A Combination of UV-Vis Spectroscopy and Chemometrics for Detection of Sappanwood (*Caesalpinia sappan*) Adulteration from Three Dyes

(Embun Spektroskopi UV-Vis dan Kemometrik untuk Pengesanan Pencemaran Kayu Sappan (*Caesalpinia sappan*) daripada Tiga Pewarna)

IRMANIDA BATUBARA1,2*, SAADATUL HUSNA1, MOHAMAD RAFIF1, TONY SUMARYADA1, SUSUMU UCHIYAMA4, BERRY JULIANDI1, SASTIA PRAMA PUTRI2 & EIICHIRO FUKUSAKI6

1Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Tanjung Street, IPB Campus Dramaga, Bogor 16680, Indonesia
2Tropical Biopharmaca Research Center, Institute of Research and Community Services, IPB University, Taman Kencana Street No. 3, IPB Campus Taman Kencana, Bogor 16128, Indonesia
3Department of Physics, Faculty of Mathematic and Natural Sciences, IPB University, Meranti Street, IPB Campus Dramaga, Bogor 16680, Indonesia
4Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
5Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Tanjung Street, IPB Campus Dramaga, Bogor 16680, Indonesia

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ABSTRACT

Sappan wood (*Caesalpinia sappan*) is very well known as a natural dye for traditional food and beverage in many countries. Recently, there are many reports of sappan wood adulteration by adding synthetic or natural dyes to obtain quality color and better appearance. In this study, UV-Vis absorption spectra coupled with chemometrics were used to develop rapid detection of sappan wood raw material adulteration (authentication) from three dyes, i.e., sudan III, commercial textile dyes, and red yeast rice. Absorption spectra of 13 sappan wood raw material and adulterated sappan wood with the three dyes in two different concentrations which resulted about 78 adulterated samples were measured with UV-Vis spectrophotometer at a wavelength range of 200-800 nm. A principal component analysis followed by discriminant analysis was used to construct a model for the authentication of sappan wood from the three dyes used in this study. The combination of both methods was successfully classified sappan wood as non-adulterated and adulterated with the dyes. Cross-validation results of the authentication model of sappan wood from sudan III, commercial textile dyes, and red yeast rice were 94.12%, 94.12%, and 92.16% correctly classified into their groups, respectively.

Keywords: *Caesalpinia sappan*; chemometrics; detection; dyes; UV-Vis spectroscopy

ABSTRAK

Kayu sappan (*Caesalpinia sappan*) sangat terkenal sebagai pewarna semula jadi untuk makanan dan minuman tradisi di banyak negara. Baru-baru ini, terdapat banyak laporan mengenai pemalsuan kayu sappan dengan penambahan pewarna sintetik atau semula jadi untuk mendapatkan warna yang berkualiti dan penampilan yang lebih baik. Dalam kajian ini, spektrum penyerapan UV-Vis yang digabungkan dengan kemometrik digunakan untuk pengembangan pengesan cepat pemalsuan bahan mentah kayu sappan (pengesahan) daripada tiga pewarna, iaitu, sudan III, pewarna tekstil komersial dan beras ragi merah. Spektrum penyerapan 13 bahan mentah kayu sappan dan 78 kayu sappan yang dipalsukan dengan pewarna tersebut dalam dua kepekatan yang berbeza dengan spektrofotometer UV-Vis pada jarak gelombang 200-800 nm. Analisis komponen utama diikuti dengan analisis diskriminan digunakan untuk membina model pengesahan kayu sappan daripada tiga pewarna yang digunakan dalam kajian ini. Gabungan kedua-dua kaedah tersebut berjaya mengelaskan kayu sappan sebagai tidak tercemar dan tercemar dengan pewarna. Hasil pengesahan silang model pengesahan kayu sappan daripada sudan III, pewarna tekstil komersial dan beras ragi merah didapati masing-masing ialah 94.12%, 94.12%, dan 92.16% dikelaskan dalam kumpulan mereka.

