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Identification of *Coptis chinensis* and Its Counterfeits via Electroanalysis-based Fingerprint

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The identification and analysis of Chinese herbal medicines has been an important challenge. Electrochemical fingerprinting is an emerging technique that can be used to identify herbal medicines. In this work, *Epimedium brevicornum* and *Berberis julianae* were investigated as counterfeits of *Coptis chinensis*. This work establishes an analytical method for the quantitative determination of the content of Chinese herbal medicines and their mixed counterfeits by electrochemical fingerprinting. Partial least squares regression (PLSR) and radial basis function artificial neural networks (RBF-ANN) were used for multivariate correction. Satisfactory results were obtained for both PLSR and RBF-ANN models. This study demonstrates that it is feasible to use electrochemical fingerprinting combined with multivariate data analysis to quantify the content of one or two counterfeits mixed in traditional Chinese medicine.

Keywords: *Coptis chinensis*; Electrochemical fingerprint; *Epimedium brevicornum*; PLSR; RBF-ANN

1. INTRODUCTION

The rhizome of *Coptis chinensis* is an important herbal medicine. In recent years, there has been a trend of increasing number of related studies on *Coptis chinensis* and its alkaloid berberine. Its pharmacological research mainly focuses on anticancer, cardiovascular, gastrointestinal and antibacterial and anti-inflammatory aspects [1–3]. Eight Chinese herbs, including *Coptis chinensis*, *Andrographis paniculata* and *Panax notoginseng*, were tested for *in vitro* antibacterial activity against conditionally pathogenic bacteria that frequently cause hospital-acquired infections, and *Coptis chinensis* was found to have the strongest antibacterial activity. *Coptis chinensis* can significantly inhibit the growth of 7 kinds of bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and

Escherichia coli. The protective effect of *Coptis chinensis* on gastric mucosa may be related to the inhibition of gastric acid secretion, improvement of gastric mucosal barrier function, improvement of gastric mucosal blood supply, regulation of plant nervous system function, inhibition of the production of inflammatory factors and anti-lipid peroxidation [4,5]. The antidiarrheal and detoxifying effects of *Coptis chinensis* may be related to its antioxidant and free radical scavenging activities. Schinella et al. [6] examined the antioxidant activity of 20 traditional herbs from China and the Mediterranean coast and found that the scavenging activity of 100 μg/mL of *Coptis chinensis* was higher than that of other plant extracts against hydroxyl radicals [7,8]. Two free radical scavengers (+)laricin and trans-ferulic acid p-hydroxyphenethyl ester were isolated from the methanolic extracts of *Coptis chinensis*. Among them, (+)laricin has more free radical scavenging activity than ascorbic acid, and both have superoxide dismutase-like effects [9].

The modern technique of "fingerprint" identification has its roots in criminology and forensic medicine in the late 19th and early 20th centuries. With the development of genetics in recent years, the concept of fingerprinting and the application of biotechnology have been combined to extend to DNA fingerprinting, and the applications have evolved from criminology to medicine and life sciences [10]. Food quality fingerprinting technique refers to the analysis of the common and individual components of the chemical composition of a food product [11]. The sample is properly processed and then analyzed to obtain an extract that can contain its main components [12]. Different characteristic spectra (including spectral, chromatographic, electrochemical and other spectra, etc.) are expressed according to the different components [13–16]. By measuring the spectral characteristics of such extracts, it is possible to get a good representation of their chemical composition and properties. For the quality control of food products, if the quality fingerprinting of food products is combined with the existing food quality control methods for comprehensive analysis, the intrinsic quality of food products can be evaluated more objectively and comprehensively [17,18].

Chinese herbal medicines and herbal products have been used for thousands of years to prevent and treat human diseases. The rapidly growing TCM and herbal medicine industry has been prompted by a lack of regulations and legislative measures, which has led the World Health Organization and other regulatory authorities to pay increasing attention to the safety and efficacy of TCM [19–21]. Quality control of herbal medicines is particularly important because their potency and quality depend on the type and corresponding concentration of their intrinsic active ingredients [22]. Many factors such as climate, cultivation conditions, harvesting time, drying methods, storage conditions, extraction methods and intentional or unintentional adulteration can cause large variations in the active ingredients [23]. In order to effectively overcome these problems, the World Health Organization has accepted fingerprinting as a method for natural product quality evaluation. Fingerprinting technology was born in this context and is considered as a fast and convenient method for quality control studies of food and herbal medicines [24].

