall differences in the contents of these components.

A discriminant analysis was subsequently applied to the GC/MS dataset to develop a statistical model for classification of the *A. radix* samples. The developed model was validated using 10 validation samples randomly selected from 3 different origins. As a result, all of the validation samples were successfully classified, with 100% accuracy (Fig. 4), which suggests the method developed based on the GC/MS data can be applied for the quality control of Korean *A. radix* from an assessment of the adulteration of different *Angelicae radix* species.

In conclusion, the GC/MS fingerprinting analysis successfully characterized three different *Angelicae radix* samples. A GC/MS-based approach exhibited the characteristic active components of the root of *A. gigas*. Furthermore, a pattern recognition system was able to facilitate discrimination of the fingerprint patterns from different *Angelicae radix* samples. This GC/MS fingerprint-pattern recognition analysis would provide a rational and practical analytical strategy for assessing the authenticity or quality of *Angelicae radix*, including Korean Angelica root.

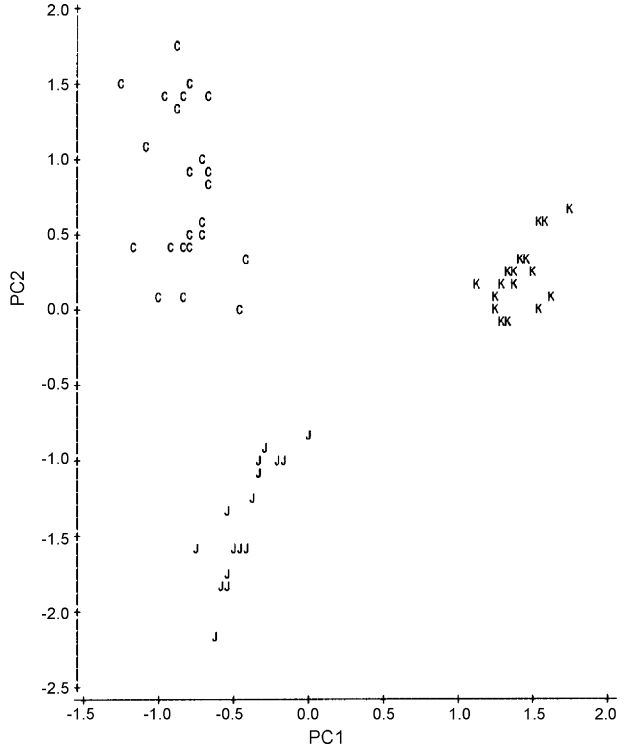

Fig. 2. PC scores plot for *A. gigas*, *A. sinensis* and *A. acutiloba* samples. Capital letters; K, C and J, represent *A. gigas*, *A. sinensis* and *A. acutiloba* samples, respectively.

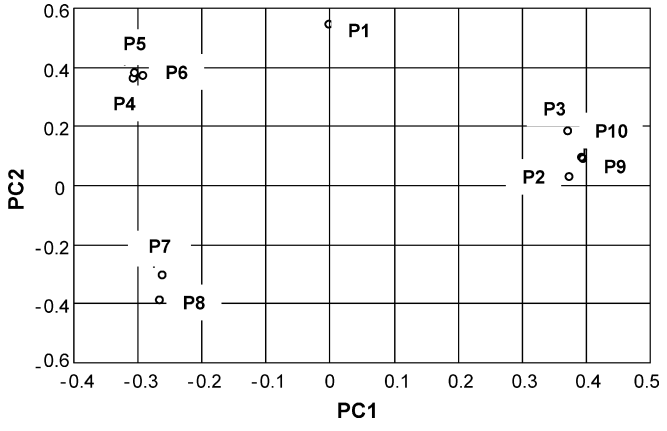

Fig. 3. PC loading plot for the 10 common peaks of the *Angelica radix* samples. The information for each peak is summarized in Table 1.

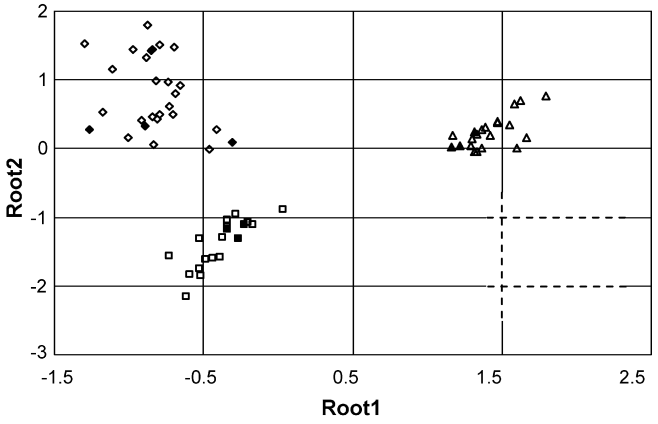

Fig. 4. Discriminant model for classification of three different *A. radix* samples. The open triangle, diamond and square represent *A. gigas*, *A. sinensis* and *A. acutiloba* samples, respectively; the closed triangle, diamond and square represent the validation sets corresponding to *A. gigas*, *A. sinensis* and *A. acutiloba* samples, respectively.

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