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Near Infrared Spectroscopy Evaluation and Regional Analysis of Chinese *Pogostemon cablin* and *Agastache rugosa*

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Abstract: *Pogostemon cablin* (Blanco) Benth. and *Agastache rugosa*(Fisch. et Mey.) O. Ktze., are commonly used traditional Chinese medicines. They belong to the family Lamiaceae and have very similar macroscopical features, which makes it difficult to differentiate them. In this study, the use of near-infrared (NIR) spectroscopy combined with chemometrics as a rapid and non-destructive tool for the discrimination of *P. cablin* from different origins has been preliminarily investigated. NIR spectra were collected in transmission mode in the wavelength range of 4000-12000cm⁻¹. Discriminant models were developed by principal component analysis (PCA) and cluster analysis (CA). The results showed that good classification could be obtained after spectral pre-treatment. The percentage of samples correctly classified by PCA methods in calibration and validation set was 99.9%. The results demonstrated that NIR could be used as a simple and rapid technique to distinguish *P. Cablin* from none-*P. Cablin* and *A. Rugosa*. To further validate the ability of NIR spectroscopy, more samples should be incorporated to build a more robust model.

Keywords: Near-infrared spectroscopy; *Pogostemon cablin*; *Agastache rugosa*; Evaluation; Regional analysis

1. Introduction

Pogostemon cablin is a traditional Chinese medicinal plant that mainly grows in Guangdong, China. Its stems slightly square column, and multi-branched, branches slightly tortuous and it's gas smell specific and slightly bitter taste. The dried above ground part of *P. cablin* is used as herbal Pogostemonis, commonly known as 'Guang-Huo-Xiang' in Chinese or Patchouli in English. It is pungent in flavor, slightly warm in property. It can be used in the area of spleen, stomach and lung channels. It can also be used to remove dampness, relieve exterior syndrome and summer heat, promote appetite and stop vomiting. *Agastache rugosa* is another common medicinal plant that can be found in most areas of China. It is Labiatae Agastache, which is tasteless and slightly cool. The dry aboveground part from *A. Rugosa* can be used to dispel summer-heat and dampness for acute gastroenteritis in summer (Xie et al., 1995).

Due to their morphological similarity, it is difficult to distinguish *P. cablin* and *A. rugosa* by microscopical characteristics, especially in Chinese materia medica preparation. However, they have different chemical component profiles (Fujita et al., 1973; Luo et al., 2003; Yang et al., 2000). Patchouli oil and Agastache oil extracts from different genera of the same family of plants, the chemical composition and pharmacological effects obviously not the same. Patchouli oil mainly containing patchouli alcohol and pogostone (more than 40% of the total), excluding methyl chavicol. etc which has anti-inflammatory, anti-allergy, enhance immunity, anti-bacterial, analgesic, anti-spasmodic, anti-oxidation, antiemetic effects, and no adverse reactions are reported in the large number of applications in medicine. However, Agastache oil mainly containing methyl chavicol (more than 80%), excluding alcohol and patchouli ketone, although antibacterial effect, but inferior to patchouli oil; which has antibacterial, antispasmodic, sedative and elevated white blood cell in the role, but the presence of carcinogenic and mutagenic security issues, no application is currently in drugs (Mo et al., 2009; Ilona et al., 2013; Siavash et al., 2008).

Chinese herbs as well as patent drugs are very important parts of traditional Chinese medicine. It is not only use of China but all over the world. Despite this widespread use, there are many similar TCMs produce different pharmaceutical effects, one TCM can have two or more names and one name can apply to more than one TCM. These problems lead to difficulty in the quality control of TCM and call for urgent attention (Li, 2009).

In recent years, NIRS as an alternative to wet chemistry for quantitative and qualitative analysis which has been widely used in the fields of agriculture (Prieto et al., 2015), petroleum (Chakraborty et al., 2015; Liu et al., 2015) and tobacco (Tang et al., 2015) etc, especially in the quality control and analysis of active ingredients in traditional Chinese medicine and Chinese materia medica preparation (Ding et al., 2015; Luo et al., 2015; Schulz et al., 2002), due to its rapid, non-destructive

and accurate nature. The aim of this study is to establish a method to discriminate *P. cablin* and *A. rugosa* by using the NIRS technology.

