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Determination of classification model and phytochemical content of methanol extract of *Andrographis paniculata* leaves from different altitude regions using near infrared spectroscopy and chemometric

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Abstract**.**
Context: *Andrographis paniculata* is one of the plants from Acanthaceae family that usually used as a traditional medicine in Indonesia. **Aims**: This research aims to explore the capability of Near Infrared (NIR) spectroscopy and chemometrics to classify the *A. paniculata* leaves extracts which were planted in different altitude regions and determine phytochemical content of the leaves extracts. **Settings and Design**: A model for determining calssification of plant extracts was formed using NIR spectra and chemometric then the model was applied on real samples. Phytochemical content of the leaves extracts were determined by partial least square model. **Methods and Material**: Samples were extracted by methanol then scanning the extract with NIR spectrophotometer at (780 to 2 500) nm. NIR spectra was analyzed with The Unscrambler software to formed classification model. **Statistical analysis used**: The samples was classified using LDA (Linear Discriminant Analysis), SIMCA (Soft Independent Modelling of Class Analogies), SVM (Support Vector Machines) and CA (Cluster Analysis). **Results**: LDA, SIMCA and SVM has formed an accuracy value of 100 % while the CA classification model produces two clusters, cluster A consist of extracts from Malang and Madura region, Indonesia, and cluster B consist of extracts from Jember region, Indonesia. *A. paniculata* from Malang region, Indonesia has the highest concentration of phenolic, flavonoid and alkaloid that were 164.3 mg GAE g^{-1}; 34.5 mg QE g^{-1}; 66.2 mg CE g^{-1}. **Conclusions**: This study showed that NIR spectroscopy coupled with chemometric could be used for classified the *A. paniculata* which is planted in different altitude regions.

Keywords: Determine phytochemical, sambiloto, traditional medicine

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Introduction

Andrographis paniculata Burm.f. known as Sambiloto is one of the traditional medicinal plants in Indonesia. Leaves of *A. paniculata* uses for treatment of fever, dysentery, tuberculosis, and antidiabetic[1]. Near Infrared (NIR) spectroscopic technology is a non–destructive method, analyzing at high speed, not cause pollution, uses simple sample preparations and does not require chemicals. NIR spectroscopy uses electromagnetic waves with a wavelength of 780 nm to 2 500 nm[2]. Chemometric technique utilize the characteristic of NIR absorption from each molecule to classify samples or to make a calibration model. The combination of NIR spectra and chemometric can be used to classify plants, even though the composition of their chemical compounds still not known exactly[3]. This research will determine the classification model and phytochemical content of *A. paniculata* from three regions with different altitude Madura (low altitude), Jember (middle altitute) and Malang (high altitude) using combination of NIR and chemometric method based on last own research about determination of the flavonoid, phenolic, and alkaloid content in medicinal plants[4]. Some analytical methods commonly used to determine the content of active compounds in plants, including the HPLC method, fluorimetry method, gas chromatography method, and uv vis spectroscopy method[5],[6],[7],[8]. These methods require a long analysis phase and a long analysis time.

This study developed an alternative method using NIR spectroscopy. NIR spectroscopy technology is a technology that can replace conventional methods and has been successfully applied to agricultural, pharmaceutical, petrochemical and environmental products analysis[9]. NIR spectroscopy technology was developed as one method that is non–destructive, can analyze at high speed, does not cause pollution, simple sample preparations and do not require chemicals. NIR spectroscopy uses electromagnetic waves with wave numbers of 12 800 cm^{-1} to 4 000 cm^{-1} or wavelengths of 780 nm to 2 500 nm[10]. Due to the complexity of the spectra, processing of NIR spectrum data was performed using multivariate statistical methods. The benefit of the multivariate statistical method is its ability to extract the spectra information needed from the infrared spectra and use that spectra information for qualitative and quantitative applications[11].

Materials and Methods

Sample Preparation

Andrographis paniculata leaves collected from three regions (Madura, Jember, and Malang) Indonesia. In each district sample colected from three sub–regions. Sampling based on consideration of the researchers who consider the desired elements already in taken sample.

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Extraction

Leaves were air–dried then put it on the microwave and powdered. Amount 50 g powder was added by 150 m methanol 96 % and extracted using ultrasonification method for 1 h then macerated for 24 h. Filter the extract and concentrated on a rotary evaporator at 50 °C. Concentrated extract 50 mg dried with aerosil, smooth up and stored in vial.

