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FINGERPRINT ANALYSIS IN DETECTING PLANT-BASED ADULTERANT IN HERBAL PREPARATIONS FROM INDONESIAN CURCUMA AERUGINOSA ROXB. USING FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

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ABSTRACT

Curcuma aeruginosa Roxb. usually consume as traditional medicine by processing themselves traditionally while purchasing instant traditional medicinal products on the market. But the lack of standard quality control of herbal medicine resulted in a lot of fraud in this regard. So, it is necessary to do quality control because herbal medicine usually consists of a mixture of several components. Fingerprint analysis is one method that can be utilized for evaluation and control of multicomponent quality of raw materials of herbal medicine. This study aims to detect turmeric adulterant on curcuma aeruginosa instant product inventory using fingerprint analysis via FTIR spectroscopy. Research stages include raw simplicial and instant sample extraction using maceration method with ethanol 96%. Furthermore, FTIR spectrum measurement was performed on wave number 4000-650 cm^{-1} and resolution of 4 cm^{-1} using Micro Lab Expert application, reflecting handling technique. Measurement results are analyzed by chemo metric method Principal Component Analysis (PCA) to produce data in the form of score and loading. Of the three samples of instant black Curcuma extract tested, the results of two samples had the same characteristics as Curcuma aeruginosa Roxb. This indicated two negative samples containing turmeric adulterant while one sample did not have the same characteristics as black Curcuma or turmeric allegedly there were another adulteress. Fingerprint analysis using FTIR combined

using the PCA method is capable of detecting adulteries in instant black dosage form and is able to detect the characteristic equations of raw Curcuma aeruginosa Roxb.and turmeric raw extract.

Keywords: Chemo metric, Curcuma aeruginosa Roxb., Fingerprint analysis, Fourier Transform Infra Red (FTIR), Turmeric, Principal Component Analysis (PCA)

1. INTRODUCTION

Curcuma aeruginosa Roxb. has a distinctive flavour that produced from the essential oils wich is contained,and itsused as an traditional medicine because it contains bioactive compounds such as saponins, flavonoids, polyphenols, triterpenoids, and glucans **[1, 2]**. *C. aeruginosa* Roxb has properties to arouse appetite, skin diseases such as scabies, colic, mouth sores, cough, shortness of breath, worms, gout, and body fatness **[3]**. People usually consume traditional medicine by processing themselves by buying instant traditional medicine products on the market.

Adulterant is the counterfeiting of a product or mixing with the addition of dangerous substances or compounds, deliberately replacing, adding, changing or misrepresenting a food ingredient or product. The lack of standard quality control from drugs and food results in a lot of cheating in this case so that the use of herbal medicines becomes marginalized **[4]**.

Quality control is needed for herbal remedies because herbal medicines usually consist of a mixture of several components. Drying, shipping and processing can cause changes in the quality of herbal medicines. The purpose of quality control is to maintain and direct the quality of products to be maintained in accordance with the desired quality standards **[5]**.

Fingerprint analysis can help in the identification and authentication of plant species for their quality control **[6]**. Chemical compounds contained by medicinal plants can be displayed in chromatographic fingerprints so that the chemical characteristics of the medicinal plants can be described thoroughly **[7]**.

Determination of chemical quality in *Curcuma aeruginosa* Roxb. Based on fingerprints has been carried out using various methods such as Liquid Chromatography Mass Spectroscopy and Thin Layer Chromatography **[8]**. Methods of Fourier Transform Infrared (FTIR) spectroscopy can be used for fingerprint analysis in herbal medicines. Fourier Transform Infrared (FTIR) spectroscopy techniques are infrared spectroscopy

equipped with Fourier transforms for analysis of spectrum results.

The aim of this research is to detect turmeric adulterant in the supply of Herbal preparation *Curcuma aeruginosa* Roxb. products that are circulating in the market with FT-IR fingerprint analysis method, and to find out whether or not there is a mixture of turmeric in *Curcuma aeruginosa* Roxb. preparations on the market

2. DATA/MATERIALS AND METHODS

The *Curcuma aeruginosa* Roxb. rhizome was taken from three different regions, namely Lampung, West Java, Central Java, and Herbal preparation *Curcuma aeruginosa* Roxb. products in the market with three different producers, 96% Ethanol

Maceration of *Curcuma aeruginosa* Roxb.

A total of 150 grams of dried Curcuma simplicial powder were extracted by maceration method using 500mL 96% ethanol. Soak for 6 hours while stirring occasionally, then let stand for 24 hours. The obtained macerate was separated and transferred to another glass beaker, while the pulp was treated equally 2 times maceration. Macerate obtained from the extraction results is put together then concentrated with a rotary evaporator until a concetrated extract is obtained.

