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UV-Vis spectroscopy and chemometrics as a tool for identification and discrimination of four *Curcuma* species

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Abstract

Curcuma species such as *Curcuma longa* (turmeric), *C. xanthorrhiza* (java turmeric), *C. aeruginosa* (black turmeric) and *C. mangga* (mango ginger) are widely used in jamu, Indonesian traditional medicines as well as herbal drink and food supplement. The identification and discrimination of these closely-related plants is a crucial task to ensure the quality and to prevent adulteration of their raw materials. Therefore, we developed a feasible and rapid method using UV-Vis spectra in combination with chemometrics for discrimination of the four species. Firstly, we extract all of the samples using sonication method with methanol as the solvent for 40 minutes. The UV-Vis spectra of this four species were acquired in the interval of 200-800 nm and then standard normal variate was used for preprocessing the spectral data. Principal component analysis (PCA) and discriminant analysis (DA) were used for classification of the four species. It turned out that the discrimination of the four species was achieved through the combination of the pre-processed UV-Vis spectra with PCA and DA, in which DA gave clearer classification according to the species because of 95.5% of the sample correctly classified into their groups by leave-one-out-cross-validation.

Introduction

Indonesia is one of the largest countries for biodiversity, especially plants, with around 900 plants potential to prevent and treat various diseases as traditional and modern medicines. *Curcuma* genus as one of the genus from Zingiberaceae family is widely used in jamu (Indonesian traditional medicine), herbal drink and food supplement such as *Curcuma longa* (turmeric), *C. xanthorrhiza* (java turmeric), *C. aeruginosa* (black turmeric) and *C.mangga* (mango ginger). Traditionally, the four species primarily used for increasing appetite, tonic, and to prevent or treat stomach diseases, diarrhea, liver disorders, diabetes, slimming agent, etc. There are some biological activities which are commonly derived from the four species, such as anti-atherosclerosis, antibacterial, antifungal, antioxidant, anti-inflammatory, and hepatoprotective activities (Kamazeri *et al.*, 2012; Mary *et al.,* 2012; Simoh and Zainal, 2015).

In *Curcuma* species, the rhizome is the most widely used and commonly available in powdered form. Not infrequently, adulteration (substitution or mixing) occurs in this powdered form because this species has some similarities in the color of the rhizomes and also the biological activities. When this happens, it will lead to an inconsistency of quality, safety, and efficacy of the end products. Consequently, we need a reliable method of quality control for the identification and discrimination of the four species to ensure the quality, safety, and efficacy of their raw materials and end products. This quality control method should meet some of these criteria such as fast, simple and accurate.

Several analytical techniques, such as chromatography (thin layer chromatography, high performance liquid chromatography and gas chromatography) and spectroscopy (ultraviolet-visible, Fourier transform infrared, Raman, nuclear magnetic resonance and mass spectrometry), have been used in the development of methods for the identification and discrimination of medicinal plants and food (Ogegbo *et al*., 2012; Chandra *et al*., 2014; Alves *et al*., 2015; Rohaeti *et al*., 2015; Buyukgoz *et al.,* 2016). Some of these analytical techniques require expensive equipment with substantial operational costs. Ultraviolet-visible (UV-Vis) spectroscopy is a good alternative for the identification and

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Table 1. Sources of samples

Sources (subdistrict, regency, province)	Number of sample			
	C. aeruginosa	*C. longa*	*C. xanthorrhiza*	*C. mangga*
Leuwiliang, Bogor, West Java	1	1	1	1
Tegalwaru, Bogor, West Java	1	1	1	-
Ciampea, Bogor, West Java	-	1	1	-
Ciemas, Sukabumi, West Java	-	1	1	-
Nagrak, Sukabumi, West Java	-	-	1	-
Cimalaka, Sumedang, West Java	-	1	-	-
Tanjungkerta, Sumedang, West Java	-	-	1	-
Balidono, Purworejo, Central Java	1	1	1	1
Kutoarjo, Purworejo, Central Java	1	1	1	1
Tirtomoyo, Wonogiri, Central Java	1	1	-	-
Wonogiri, Wonogiri, Central Java	2	2	2	2
Baturetno, Wonogiri, Central Java	1	1	-	-
Ngadirojo, Wonogiri, Central Java	-	1	-	-
Tawangmangu, Karanganyar, Central Java	1	1	1	1
Karangpandan, Karanganyar, Central Java	1	1	1	1
Gondang, Sragen, Central Java	-	1	1	-
Songgolangit, Ponorogo, East Java	1	1	1	1
Pulung, Ponorogo, East Java	1	2	1	-
Minulyo, Pacitan, East Java	1	1	1	1
Tegalombo, Pacitan, East Java	1	1	1	1
Arjosari, Pacitan, East Java	-	1	-	-
Kuniran, Ngawi, East Java	-	1	1	1
Total samples	14	22	18	12

discrimination of these closely-related plants. This technique has some advantages such as quick, cheap, simple and accurate with simple sample preparation. It is, therefore, more efficient when utilized in quality control processes (Sanchez *et al.*, 2008).

