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Identification of Giant Calotrope (Calotropis Gigantea) in Alue Naga and Ulee Lheu Coast Using Combination Method of Infrared Spectroscopy and Principal Component Analysis

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ABSTRACT
This research aimed to identify giant calotrope leaf (*Calotropis gigantea*) originated from Alue Naga and Ulee Lheu coast using combination method of Fourier Transform Infrared (FTIR) spectroscopy and Principal Component Analysis (PCA). FTIR spectra analyzed with PCA to identify the effect of the treatments against the sample which are dry leaf maceration, fresh leaf maceration, and plain fresh leaf against similarity and difference pattern between giant colatrope leaves from different areas, namely Alue Naga coast and Ulee Lheu coast. The result showed that samples were grouped into 4 groups, namely Alue Naga and Ulee Lheu macerated dry leaf group, Alue Naga and Ulee Lheu macerated fresh leaf group, Alue Naga fresh leaf group, and Ulee Lheu fresh leaf group with a variance of 97%. It can be concluded that giant calotrope fresh leaf can be identified according to its location with FTIR-PCA method, but the macerated leaf cannot.

Keywords: *Giant calotrope leaf, FTIR spectroscopy, Chemometrics, PCA*

1. INTRODUCTION

Giant calotrope (*Calotropis gigantea*) is a plant that is rich in secondary metabolites. Based on GC-MS analysis, there are 46 active compounds in giant calotrope (24 in the leaf and 22 in the sap) [1]. Giant calotrope fruit contains cardenolide type compound [2], and giant calotrope flower contains alkaloid, tannin, phenol, flavonoid, sterol, anthraquinone, protein, and quinone [3] [4] ,which can be used as medicine, for example, dysentery medicine [5]. Giant calotrope often used as traditional medicine by Asians [2] [5]. Recent report stated that this plant also potentially used as an anti-bacterial in the prevention of tooth decay bacteria, such as *Streptococcus* and *Lactobacillus sp.*[6].

Secondary metabolites of plants is often affected by its geographical properties. Plants that grow in high salinity site are predicted to have higher secondary metabolites such as phenol and terpene [7]. Giant calotrope is a plant that could grow in many environments, from plateau to coast, and soils with high lime content. The coast is an area with many giant calotrope plants grow and this research will carry out a quick and accurate analysis on the difference of giant calotrope locations.

Identifications of organic samples have been introduced by several spectroscopy methods such as Uv-Vis for protein [8] and fish meat [9], laser for food [10] and coal [11], and FTIR for char [12] and bone [13] analysis. Specifically for FTIR, it is a device to identify types of functional group in a compound [14]. The wavelength of light absorbed is a characteristic of the functional group of the chemical compound, it is because the infrared spectra pattern produced is very complex. Therefore, chemometric is needed to identify the sample spectra pattern produced by chemical instrument to collect more information. Chemometric analysis of chemical instrumental data have been widely used for classifying of retention index similarity [15], measuring of binding affinity between protein and fatty acids [16] [17], distinguishing of gelatin sourced from pork or bovine [18], grouping of dead time from different methods [19], differentiating of cattle bond from variety of cow [20] and so on.

As FTIR spectroscopy combined with a suitable multivariate statistical method, then it could become a connecting technique with specific spectral marker [21]. The statistical method that can be used to identify the pattern difference between each FTIR spectra from several samples of the same type is *Principal Component Analysis* (PCA). PCA is a statistical method that is used to reduce data from a complex data set [22].

The combination method of FTIR and chemometric had been used to identify and authenticate various samples. According to report by Sanchez [23], FTIR spectra that were analyzed with chemometric showed a good, powerful, and simple procedure in identifying coffee leaves that were collected from various climates. Giannetti [24] also reported that PCA can identify volatile compounds from the drying process in wheat production proses. Then, Wadood [25] reported that NIR spectroscopy combined with chemometrics (PCA, LDA) showed potential to determine the geographical difference, production year, and genotypic classification of wheat. Based on the reports above, this research will carry out identification on the difference of the sample preparation method and the difference in giant calotrope sample locations.

2. METHODOLOGY

2.1. Tools and Materials

Materials used were giant calotrope leaves and ethanol 70%. Giant calotrope leaves were obtained from the coast area of Alue Naga, Syiah Kuala Sub-district, Banda Aceh and Ulee Lheu coast, Meuraksa Sub-district, Banda Aceh.

Tools used were a set of maceration device, Rotary evaporator FTIR spectroscopy, and *XLSTAT* software.

2.2. Sample Preparation

Giant calotrope leaf samples were prepared with several methods: without extraction (fresh leaf), fresh leaf with maceration, and dry leaf with maceration. As for dry leaf with maceration, giant calotrope leaf was dried in room temperature for 5 days and then the leaf was mashed up until it became powder. Next, the leaf powder was macerated with ethanol 70% for 48 hours. As for fresh leaf with maceration, the leaf was mashed up with mortar and pestle. Next, the leaf powder was macerated with ethanol 70% for 48 hours.

Table 1 Types of sample preparation methods.

