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Feasibility of UV-Vis Spectral Fingerprinting Combined with Chemometrics for Rapid Detection of *Phyllanthus niruri* Adulteration with *Leucaena leucocephala*

(Kebolehlaksanaan Gabungan Spektrum Cap Jari UV-Vis dengan Kemometri untuk Pengesanan Pantas *Phyllanthus niruri* Dicemarkan dengan *Leucaena leucocephala*)

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ABSTRACT

Phyllanthus niruri *is widely used in Indonesia as immunostimulant. The morphology of* Leucaena leucocephala *leaves is similar to that of* P. niruri *leaves*. L. leucocephala *is easy to find and collect because it is widely distributed in the world. Therefore, it is likely* P. niruri *could be adulterated with* L. leucocephala*. Therefore, identification and authentication of* P. niruri *is important to ensure the raw materials used are original without any substitution or mixture with other similar plants causing inconsistencies in their efficacy. In this paper, we described feasibility used of UV-Vis spectral fingerprinting and chemometrics for rapid method for the identification and detection of* P. niruri *leaves adulterated with* L. leucocephala *leaves. UV-Vis spectra of samples measured in the interval of 200-800 nm and signal smoothing followed by standard normal variate were used for pre-processing the spectral data. Principal component analysis (PCA) with the absorbance data from the pre-processed UV-Vis spectra in the range of 250-700 nm as variables could distinguish* P. niruri *from* L. leucocephala*. PCA followed by discriminant analysis (DA) could successfully classified* P. niruri *mixed with 5, 25, and 50%* L. luecocephala *into their respective groups (96.81%). We also employed soft independent modelling of class analogy (SIMCA) for authentication of* P. niruri *and found that 88.3% of the samples were also correctly classified into their respective groups. A combination of UV-Vis spectroscopy with chemometrics, such as PCA-DA and SIMCA, were used for the first time for the identification and detection of* P. niruri *adulterated with* L. leucocephala.

Keywords: Authentication; chemometrics; Leucaena leucocephala*;* Phyllanthus niruri*; UV-Vis spectroscopy*

ABSTRAK

Phyllanthus niruri *banyak digunakan di Indonesia sebagai imunostimulan. Morfologi daun* Leucaena leucocephala *menyerupai daun* P. niruri. L. leucocephala *mudah dijumpai dan dikumpulkan kerana boleh diperoleh secara meluas di seluruh dunia. Oleh itu, berkemungkinan* P. niruri *boleh disatukan dengan* L. leucocephala*. Oleh itu, pengenalan dan pengesahan* P. niruri *adalah penting untuk memastikan bahan mentah yang digunakan adalah asli tanpa penggantian atau campuran dengan tanaman lain yang dapat menyebabkan ketidaktekalan dalam keberkesanannya. Dalam kajian ini, kami menerangkan kemungkinan penggunaan cap jari spektrum UV dan Vis dan kemometri untuk kaedah yang cepat untuk mengenal pasti dan mengesan daun* P. niruri *yang disatukan dengan daun* L. leucocephala*. Sampel spektrum UV-Vis yang diukur dalam selang 200-800 nm dan kelancaran isyarat diikuti dengan variasi piawai digunakan untuk memproses data spektrum. Analisis komponen utama (PCA) dengan data serapan daripada spektrum UV-Vis yang telah diproses pada julat pada 250-700 nm kerana pemboleh ubah dapat membezakan* P. niruri *daripada* L. leucocephala*. PCA diikuti dengan analisis diskriminan (DA) berjaya mengkelaskan* P. niruri *bercampur dengan 5, 25 dan 50%* L. luecocephala *ke dalam kumpulan masing-masing (96.81%). Kami juga menggunakan model bebas analogi kelas (SIMCA) untuk pengesahan* P. niruri *dan mendapati bahawa 88.3% sampel juga dikelaskan dengan betul ke dalam kumpulan masing-masing. Gabungan spektroskopi UV-Vis dengan kemometri, seperti PCA-DA dan SIMCA digunakan untuk pertama kalinya dalam pengenalan dan pengesanan* P. niruri *yang disatukan dengan* L. leucocephala*.*

Kata kunci: Kemometri; Leucaena leucocephala*; pengesahan;* Phyllanthus niruri*; spektroskopi UV-Vis*