2. Materials and Methods

2.1. Plant Materials

35 samples of *P. cablin* were collected from different regions in Guangdong province of China, whereas 5 samples of *A. rugosa* were collected in Henan province of China. All samples were conserved in the laboratory of Pharmacognosy, Guangdong Pharmaceutical University, Guangdong, China. The information of samples is shown in Table 1. All the samples were dried and ground to powders.

Table 1: Source of materials

Species	Sample No.	Voucher/Collection No.	Collection Time	Locality
<i>Pogostemon cablin</i>	No.1- No.3	SG Ji/ CJL-1003001- CJL-1003003	2010.03	Guangzhou, Guangdong Prov., China
	No.4- No.5	SG Ji/ CJL-1004001- CJL-1004002	2010.04	Guangzhou, Guangdong Prov., China
	No.6- No.10	SG Ji/ CJL-1008001- CJL-1008004	2010.08	Chaozhou, Guangdong Prov., China
	No.11- No.15	SG Ji/ CJL-1007001- CJL-1007005	2010.07	Zhangjiang, Guangdong Prov., China
	No.16- No.20	SG Ji/ CJL-1007006- CJL-1007010	2010.07	Zhaoqing, Guangdong Prov., China
	No.21- No.25	SG Ji/ CJL-1007011- CJL-1007015	2010.07	Yangjiang, Guangdong Prov., China
	No.26- No.30	SG Ji/ CJL-1006001- CJL-1006005	2010.06	Wanning, Hainan Prov., China
	No.31- No.35	SG Ji/ CJL-1007016- CJL-1007020	2010.07	Maoming, Guangdong Prov., China
<i>Agastache rugosa</i>	No.36- No.40	SG Ji/ CJL-1012001- CJL-1012005	2010.12	Zhengzhou, Guangdong Prov., China

2.2. NIRS Spectra Collection

4 g of 60 mesh powder from each batch of samples was transferred into the quartz sample cup and then slightly shaken to allow the samples to distribute evenly in the cup. The NIRS data were

acquired in a Fourier transform near infrared spectrometer (Nicolet 6700, USA) with the integrating sphere diffuse reflectance method. All the NIRS measurements were conducted using the following settings: scanning region at 12000 cm^{-1} - 4000 cm^{-1} for 64 times, resolution: 8 cm^{-1} , temperature: $25 \pm 2\text{ }^{\circ}\text{C}$, relative humidity: 40%-45%. Every sample was measured five times, followed by averaging the results.

2.3. Spectral and Discriminate Analysis

40 samples (include *P. cablin* and *A. rugosa*) were divided into two classes (Fig. 1), the calibration set consisted of stochastic 22 *P. cablin* samples and 3 pieces of *A. rugosa*, while the remaining 13 *P. cablin* samples and 2 pieces of *A. rugosa* were used as the validation class, and then analyzed with Constant, Multiplicative signal correction(Wang et al., 2014; Dou et al., 2006) (MSC, the method in solving the particle size of the sample container uneven or inconsistent test sample has a good effect on the impact of spectrum), Standard normal variate(Luypaert et al., 2004; Qu et al., 2007; Yoon et al., 2004; zhao et al., 2014) (SNV, SNV technique can be removed due to the influence of this light scattering caused. It is the strength of the original data by subtracting the average intensity of each wavelength where the wavelength spectrum, and then divided by the standard deviation of the intensity spectrum at all wavelengths, the role is of the same scale between the spectral data for each wavelength intensity on comparable.