NIR scanning and determination classification model

Power on the NIR Instrument and wait for 30 min. Open Brimrose software and put the sample on sample plate equally and slightly pressed. One sample was scanned five times and shoots three times on each scan, repeat this step for all samples. Scanning spectra were observed with Prospect software. All collected spectra data was prosseced with The Unscrambler × 10.2 (Camo software) to form classification models. Classification model (qualitative) used in this research were Partial Least Square (PLS), Linear Discriminant Analysis (LDA), Support Vector Machines (SVM), Soft Independent Modelling of Class Analogies (SIMCA), and Cluster Analysis (CA).

Determination of phytochemical content

Method for determination of phytochemical content based on last research[4]. First, determine phytochemical content of test sets sample using with UV–Vis Spectrophotometer as reference method.

Amount phenolic 400 µL extract added by 400 µL Folin–Ciocalteu in aquadest (1 : 10 v/v) and rested for 6 min then added 3 200 µL Na_2CO_3 (75 g L^{-1} aquadest). This mixture rested at room temperature for 30 min and measured the absorbance at a wavelength 628 nm. Using standard Gallic Acid.

Amount flavonoid: 0.5 mL extract solved in 3 mL ethanol, add 0.2 mL $AlCl_3$ 10 %, add 0.2 mL potassium acetate 1 M, and add 10 mL aquadest until the mark. This mixture rested at room temperature for 30 min and measured the absorbance at a wavelength 432 nm. Using standard Quercetin.

Amount alkaloid 200 µg mL^{-1} extract added by 2 mL phosphate buffer pH 4.7 and 2 mL BCG (Bromocresol Green) then extracted with 3 mL chloroform three times using vortex. Use the chloroform phase and put it on 10 mL volumetric flask then added chloroform until the mark. This mixture measured the absorbance at a wavelength 432 nm. Using standard Caffein.

Then the data of phytochemical content used for revalidating the calibration model (PLS model). After the model was valid, it can be used to determine the phytochemical content of samples.

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Statistic analysis

The samples was classified using LDA (Linear Discriminant Analysis), SIMCA (Soft Independent Modelling of Class Analogies), SVM (Support Vector Machines) and CA (Cluster Analysis)

Result

Leaves of *A. paniculata* from different altitude extracted and percentage yields after extraction are shown in Table 1. *Andrographis paniculata* leaves from Lawang Sub–region has the highest % yield compared to the others. Phytochemical content from all extract (phenolic, flavonoid and alkaloid) were determined by PLS model and the result were shown in Table 2. Classification models (LDA, SIMCA, SVM) of *A. paniculata* leaves from three different altitude (low, medium, and high) have formed an accuracy value of 100 % and CA model produces two clusters, that were cluster A consist of extracts from Malang and Madura region Indonesia, and cluster B consist of extracts from Jember region Indonesia.

Table 1. Altitude of each sub–regions and percentage yield of dry methanolic extract per 50 g of *A. paniculata* leaves powder

Regions	Sub-regions	Altitude (m asl)	% yield after extraction
Madura (Low Altitude)	Kamal	7	4.24
	Lenteng	22	3.45
	Pakong	44	4.31
Jember (Medium Altitude)	Jubung	59	5.39
	Sumberbaru	73	5.78
	Sumbersari	99	5.57
Malang (High Altitude)	Lawang	512	10.2
	Singosari	559	3.81
	Batu	1 040	4.49

Table 2. Determination of phytochemical content using PLS model

Regions	Sub–Regions	Phenolic (mg GAE g^{-1} extract ± SD)	Flavonoid (mg QE g^{-1} extract ± SD)	Alkaloid (mg CE g^{-1} extract ± SD)
Madura	Kamal	53.3 ± 0.47	19.1 ± 0.28	24.2 ± 0.06
	Lenteng	54.9 ± 0.28	19.2 ± 0.34	35.4 ± 0.88
	Pakong	55.4 ± 0.34	19.4 ± 0.47	47.2 ± 1.85
Mean		**54.5**	**19.2**	**35.6**
Jember	Jubung	85.9 ± 0.61	28.7 ± 0.74	57.8 ± 1.58
	Sumberbaru	89.9 ± 0.74	28.8 ± 0.46	59.1 ± 1.15
	Sumbersari	90.8 ± 0.88	29.1 ± 0.61	65.2 ± 1.36
Mean		**88.9**	**28.9**	**60.7**