Introduction

Phyllanthus niruri, which belongs to the family of Phyllanthaceae, is distributed worldwide, especially in the tropical and subtropical regions. This small shrub is known as a stone breaker, or in Indonesia, it is known as *meniran hijau*. The leaves of *P. niruri* have an even number of fins, which are like the *Leucaena leucocephala* leaves. *L. leucocephala* is a shrub tree with the pods more widely used than the other parts of this plant. *P. niruri* has a long history of use in some herbal medicine systems such as Ayurveda, Traditional Chinese Medicine and *Jamu* (Traditional Indonesian Medicine) for various therapeutic purposes, such as dysentery, influenza, vaginitis, tumor, diabetes, diuretics, jaundice, kidney stones, and dyspepsia (Bagalkotkar et al. 2006). *L. leucocephala* has been known to control stomachache, contraception, and abortifacient (Zayed et al. 2018).

Previous reported of biological activities commonly derived from *P niruri*, such as antihyperuricemics (Murugaiyah & Chan 2009), gastroprotective effect (Abdulla et al. 2010), antidiabetic (Okoli et al. 2011), antisplasmodial (Ifeoma et al. 2012), anti-inflammatory (Couto 2013), antimicrobials (Ibrahim et al. 2013), immunomodulatory effect (Jose et al. 2014), antioxidant (Rusmana et al. 2017; Zain & Omar 2018), antiviral (Wahyuni et al. 2019), and hepatoprotective activity (Ezzat et al. 2020). Meanwhile, *L. leucocephala* has been known to control stomachache, as contraception and abortifacient (Zayed et al. 2018). Meanwhile, *L. leucocephala* have been reported as anthelmintic (Soares et al. 2015), antioxidant, antidiabetic (Chowtivannakul et al. 2016; Pujangga et al. 2019), cytotoxic (Ibrahim 2017), anticancer (She et al. 2017), and antibacterial (Saptawati et al. 2019). These pharmacological effects were caused by the presence of some bioactive compounds in both plants. Lignans, tannins, coumarins, terpenes, flavonoids, alkaloids, saponins, and phenylpropanoids class have been identified in *P. niruri* (Bagalkotkar et al. 2006). A chemical component found in *L. leucocephala* such as alkaloid, cardiac glycosides, tannins, flavonoids, saponins, and glycosides (Awe et al. 2013; Xu et al. 2018).

P. niruri is commonly found growing wild and not yet appropriately cultivated. An adulteration of *P. niruri* raw material with a plant with similar morphology like *L. leucocephala* could occur because the supply of the raw material only depends on the wild sources. The leaves of *P. niruri* have the same shape as the leaves of *L. leucocephala* (Figure 1). Adulteration (substituting or mixing) of *P. niruri* leaves with *L. leucocephala* leaves may occur and will ultimately lead to the inconsistency of the *P. niruri*-based commercial products. Consequently, a reliable method for the identification and detection of *P. niruri* leaves adulteration with *L. leucocephala* leaves is needed. One approach that could be employed to develop a method of identification and authentication is a multicomponent approach, such as fingerprint analysis, which is now commonly used in herbal medicines' quality control. Fingerprint analysis, either using spectroscopy or chromatography, have some advantages like more comprehensive or realistic as the entire information signals from the chemical compounds present in the medicinal plants are used (Septiyanti et al. 2016). There are several reported papers for identification and authentication of *P. niruri* from related species such as DNA barcoding (Inglis et al. 2018), HPLC fingerprinting combined with multivariate analysis (Guo et al. 2015; Martins et al. 2011; Nasrulloh et al. 2018), and TLC fingerprint analysis (Wahyuni et al. 2020). However, these techniques require many preparation steps and lengthy analysis time. Another technique that can be a choice is spectroscopic techniques such as UV-Vis spectroscopy. UV-Vis spectroscopy has several advantages, including fast, inexpensive, and easy sample preparation, so it is considered more efficient in quality control (Sanchez et al. 2008).