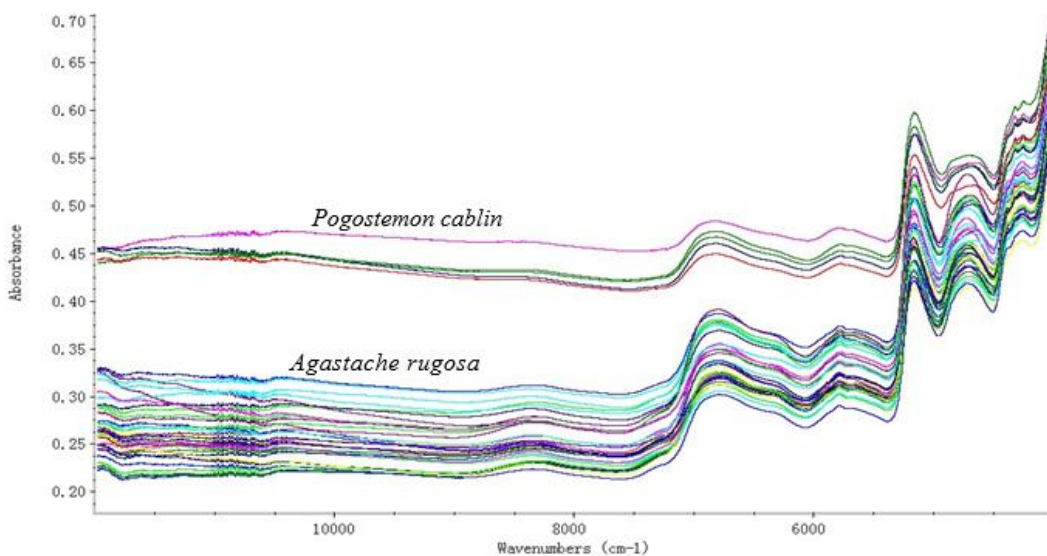
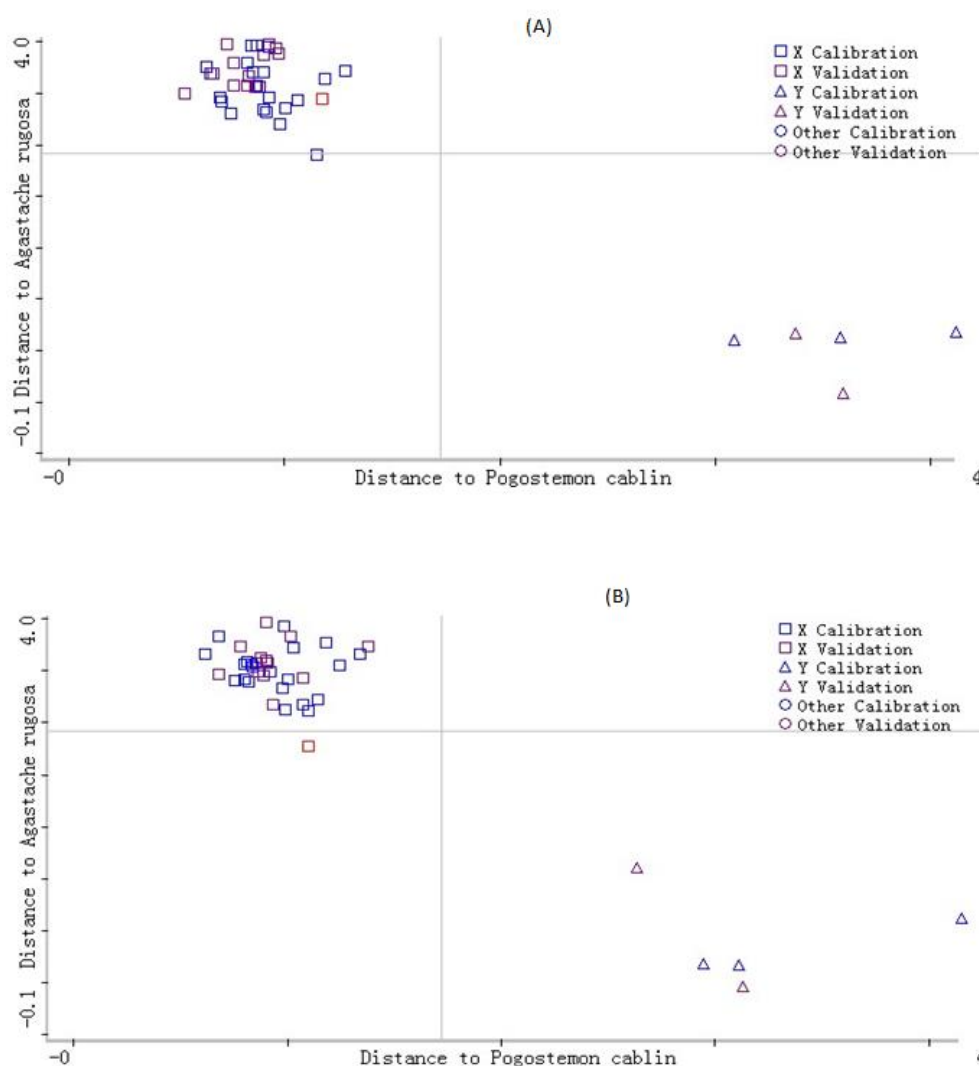


