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A Simple Photometer and Chemometrics Analysis for Quality Control of Sambiloto (*Andrographis Paniculata*) Raw Material

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ABSTRACT

In this paper, we described the use of a light emitting diode (LED)-based photometer and chemometric analysis for quality control of king of bitter or *sambiloto* (*Andrographis paniculata*) raw material. The quality of medicinal plants was determined by their chemical composition. The quantities of chemical components in medicinal plants can be assessed using spectroscopic technique. We used an in-house photometer to generate spectra of *sambiloto*. The spectra were analyzed by chemometric methods, *i.e.* principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA), with the aim of herbal quality classification based on the harvesting time. From the results obtained, based on thin layer chromatography analysis, *sambiloto* with different collection times (1, 2, and 3 months) contained different amounts of active compounds. Evaluation of *sambiloto*, using its spectra and chemometric analysis has successfully differentiated its quality based on harvesting time. PCA with the first two PC's (PC-1 = 60% and PC-2 = 35%) was able to differentiate according to the harvesting time of *sambiloto*. Three models were obtained by PLS-DA and could be used to predict unknown sample of *sambiloto* according to the harvesting time.

Key word: *sambiloto*, *Andrograpis paniculata*, photometer, chemometrics

INTRODUCTION

There is increasing trend globally for the use of bio-resources products, in particular, herbal medicines for diseases treatment or prevention. In Indonesia, the use of herbal medicines or *jamu* has been practiced throughout history. Currently, there are almost 5,310 jamu products available on the market. These products are derived from more than 500 medicinal plants [1].

Despite the long history of *jamu*, there are some aspects or supporting areas in the herbal medicines system which is not being developed. For example, the raw materials supplies of some medicinal plants are collected from wild populations and some are from cultivation. Farmers as the main suppliers of the medicinal plant material obtain small profits from its economic value. Farmers do not earn enough income, often just owe the low fractions of price paid by the final consumer. It appears that the medicinal plant raw materials are sold without any prior quality sorting process and paid according to weight, regardless the

quality [2]. Therefore, introducing raw material analysis and classification procedure for the farmers will alleviate the problem of payment solely by weight, and the implementation of such measures will eventually increase the farmer's revenue. Therefore, it is important to develop simple quality control tool to be applied by the farmers.

Commonly, the quality control of medicinal plant from raw materials is performed using chromatographic techniques, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), and thin layer chromatography (TLC) [3-5]. These techniques will generate fingerprint chromatograms, which characterize materials by showing specific chemical compounds contained in the extracts [6]. Spectroscopic techniques also can be employed to check quality of herbal raw materials [4]; and offer some advantages compare to chromatographic techniques, such as simpler and easier to use. Several studies have been reported using spectroscopic methods for determining quality control of medicinal plants [7-9]. The instruments used are typically FTIR or UV-Vis spectrophotometer. However, these tools are costly and not easy to operate in the field. Therefore, in this study, we build an in-house portable photometer using electronic parts that were locally available. The photometer utilizes light emitting diodes (LED) as radiation sources, light dependent resistors (LDR) as transducers, and a voltmeter as readout and display. This photometer has been used for classification of cancerous and healthy skin tissue [10].

In this study, the ability of the photometer to check the optical properties of samples which obtained from sambiloto (*Andrographis paniculata*) of different quality levels (harvesting time) was tested. *Sambiloto* is well known for its anticancer, antidiabetic, antipyretic, and antibacterial activity [11]. The data obtained using from the photometer was then evaluated using pattern recognition techniques, namely Principle Component Analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA). PCA was used to perform classification pattern according to the harvesting time of *sambiloto*, whereas PLS-DA was used to build a predictive model that can be utilized for the classification of unknown samples.

