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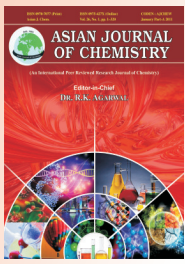

UV Spectra Fingerprint and Multivariate Analysis Approach for Identification of the Species of *Panax*

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The genus *Panax* plants are important herbal remedies and dietary supplements. Species identification and source authentication of this genus are important to ensure their safety and efficacy. The aim of this study to identify *Panax japonicus* var. *major*, *Panax stipuleanatus*, *Panax pseudo-ginseng* var. *bipinnatifidus*, *Panax* sp. and *Panax notoginseng* by the use of UV spectra fingerprint and multivariate analysis methods, such as hierarchical cluster analysis (HCA) and partial least squares discriminant analysis (PLS-DA). The proposed method could divide the five genus *Panax* species into four classes, in which the *Panax* sp. and *Panax notoginseng* have been got together. The result indicated that this method was able to discriminate the five genus *Panax* species and provided reference for quality evaluation of Chinese herbal medicine.

Keywords: Genus *Panax*, UV spectra fingerprint, Hierarchical cluster analysis, PLS-DA, Species identification.

INTRODUCTION

The genus *Panax* (Araliaceae) is an important medicinally genera and consists of 12 species with 10 from eastern Asia and 2 from eastern North America[1]. The species of genus *Panax* contain dammarane-type and oleanane-type saponins[2]. The *Panax* genus has been reported to have the functions of reducing pain, curing cardiovascular and cerebrovascular diseases, improving working memory, preventing cancer and so on[3-5]. The genus *Panax* has been used as a dietary supplement by the US and other countries[6,7]. The properties of genus *Panax* directly depended on the chemical constituents. However, the different species or same species that cultivated in different geographical locations can result in differences in chemical compositions[8,9].

The morphological appearance and some compositions of the genus *Panax* are similar[10], so the species of genus *Panax* have been confused by herbal markets. The common phytochemical methods are difficult to identify different species because they are tedious and expensive and the chemical compositions of genus *Panax* are highly complex. Spectral fingerprinting is a simpler approach to research the chemical composition of plant materials[11]. Spectral fingerprinting techniques include ultraviolet (UV), mass (MS), nuclear magnetic resonance (NMR), infrared (IR), near-infrared (NIR) and other spectrometry[12-16]. Chen *et al.*[17] developed the UV, NIR and MS spectral fingerprints for discriminating the three species of genus *Panax* (*P. quinquefolius*, *P. ginseng* and *P. notoginseng*). Lee *et al.*[18] analyzed the ^{1}H NMR spectral fingerprints of different ginseng species and established quality control code protocol of ginseng products. To date, the special UV spectral fingerprint (UV SFP) analysis of the genus *Panax* has not been intensively used, because distinguishing contributions of specific components are influenced by the broad width of molecular absorption (UV spectrophotometry) bands.

Recently, the multivariate analysis methods extensively used to classify plant material according to their geographical origin, variety and other properties. One of the most remarkable multivariate analysis methods is the principal component analysis (PCA) coupled with other analysis methods. For instance, Sârbu *et al.*[19] recommended PCA and linear discriminant analysis (LDA) were able to discriminate different types, species and subspecies of kiwi and pomelo fruits. Moura *et al.*[20] applied the UV/visible spectrophotometry along with PCA and hierarchical cluster analysis (HCA) for distinguishing species of the genus *Solanum*. Ding *et al.*[21] applied PCA and common and variation peak ratio dual index sequence analysis method to analysis the UV spectral fingerprint of *P. notoginseng*. The partial least squares discriminant analysis (PLS-DA) which had been successfully used to discriminate

the species of plant samples. Hobro *et al.*[22] applied the mid-IR spectroscopy coupled with PLS-DA could discriminate between species and between steam-treated and non-stream-treated wood from *Juglans nigra*. However, the combination of PLS-DA with HCA has never been used to analyze the data of fingerprinting. Up to now, PLS-DA and HCA is a new direction in analysis of UV spectral fingerprint of genus *Panax*.