Fig. 1. Spectrums of *Pogostemon cablin* and *Agastache rugosa* samples

Normalized by the standard technique processed spectral data, the right to the same variable weight, with mean zero and standard deviation one), First derivative and Second derivative(Pande et al., 2015; Tang et al., 2014) are adjusted for baseline and chemical signal processing most commonly used

method for improving spectral resolution, after deducting the background signal has important significance. (Fig. 2). It was showed that five different preprocessing methods could influence the distances. The farthest distance from P. cablin and A. rugosa could be proved that the SNV was the best processing method, which was used in this research. TQ analyst 8.0 software (Nicolet 6700, USA) and many tests proved that the best wavelength was range from 4157.77 cm^{-1} to 11840.79 cm^{-1} , which could be used to establish the NIRs quantitative model. PCA was used to choose principal components (PCs), the ten PCs were selected and the cumulative relative value was 99.9% (Table 2). It means these 10 PCs could explain 99.9% variables of the sample spectra.



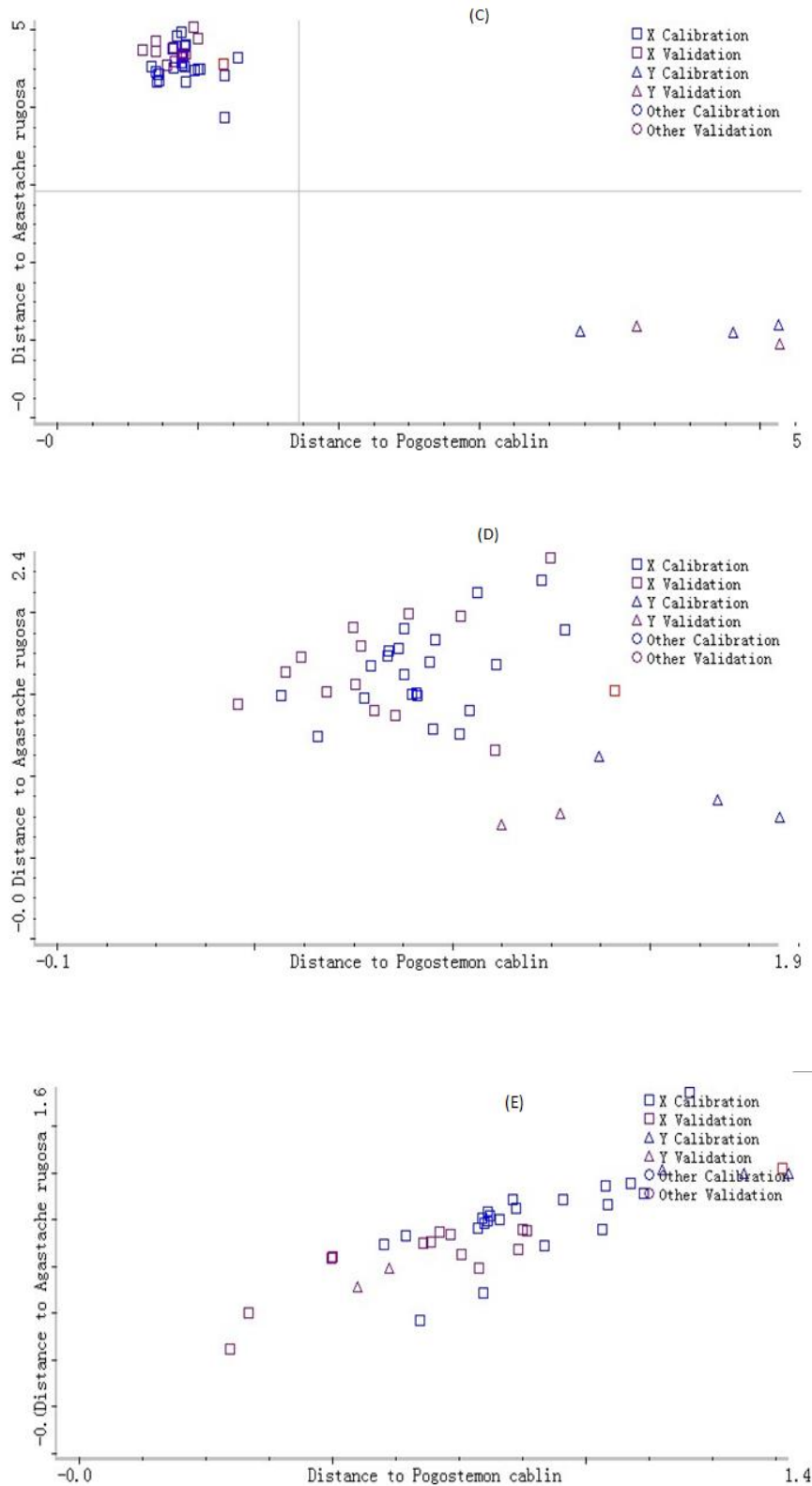


Fig. 2. Distance to *Pogostemon cablin* and *Agastache rugosa* (□: *Pogostemon cablin*; △: *Agastache rugosa*)

Processing methods: (A):Constant, (B):MSC, (C):SNV, (D):First Derivative, (E):Second Derivative.

