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Chemometric Analysis on Fingerprints of *Acalypha Indica* L Based on the Different Drying Methods

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Abstract

The use of an anting-anting plant (Acalypha indica Linn) as a raw material of herbal medicine requires a standardized process in its production. This research aims to determine the fingerprint pattern of active compounds using a thin-layer chromatography method with specific samples that applied various drying methods, such as direct sunlight drying, greenhouse drying, and oven drying at 50°C. The chemometrics data interpretation with the Principal Component Analysis (PCA) method was applied to analyze the obtained fingerprint patterns to identify the differences among the variations. The separation of active compounds in the anting-anting plant using ultrasonic extraction for 20 minutes with ethyl acetate as the solvent. Consecutively, the thin-layer chromatography fingerprint was investigated by applying a subsequent comparison of mobile phase cyclohexane: toluene: diethylamine by 75:15:10. The result from the TLC plate spots was examined to detect the differences among the fingerprints, with the help of ImageJ and Orange software. The fingerprints with various drying methods resulted in the possession of 11 stains of active compounds. The ImageJ software generated chromatograms with Area Under the Curve (AUC) values, further subjected to chemometrics analysis using PCA. The PCA results showed simple grouping patterns for each variation, with a total principal component (PC) of 86.03% (PC1 = 52.78%, PC2 = 33.25%), indicating the success criteria of PCA chemometrics analysis.

Keywords: *the anting-anting plant; the different drying methods; chemometrics*

Introduction

The herbal plants are standardly utilized for biodiversity because they are abundant to stock, easy to obtain, affordable to cost, and safe to cause no side effects. One of these herbal plants is the *anting-anting* plant (*Acalypha indica* L.), which has erect stems around 30–50 cm in height, branching trees, tap roots, green round-oval leaves with a diameter of 2.5–8 cm, serrated leaf edges, and trumpet-shaped flowers (Ameilia, 2018). The *anting-anting* plant can be used as herbal medicine because it contains active compounds such as alkaloids, flavonoids, saponins, steroids, quercetin, and tannin (Handayani et al., 2018).

A post-harvest process of the *anting-anting* plant should be considered to make it a standardized herbal medicine. One necessary post-harvest process is the drying process, which can impact the compound quality. The drying process has various methods, such as direct sunlight, oven, and

greenhouse drying. The latest is sourced from sunlight but located in a room that can control the microclimate, such as light and temperature, and manipulate conditions for plant optimization (Ardika et al., 2018). The purposes of drying are to reduce the water contained within the samples, prevent mold growth causing spoilage, and increase the sample's storage time (Kurniawati & Fitriyya, 2018).

The quality of the active compounds of the *anting-anting* plant can be identified by fingerprint analysis using the *Thin-Layer Chromatography* (TLC) method, which can analyze and provide information to compare pattern results for each sample (Hawrył et al., 2020). The chemometrics analysis on the characteristics of active compounds was conducted to investigate similarities or the falsification of herbal medicines. The analysis showed a statistical correlation of TLC results by applying mathematics, statistics, and logic to process data chemically (Rochman et al., 2021). This analysis can apply the *Principal Component Analysis* (PCA) method by simplifying the observed extensive data with the implementation of reducing variables without eliminating the original variables, thus producing data in the form of images.

Further, Andriansyah et al. (2022) examined the chemometrics analysis on fingerprints of *Centella asiatica* L. based on regional diversity in West Java and showed the differences in each region. However, there has been no research regarding the chemometrics analysis on fingerprints of *Acalypha indica* L which is based on different drying methods, so this research uses the same method but uses different samples. Therefore, this research aims to analyze the chemometrics of *anting-anting* plants based on the different drying methods; sunlight, greenhouse, and oven drying.