Location	Sample code	Preparation method
Alue Naga	Alue N 1	Macerated (dry leaf)
	Alue N 2	Macerated (fresh leaf)
	Alue N 3	Fresh leaf
Ulee Lheu	Ulee L 1	Macerated (dry leaf)
	Ulee L 2	Macerated (fresh leaf)
	Ulee L 3	Fresh leaf

2.3. FTIR Analysis

The prepared samples were analyzed with FTIR spectroscopy on a wavelength range of 400-4500 cm^{-1}. FTIR spectra were saved in *Microsoft Excel* format for further PCA process.

2.4. PCA analysis

The PCA analysis was carried out with *XLSTAT* software. Before PCA, the data needed to go under *preprocessing* with *smoothing*, *baseline*, and SNV method.

3. RESULTS AND DISCUSSIONS

3.1. The Infrared Spectrum of the Giant Calotrope Leaves

FTIR analysis result showed that all samples from maceration treatment have the similar functional group, except samples without maceration. The infrared spectra of the sample can be seen in figure 1.

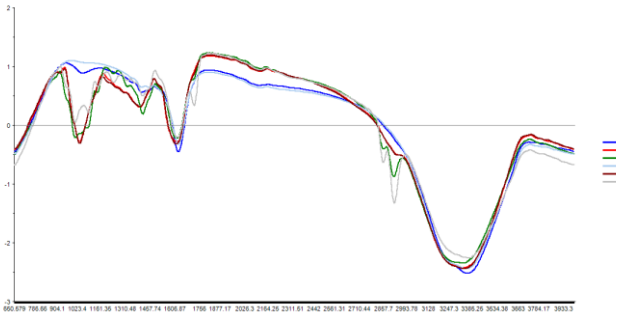

Figure 1. FTIR spectra of fresh leaf, macerated dry leaf, and macerated fresh leaf from giant calotrope leaf sample obtained from Alue Naga and Ulee Lheu.

The IR spectra of fresh giant calotrope leaf (Alue N3 dan Ulee L3) showed the functional groups on several wavelength numbers: 3280 cm^{-1} (O-H acid), 2917 cm^{-1} (C-H stretching), 2853 cm^{-1} (O-H acid), 1726 cm^{-1} (C=O), 1632 cm^{-1} (C=C aromatic ring), 1426 cm^{-1} (C-H bending), and 1019 cm^{-1} (C-O). It was very difficult to identify the type of compound that contained in giant calotrope leaf based on the fresh leaf spectra. Moreover, on *fingerprint* wavelength (1500-500 cm^{-1}), almost all types of functional groups showed up. This might be caused by the impurities that still contained in the samples.

On the fresh extract spectra, it was detected the several function groups on wavelength numbers: 3230 cm^{-1} (O-H acid), 2932 cm^{-1} (C-H stretching), 1612 cm^{-1} (C=C aromatic ring), 1401 cm^{-1} (C-H bending), and 1043 cm^{-1} (C-O). Generally, it can be estimated that the contained compounds were cardenolides [2] which was identical with an aromatic ring. As for spectra from dry leaf extract, the functional groups were known on wavelength numbers: 3334 cm^{-1} (O-H acid), 2932 cm^{-1} (C-H), 1627 cm^{-1} (C=C), 1403 cm^{-1} (C-H *bending*), and 1034 cm^{-1} (O-H).

3.2. Principal Component Analysis (PCA) Analysis

Before the PCA analysis was carried out, the data were going through preprocessing, namely smoothing with Savitzky–Golay method, baseline method, and SNV method. Preprocessing was done in order to obtain an optimum result on PCA analysis, dan selecting outlier values [26].

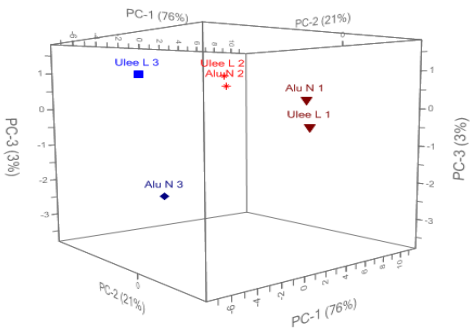

Figure 2. PCA plot of fresh leaf macerated dry leaf, and macerated fresh leaf from giant calotrope leaf samples obtained from Alue Naga dan Ulee Lheu.

The PCA plot result showed a variance of 97 % (PC1 76% + PC2 21%). Statistically, the variance obtained is good enough to explain the linkages and groups between measured variables. The PCA plot data showed that samples are grouped into four groups, namely Alue Naga and Ulee Lheu macerated dry leaf group, Alue Naga and Ulee Lheu macerated fresh leaf group, Alue Naga fresh leaf group, and Ulee Lheu fresh leaf group. This showed that the PCA method can differentiate the types of preparation methods used. Besides that, for fresh leaf samples, the sample that was obtained from different locations, namely Alue naga dan Ulee Lheu is forming a different group, but the extracted samples are not.

4. CONCLUSION

The combination method of FTIR and PCA can be applied to differentiate samples from different preparation methods used which are fresh leaf, macerated fresh leaf and macerated dry leaf. This method can also differentiate sample locations, which are from Alue Naga coast and Ulee Lheu coast for fresh leaf samples. The PCA analysis showed a good level of variance with the variance of 97% with PC1 76% and PC2 21%.

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