As we know, UV-Vis spectra will contain complex information describing the overall signal from many chromophores in the sample. We can see in the UV-Vis spectra if there are any changes in the intensities or positions of bands that will be associated with the variations in the compound composition present in a sample. However, discrimination only by visual inspection in the UV-Vis spectra will be difficult because not many bands will appear in the spectrum, like the IR spectra. The difference in the sample or from another sample only comes from the intensities of the bands. Accordingly, to address these issues, we use chemometrics to extract particular information from the sample. The combination of UV-Vis spectra and chemometrics has been extensively used for identification, discrimination, and authentication of medicinal plants. This combination have been used for classification of coffees (Souto *et al.*, 2010), authentication of medicinal plants from the genus of Thymus (Gad *et al.*, 2013), differentiation of tea varieties (Morillo *et al.,* 2013) and as a screening tool to identify adulteration of culinary spices with Sudan I and blends of Sudan I + IV dyes (Di Anibal *et al.*, 2014).

In this study, we attempted to develop and evaluate the potency of UV-Vis spectroscopy in combination with chemometrics to discriminate four species of *Curcuma* from Indonesia. A comparison of two chemometrics method, namely principal component analysis (PCA) and discriminant analysis (DA), has been performed to determine an adequate discrimination model. The developed method was able to and successfully applied to identify and discriminate the four samples used in this study.

Materials and Methods

Solvent and samples

Methanol pro analysis from Merck (Darmstadt, Germany) was used as the extraction solvent. A total of 66 samples consisting of *C. longa* (n = 22), *C. xanthorrhiza* (n = 18), *C. aeruginosa* (n = 15) and *C. mangga* (n = 11) were collected from a various location in three provinces namely West Java, Central Java and East Java (Table 1). All of the samples were sieved, dried and pulverized prior to use.

Sample preparation

About 10 mg of powdered samples were extracted in 10 mL methanol with ultrasonication device (As-one, Osaka, Japan) for 40 minutes and then left to cool to room temperature. The extracts were then filtered and transferred to 10 mL volumetric flask. Subsequently, 1.5 mL of the extract stock solutions were transferred to 10 mL volumetric flask and diluted with methanol. These extract solutions were used for UV-Vis spectra measurement.

UV-Vis spectral measurement and preprocessing

The UV-Vis spectra of the diluted extracts were measured using UV-Vis double beam spectrophotometer 1700 PC (Shimadzu, Kyoto, Japan). The UV-Vis spectrophotometer equipped with a quartz cell with an optical path of 1 cm. Measurement of UV-Vis Spectra was carried out in the range of 200-800 nm with spectral resolution of 0.5 nm, which represents 1201 variables. All UV-Vis spectra were preprocessed using standard normal variate (SNV) as scatter correction.

Data analysis

Data matrix from 210-500 nm, consisting of 581 variables, was used to build a discrimination model using PCA and DA. PCA and DA were performed in XL-Stat software version 2012.2.02 (Addinsoft, New York, USA).

Results and Discussions

UV-Vis spectral analysis

The UV-Vis absorbance spectra of methanolic extracts of the samples were recorded in the range of 200-800 nm. The UV-Vis absorbance spectra of the four samples have different spectral shapes and intensities. As can be seen in Figure 1, the UV-Vis spectra of *C. longa* is very different with other samples because of the high absorbance intensity in the range of 350-500 nm with maximum absorbance at 425 nm. In this region, the π-π electronic transitions are responsible for exhibiting the absorption of UV-Vis radiation (Kim *et al*., 2013). These transitions are due to the presence of high amount of curcuminoids as major secondary metabolite compounds present in *C. longa. C. xanthorrhiza* also gives an absorption in this region, but the intensity is not very high because curcuminoids content in *C. xanthorrhiza* is much lower than in *C. longa* (Rafi *et al.*, 2015). The absorbance in the range of 200-350 nm is associated with the n-π electronic transitions and usually comes from aromatic compounds and other chromophores, such as hydroxyl and carbonyl. In this region, all samples give similar absorption bands but only differ in their intensities. This similarity may be due to the same composition of their chemical components.