Table 2: Compare of different processing

Processing	Mean of <i>Agastache rugosa</i> . Distant to <i>Pogostemon cablin</i>	Mean of <i>Pogostemon cablin</i> Distant to <i>Agastache rugosa</i>	Principle compounds	Variability described (%)	Numbers of misclassify
Constant	3.19	3.51	9	100	0
MSC	3.55	3.62	10	99.9	0
SNV	4.58	4.60	10	99.9	0
First derivative	1.45	1.66	10	89.7	0
Second Derivative	1.00	0.95	10	84.4	9

2.4. Cluster Analysis

All NIRS must be processed by First Derivative and Vector Normalization. OPUS 6.5 software (Bruker, Germany) was used in the range of 6499 cm⁻¹-11999 cm⁻¹ as ward’s algorithm standard method. The dendrogram (Fig.3) was obtained.

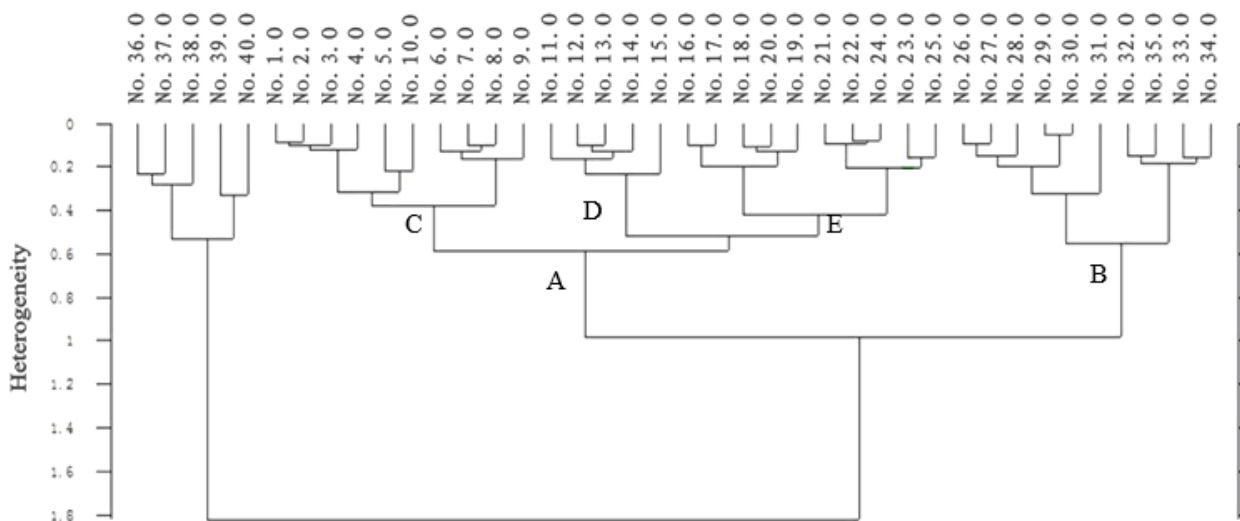


Fig. 3. Dendrogram from cluster analysis of *Pogostemon cablin* and *Agastache rugosa*

3. Results and Discussion

3.1. Acquisition of the Principal Component

PCA is an effective data mining technique, which can choose some PCs that represent the huge original data of medicine’s NIRS. After SNV was confirmed to be the reasonable processing method for

optimum separation, the target of PCA was to reduce the data dimensions and transform the original variables into several new variables, so as to build a linear combination of original ones. Moreover, these new variables should express the primary variables as much as possible, so that we could avoid losing any information of NIRS(Tian et al., 2005).