In this study, UV spectrophotometry (190-400 nm) was used to obtain spectral fingerprints of different species and multivariate analysis methods were used to determine the relationships among various species of genus *Panax* samples. The aim of this study was to discriminate different species and geographical origin of genus *Panax* and provided reference for the quality control of the genus.

EXPERIMENTAL

All of genus *Panax* samples were collected in the Xiaoshao field base in Kunming, Yunnan province on October 11, 2012. All samples of the genus *Panax* were authenticated by Dr. Jinyu Zhang, Institute of Medicinal Plants, Yunnan Academy of Agricultural Sciences. The code, species and source of each group of genus *Panax* samples were listed in Table-1.

TABLE-1
CODES, SPECIES AND SOURCE OF GENUS *Panax*

Codes	Species	Source
G1	*Panax japonicus* var. *major*	Hexi, Lanping, Nujiang, Yunnan
G2	*Panax stipuleanatus*	Wenshan, Yunnan
G3	*Panax pseudo-ginseng* var. *bipinnatifidus*	Wenshan, Yunnan
G4	*Panax japonicus* var. *major*	Fuhe Mountain, Lanping, Nujiang, Yunnan
G5	*Panax* sp.	Tiantang forest, Changning, Baoshan, Yunnan
G6	*Panax japonicus* var. *major*	Liangwang Mountain, Chengjiang, Yuxi, Yunnan
G7	*Panax japonicus* var. *major*	Xinshengqiao, Lanping, Nujiang, Yunnan
G8	*Panax japonicus* var. *major*	Mading, Heqing, Dali, Yunnan
G9	*Panax notoginseng*	Ameng, Yanshan, Wenshan, Yunnan

UV-2500 UV/visble spectrometer (Shimadzu, Japan) with spectral range: 190-400 nm; DFT-100 type grinder (Zhejiang wenling city linda machinery Co., Ltd., China); SY3200-T type ultrasonic washer (Beijing zhongxi taian technology services company, China); 100 mesh stainless steel sieve (Shanghai shenyuan ultrasonic equipment Co., Ltd., China); AR1140 electronic analytical balance (NJ, USA); UPT-I-10 Millipore Waters Milli-Q system (Ulupure, China).

Ethanol and chloroform were analytical grades. Double distilled water was obtained from UPT-I-10 Millipore Waters Milli-Q system (Ulupure, China).

Sample processing: Nine different genus *Panax* samples were freeze-dried and ground to fine powder by grinder. Fifteen hundred milligram of each of the powder of samples was extracted with 20 mL chloroform and ultrasonic treatment for 20 min. The solid residue powder was isolated and air dried and then extracted with 20 mL ethanol and ultrasonic treatment for 20 min. The preparation of water extracts were just like ethanol.

Data acquisition : Each genus *Panax* sample extracts 5 mL was analyzed by UV-2500. The UV spectra were scanned by UV-2500 at 1 nm sampling interval and 0.2 nm slit width between 190 and 400 nm. For UV spectra, the data were treated by the three groups of average, the first derivative and the two points smoothing.

Multivariate analysis: The data were analyzed by HCA and PLS-DA. The recorded wavelength and absorbance data were exported to an Excel spreadsheet and analyzed using HCA and PLS-DA. The HCA and PLS-DA were performed using the SPSS (version 20) software package and SIMCA-P+ (version 11.0) software, respectively. Data were visualized using the HCA and PLS-DA. HCA is an unsupervised data analysis method that allowed the clustering of the samples according to intrinsic variance between them without being biased by desired outcomes. PLS-DA is a supervised technique that used to develop models to discriminate samples according to their absorbance. Each point on the PLS-DA represented an individual samples. HCA and PLS-DA were possibility to discriminate the different species and geographical origin of genus *Panax* samples.