In recent years, with the rising price of *Coptis chinensis*, there is a phenomenon of using *Epimedium brevicornum* and *Berberis julianae* as counterfeit products in the market. In this work, 60 samples were tested using electrochemical fingerprinting techniques developed in recent years. The aim of this work was to establish a rapid electrochemical analysis method. Unsupervised principal component analysis (PCA) and supervised linear discriminant analysis (LDA), partial least squares

discriminant analysis (PLS-DA) and back-propagation artificial neural network (BP-ANN) were used to build models to distinguish *Coptis chinensis*, *Epimedium brevicornum* and *Berberis julianae*. In addition, we further considered the case of adding *Epimedium brevicornum* or *Berberis julianae* to *Coptis chinensis*.

2. EXPERIMENTAL

2.1. Sample collection and treatment.

Twenty samples of *Coptis chinensis*, *Epimedium brevicornum* and *Berberis julianae* each were acquired from the internet and identified by Hubei University of Chinese Medicine. The dried samples were crushed using a herb crusher. The powdered specimens were in a dry, light-proof environment at a temperature of 4C and a relative humidity of 85% until analysis.

2.2. Electrochemical fingerprints collection

Shanghai T&H CHI760E electrochemical workstation is used to detect electrochemical fingerprints of samples. A three-electrode system was used to detect the electrochemical fingerprints of plants. Glassy carbon electrode, platinum wire electrode and Ag/AgCl electrode were used as working electrode, counter electrode and reference electrode respectively. Differential pulse voltammetry was used to scan the samples. The electrolytes were 0.1 M phosphatic buffer solution (PBS, pH 7.0) and acetate buffer solution (ABS, pH 4.5).

2.3. Data treatment

The electrochemical fingerprint data of the samples were exported in Text form and then imported into Origin software for multivariate analysis. Multiple scattering correction (MSC), standard regular transform (SNV), detrending, and Savitzky-Golay filtering were used for comparison when applying fingerprint data for modeling. Based on the best model forecast result parameters: the correlation coefficient of the forecast set (Rpre) and the root mean square error of the forecast set (RMSEP).

2.4. Chemometrics modeling and validation

Chemometric modeling is based on first-order derivative processed electrochemical plant data, for sample differentiation. Fingerprint data matrices were submitted to unsupervised pattern recognition techniques, principal component analysis (PCA) and supervised pattern recognition techniques. Linear discriminant analysis (LDA), partial least squares discriminant analysis (PLS-DA) and back propagation artificial neural network (BP-ANN).

3. RESULTS AND DISCUSSION

Electrochemical fingerprints of twenty samples of *Coptis chinensis*, *Epimedium brevicornum* and *Berberis julianae* were shown in Figure 1. The fingerprint profiles of each species are very similar and overlap each other, representing that the substances involved in electrochemical oxidation are very similar for each specie. The spectra of all samples pre-treated with first-order derivatives are shown in Figure 2. These oxidation peaks are associated with the electrochemical oxidation of phenolics, nitrogenous compounds and glucose [25–27]. The electrochemical fingerprinting of the three species shows that the differences between them are difficult to distinguish between samples of different origins by visual observation [28]. Therefore, electrochemical fingerprinting data need to be further analyzed by pattern recognition methods.