UV-Vis spectroscopy is one technique that can be used in the development of identification and authentication methods for medicinal plant raw materials. Complex information from the overall signal of many chromophores in the sample will be shown in the UV-Vis spectra. In the UV-Vis spectra, the compound composition changes will be associated with the changes in the bands' intensities or position. However, visual inspection of the UV-Vis spectra cannot be used to identify and authenticate the medicinal plants in the sample since only some bands appear and differ in their spectra. Accordingly, to address these issues, we use chemometrics to extract unique information from the samples to obtain the desired information to identify and authenticate one plant from their counterfeits (Bansal et al. 2014). The combination of UV-Vis spectroscopy and chemometrics has been widely applied in medicinal plants' quality control for identification, authentication, and discrimination. This combination has been used for the authentication of medicinal plants from the genus of Thymus (Gad et al. 2013a), discrimination of peaberry coffee (Suhandy & Yulia 2017), discrimination of four Curcuma species (Rafi et al. 2018), tea types classification (Dankowska & Kowalewski 2019) and authentication of chili powder (Rohaeti et al. 2019).

In the present study, we have studied a feasibility of UV-Vis spectroscopy combined with chemometrics for detection of *P. niruri* adulteration with *L. leucocephala*. We compared the use of three chemometrics methods, i.e. principal component analysis (PCA), discriminant analysis (DA) and soft independent modeling of class analogy (SIMCA), to determine the most accurate identification and authentication model. PCA works to find the principal

components (PCs) and produces a linear combination of the original variables (Custers et al. 2016). The discriminant analysis could maximize the spread of data between different classes and minimize the data spread in the same class (Berrueta et al. 2007). While in SIMCA, this method based on making the PCA model for each class and then classifying each sample to each PCA model obtained (Yang et al. 2013). A combination of UV-Vis spectroscopy and chemometrics analysis successfully applied to identify and detect *P. niruri* adulteration with *L. leucocephala*.

Materials and Methods

CHEMICALS AND MATERIALS

Methanol p.a. for solvent extraction was purchased from Merck (Darmstadt, Germany). A total of 46 samples consisting of *P. niruri* (n = 30, M1-M30) and *L. leucocephala* (n = 16, P1-P16) were collected from different locations in Java Island, Indonesia (Table 1). All samples were identified at the Herbarium Bogoriense, Indonesian Institute of Sciences and voucher specimens were deposited at the Pusat Studi Biofarmaka Tropika, Institut Pertanian Bogor, Indonesia. All samples were sieved, dried, and pulverized before use.

PREPARATION OF SAMPLES

About 10 mg of an individual (*P. niruri* and *L. leucocephala*) and mixture (5, 25, and 50% *L. leucocephala* mixed with *P. niruri*) samples were weighed. Ten mL of methanol p.a. was added to the samples for extraction in an ultrasonication device (As-one, Osaka, Japan) for 60 min and then left to cool to room temperature. The extracts were filtered in a 10 mL flask and transferred into a 10 mL volumetric flask. A 2.5 mL extract solution was diluted with methanol in a 10 mL flask to make a concentration 250 µg/mL of extract solution. The extract solutions were used for UV-Vis spectra measurement.

UV-VIS SPECTRA MEASUREMENT

The UV-Vis spectra of the diluted extracts were recorded using a UV-Vis spectrophotometer 1700 PC (Shimadzu, Kyoto, Japan). The measurement of UV-Vis spectra was carried out in the 200-800 nm in a quartz cell with an optical path of 1 cm and a spectral resolution of 0.5 nm.

CHEMOMETRICS ANALYSIS

Before starting the chemometrics analysis, all UV-Vis spectra were pre-processed using the smoothing and standard normal variate (SNV) to remove noise and correct the scatter with The Unscrambler X version 10.1 (CAMO, Oslo, Norway). Data matrix from 250-700 nm, consisting of 901 variables, was used to build an identification and authentication model using PCA and DA. PCA and DA were performed in XL-STAT software version 2012.2.02 (Addinsoft, New York, USA). SIMCA was performed in The Unscrambler X version 10.1.