EXPERIMENT

Chemicals

All chemicals and solvents were analytical grade and obtained from Merck (Darmstadt, Germany), they were used without further treatment. Dried material samples of *sambiloto* were cultivated and collected from Conservation and Cultivation Unit of Tropical Biopharmaca Research Center, Bogor Agricultural University, Indonesia. Samples were collected at different harvesting times (1 month (Q1), 2 months (Q2) and 3 months (Q3) after planting) that represent different chemical quality levels.

Procedure

TLC Analysis

One gram of dried sample powder was macerated using 10 mL ethanol for 3 hours. After filtering the mixture, solvent from filtrate was removed using rotary evaporator. A total of 0.1 g of the extract was dissolved in 5 mL of ethanol. An aliquot was spotted onto silica gel F_{254} plates using a Linomat 5 (Camag, Muttez, Switzerland). The TLC plate was developed using a chloroform-methanol eluent with a ratio of 9:1. Finally, detection of spot was carried out using a Reprostar 3 TLC documentation (Camag, Muttez, Switzerland) with 245 nm UV light. The image was then further processed using ImageJ software [12].

Measurement of the Optical Properties of the Samples

The optical properties of the samples were measured using a photometer (Figure 1) as employed by Zain *et al*. [10].. Firstly, 400 mg of dried sample powder was formed into a solid pellet. Before the sample analysis, the intensity of the photometer was adjusted using a standard white color. The sample was placed in the bottom of the optical probe, and measurement results (in V) appeared immediately on the voltmeter display. The photometer measured three types of samples (Q1, Q2, and Q3), with ten replicates for each sample. In order to extend the variability of optical properties of the samples, measurements were also carried out using different LED sources and different filters. The photometer used UV LED, blue LED, red LED, and 17 different filters. There was a total of 51 measurements for each sample carried out by the photometer.

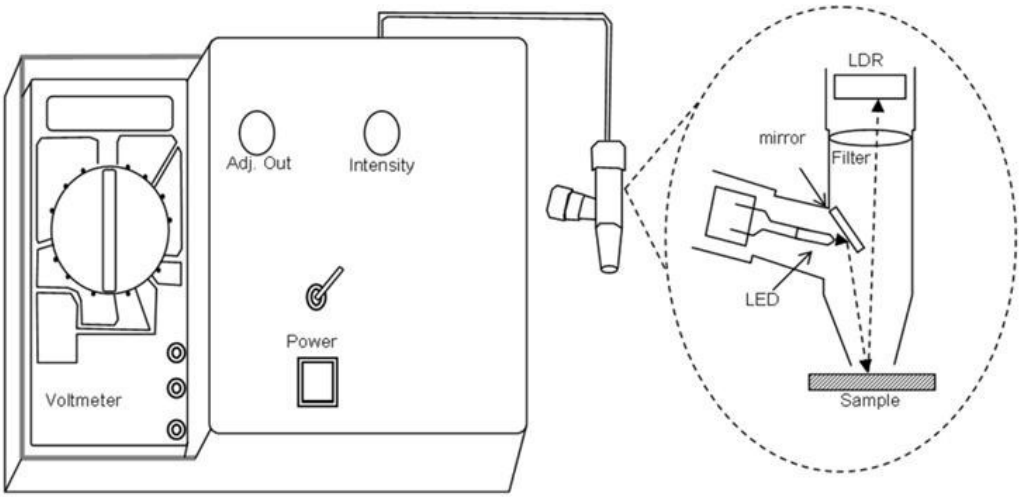

Figure 1. Schematic diagram of in-house photometer.

Chemometric Analysis

The data collected was tabulated as shown in the Supplementary information. In order to explore the variation patterns of material quality based on its chemical composition, PCA analysis was performed using Unscrambler 9.7 software [13]. A model for the following prediction was generated by PLSDA analysis, using the same software.

RESULT AND DISCUSSION

This research consisted of three experimental parts. The first part was the analysis of material quality based on its chemical composition by TLC analysis. It was carried out to confirm that different harvesting times resulted in different chemical compositions, and therefore different harvesting times represented different levels of sample quality. The second part was generation of optical data curves of the samples, in order to demonstrate that the photometer was able to produce characteristic values for the samples. The last part was data evaluation using chemometric techniques. It was carried out to confirm that the experimental data, combined with chemometric analysis, was able to classify the samples based on their level of quality.