RESULTS AND DISCUSSION

Validation of UV methodology: The ultraviolet spectrum gives integrative information on samples and involves a lot of variable. It is important to validate efficacy of UV method. The sample G9 was prepared according to the UV method and recorded spectrum after storage of 1, 5, 10, 20 and 30 h, respectively, to evaluate stability. The stability was expressed as relative standard deviations (RSD) and calculated based on wavelength of peaks. The RSD % of chloroform, ethanol and water extracts were arranged from 0.10-3.74, 0.42-1.25 and 0-0.53, respectively. The precision was assessed by the spectrum of the same sample solution six times. The RSD % of the wavelength of peaks were arranged from 0-3.13, 0-0.37, 0-1.10, respectively. The reproducibility was performed through wavelength of peaks from spectrum of six same weight samples which were prepared with the same sample preparation program. The RSD % of reproducibility were 0-2.03, 0-0.96 and 0-2.75, respectively. The results indicated that this method was reasonable.

Selection of the solvent: The UV spectrum of genus *Panax* samples extracted by chloroform, ethanol and water show difference in their UV absorbance due to difference in their metabolites and polar solvent (Fig. 1). Fig. 1 shows that different absorbance peaks between different locations and species of genus *Panax* samples. It shows that the compositions are different. The figure of chloroform extracts shows that the absorbance peaks arrange from 190-240 nm regions. The figure of ethanol extracts shows that most of the absorbance peaks arrange from 190-210 nm regions. Only the absorbance peaks of G9 arrange from 190-240 nm. However, the absorbance peaks are less in the figure of ethanol extracts. The water extracts of spectrum can be divided into two characteristic spectral regions: the low field region between 190 and 200