Results and Discussion

ANALYSIS OF UV-VIS SPECTRA SAMPLES

P. niruri and *L. leucocephala* have similarities in the form of leaves, so there is concern that there is *P. niruri* products' adulteration. Therefore, the detection of *P. niruri* adulterated with *L. leucocephala* is needed to avoid adulteration of other products. The shapes and the intensities of the UV-Vis spectra from *P. niruri* and *L. leucocephala* were different (Figure 2). The UV absorption bands shown are usually associated with the presence of different chromophores from various components such as phenolics, flavonoids, and other conjugated systems and UV absorbers. In Figure 2, the UV-Vis spectra of *P. niruri* showed a peak in the range of 245-330 nm with maximum absorbance at 280 nm. While *L. leucocephala* showed double peaks at 265 and 280 nm in the range of 240-305 nm and another peak at 350 nm, in these regions, all samples gave similar absorption bands and only differed in their intensities. This similarity may be caused by the same composition in their chemical components. The electronic transition of π-π* (conjugated C=C), n-s* (aromatic compounds), and n-π* (O-H or C=O) may contribute to the absorption of UV-Vis in the region of 240-400 nm of the samples, and the peak may appear due to the presence of flavonoid compound. The spectral regions in the range of 250-700 nm will be feasible and useful in identifying and authenticating *P. niruri* and *L. leucocephala* and the mixture of the two species because of the differences in the absorption bands. Using these differences, we could further identify and authenticate *P. niruri* from *L. leucocephala* with the help of chemometrics analysis.

IDENTIFICATION AND DETECTION OF *P. niruri* ADULTERATION WITH *L. leucocephala* USING UV-VIS SPECTRUM AND CHEMOMETRICS

A combination of UV-Vis spectroscopy with chemometrics analysis is now widely used for rapid identification, discrimination, and authentication of closely related medicinal plants (Bian et al. 2020; Dankowska & Kowalewski 2019). In this study, we used absorbance from 250-700 nm as variables so that we would have about 901 variables for one sample. This range of wavelengths were used to maximize the classification between the concentrations of the *L. leucocephala* added. This is

because in the range of wavelengths the UV-Vis spectra pattern of the mixed sample gives a significant value. Before subjected to the chemometrics analysis, spectral data pre-treatment for minimizing light scattering, baseline variations, and systematic noise was performed. In this work, we used signal smoothing and SNV as the data pre-treatment. Smoothing is a method that can be used to reduce noise. SNV is preliminary process carried out to improve data information which will eliminate the scatter effect from the spectrum data. We used principal component analysis and soft independent modeling of class analogy for the identification and authentication of *P. niruri* from *L. leucocephala*.

PRINCIPAL COMPONENT ANALYSIS (PCA) AND DISCRIMINANT ANALYSIS (DA)

PCA is one of the chemometrics methods which can be used to reduce data and group the samples tested. PCA belongs to the unsupervised pattern recognition technique and is widely employed in the making of identification, discrimination, and authentication model for quality control of medicinal plant raw materials. PCA works to find the principal components (PCs) and produces a linear combination of the original variables. The first principal component has the highest variance in the data group, while the second PC is perpendicular to the first principal component and has the next largest variance (Miller & Miller 2010). By plotting the first two largest variances of PCs in the PCA plot, we could obtain similar grouping for all samples. Typically, the first two PCs with a variation of more than 70% are used to make a PCA plot because they are the most useful components for analysis, and these PCs capture most of the variations in the data. The closer the PC values, the higher the similarity between the samples. From the PCA plot, we could obtain a sample pattern, groupings, and similarities and differences (Lucio-Gutierrez et al. 2012).

Figure 3(a) shows the score plot from PCA analysis after performing a signal smoothing and SNV in the samples' UV-Vis spectra. The plot of the first two PCs includes 92.54% of the total variance (PC-1 = 57.57%, PC-2 = 34.97%). From the score plot, we can see that PCA distinguishes the two medicinal plants indicating that the two species are relatively different in the chemical compositions. *P. niruri* samples (PN-1 to PN-30) were grouped in the diagonal axis (PC1 and PC2) and well separated from *L. leucocephala* samples (LL-1 to LL-16), which were grouped on the left of the score plot. In this study, we developed a rapid detection method for *P. niruri* leaves adulteration with *L. leucocephala* leaves because of their similar leaves shape. We used a mixture of samples (5, 25, and 50% *L. leucocephala* mixed with *P. niruri*) to build a model for the detection of adulteration in *P. niruri*. The samples of *P. niruri* used for making the mixture were from Benda, Sukabumi (PN-10), Padalarang, West Bandung (PN-19), Guyangan, Purworejo (PN-25), and Ngampel, Ponorogo (PN-30). While the samples of *L. leucocephala* used as adulterant were from Sindangbarang, Bogor (LL-1), Cibeureum, Cianjur (LL-8), Cibubur, Jakarta (LL-11), and Tawangmangu, Karanganyar (LL-12). PCA was performed to build an authentication model of *P. niruri* from *L. leucocephala* but had not distinguished the mixture from the individual samples of *P. niruri* (Figure 3(b)). *P. niruri* mixed with *L. leucocephala* tended to cluster with individual *P. niruri*, indicating that the samples claimed to be *P. niruri* could not provide information on the existence of *L. leucocephala* adulteration. Therefore, we tried another chemometrics methods, i.e. DA and SIMCA, for this purpose.