Taking into account the above explanation, the spectral region in the range of 210-500 nm will be very useful to discriminate the four species. In order to obtain a good observation in this region we made an appropriate dilution of the extracts. The reason why we should also pay attention to this region is that when we performed some data reduction, the total variance of the data were affected (Morillo *et al.*, 2013). We can see in Figure 1 that *C. longa* has the highest absorbance in the region of 210-500 nm compared to the other samples, which is followed by *C. xanthorrhiza, C. mangga,* and *C. aeruginosa.* By using these differences from the obtained UV-Vis spectra, we could further discriminate the samples according to the species with the help of chemometrics analysis.

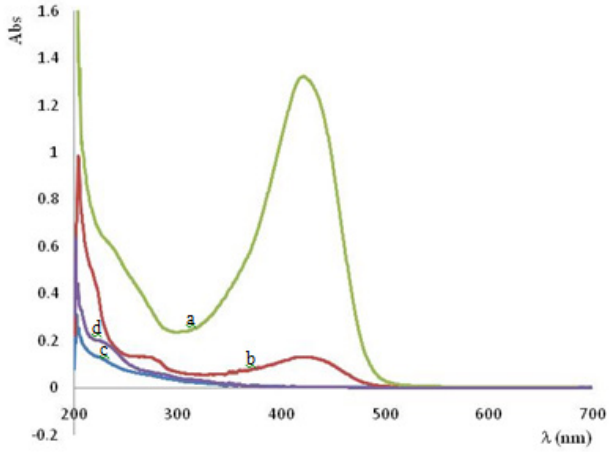

Figure 1. Representative UV-Vis spectra of *C. longa* (a), *C. xanthorrhiza* (b), *C. aeruginosa* (c) and *C. mangga* (d)

Discrimination of the four species with chemometric analysis

Currently, UV-Vis spectra combined with chemometrics is increasingly used for the purpose of identification and discrimination of closely-related medicinal plants. There are variations in peak positions and intensities from the 66 samples used in this study and chemometric analysis was used further to discriminate the four species. In order to build an identification and discrimination model, we used absorbance from 210-500 nm as variables, which contained 581 variables, so we had 66 objects x 581 data matrices.

In general, spectral data pre-treatment is needed before the application of chemometric analysis in order to minimize light scattering, baseline variations, and systematic noise, etc. (Chen *et al*., 2008). Data pre-treatment is a standard procedure in developing identification and discrimination models using chemometric analysis to avoid incorrect or trivial conclusions (Berrueta *et al*., 2007). In this study, we used standard normal variate (SNV) as data pre-treatment, which is usually used to remove the scatter effect. It is transformation process line applied to every spectrum individually. In SNV, the average and standard deviation of all data points for a given spectrum are calculated. The absorbance at every data point is subtracted with the mean value of absorbance, and the result is divided by the standard

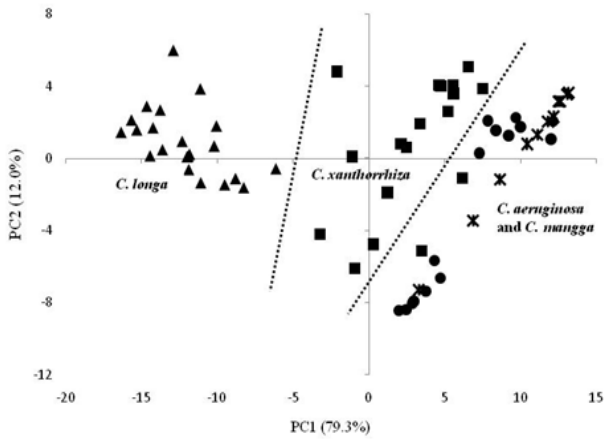

Figure 2. PCA plot of samples

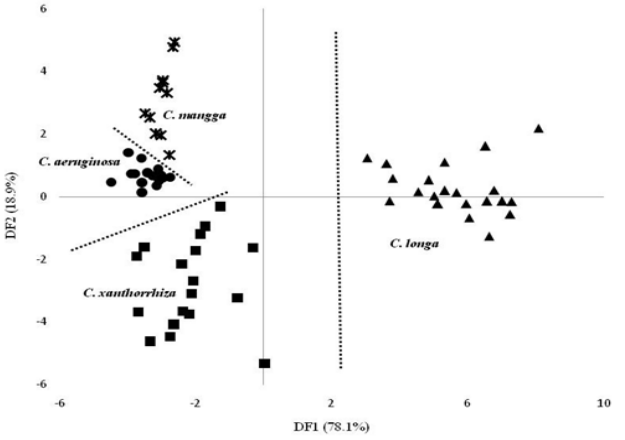

Figure 3. DA plot of samples

deviation, thus giving UV-Vis spectra a unit standard deviation (Barnes *et al*., 1989).