Kata kunci: *Caesalpinia sappan*; kemometrik; pengesahan; pewarna; spektroskopi UV-Vis
**INTRODUCTION**

Sappan wood (*Caesalpinia sappan*) is known as *kayu secang* in Indonesia and belongs to the Caesalpinaceae family. Sappan wood is mainly distributed in Southeast Asia, Africa, and United States of America. The dry sappan wood has been used as a traditional herbal drink in many Asian countries such as India, Indonesia, Korea, and Thailand (Lioe et al. 2012; Nirmal & Panichayupakaranant 2014). In Indonesia, it is very well known as a natural red dye for traditional beverages such as in secang tea in South Sulawesi, *bir pletok* by Betawi people in Jakarta as well in *wedang uwuh* by Javanese people. It has also been used as traditional medicine.

An aqueous decoction of sappan wood could be used to strengthen the blood system, promote menstruation, reduce pain, and inflammation (Nirmal et al. 2015) and to treat arthritis (Wang et al. 2011). Previous studies reported that sappan wood exhibits some biological activity such as antiacne, antibacterial, antioxidant (Batubara et al. 2010), anti-inflammatory (Wu et al. 2011), anti cervical cancer (Kitdamrongtham et al. 2013), antiallergy (Yodsa Aeue et al. 2009), and anticonvulsant (Baek et al. 2000). The main active compound in this plant is brazilin, which is also a unique marker for this plant. Various chemical compounds have been isolated from sappan wood, including flavonoids and phenolic compounds such as 4-O-methylsappanol, protosappanin A, protosappanin B, protosappanin E, brazilin, caesalpinia J, brazileide A, neosappanone, caesalpinia P, sappanchalcone, 3-deoxy sappanone and 7,3,4-trihydroxy-3-benzyl-2H-chromene (Batubara et al. 2010).

Sappan wood could be harvested 6-8 years after being planted. It is marketed as wood shavings or small pieces, with a more extended storage period allowing the wood color changes due to exposure to light, air, or contamination of microorganisms. In the natural habitat, it grows in clay and stony soil. At low and mid-elevations, it does not tolerate wet soils (Orwa et al. 2017), so that the sappan wood tends to be colorless despite having reached the age of harvest. Both these conditions resulted in the adulteration of sappanwood with the addition of synthetic or natural dyes to obtain quality color and better appearance.

Some dyes could be used as color enhancers in sappan wood are Sudan III, red textile dye, and red yeast rice. Sudan III is used as a dye in textile products, plastics and is often found illegally as an additive in foodstuffs. The dye is selected because it is cheap, produces a bright red, and stable in storage (Anibal et al. 2009). A commercial textile dye is widely used as a counterfeit on a product because it is easy to obtain, provides various choices of color, and cheap. Another dye that can be used as a counterfeit is red yeast rice. Red yeast rice is a fermented rice product with various color pigments of yellow, orange, and red. It is often applied as a dye in food, beverage, and personal care products. Substitution of dye on raw materials used to make food and herbal medicinal products can be a serious problem because it causes inconsistencies in the safety, efficacy, and stability of the product. Therefore, developing a rapid detection or authentication method of sappan wood raw material becomes critical as part of quality control.

Quality control of raw materials can be performed by a detection or authentication method using a multi-component approach. UV-Vis spectroscopy could be used to develop a detection or authentication method because it is low-cost, simple, easy in sample preparation, and a non-destructive technique (Aroca-Santos et al. 2015). UV-Vis spectrophotometer will produce large data as well as a non-selective signal. Thus, a chemometrics technique such as multivariate analysis for data interpretation is required (Boggia et al. 2012). The combination of UV-Vis spectroscopy and chemometrics has been widely reported to detect adulteration or authentication purposes rapidly. An example of a combination such as identification of adulteration in spices with sudan dyes (Anibal et al. 2014, 2009), identification of four Curcuma species (Rafi et al. 2018), detection of adulteration in chili powder (Rohaeti et al. 2019), and differentiation of *Mimosa scabrella* Bentham honeydew honey (Bergamo et al. 2020). In this study, we developed a rapid detection of adulteration (authentication) in sappan wood raw material with Sudan III, commercial textile dye, and red yeast rice by UV-Vis spectra of samples combined with chemometrics analysis. The developed method could be used for quality control of sappan wood raw materials to detect adulteration from the three dyes tested.