Chemical Profiling using TLC

The TLC chromatogram shows the variability of the chemical profiles of the samples in Figure 3. All of samples resulted in similar TLC profiles (number of spots), but with different spot intensities. From the TLC chromatogram, spot intensities were correlated with compound concentrations. Image transformation from each chromatogram was performed using ImageJ software to produce more detailed chemical quality comparison between samples. One spot in the chromatogram, in R_f value of 0.58, was characterized as *andrographolide* (Figure 2). *Andrographolide* is one of the most abundant active compounds found in *sambiloto*, and usually, it is used as a marker compound. This compound is a diterpene lactone and caused the bitter taste of *sambiloto* [14,15]. The densitogram showed that the Q3 sample has more *andrographolide* compared to the others. A study conducted by Pandey and Mandal [16] showed that the highest concentrations of *andrographolide* were found in *sambiloto* at the age of 3-4 months after planting when 50% of the crop starts to flower.

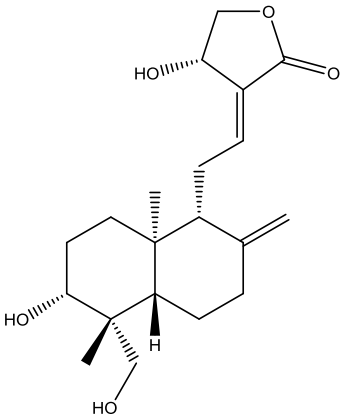

Figure 2. The chemical structure of *andrographolide*

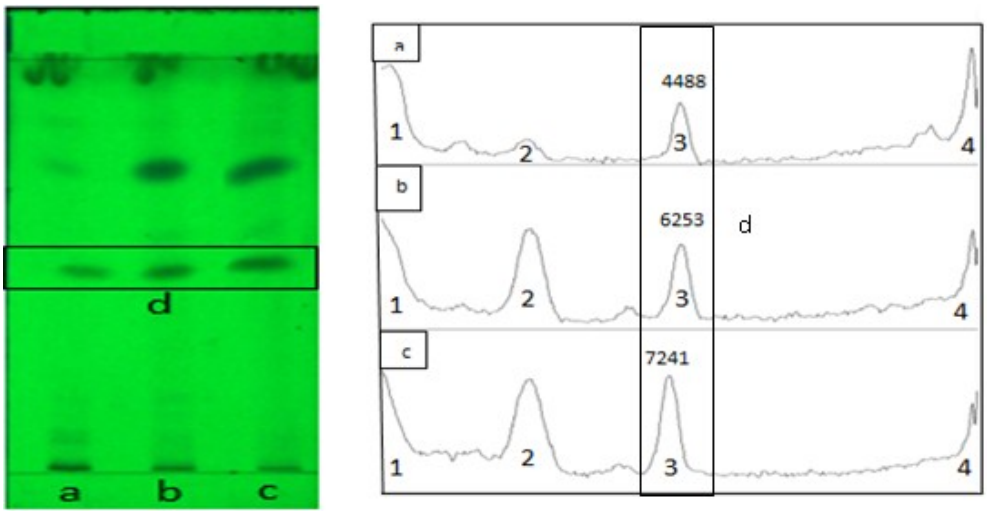

Figure 3. TLC analysis for dried sample of *A. paniculata*, (a) Q1; (b) Q2; (c) Q3 and (d) spot and peak for *andrographolide*.

Classification of *sambiloto* by PCA

Thirty samples of dried material of *sambiloto* were measured using the photometer. Three LED and 17 filters were used to measure the optical properties of each sample. In total, there were 30 x 51 matrix data results in this experiment. Figure 4 showed an example of the curve plot of the data. Pattern of the curve seems similar for the different harvesting times. The differences were shown from the absolute value of the measurements. Singh *et al*. [4] explained that samples from the same species or family would have same spectral pattern. The differences will be seen from the peak intensity from each sample. Therefore, pattern recognition like PCA is needed to depict the differences between samples.