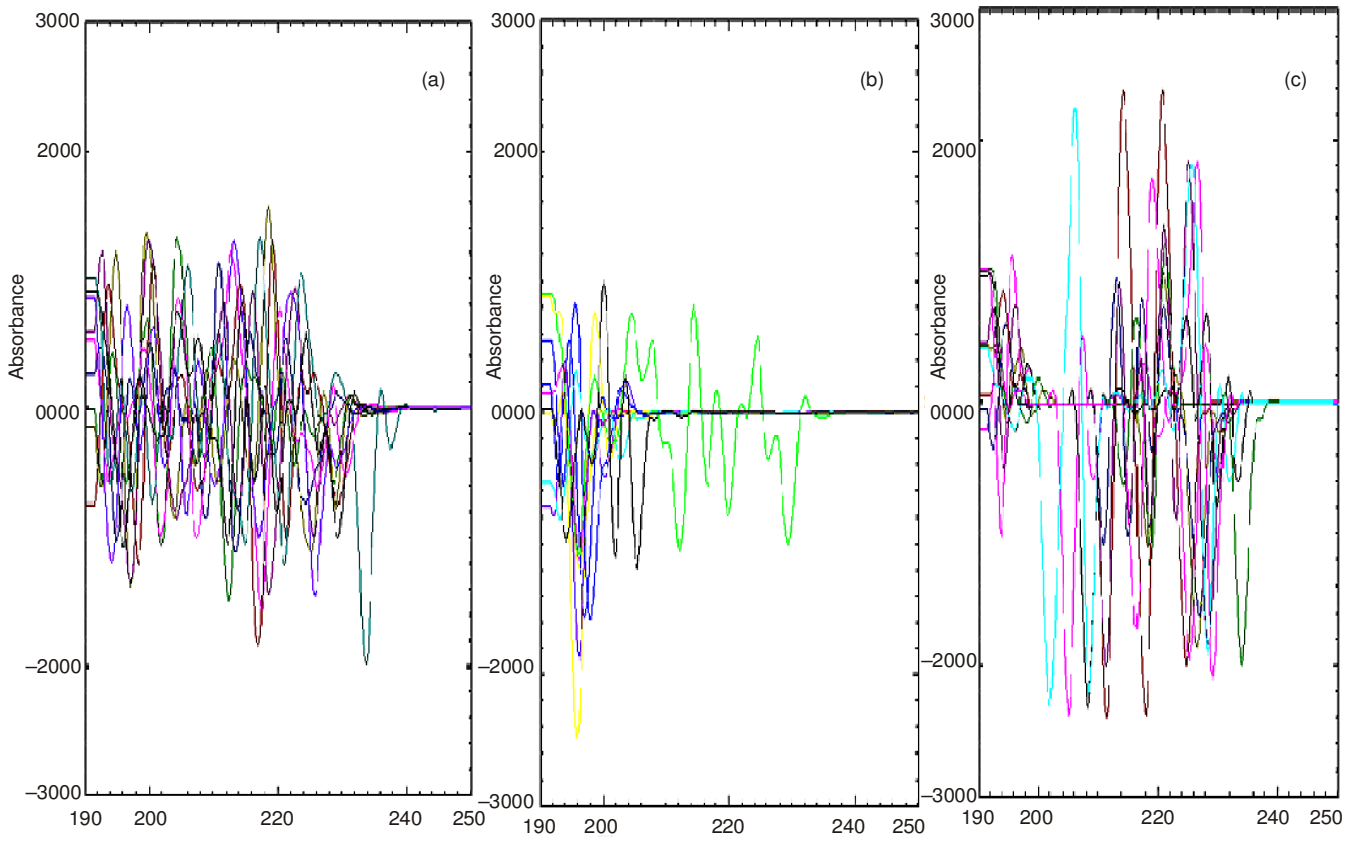

Fig. 1. UV fingerprint spectra of the genus *Panax* samples: (a) solution extracted with chloroform, (b) solution extracted with ethanol, (c) solution extracted with water

nm, the high field region between 200 and 240 nm. The absorbance peaks of the water extracts are less than the chloroform extracts. The result indicated that the chemical compositions were asymmetric relationship among the different locations and variety of genus *Panax* samples. The chloroform extracts show absorption maxima characteristic of the presence of compositions.

UV fingerprint spectra of the genus *Panax* solution extracted with chloroform: The UV spectra fingerprints of the nine genus *Panax* samples show in Fig. 2. We only illustrate UV spectra fingerprints of chloroform extracts. Because the absorbance peaks of chloroform extracts are most. In the UV spectra fingerprints of the genus *Panax* solution extracted with chloroform, one band is observed for the region characteristic of saponins. The UV spectra fingerprints of the G1, G2, G3, G5 and G9 samples are different to G4, G6, G7 and G8 sample in range of 200-240 nm. There are same absorbance peaks between G1 and G4 sample. The G1, G4, G6, G7 and G8 samples are *P. japonicus* var. major, which came from different origins. The UV spectra fingerprints of the samples show difference among G2, G3 G5 and G9 samples in region of 190-240 nm. The G2, G3 and G9 samples are different locations and species of genus *Panax* samples. The UV spectra fingerprints of the G5 and G9 samples are similar in range of 190-240 nm. The G5 and G9 are *Panax* sp. and *Panax notoginseng*, respectively.

Hierarchical cluster analysis (HCA): The HCA of the data obtained by all the genus *Panax* solution extracted with chloroform used generated coefficients of correlation above 151448.920 (Table-2). The table revealed the cluster stage and relationship among the genus *Panax* samples. The formation of two groups is clearly observed (Fig. 3). The Group 1 consisting of the species *P. japonicus* var. major and *P. notoginseng*. The G6, G8, G4 and G7 that belong to the same

TABLE-2
AGGLOMERATION SCHEDULE

Stage	Cluster Combined		Coefficients	Stages Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	6	8	151448.92	0	0	2
2	4	6	220553.78	0	1	3
3	4	7	321122.75	2	0	5
4	1	3	395332.52	0	0	7
5	4	5	423360.72	3	0	6
6	4	9	461561.33	5	0	8
7	1	2	536535.30	4	0	8
8	1	4	679876.25	7	6	0