Methods

Tools and materials

The tools applied for this research were drying containers, grinding machines, sieving machines, sample powder storage, ovens, beaker glass (Iwaki), analytical balance, porcelain cup (Iwaki), desiccator, UV lamp (254 nm and 366 nm), ultrasonic, glass funnel (Iwaki), stir bar, filter paper, capillary tube, vial, measuring pipette (Iwaki), suction bulb, DSLR camera (Canon), ImageJ software and Orange software. This study also involved several materials, such as the *anting-anting* plants (*Acalypha indica* L.), ethyl acetate (p.a., Merck), cyclohexane (p.a., Merck), toluene (p.a., Merck), diethylamine (p.a., Merck), and TLC plat of silica gel $G_{60}F_{254}$ (Merck).

Work Procedures

Preparing the Anting-anting Plant

The *anting-anting* plants were brought from the Glagahagung Village, Purwoharjo District, Banyuwangi Regency, East Java. Those wild plants were then washed with running water. Next, the samples were dried with various drying methods, such as direct sunlight drying for 18 hours, greenhouse drying for 36 hours, and oven drying at 50°C for 8 hours. After that, the samples were crushed to produce the powder of an *anting-anting* plant.

Water Content

The following step was that the water content was measured using 1 gram of the sample placed in a porcelain cup whose curb weight was already identified. Then, it was heated in the oven for 15 minutes at 105°C, cooled in a desiccator for 30 minutes, and weighed until obtaining a constant weight.

The Extraction and The Thin-Layer Chromatography

The extraction stage required 1 gram of the *anting-anting* plant powder, which was dissolved in 10 mL of ethyl acetate and then extracted with the help of an ultrasonic instrument for 20 minutes at a frequency of 20 kHz. The next was the fingerprint process applying the thin-layer chromatography

method. During this process, a 6x10cm $G_{60}F_{254}$ silica plate with a margin on the top and bottom of 1 cm for each. An activation followed the process by placing it in the oven at 105°C for 30 minutes. Meanwhile, the mobile phase in the form of an eluent consisting of cyclohexane : toluene : diethylamine = 75:15:10, had been saturated for an hour in the vessel. Next, the extract was spotted 15 times on the plate. The plate was eluted in the twin-trough vessel until the eluent reached the upper line limit. The fingerprint silica plate was observed using 254 nm and 366 nm UV light. After all, the Rf value was calculated.

Data Processing with The Image J

Image J software processes the fingerprint images in files (JPEG/PNG). The image input was done by clicking "File" or "Open" and selecting the image. The part of the selected image was marked with a box-shaped icon (rectangular). After that, select the menu "Analyze," "Gels," and "Select first line" or "Select next line" for the next band. Next, select the menu "Analyze," "Gels," and "Plot Lane" again. Then, the chromatogram of each TLC band was displayed. At the base of the peak, a baseline was created by selecting the "Straight" icon and the stick-shaped icon "Wand Tool" to produce an AUC value.

Chemometrics Analysis

The AUC value was inputted in Microsoft Excel and then analyzed using the PCA (*Principal Component Analysis*). The Orange software was applied to do this. The first step was to open the Orange software, drag the "File" widget, and click twice to select the file. Next, the preprocessing stage was conducted by clicking "Add preprocessor," "Normalize spectra," "Final preview," and "Commit Automatically." Then, drag a line from the "Preprocess Spectra" widget and select "Spectra." Click it twice to see the spectrum of the AUC value. The next step was to drag a line from the "Spectra" widget, select "PCA", drag a line to select "Score Plot" and click twice on "Linear Projection".

Results and Discussion

Sample Preparation and Water Content

The sample of *Anting-anting* plant (*Acalypha indica* L.) with a wet weight of 1220 g was dried using various drying methods, such as drying in direct sunlight for 18 hours, drying with the heat of a green*house* at room temperature 40^0C for 36 hours, and drying in an oven at 50^0C for 8 hours. The time and method showed a good quality water content (<10%), which meets a requirement for a simplicial (Lady Yunita Handoyo & Pranoto, 2020). Then, the sample is ground using a grinding and sieving machine to equalize and expand the surface of the sample particles to make it easier to interact between the sample and the solvent. The results of measuring the water content in Table 1 show that the water content in samples using various drying methods still meets the quality requirements for simplicia preparations with a maximum limit of ≤10%, which means that the samples are not quickly overgrown by mold, microbes, or insects (Kurniawati & Fitriyya, 2018).