Discriminant analysis works by making a discriminant function (DF) for each group by finding a linear combination of data that will provide the separation of two or more groups of observations (Gad et al. 2013b). The high correlation in the UV-Vis spectra because of absorption from all chemical compounds present in the sample causing the DA could not be used directly to the absorbance data matrix from the UV-Vis spectra. DA will work effectively when the number of samples is more than the number of variables. Thus, reducing the number of variables is required before the use of DA. We used PCA to reduce the number of variables from the initial data matrix to obtain PCs. The number of PCs as variables in DA is selected with the criteria established by Kaiser (1960). PC with an eigenvalue of greater than one is used in DA based on the Kaiser criterion. Using this criterion, we used 12 PCs to build an authentication model of *P. niruri* from *L. leucocephala* using DA.

Based on the 12 PCs from the result of PCA, a predictive DA model was developed to get clear separation of the *P. niruri*, *L. leucocephala*, and the mixture of the two (5, 25, and 50% *L. leucocephala* mixed with *P. niruri*). We found that the total variance from the two discriminant functions (DFs) obtained by using DA was 99.85% (DF-1 75.29% and DF-2 24.56%). From the DA plot (Figure 4), it is shown that 100% of samples were correctly classified according to the observation groups. This result indicated that the DFs obtained could separate *P. niruri*, *L. leucocephala*, and *P. niruri* adulterated with *L. leucocephala* better and more clearly compared to PCA. The predictive capability of the discriminant model was evaluated by the Leave-one-out cross-validation (LOOCV) test. We found that about 96.81% were correctly classified using the LOOCV method. Based on these results, the 12 PCs obtained from the absorbance

data of the UV-Vis spectra by PCA are considered good predictors for the identification and authentication of *P. niruri* from *L. leucocephala*.

SOFT INDEPENDENT MODELLING OF CLASS ANALOGY (SIMCA)

We also used SIMCA as one classification method to classify each sample in this study. SIMCA is one of the supervised multivariate analysis methods widely used to classify or discriminate samples. This classification method is based on the making of the PCA model for each class and then classifying each sample to each PCA model obtained. The absorbance values were used for making a PCA model for each class in the wavelength range of about 250-700 nm. The output from the SIMCA is a classification table where samples can be classified into their groups, which subsequently will be classified into several classes, or even cannot be classified into any available classes of samples.

Cooman plot was used to evaluate the classification results with class membership at a significant limit of 5% (Figure 5). As shown in Figure 5, the two samples, *P. niruri* and *L. leucocephala*, were separated using SIMCA. A total of 94 samples were used, and based on the evaluation of the plot, *P. niruri* (upper left quadrant) was far apart from the *L. leucocephala* (lower right quadrant). *P. niruri* sample adulterated with *L. leucocephala* scattered in the right upper quadrant. From this classification, we obtained 83 samples (88.30%) were correctly classified into their respective groups, and 11 samples were classified into both groups and even not classified into any of the groups (Table 2).

a b

FIGURE 1. *P. niruri* (a) and *L. leucocephala* (b) leaves

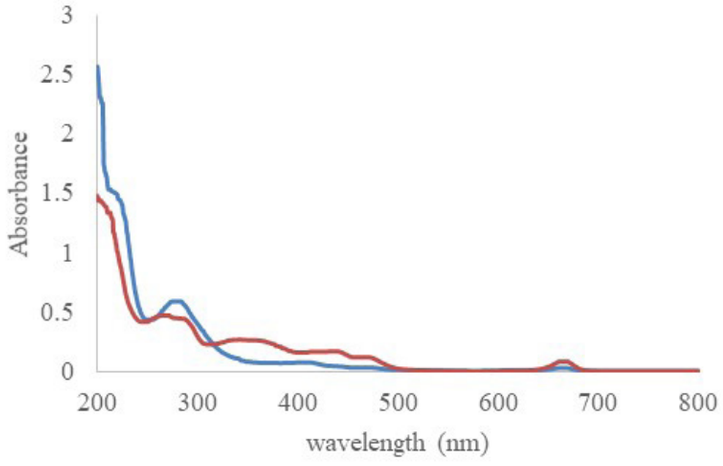

FIGURE 2. Representative UV-Vis spectra of *P. niruri* (blue line) and *L. leucocephala* (red line)