In this study, SNV was used for pretreating the original spectrums, and the ten PCs were chosen by PCA (TQ analyst 8.0 software). The cumulative contribution rate of 10 PCs was 99.9% (Fig.4). So the ten PCs were totally explained the 99.9% of raw spectral data, among which the first two principle components were accounted for 89.09% of the total variables (Fig.5). Hence, the ten PCs established the classification model and could be used to identify *P. Cablin*.

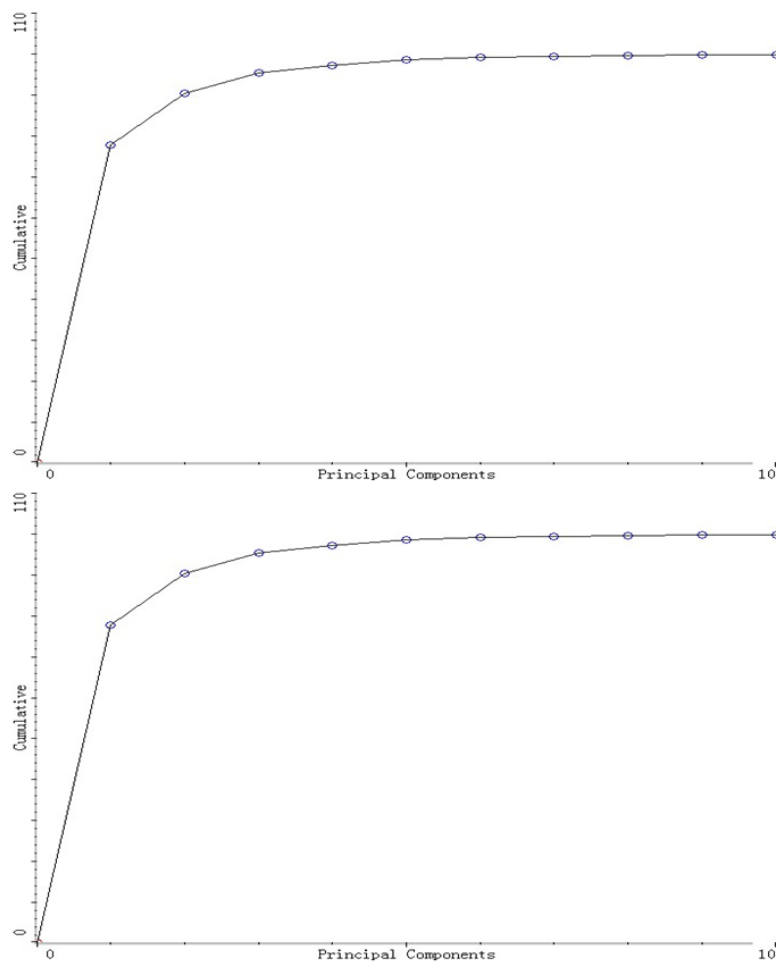


Fig. 4. The cumulative relative variance of the initial 10 PCs

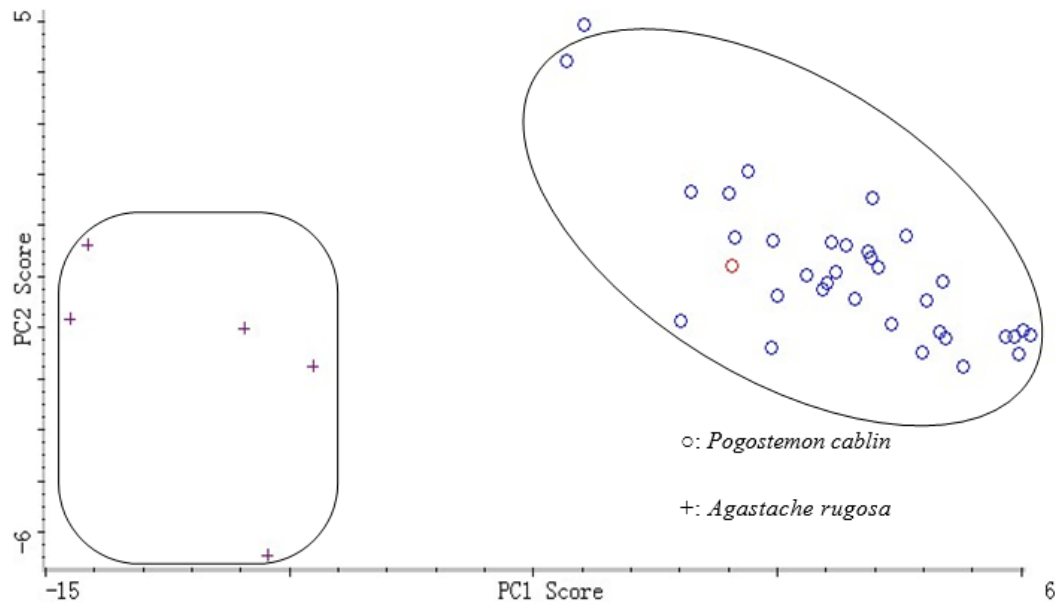


Fig. 5. The first two principle components score of the classification model

3.2. Model of Discrimination Analysis

Discrimination analysis was performed for examining the differences between *P. cablin* and *A. rugosa*. 25 samples were selected from 40 calibration class samples and analysed by TQ analyst 8.0 software in the range of 4 157.77 cm^{-1} to 1 1840.79 cm^{-1} . The raw spectra must be corrected, to remove spectral baseline shift caused by the different color appearance, thickness and unregulated distribution of samples. Fig.2 and Table 2 showed that all the five pretreatment methods could be discriminated *P. cablin* by accurately except the Second Derivative processing method. Under the condition of SNV pretreatment, model's mean distance from *P. cablin* to *A. rugosa* was 4.58 and the mean distance from *A. rugosa* to *P. cablin* was 4.60, they were the farthest in five methods. It means that the SNV pretreatment was the best for establishing the model. After the model was set up, 15 samples of validation set were tested and the results showed that the discriminate accuracy of the model reached 100%, which indicated this model could be used as a standard discriminate method.

3.3. Cluster Analysis Discrimination

Cluster analysis, the unsupervised classification of samples based on their similarity, was performed by OPUS 6.5 software. Samples with similar NIR spectra are grouped into the same cluster, thus allowing differentiation of distinct samples. The dendrogram (Fig.3) reveals two major clusters, indicating that the samples can be classified into two distinct classes. One cluster includes all sample derived from *P. cablin* (ie., samples No. 1 to 35), while the other cluster comprises samples from *A.*