(Continued on next page)

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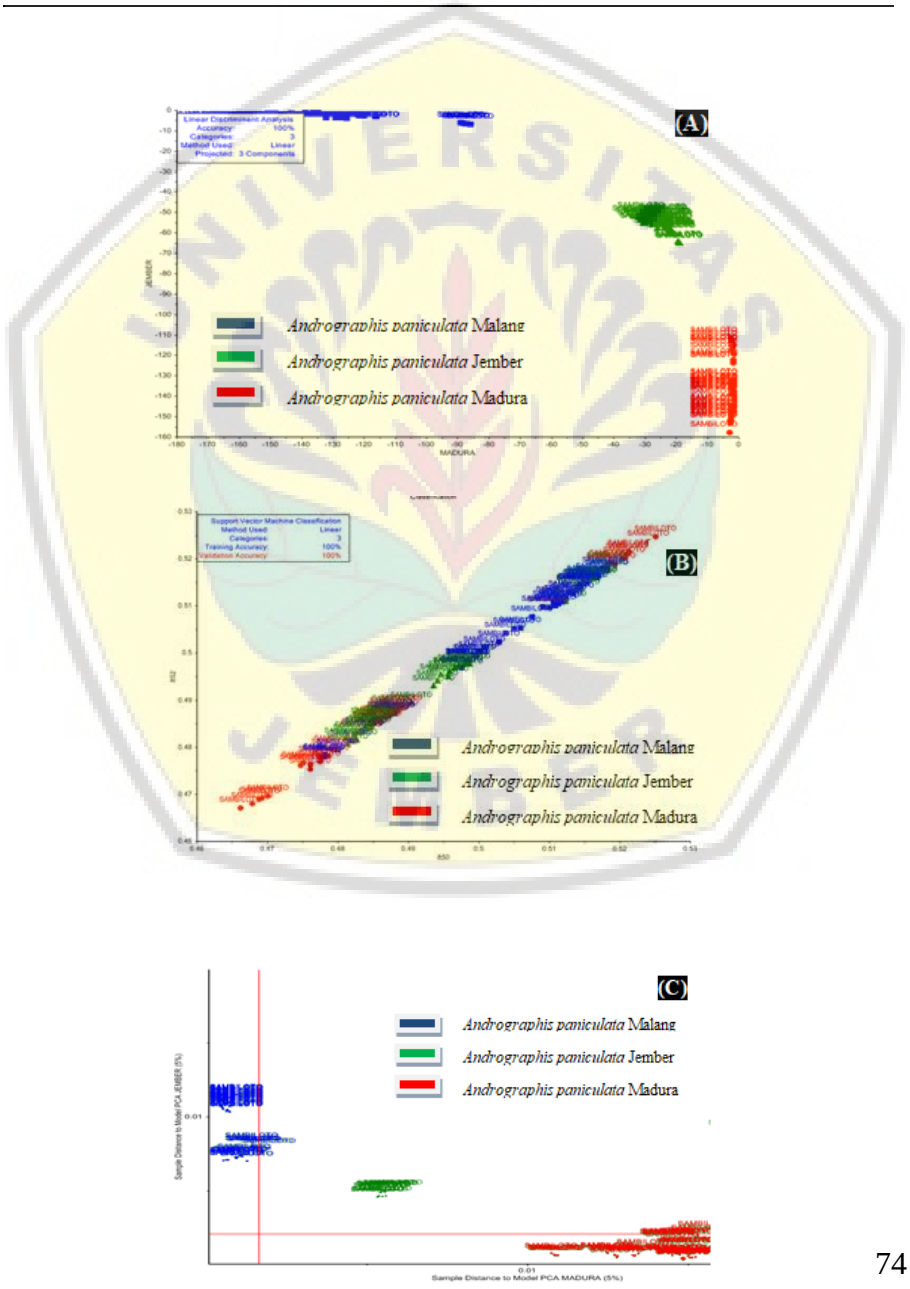
Table 2. Continued

Regions	Sub–Regions	Phenolic (mg GAE g^{-1} extract ± SD)	Flavonoid (mg QE g^{-1} extract ± SD)	Alkaloid (mg CE g^{-1} extract ± SD)
	Lawang	164.1 ± 2.77	34.2 ± 0.25	66.2 ± 1.02
Malang	Singosari	164.2 ± 3.35	34.6 ± 0.23	66.2 ± 1.00
	Batu	164.5 ± 7.92	34.6 ± 0.23	66.3 ± 0.65
Mean		**164.3**	**34.5**	**66.2**