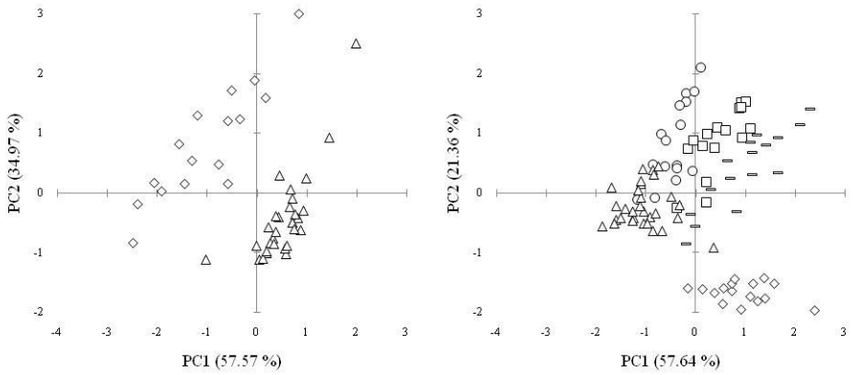

FIGURE 3. PCA plot of samples for distinguishing *P. niruri* from *L. leucocephala* (a) and authenticating of *P. niruri* and *L. leucocephala*, and (b): *P. niruri* (Δ), 5% *L. leucocephala* mixed with *P. niruri* (○), 25% *L. leucocephala* mixed with *P. niruri* (□), 50% *L. leucocephala* mixed with *P. niruri* (-), and *L. leucocephala* (◊)

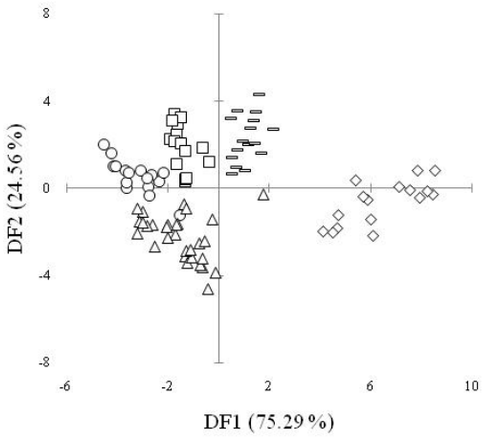

FIGURE 4. DA plot of samples. *P. niruri* (Δ), 5% *L. leucocephala* mixed with *P. niruri* (○), 25% *L. leucocephala* mixed with *P. niruri* (□), 50% *L. leucocephala* mixed with *P. niruri* (-), and *L. leucocephala* (◊)

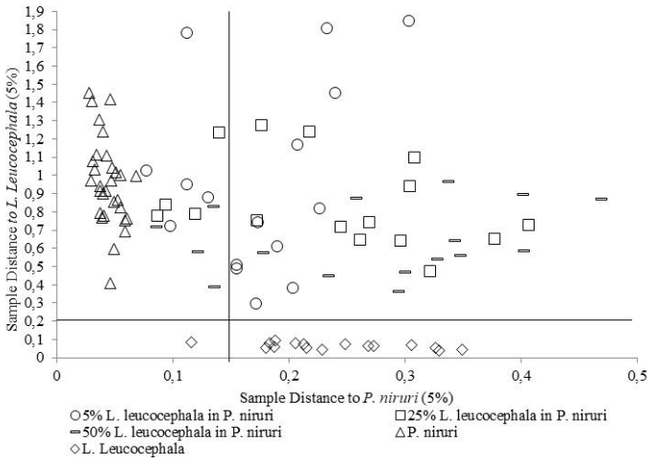

FIGURE 5. Cooman plot of *P. niruri* (Δ) vs *L. leucocephala* (◊) for sample classification