**MATERIALS AND METHODS**

**CHEMICALS AND MATERIALS**

We collected 13 sappan wood from different areas in Indonesia (Table 1). Methanol was used for solvent extraction (Merck, Darmstadt, Germany). Sudan III (95%) was obtained from Sigma-Aldrich (St. Louis, USA). Commercial textile dye was obtained from Mardha (Surabaya, Indonesia) and red yeast rice from Sari Utama Food Industry (Jakarta, Indonesia).
PREPARATION AND EXTRACTION OF SAMPLES

The samples were dried, ground, and sieved using an 80 mesh sieve. About 250 mg of each sappan wood powder was extracted with 10 mL of methanol for 30 min using an ultrasonicator (Branson, Danbury, USA). Sappan wood extracts were filtered and transferred to a 10 mL volumetric flask. There are about 13 sappan wood extracts from this extraction work.

MEASUREMENT OF UV-VIS SPECTRUM

A total of 250 µL of each sappan wood extract was added in 5 mL flask and also put red yeast rice solution with a final concentration of 10 µg/mL and 20 µg/mL and diluted with methanol. Also, the sappan wood extract solution was adulterated with commercial textile dye (final concentration of 0.5 µg/mL and 2 µg/mL) and sudan III (final concentration of 0.5 µg/mL and 2 µg/mL). About 78 adulterated samples was produced from the 13 sappan wood extracts adulterated with three dyes in two different concentration. UV-Vis spectra of all pure and adulterated sappan wood extracts solutions were measured using Hitachi U-2800 ultraviolet-visible spectrophotometer (Hitachi, Tokyo, Japan) in a 1 cm quartz cuvette. UV-Vis spectra were measured in the range of 200-800 nm in a quartz cuvette with an optical path of 1 cm and a scan speed of 200 nm/min.

CHEMOMETRICS ANALYSIS

The preprocessing signal was applied to the UV-Vis spectra of all samples by Savitzky-Golay smoothing to reduce noise. Principal component analysis (PCA) alone or followed by discriminant analysis (DA) was used for the detection of adulteration (authentication method) of sappan wood from three dyes tested using XLSTAT software version 2012 (Addinsoft, New York, USA).

RESULTS AND DISCUSSION

THE ULTRAVIOLET-VISIBLE SPECTRUM OF SAMPLES

Sappan wood samples were extracted using methanol because most chemical compounds could dissolve in this solvent, especially for secondary metabolites. The extraction method used was ultrasonication because it is simple, cheap, has good repeatability, produces maximum extract yield, and shorter extraction time (Chemat et al. 2011). UV-Vis spectra of sappan wood methanolic extract (Figure 1) were characterized by the maximum absorption in the ultraviolet region at a wavelength range of 250-300 nm, which is predicted to be the absorption of flavonoids. In the flavonoids, electron transition of $\pi \rightarrow \pi^*$ from the conjugated aromatic group $-\text{C}=\text{C}=-n \rightarrow \sigma^*$ from $-\text{OH}$ group and $n \rightarrow \pi^*$ from $-\text{C}=\text{O}$- occurred. Brazilin, known as a natural red dye, is the main flavonoid contained in sappan wood. Broadening in the UV-Vis spectra at 400-500 nm was observed. It can be caused by the electronic transition of conjugated $-\text{C}=\text{C}$ and $-\text{OH}$ groups on brazilein, which results from oxidation of the brazilin.