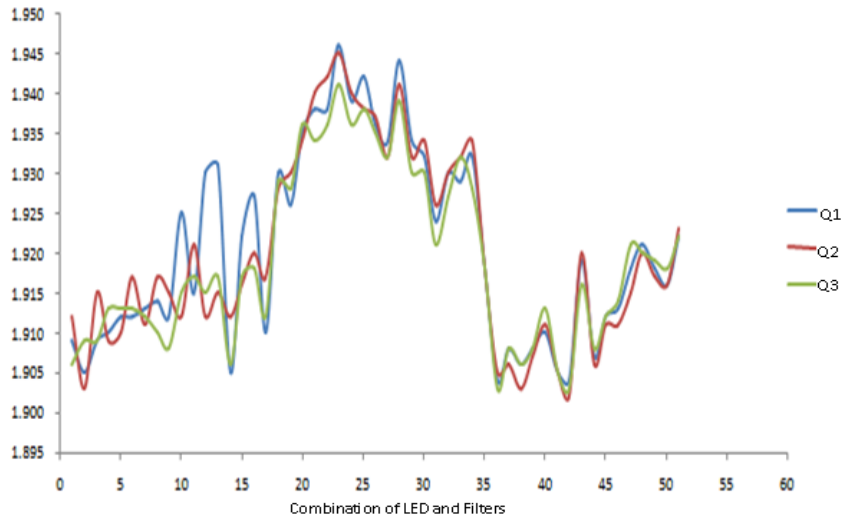

Figure 4. Data curve from photometer measurement. In x axis, the numbers 1-17 represent data measurement using UV LED and 17 filters, 18-34 was for blue LED and 17 filters, and 35-51 was for red LED and 17 filters.

PCA is an unsupervised pattern recognition technique, which can summarize multivariate variation. PCA was employed to assess the discrimination ability of photometer measurement. By using PCA, a large set of data further reduced to a new primary variables or principle components (PCs), which can represent the structure and variation in the data [17]. Figure 5 describes PCA results from data. The first and second PCs explained 96% of the total variability in original data. Hence, the first two PCs reduce the multivariate data into a two-dimensional dataset to cluster the samples. The scores plot of these first two principal components showed a clear differentiation of samples based on their chemical composition. The score points of the samples were scattered to become three groups which were marked as groups Q1-Q3, according to harvesting time of the *sambiloto*.

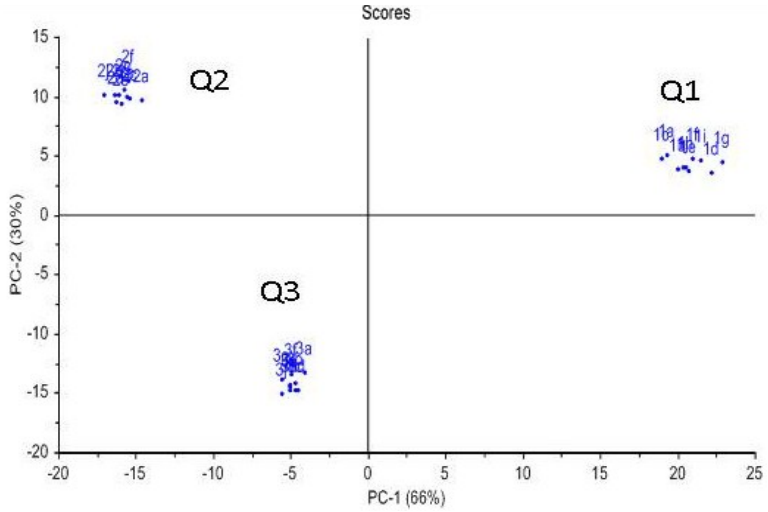

Figure 5. Plot score of the first two principal components, PC1 represents 66% of the variation of the data and PC2 represents 30% of the variation of the data.