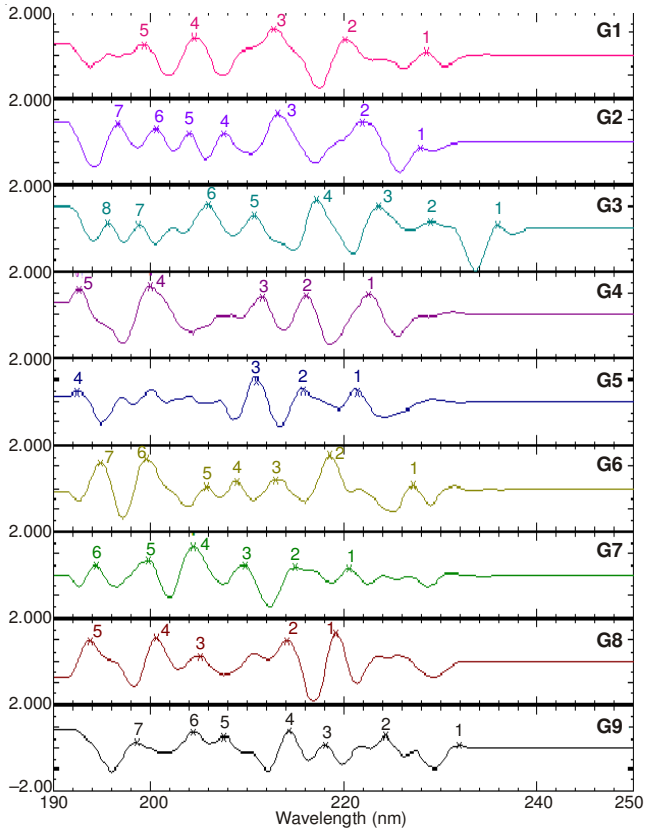

Fig. 2. Comparative UV fingerprint spectra of the genus *Panax* solution extracted with chloroform (G1) Chloroform extracts of *Panax japonicus* var. *major*; (G2) Chloroform extracts of *Panax stipuleanatus*; (G3) Chloroform extracts of *Panax pseudo-ginseng* var. *bipinnatifidus*; (G4) Chloroform extracts of *Panax japonicus* var. *major*; (G5) Chloroform extracts of *Panax* sp; (G6) Chloroform extracts of *Panax japonicus* var. *major*; (G7) Chloroform extracts of *Panax japonicus* var. *major*; (G8) Chloroform extracts of *Panax japonicus* var. *major*; (G9) Chloroform extracts of *Panax notoginseng*

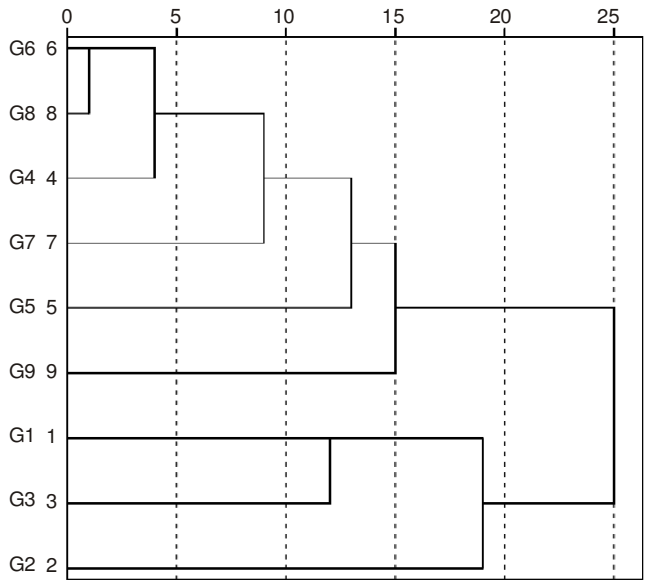

Fig. 3. Dendrograms obtained from HCA of the UV fingerprint spectra the genus *Panax* solution extracted with chloroform

species are clustered together. Group 1 revealed correlation between the metabolome of the genus *Panax* and their species. From the group 2, the G2 and G3 that come from same locations are clustered together. The group 2 indicated that there are correlation between the composition of the genus *Panax* and their geographic distance. The result of HCA offers useful information about the different locations and species of the genus *Panax* samples.

Partial least squares-discriminant analysis (PLS-DA): The PLS-DA is a supervised method that performed on the values of UV absorbance of the genus *Panax* solution extract with chloroform. PLS-DA shows the majority of extract information from multicomponent measurements on two-dimensional spaces. From the Fig. 4, we know that the values of DModX of all samples under 1.4 and the statistical significance of $P < 0.05$. The DModX is the distance of the observation in the training set to the X model plane or hyper plane. The Fig. 4 revealed that the result of PLS-DA is reasonable. The genus *Panax* samples on the first and second components of PLS-DA analysis are presented in Fig. 5. Four groups of samples can be clearly defined: the first group for the samples of *P. japonicus* var. major, the second group for the samples of *P. stipuleanatus*, the third group for the samples of *P.* sp and *P. notoginseng*, the last group for the samples of *P. pseudo-ginseng* var. *bipinnatifidus*. The *Panax* sp and *Panax notoginseng* are very similar. The result indicated that the PLS-DA can be utilized to distinguish the genus *Panax* samples than HCA. PLS-DA is a preferred alternative to HCA.