Table 1. Results of the Water Content Analysis

No	Drying Method	Water content (%)					% mean
		U1	U2	U3	U4	U5	
1	Direct sunlight	4.23	4.77	5.14	4.23	5.90	4.65±0.7
2	Heat of greenhouse	4.87	4.32	4.30	4.79	4.77	4.61±0.2
3	Heat of oven (50^oC)	4.62	3.98	4.70	4.77	5.73	4.76±0.6

Separating of Active Compounds by Fingerprint Method. The *anting-anting* plant, which is extracted by ultrasonic wave, works quickly. It doesn't require much solvent and extracts well. It is assisted by ultrasonic waves, which cause vibrations that cause the formation of cavitation bubbles in the cell wall area of the active compound and interfere with the penetration of the solvent into plant cells through ultrasonic emissions (Ariska Damanik & Pandia, 2019). The extraction process is assisted by the ethyl acetate solvent, which is semipolar with a polarity index of 4.4 and can bind compounds with a wide polarity range from polar to non-polar. The anting-anting plant causes it to have active compounds with various polar properties.

The active compound can separate the extraction results using the thin-layer chromatography fingerprint method. The results of which are

observed under UV light at wavelengths of 366 nm and 254 nm. This wavelength is used to clarify the active compound stains on the plate for the most precise stain results found in the results.