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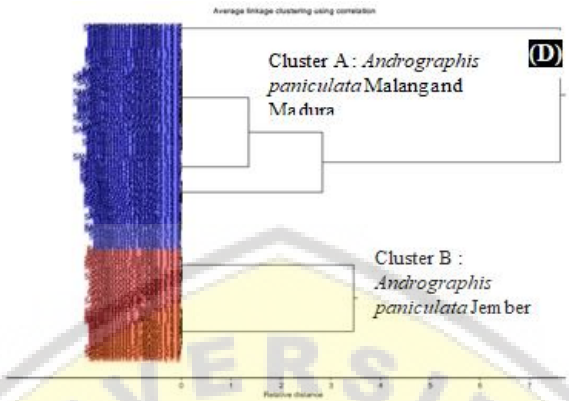

Figure 1. Classification models result (A) LDA ; (B) SVM ; (C) SIMCA ; (D) CA

Discussion

Analysis using NIR spectroscopy will produce a spectra. Molecular movements from polyatomic molecules will form a absorption bands that are specific to each molecule. This makes IR spectroscopy a very useful for qualitative analysis method, but it is difficult to do due to the similarity response of each spectrum. Quantitative analysis of IR spectra is also very difficult because of the overlapping absorption spectrum of the molecules in the sample[12]. Chemometric is a solution for all types of chemical problems with application of mathematical methods, to design or choose optimal procedures and experiments, and to provide maximum information with chemical data analysis[13]. Chemometric can be used to analyze multivariate data. Multivariate data is a data that has many variables and from each of these variables can be correlated with each other. The advantage of multivariate analysis is that more information will be obtained because multivariate analysis considers many variables simultaneously, another advantage is that multivariate analysis can be more selective in a measurement, and can detect false samples[14].

Multivariate analysis can be used to make a classification model of an object. Classification models used in this research are LDA, SVM and SIMCA. Accuracy value of each model can be used to determine the ability of the model in grouping samples into three categories that have been formed (Madura, Jember, and Malang). The results of the accuracy value in this research is 100 %, which means that the model can correctly classify the nine samples from various sub–regions.

CA model is based on the discrimination ability to measures character similarity of the samples. The results of CA model showed that the lower the distance, the lower the inequality or high similarity. CA model result in this research divided into two Clusters, namely Cluster A and Cluster B, that were cluster A consist of extracts

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from Malang and Madura region, and cluster B consist of extracts from Jember region.

PLS is one of the multivariate calibration methods used in chemometric. PLS is able to analyze a large amount of data, has a high level of collinearity, reduces data dimensions, looks for the most relevant factors in predicting and interpreting data[15]. Result of revalidating the PLS model for phenolic, flavonoid, and alkaloid obtained R^2 value 0.97 ; 0.98 ; 0.99. If the value of R^2 is 0.8 to 0.95 then the analysis can be stated as good and if R^2 at the interval of 0.7 to 0.8 then the analysis can be stated to be quite good. So this study can concluded that the model has good reliability to be implemented in our research.

The determination of phytochemical content result *A. paniculata* leaves in methanol extract (Table 2) showed that the highest total phenolic, flavonoid and alkaloid were produced in high altitude, Malang region, Batu sub–region (1 040 m asl) that were 164.5 mg GAE g^{-1} ± 7.92 mg GAE g^{-1} ± SD; 34.6 mg QE g^{-1} ± 0.23 mg QE g^{-1} ± SD; 66.3 mg CE g^{-1} ± 0.65 mg CE g^{-1} ± SD. While *A. paniculata* leaves in methanol extract from Malang region has the highest mean concentration of phenolic, flavonoid, and alkaloid that were 164.3 mg GAE g^{-1}; 34.5 mg QE g^{-1}; 66.2 mgCE g^{-1}. This is caused by several factors that can affect, e.g. temperature, moisture, rainfall and the height of the place to grow. Malang region is high altitude compared to Madura region. More high the altitude, plants will receive more environmental stress, for example highland or mountainous areas have higher rainfall with lower temperatures. Therefore in the highlands or land mountains are relatively more fertile.

Conclusion

This study showed that NIR spectroscopy coupled with chemometric could be used for classified the *Andrographis paniculata* which is planted in different altitude regions.

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