Quality Prediction Model by PLS-DA

For identification of the quality of unknown samples, a prediction model of the data was generated using PLS-DA. PLS-DA is a supervised recognition technique and used for classification of the priority given classes of objects. Furthermore, PLS-DA is a regression extension of PCA, which develops a calibration model from the two data matrices. Matrix X is the predictor matrix that contains the original data derived from sample measurements and matrix Y is the response matrix which is usually consist of dummy variables from the class of the objects (1 represents "belongs to the class" and 0 represents "not"). Then, PLSDA calculates scores of the X and Y matrix and uses them to create regression models between these values [8].

In this analysis, the whole data set was divided into two groups, calibration, and testing groups. The calibration set was used for developing a prediction model, while the testing set was utilized for the evaluation of the prediction model performance. From the total of 30 datasets available, 24 datasets were used in calibration (8 from each sample Q1, Q2, Q3) and six datasets were used for model validation (2 from each sample Q1, Q2, Q3). PLSDA generated three calibration models from the samples. Table 1 showed the PLS-DA statistical performance for each model. Parameters related to the criteria of goodness of fit for these models are R2, root mean square error of calibration (RMSEC), and root means square error of prediction (RMSEP). All the models provide correlation coefficient near unity (>0.99) with the error less than 5%, which means that the models resulted in a good fit and were reliable for utilization for unknown sample prediction [18]. The validation results of the quality prediction model for *sambiloto* samples are shown in Figure 6. The prediction models calculated response values near 1 for each of type of samples. It means that the models were able to classify all testing dataset samples to their appropriate sample types.

Table 1 The goodness of fit parameters from PLS-DA calibration and prediction model

Model	Calibration		Prediction	
	R^2	RMSEC	R^2	RMSEP
Q1	0.9974	0.0238	0.9972	0.0269
Q2	0.9972	0.0248	0.9970	0.0247
Q3	0.9975	0.0235	0.9965	0.0263

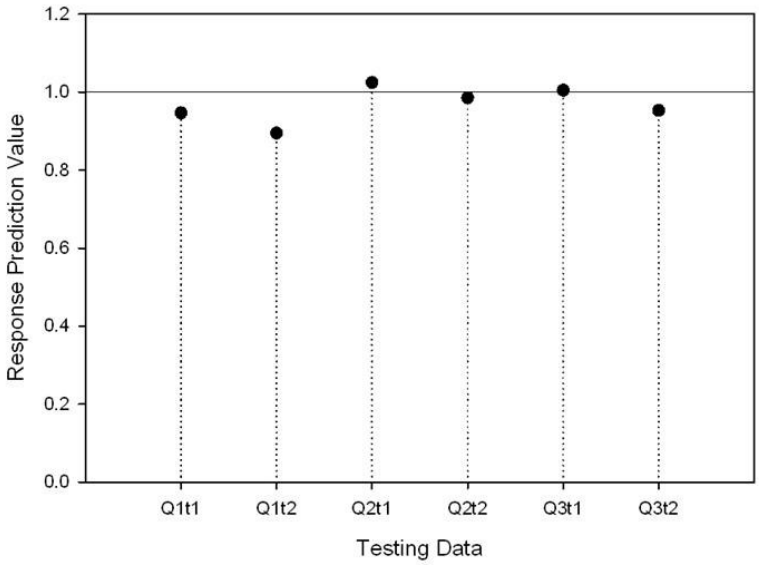

Figure 6. Response values of testing dataset (Reference value is 1).

CONCLUSION

The chemical composition and quality of medicinal plant raw materials were influenced by planting age of the plants. The research demonstrated that in-house photometer, combined with pattern recognition techniques, successfully used to classify *sambiloto* raw materials with different chemical quality levels.

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