Mixing the sappan wood with all three types of dyes gave different spectrum patterns for each dye type (Figure 1). It is due to the differences in chemical components contained in each dye. Based on the results obtained, all three spectra of adulterated samples showed an increase in absorbance at 250-300 nm region, and peak broadening was observed in the visible region at a wavelength range of 400-600 nm. A mixture of sappan wood methanolic extracts with sudan III gave an increase in absorbance value in the range 500-550 nm because of the electron transition of $n \rightarrow \pi^*$ from conjugated $-\text{N}=\text{N}$- with $-\text{OH}$. Meanwhile, in the spectrum of sappan wood methanolic extracts adulterated with commercial textile dye, peak broadening and increasing absorbance in the range 500-600 nm region occurred. These phenomena are due to the absorbance of complementary colors from the color observed in textile dyes. Increases in the absorbance of sappan wood-red yeast rice adulteration in the wavelength range of 400-500 nm may occur due to the absorbance of the yellow pigment called ankaflavin and monascin, an orange pigment called rubropunctatin and monascorubrin and a red pigment called rubropunctamin contained in the red yeast rice (Wang & Lin 2007). Maximum absorbance of yellow, orange and red pigment is at 410 nm, 470 nm, and 500 nm, respectively (Huang et al. 2014; Mukherjee & Singh 2011; Zhong et al. 2015).

DETECTION OF SAPPAN WOOD ADULTERATION FROM THREE DYES

Authentication of sappan wood raw material is required to ensure that the right sappan wood raw material is used. In this work, we developed an authentication method of sappan wood as rapid detection of three dyes in sappan wood, i.e. sudan III, textile dye, and red yeast rice used as a color enhancer for sappan wood. We combined the UV-Vis spectra with chemometrics methods to develop a detection and authentication method for sappan wood from a low concentration of adulteration dyes.
Single and adulterated sappan wood spectrum data used for multivariate analysis were selected based on the region producing maximum absorbance to obtain the different wavelength range for each type of dye-adulterated sappan wood. Before analyzing the data, the data matrix used was pretreated using a smoothing analysis. Data smoothing was used to reduce noise by improving the signal-noise ratio without distorting the signal. This smoothing data was used to build an authentication model with PCA and PCA-DA.

PCA is an unsupervised pattern recognition method that is commonly used to reduce the number of variables and extract all information to find the combination of variables or factors that may explain the major tendency in a data set (Gad et al. 2013). The reduction is a new variable that is not correlated and so-called principal component (PC). Each PC is a linear combination of the observed variables. Initial data in the PCA are transformed into two matrices, and those are scores and loading. Scores illustrate sample information while loading focuses on the most influential variables in the sample separation between groups (Yudthavorasit et al. 2014). The two main components most commonly used in the PCA (PC-1 and PC-2), because both PCs have a large variety of data and information. The PCA of the sappan wood adulterated with sudan III produced two PC that could explain about 94.78% of the total variance (PC-1 = 65.55%, PC-2 = 29.23%) (Figure 2(a)). Sappan wood adulterated with commercial textile dye and red yeast rice has 91.96% (PC-1 = 59.96%, PC-2 = 35.00%) and 94.35% (PC-1 = 64.42%, PC-2 = 29.83%) of the total variance (Figure 2(b) and 2(c)), respectively. Based on the classification result by PCA, sappan wood and adulterated sappan wood could not be separated into their groups because the characteristics of the spectrum are similar. Therefore, the samples were further analyzed using discriminant analysis (DA).