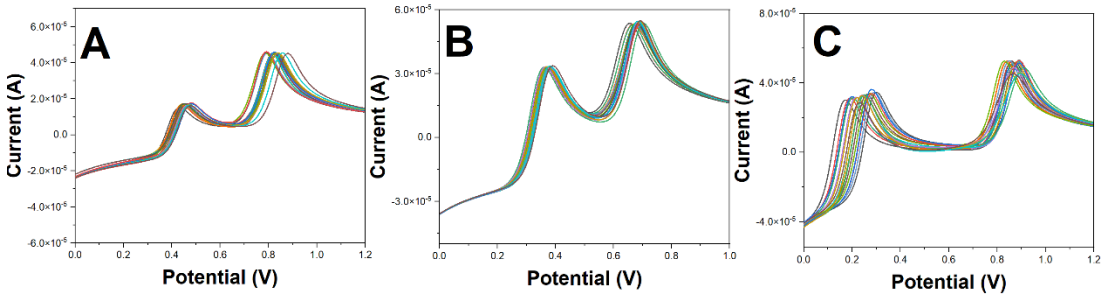

Figure 1. Electrochemical fingerprints of twenty samples of (A) *Coptis chinensis*, (B) *Epimedium brevicornum* and (C) *Berberis julianae.*

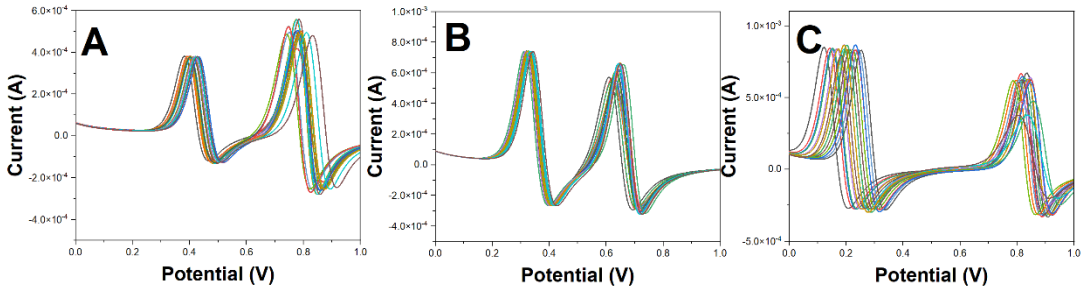

Figure 2. First derivative of electrochemical fingerprints of twenty samples of (A) *Coptis chinensis*, (B) *Epimedium brevicornum* and (C) *Berberis julianae.*

PCA was first used to analyze the spectral data matrix after first-order derivative processing. The PC1-PC2 score projection diagram is shown in Figure 3. As can be seen from the figure, when all samples were projected to PC1, most *Coptis chinensis* scored negative while most *Epimedium brevicornum* samples scored positive. Thus *Coptis chinensis* and *Epimedium brevicornum* samples can be better separated in the PC1 direction [29]. However, when *Berberis julianae* samples were projected onto PC1, they mostly overlapped with *Coptis chinensis* and *Epimedium brevicornum* samples, indicating that samples from the three origins could not be distinguished in the PC1 direction. When all

samples were projected to the PC2 direction, *Berberis julianae* samples scored positive, while *Coptis chinensis* and *Epimedium brevicornum* samples scored mostly negative and overlapped each other [30]. Thus, *Berberis julianae* samples were well distinguished from *Coptis chinensis* and *Epimedium brevicornum* samples in the PC2 direction. That is, there is no clear demarcation between the three samples. However, the combined properties of the three groups of samples in the PC1-PC2 plane based on the first two principal components can be more or less distinguishable.