Principle component analysis

PCA is one of the unsupervised pattern recognitions which is aimed to reduce data dimensionality and extract information in order to describe the patterns in the measured data. PCA will transform the original variables into principal components (PCs), which are uncorrelated (Berrueta *et al*., 2007). By plotting the first two PCs known as PCA plot, we could obtain groups of similar patterns for samples tested. Typically, the first two PCs are used to make a PCA plot for they are the most useful components for analysis and because these PCs capture most of the variations in the data.

In our study, PCA was performed to the UV-Vis spectra in the region of 210-500 nm to investigate the possible classification of all samples. The full 66 objects x 581 variables data matrices were subjected to PCA. Figure 2 shows the PCA score plot using the first two PCs that is accounted for 91.3%, with PC1 about 79.30% and PC2 about 12.0%. From the PCA plot, we can see that there are three groups obtained for all samples tested. *C. longa* separated clearly from other species at a negative value of PC1. *C. xanthorrhiza* also separated from other species, but two samples were not well separated and both found in one group consisting of *C. aeruginosa* and *C. mangga*. The clear separation of *C. longa* may be due to the high content of curcuminoids, which is then followed by *C. xanthorrhiza,* but this is not the case of the two other species used in this study, i.e. *C. aeruginosa* and *C. mangga,* that they could not be well separated from each other. This poor result may be due to the similar chemical profiles of the two species (*C. aeruginosa* and *C. mangga*).

Discriminant analysis

From the PCA model, only *C. longa* and *C. xanthorrhiza* could be separated into different groups while *C. aeruginosa* and *C. mangga* still overlap in one group. So we tried another chemometric analysis, namely discriminant analysis (DA) that belongs to the supervised pattern recognition, in order to discriminate the four species more clearly. The discriminant analysis finds a set of linear combinations of the variables, whose values are as close as possible within groups and as far apart as possible between groups. The linear combinations are called discriminant functions. Thus, a discriminant function is a linear combination of the discriminating variables. The discriminant function is generated to give better separation in two or more groups of observations.

In this study, we employed PCA prior to DA. In order to work effectively, the number of samples must be higher than the number of variables in DA. So, we performed PCA on the UV-Vis spectra data of all samples to get PCs as input variables in DA. The number of PCs used as variables can be chosen with Kaiser criterion. In Kaiser criterion, the PCs with eigenvalue greater than 1 must be retained because these PCs explain as much variance as are observed in the variables (Kaiser, 1960). By using this criterion, we chose the first four PCs with a cumulative variation of about 99.44% to construct a DA for discrimination of the four samples.

Based on the four PCs obtained from PCA, a predictive DA model was developed to get clearer discrimination of the four species used. There are differences in the within-class covariance matrices from the Box test. Therefore, separate covariance matrices were employed in this classification model according to the species. We found the total variance from the two DFs obtained by use of DA was 97.0% (DF1 = 78.1% and DF2 = 18.9%). As can be seen in Figure 3, 100% of the original groups were correctly classified into their group, indicating that the DFs obtained could more clearly discriminate

the four samples compared to PCA. *C. aeruginosa* and *C. mangga* were completely separated in this discrimination model of DA in the positive value of DF2. *C. longa* was far away from the other groups in the positive value of DF1, while *C. xanthorrhiza* was separated by the negative value of DF2.

Evaluation of the prediction ability of this model was evaluated by cross-validation technique. Cross-validation is commonly used, especially when the sample numbers are low. We used leave-one-out cross-validation (LOOCV) method for this purposes. LOOCV works by a single training set; one sample is removed at a time from the training set, and the remaining samples are used to build a model. Then the removed sample is treated as an unknown, and its class membership is predicted (Brereton, 2003). Approximately 95.5% of the samples used in this study were correctly classified into their group. Only three samples (2 samples of *C. aeruginosa* and 1 sample of *C. mangga*) were misclassified. Accordingly, this discrimination model of DA gives satisfactory predictions for the samples tested.

Conclusion

In this study, the combination of UV-Vis spectroscopy and discriminant analysis can well discriminate *C. longa, C. xanthorrhiza, C. aeruginosa* and *C. mangga* from Indonesia. Compared to the combination of UV-Vis spectroscopy and PCA, this combination performed better discrimination. By using this combination, about 63 samples (95.5%) were correctly classified into their species. Therefore, the developed method could be used as a rapid, effective and reliable method for discriminating the four samples used in this study.

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