Discriminant analysis is a multivariate analysis included in supervised pattern recognition. DA is used to separate multiple sets of data clustered by forming a discriminant function (FD). The data used for the data discriminant analysis are principal component data resulted from the reduction by PCA. The principal components resulting in eigenvalues > 1 are used for DA based on criteria established by Kaiser (Brereton 2003). Principal components used for discriminant analysis of sappan wood adulterated with sudan III, commercial textile dye, and red yeast rice were four principal components.

| No | Sampel Code | Sources (sub district, regency, province) |
|----|-------------|------------------------------------------|
| 1  | CS-1        | Pathuk, Gunung Kidul, DIY                |
| 2  | CS-2        | Malino, Gowa, South Sulawesi            |
| 3  | CS-3        | Enrekang, South Sulawesi                |
| 4  | CS-4        | Tanete, Selayar, South Sulawesi         |
| 5  | CS-5        | Serakakapi, Dompu, West Nusa Tenggara  |
| 6  | CS-6        | Donggo, Bima, West Nusa Tenggara       |
| 7  | CS-7        | Kananta, Dompu, West Nusa Tenggara     |
| 8  | CS-8        | Legara, Dompu, West Nusa Tenggara      |
| 9  | CS-9        | Sakuru, Bima, West Nusa Tenggara       |
| 10 | CS-10       | Mangkang, Kendal, Central Java          |
| 11 | CS-11       | Jatiyoso, Karanganyar, Central Java     |
| 12 | CS-12       | Allu, Bulukumba, South Sulawesi        |
| 13 | CS-13       | Kelara, Jeneponto, South Sulawesi      |
DA plot produced two initial DF values in the samples adulterated with each dye. Samples adulterated with sudan III resulted in two initial DF values with the variance of $DF-1 = 92.00\%$ and $DF-2 = 8.00\%$ (Figure 3(a)), samples adulterated with commercial textile dye led to FD values with the variance of $DF-1 = 91.44\%$ and $DF-2 = 8.56\%$ (Figure 3(b)). Samples adulterated with red yeast rice resulted in initial DF values with the variance of $DF-1 = 93.06\%$ and $DF-2 = 6.94\%$ (Figure 3(c)). Based on DA, samples adulterated with three dyes were separated into their group. This result indicates that DA could give more clear separation compared to PCA.

The evaluation of the model predictability was performed using leave-one-out cross-validation (LOOCV). The technique requires only a single training data obtained by removing one sample and the rest are used to build the model. The removed sample was then tested on the model, and the membership of samples can be predicted. This procedure was repeated until all objects have been removed (Brereton 2003). The cross-validation result for

![Figure 1](image1.png)

**FIGURE 1.** Representation of absorption spectras of sappanwood (SW), (SW-S) sappanwood adulterated with Sudan III, (SW-P) sappanwood adulterated with commercial textile dyes, and (SW-A) sappanwood adulterated with red yeast rice

![Figure 2](image2.png)

**FIGURE 2.** PCA plot scores of (a) Sappanwood and sappanwood adulterated with sudan III, (b) sappanwood and sappanwood adulterated with commercial textile dyes, (c) sappanwood and sappanwood adulterated with red yeast rice
the authentication of sappanwood from sudan III dye, commercial textile dye, and red yeast rice was 94.12%, 94.12%, and 92.16%, respectively. This result indicates that the developed model has good predictive power although not all samples can be grouped according to the cross-validation of DF. This is due to variations in the dye’s concentration being very low so that the characteristics of the resulted UV-Vis spectra are similar.

FIGURE 3. DA plot of (a) Sappanwood and sappanwood adulterated with sudan III, (b) sappanwood and sappanwood adulterated with commercial textile dyes, (c) sappanwood and sappanwood adulterated with red yeast rice

CONCLUSIONS
In this work, the authentication method of sappanwood from the counterfeit of three dyes was developed. The UV-Vis spectra and PCA-DA represent a simple and effective method for authentication sappanwood from sudan III dye, commercial textile dye, and red yeast rice. The combination of both methods was successfully classified pure sappan wood and adulterated sappan wood with the three dyes.

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*Corresponding author; email: ime@apps.ipb.ac.id*