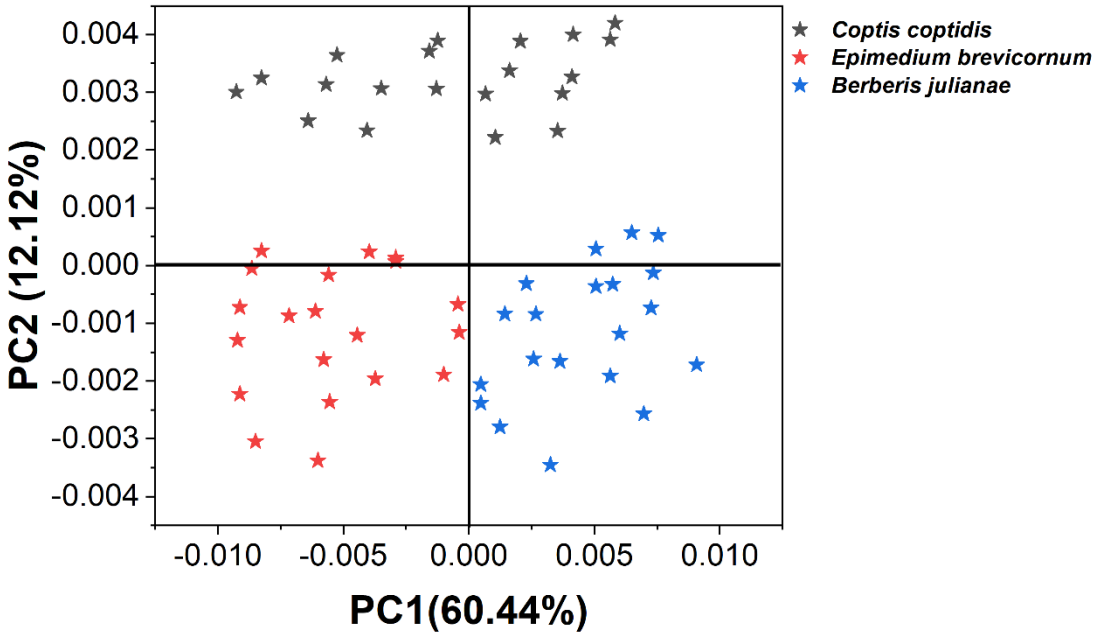

Figure 3. PCA scores plots of 20 samples of *Coptis chinensis*, *Epimedium brevicornum* and *Berberis julianae.*

From the analysis of PCA, it is clear that there are significant qualitative differences between the electrochemical fingerprints of different samples. The PCA scores were continued to be attempted as input variables to the LDA to distinguish between the three types of species [31]. The first 24 principal components of PCA with a variance contribution of 95.0% were selected for the input data. The correct classification rate has been used as the value for comparison. Table 1 shows the results of the LDA, PLS-DA and BP-ANN models, indicating good recognition rates for the calibration set samples of the three types of species [32]. In this case, the score projections of the first two discriminant functions of the LDA are shown in Figure 4. The results showed that the LDA was significantly better than PCA, and the three species could be well clustered into three classes. The PLS-DA model results in 96.7 % and 93.3% identification and prediction rates, respectively. The BP-ANN model only missed one sample, and the correct classification rate reached 95.8%.

Table 1. Identification results of *Coptis chinensis*, *Epimedium brevicornum* and *Berberis julianae* using LDA, PLS-DA and BP-ANN.

Mode	Identification rate	
	Calibration set	Prediction set
LDA	96.7	95.0
PLS-DA	96.7	93.3
BP-ANN	100	98.3

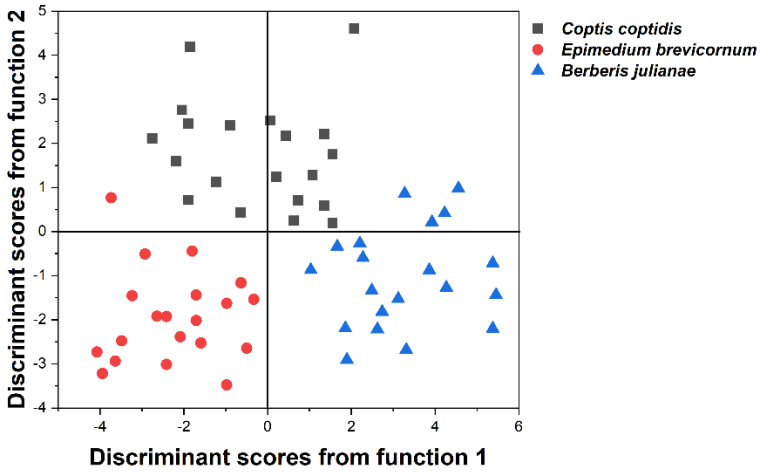

Figure 4. Identification of 20 samples of *Coptis chinensis*, *Epimedium brevicornum* and *Berberis julianae* using LDA mode.