TABLE 1. Code and source of samples

Sample code[a]	Source (Subdistrict, Regency, Province)	Sample code[a]	Source (Subdistrict, Regency, Province)
PN-1	Semplak, Bogor, Jawa Barat	PN-24	Rajapolah (II), Tasikmalaya, Jawa Barat
PN-2	Ciomas (I), Bogor, Jawa Barat	PN-25	Guyangan, Purworejo, Jawa Tengah
PN-3	Ciomas (II), Bogor, Jawa Barat	PN-26	Weleri, Kendal, Jawa Tengah
PN-4	Ciomas (III), Bogor, Jawa Barat	PN-27	Wonosari, Gunungkidul, Yogyakarta
PN-5	Cilibende, Bogor, Jawa Barat	PN-28	Kebonrejo, Kulonprogo, Yogyakarta
PN-6	Dramaga, Bogor, Jawa Barat	PN-29	Bantul, Ponorogo, Jawa Timur
PN-7	Nanggewer, Bogor, Jawa Barat	PN-30	Ngampel, Ponorogo, Jawa Timur
PN-8	Ciutara, Sukabumi, Jawa Barat	LL-1	Sindangbarang, Bogor, Jawa Barat
PN-9	Cisaat, Sukabumi, Jawa Barat	LL-2	Dramaga, Bogor, Jawa Barat
PN-10	Benda, Sukabumi, Jawa Barat	LL-3	Cipayung, Bogor, Jawa Barat
PN-11	Sukaraja, Sukabumi, Jawa Barat	LL-4	Cisarua, Bogor, Jawa Barat
PN-12	Caringin (I), Sukabumi, Jawa Barat	LL-5	Ciutara, Sukabumi, Jawa Barat
PN-13	Caringin (II), Sukabumi, Jawa Barat	LL-6	Cimacan, Sukabumi, Jawa Barat
PN-14	Cicantayan, Sukabumi, Jawa Barat	LL-7	Cibadak, Sukabumi, Jawa Barat
PN-15	Pasir Gede, Cianjur, Jawa Barat	LL-8	Cibereum, Cianjur, Jawa Barat
PN-16	Cugenang, Cianjur, Jawa Barat	LL-9	Pasir Gede, Cianjur, Jawa Barat
PN-17	Karangtengah, Cianjur, Jawa Barat	LL-10	Cugenang, Cianjur, Jawa Barat
PN-18	Jatinangor, Bandung, Jawa Barat	LL-11	Cibubur, Jakarta, Jakarta
PN-19	Padalarang, Bandung, Jawa Barat	LL-12	Tawangmangu, Karanganyar, Jawa Tengah
PN-20	Indihiang (I), Tasikmalaya, Jawa Barat	LL-13	Cepogo, Boyolali, Jawa Tengah
PN-21	Indihiang (II), Tasikmalaya, Jawa Barat	LL-14	Guyangan, Purworejo, Jawa Tengah
PN-22	Cisayong, Tasikmalaya, Jawa Barat	LL-15	Kebonrejo, Kulonprogo, Yogyakarta
PN-23	Rajapolah (I), Tasikmalaya, Jawa Barat	LL-16	Ngampel, Ponorogo, Jawa Timur

[a]PN: *P. niruri*, LL: *L. leucocephala*

TABLE 2. SIMCA classification for the detection of *P niruri* adulteration with *L. leucocephala*

Sample	*L. leucephala* as adulterant (% w/w)	% Correct classification (No. of correct classification/No. of sample)
P. niruri	0	90.0 (27/30)
	5	81.3 (13/16)
	25	81.3 (13/16)
	50	87.5 (14/16)
L. leucocephala	-	100.0 (16/16)
Total		88.3 (83/94)

Conclusion

The combination of UV-Vis spectroscopy and chemometrics analysis (PCA, DA, and SIMCA) can well-identified and authenticated *P. niruri* leaves from *L. leucocephala* leaves. We used the absorbance data of all individual and mixture samples in the region of 250-700 nm. *P. niruri* leaves from *L. leucocephala* leaves could be authenticated using a combination of PCA and DA methods with the result that 97% of the samples were identified into their respective groups by cross-validation. The SIMCA method was used in sample authentication, and the results obtained were 88.3% of the samples were successfully classified into their respective classes. PCA and DA could clearly distinguish the two species, while for the detection of adulteration of *P. niruri* with *L. leucocephala*, DA and SIMCA could give a better and clear separation between the individual and the mixture samples. Therefore, the developed method is feasible and reliable for identifying and detecting *P. niruri* leaves adulterated with *L. leucocephala* leaves.

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