rugosa (ie., samples No. 36 to 40). The segregation of *P. cablin* from *A. rugosa* demonstrates that NIRS combined with cluster analysis can discriminate *P. cablin* and *A. rugosa*.

As shown in Fig.3, samples No.1 to 25, which were collected from Guangzhou, Chaozhou, Zhaoqing, Yangjiang and Zhanjiang in Guangdong province, were grouped together into cluster A. Among them, samples of sub-cluster C in cluster A were collected from Guangzhou and Chaozhou, while the samples of sub-cluster D were collected from Zhanjiang. The sub-cluster E was made up of two smaller clusters in which the samples were collected from Zhaoqing and Yangjiang. Historically, “Shipai” *P. cablin* was known as a famous genuine drug of Guangdong province and was mainly cultivated in “Shipai” village of Guangzhou. Nowadays, the “Shipai” *P. cablin* from Guangzhou was transplanted to Zhaoqing city due to the urbanization, and later has been transplanted to different areas in Guangdong province during the past decades. Under the influence of the environments and cultivating practices of different city, the contents of some chemical constituents have changed, however, the main chemical constituents remain unchanged. Therefore, we can infer that cluster A is “Shipai” *P. cablin*. In cluster B, samples No.31 to 35 were collected from Maoming, Guangdong province, while samples No.26-No.30 were from Hai’nan province. Samples from Maoming city were actually transplanted from Hai’nan province in recently years mainly for essential oil. Hence, although Maoming city has a climate similar to Zhanjiang city and Yangjiang city, samples of Maoming city were more similar to samples from Hai’nan province.

4. Conclusion

In conclusion, the application of NIRS to classify *Pogostemon cablin* from different geographical origins is presented in this study. The results showed that good classification could be obtained after spectral pre-treatment. The percentage of samples correctly classified by PCA methods in calibration and validation set was 99.9%. It was demonstrated that NIRS could be used as a simple and rapid technique to distinguish *P. Cablin* from none-*P. Cablin* and *A. Rugosa*. Furthermore, the study is just a preliminary study and more samples from other varieties and origins will be required to build a more robust model in future.

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