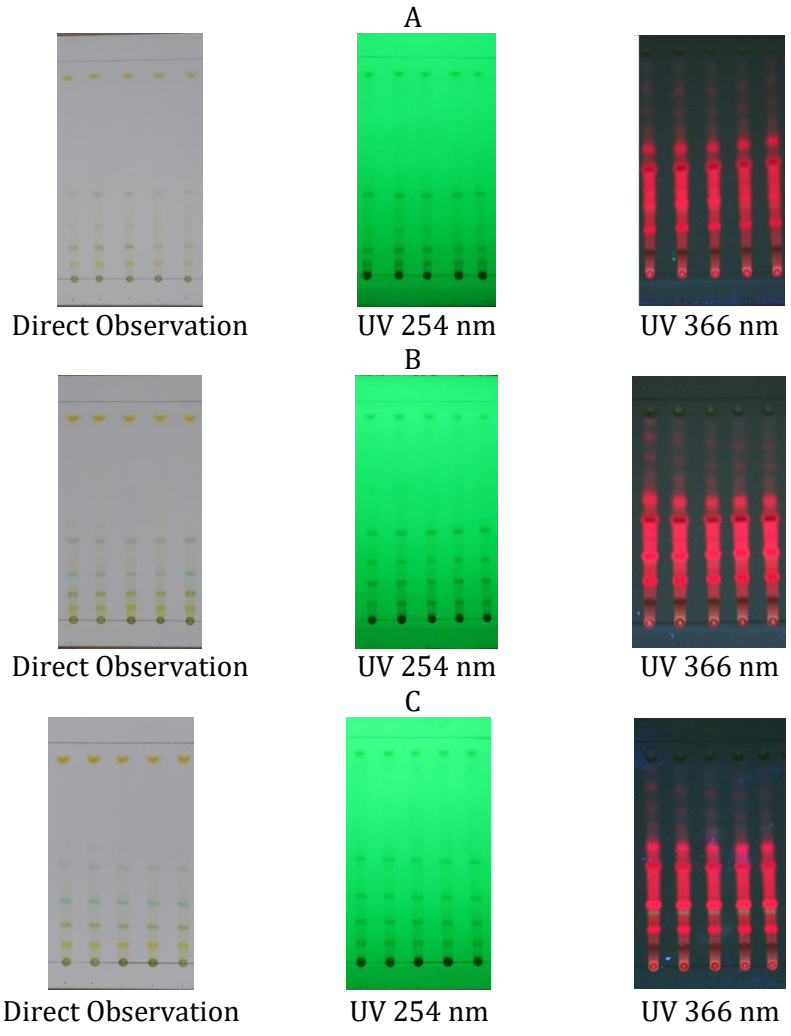

Figure 1. Results of the separation of chemical compounds (A): drying method using direct sunlight; (B): greenhouse drying method; (C): drying method using oven heat at 50°C.

At **Figure 1** produced the same number of 11 active compound spots in all drying methods. However, the intensity of the stain color for each method is different. It can be observed that the most obvious stain color is oven drying, drying in a greenhouse, and then drying in direct sunlight. Therefore, drying methods that have different lengths of time and temperature will result in different qualities of Simplicia because the active compound content is sensitive to the drying process (Azzahra & Budiati, 2022). Meanwhile, the standard deviation value of Rf is 0-0.01, so stains from all methods are assumed to have the same precision because the Rf value does not exceed the acceptance requirements, namely ΔRf ≤ 0.05 (Adawiyah, 2018).

Chemometric Analysis: PCA Method

The chemometric analysis process uses data processing software ImageJ with plate documentation input under 366 nm UV light because the results in Figure 1 show that the active compound stains are more evident compared to observations under 254 nm UV light and direct observation. Active compound bands are marked on the *Thin layer chromatography* (TLC) plate image to display the chromatogram of each active compound band. The results are AUC values for each drying method. At this stage, we cannot explain the differences, so *principal component analysis* (PCA) is assisted in the next stage.

Previously, preprocessing was carried out first in processing the AUC data results in the form of min-max normalization, a data processing method using maximum and minimum values to convert data into a value range of 0 to 1. This method obtains smaller data dimensions (Ritonga & Muhandhis, 2021).