For each binary combination (*Coptis chinensis* - *Epimedium brevicornum* or *Coptis chinensis* - *Berberis julianae*), the SNV-processed electrochemical fingerprint data matrices were input to the PCA analysis model, and the PC1-PC2 two-dimensional score projection plots (Figures 5A and 5B) represent the relationship between each sample in each data matrix. For the *Coptis chinensis* - *Epimedium brevicornum* matrix, the variance contribution of PC1 and PC2 was 95.3%. For the *Coptis chinensis* - *Berberis julianae* binary system, the variance contribution of PC1 and PC2 was 98.5%. Considering some differences in the nature of the samples, the distributions of all samples on PC1 and PC2 were very similar to those on the *Coptis chinensis* - *Epimedium brevicornum* two-dimensional score projection map [33,34]. In conclusion, in both cases, PC1 weighted for most of the data variance and the sequence distribution of the adulterated samples was as a function of the concentration of the mixed counterfeit in the authentic product. However, such qualitative analyses do not provide sufficient quantitative information about the sample distribution [35]. Therefore, electrochemical fingerprinting data combined with linear and nonlinear multivariate correction models were used to analyze the adulterant content in the mixtures.

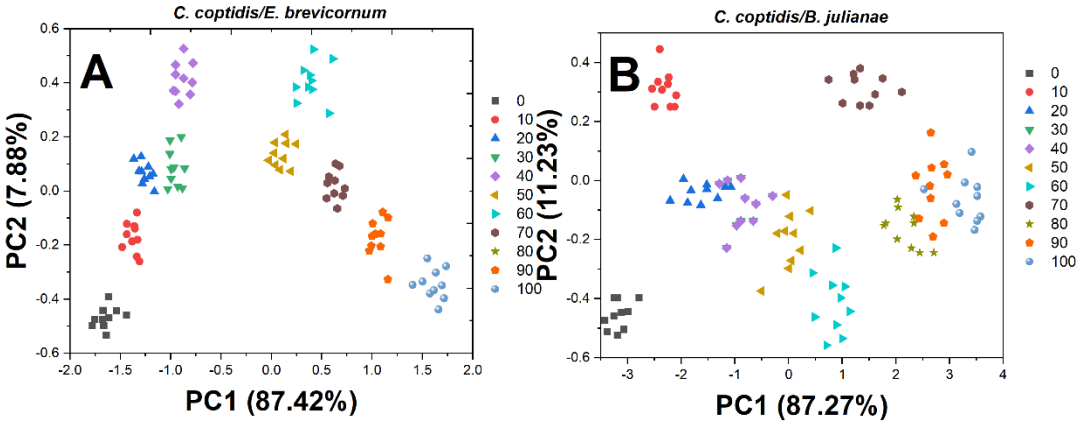

Figure 5. PCA plots for (A) *Coptis chinensis* - *Epimedium brevicornum* and (B) *Coptis chinensis* - *Berberis julianae* with different mixing proportion.

In the quantitative analysis of the binary system mixed with one adulterant, 21 samples of different proportions were prepared and each proportion was repeated three times, for a total of 63 samples. 33 samples (in the order of 0%,10%,...,100%) were used to build the model, and the remaining 30 samples (in the order of 5%, 15%,...,100%) were used to validate the model. Correspondingly, the first 33 samples were used to build a PLS model to quantify the amount of adulterants [36,37]. The performance of the model is characterized by the root mean square of cross-validation error (RMSECV), root mean square of corrected error (RMSEC), root mean square of predicted error (RMSEP), corrected correlation coefficient (Rcal), and predicted correlation coefficient (Rpre) [38]. Leave-one-out cross-validation (LOOCV) is used to optimize the model. The number of factors at the starting minimum of RMSECV was chosen as the number of factors for the electrochemical fingerprint dataset with a latent variable of 3. The results are shown in Table 2, and the PLS1 model obtained Rpre and RMSEP with 0.9991 and 0.0097, respectively.

Table 2. Parameters of PLS1 and RBF-ANN models for *Coptis chinensis* - *Epimedium brevicornum* and *Coptis chinensis* - *Berberis julianae* prediction.