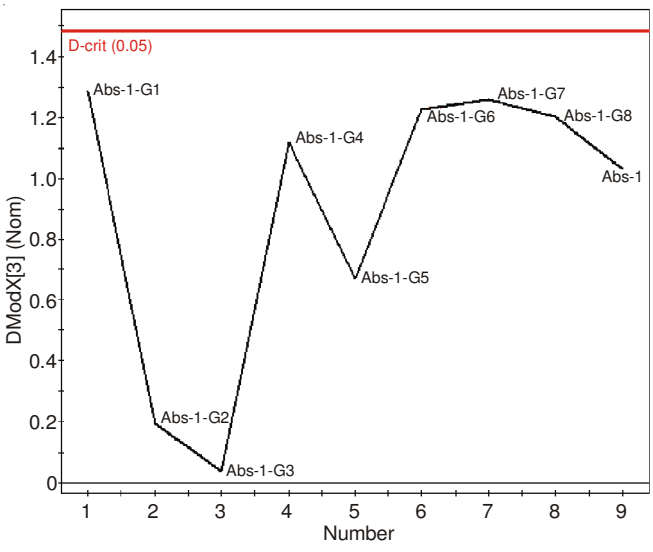

Fig. 4. DModx of PLS-DA of the genus *Panax* samples (*Panax japonicus* var. *major*, *Panax stipuleanatus*, *Panax pseudo-ginseng* var. *bipinnatifidus*, *Panax* sp., *Panax notoginseng*

Conclusion

A simultaneous fingerprinting analysis of the species of genus *Panax* has been performed by employing the UV spectroscopy. The analysis of the data has been performed by HCA and PLS-DA. Five species of genus *Panax* were analyzed in order to investigate the most efficient technique of simultaneous discrimination. The multivariate analysis methods offered reasonable result. Five different species genus *Panax* had been divided into four groups. The five *P. japonicus* var. major samples hold together. The result has indicated the UV fingerprint coupled with PLS-DA was used successfully for classification and discrimination of the species of genus Panax.

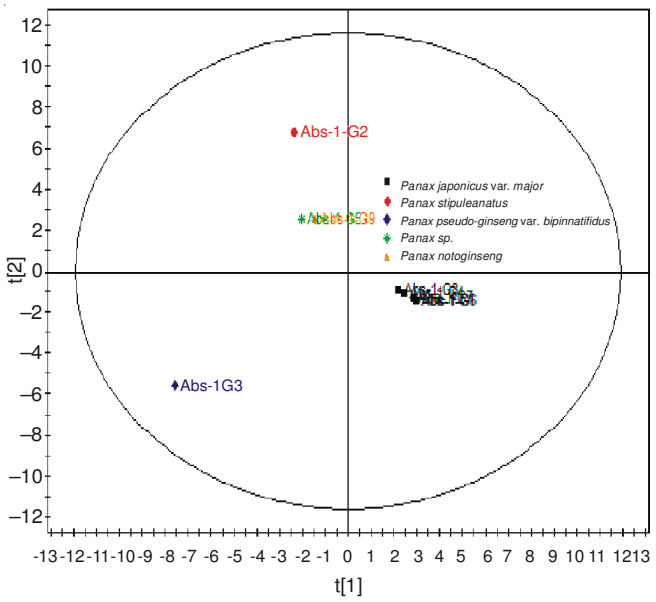

Fig. 5. PLS-DA of the genus *Panax* samples (*Panax japonicus* var. *major*, *Panax stipuleanatus*, *Panax pseudo-ginseng* var. *bipinnatifidus*, *Panax* sp., *Panax notoginseng*) given as a two-dimensional representation of the scores (t[1] and t[2]) on the first [1] and second [2] PLS components

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