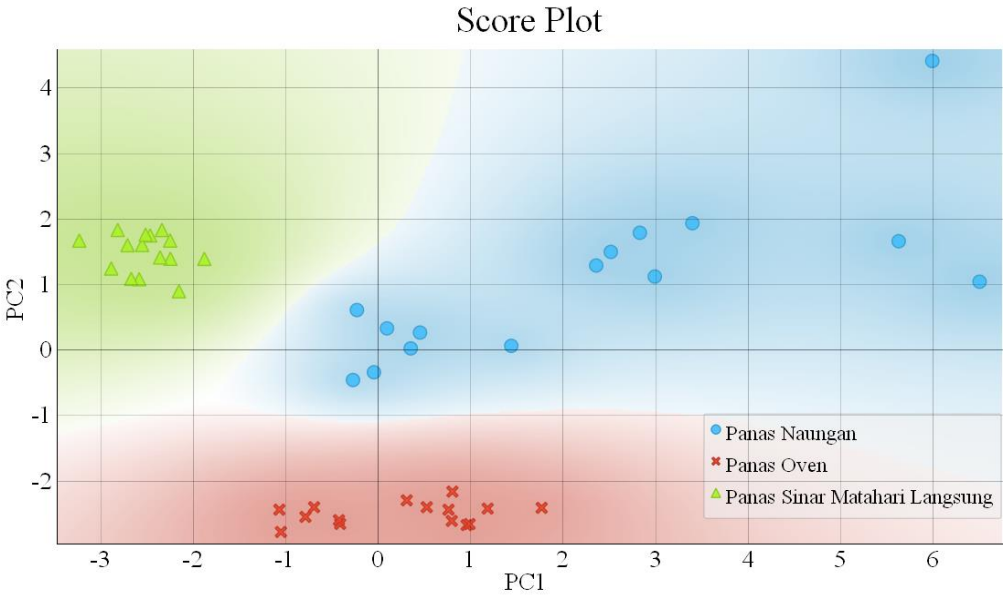

Figure 2. Interpretation score plot of *Principle Component Analysis*

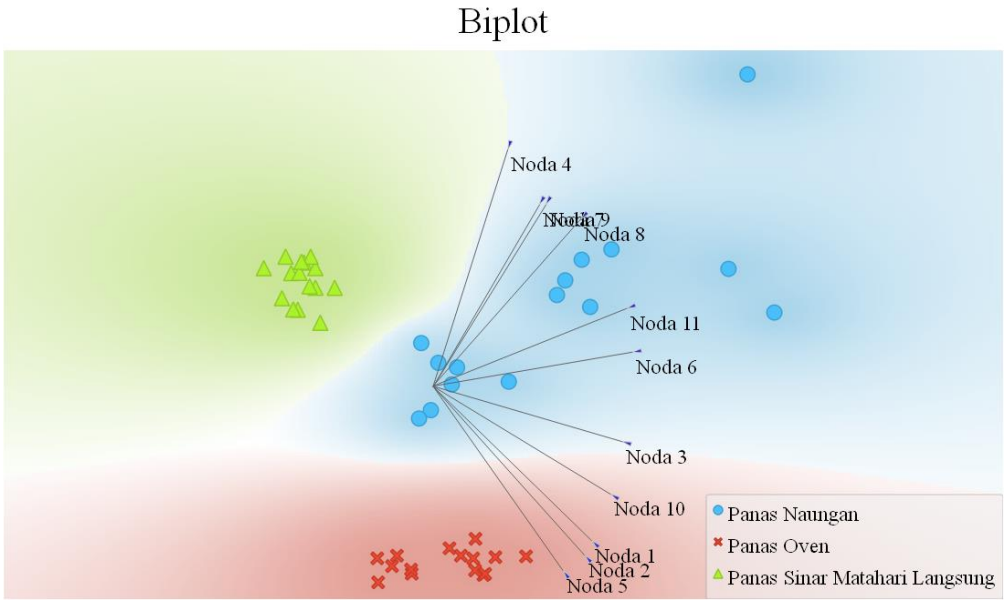

Figure 3. Interpretation biplot of Principle *Component Analysis*

Figure 2 and Figure 3 shows that in the score plot interpretation and biplot interpretation with Orange software, at score plot the pattern points for each variation are not close to each other in each drying method. It can occur due to differences in the intensity of the stain color. The results of the chemometric analysis are based on the PC (*principal component*) used, namely PC1 and PC2, with a total PC value of 86.03% consisting of PC1 = 52.78% and PC2 = 33.25%. Apart from that, the PCA results in Figure 2 show that the PC1 variable has a high contribution in the form of stain 6, while PC2 is in the form of stain 4. These results show that spots 4 and 6 are the variables that have the most influence in forming new data based on the fingerprint results of variations in various drying methods for *anting-anting* plants. This difference in drying can occur due to the influence of humidity and the consistency of a temperature, so it can affect the water content and color intensity when analyzing TLC fingerprints. When drying in direct sunlight, there is exposure to atmospheric oxygen at night, which can affect the bioactivity of the sample. Excess *ultraviolet B* (UV-B) radiation can affect active plant compounds (Triastarani, 2021). High temperatures during the drying process will cause the molecules to move at high speeds to exceed the attractive forces in liquids and solids so that the water molecules will turn into gas.

Conclusion

The results of thin layer chromatography fingerprinting on *anting-anting* plants using various drying methods produced the same number (11 active compound stains), but with different color intensities. Meanwhile, the results of the PCA chemometric analysis show that the interpretation of the score plot is in the form of grouping each variation of drying method with a total variance of 86.03% consisting of PC1 = 52.78% and PC2 = 33.25%, eigenvalues PC1 = 5.8056 and PC2 = 3.6576. Meanwhile, the biplot interpretation shows that the variables that greatly contribute to PC1 are stain 6 and PC2 stain 4.

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