Sample	Models	RMSECV	Training set		Testing set	
			Rcal	RMSEC	Rpre	RMSEP
Coptis chinensis - *Epimedium brevicornum*	PLS1	0.0077	0.9993	0.0075	0.9991	0.0097
	RBF-ANN	0.0039	0.9999	0.0038	0.9996	0.0081
Coptis chinensis - *Berberis julianae*	PLS1	0.0078	0.9994	0.0075	0.9985	0.0122
	RBF-ANN	0.0022	0.9999	0.0021	0.9988	0.0105

RBF-ANN was used as a nonlinear method to examine the quantitative analysis of *Epimedium brevicornum* as a hybrid of *Coptis chinensis*. As shown in the PLS model, the RBF-ANN model also uses the SNV processed data as the input to the network for quantitative analysis The number of nodes in the input, implicit and output layers are 851, 16 and 30, respectively. Gaussian functions were used to build the model. The target minimum mean square error was 1.0×10^{7}. The Rpre and RMSEP were 0.9996 and 0.0081, respectively. It is clearly seen that the RBF-ANN model outperforms the PLS1 model in terms of forecasting results [39]. From the scatter plot (Figure 6A) of the correlation between the actual and predicted contents of *Epimedium brevicornum* in *Coptis chinensis* in the RBF-ANN model, it can be seen that most of the sample points fall on or near the straight line y=x. This coincides with the previous RBF-ANN correction model to obtain better results [40]. Similarly. The same method and number of samples were used to build the PLS1 model and the RBF-ANN model to analyze the content of the adulterant *Berberis julianae* in *Coptis chinensis* samples. The results of the RBF-ANN model were again confirmed to be better than the PLS1 model, and the scatter plot of the correlation between the actual and predicted contents of *Berberis julianae* in the *Coptis chinensis* - *Berberis julianae* binary system (Figure 6B) reconfirmed these conclusions.

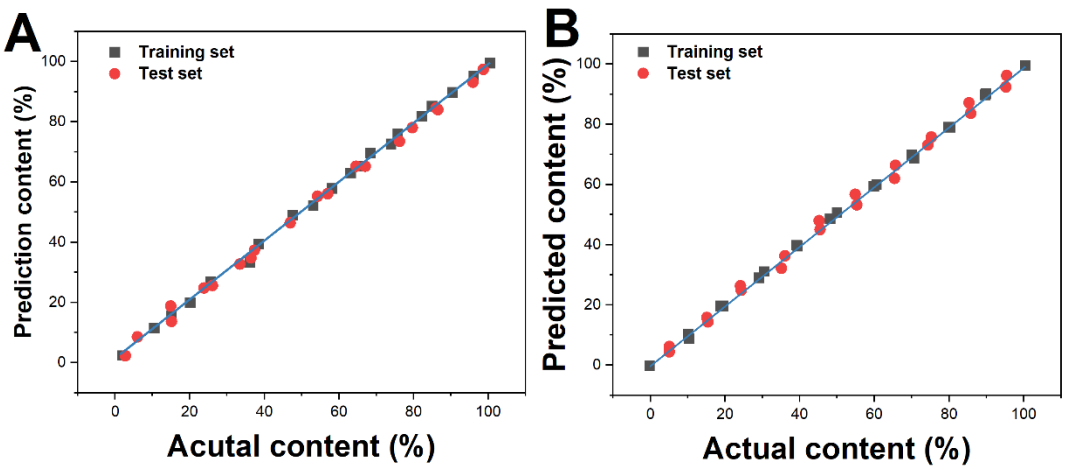

Figure 6. Plots of actual vs. predicted content in (A) *Coptis chinensis* - *Epimedium brevicornum* and (B) *Coptis chinensis* - *Berberis julianae.*

4. CONCLUSION

In conclusion, electrochemical fingerprinting was used to establish a simultaneous determination of the differences between *Coptis chinensis* with the counterfeit *Epimedium brevicornum* and *Berberis julianae*. In addition, the admixture of *Coptis chinensis* with *Epimedium brevicornum* or *Berberis julianae* was considered. The SNV processed data were subjected to principal component analysis and the results showed that the samples in the binary system were able to arrive at a better discrimination. The PLS1 model based on the parameters Rpre and RMSEP achieves satisfactory forecasting results. Compared with the PLS model, the RBF-ANN gives better results. The present work shows that this

method can be applied to complex substances of similar nature such as *Coptis chinensis* adulterated with *Epimedium brevicornum* and *Berberis julianae*, and combined with multivariate calibration methods it can be extended to the study of quality control of herbal medicines of other species.

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