Maceration of turmeric rhizomes

A total of 150 grams of dried turmeric simplicial powder were extracted by maceration method using 500mL 96% ethanol solvent. Soak for 6 hours while stirring occasionally, then let stand for 24 hours. The obtained macerate was separated and transferred to another glass beaker, while the pulp was treated equally 2 times maceration. Macerate obtained from the extraction results is put together then concentrated with a rotary evaporator until a thick extract is obtained.

Extraction of instant *Curcuma aeruginosa* Roxb.

Samples a total of 20 grams of instant *Curcuma aeruginosa* Roxb. extract samples obtained from 3 different producers were macerated using 50 mL 96% ethanol solvent. Soak for 6 hours while stirring occasionally, then let stand for 24 hours. The obtained macerate was separated and transferred to another glass beaker, while the pulp was treated equally 2 times maceration. The macerate obtained from the extraction results is put together then concentrated

Dry *Curcuma aeruginosa* Roxb. and tumeric extract was then carried out by measuring the infrared spectrum of the fingerprint area using FT-IR and Micro Lab Expert applications. The IR spectrum is read at a frequency of 4000-650 cm^{-1} and 1400-400

cm^{-1} with a resolution of 4 cm^{-1}, with reflectance sample handling techniques.

PCA chemo metric analysis

To facilitate the chemo metric analysis, use the software Unsclamber X 10.4. The data obtained from the analysis using FTIR are X and Y values. Chemo metric testing uses Principal Component Analysis (PCA) with data values of X (Wave Numbers) and Y values (Absorbance). The results of PCA analysis are scores and loading. Validation on PCA uses cross validation, eigen value.

Detection of Adulterants in the Sample

Extracts of instant *Curcuma aeruginosa*Roxb. samples from three different products available in the market were analyzed using FT-IR and Micro Lab Expert applications. The FT-IR spectrum is read at a frequency of 1400-400 cm^{-1} and 4 cm^{-1} resolutions, with reflectance sample handling techniques. The X and Y values are then tested using the Principal Component Analysis (PCA) to determine the adulterant in the sample.

3. RESULTS AND DISCUSSION

Results of Plant Determination

The results showed that the samples taken from Lampung, Central Java and West Java were *Curcuma aeruginosa* Roxb. plants and turmeric (*Curcuma Rhizoma* Roxb.) which was the Zingiberaceae tribe. The purpose of determining plants is to make the species as specific as possible and on target.

Preparation of Extracts

Simplicial of fine *Curcuma aeruginosa* Roxb. from three different regions, namely Lampung, West Java, and Central Java then extracted using maceration method. Maceration of the sample was carried out using ethanol 96% because of its ability to dissolve almost all substances, both polar, semi-polar, and non-polar **[9]**. Then through the extraction process, the macerate was concentrated using a rotary evaporator at a temperature of 50ºC until a thick extract was obtained.

Results of extraction *Curcuma aeruginosa* Roxb.

The *Curcuma aeruginosa* Roxb. rizome extract obtained from the thick extracted texture was thick Curcuma and has a distinctive aromatic odor as this content of essential oils contained in the Curcuma simplicial. The percentage of the extract yield shows the maximization of the solvent in chasing the simplicial. The simplicial percentage of *Curcuma aeruginosa* Roxb. extract obtained from Lampung, Central Java and West Java was 13.39%, 13.17%, and 13.28%, respectively.

Results of extraction of Turmeric

Turmeric rhizome extract obtained with thick reddish orange textured extract is caused by the content of curcumin available in turmeric rhizome. The color of reddish orange turmeric extract and turmeric rhizome extract also has an aromatic odor that is very typical of this because in the rhizome contains the aroma of essential oils that give a very aromatic aroma of turmeric. The yield percentage of crude turmeric extract from Lampung, West Java, and Central Java is 13.56%, 13.47%, and 12.96%, respectively.

Spectrum pattern of *Curcuma aeruginosa* Roxb. FTIR

FTIR spectroscopy is a fast, simple, and non-destructive analysis technique with all chemical properties in the sample can be expressed and raised in the FTIR spectrum. The principle of FTIR analysis is to use infrared radiation. The signal captured by the detector is converted using an analog-to-digital converter, then the digital signal is transferred to the computer for Fourier-transformation **[10]**.

In this study, using Curcuma extract standard extracts with reflecting sample handling techniques and analysis recorded in the form of absorbance. The IR spectra data of each sample were obtained from the scanning results with FTIR tools and Micro Lab Expert application with ATR (Attenuated Total Reflectance) accessories that work by measuring changes that occur in the process of reflecting infrared light when light comes to the sample. The advantage of the ATR method is that measurement is nondestructive and does not require complicated sample preparation so that the analysis process is faster. Then scanning 6 times in the wave number range 4000-650 cm^{-1} where in the wave number range 1400-800 cm^{-1} is the fingerprint area, the resolution used 4 cm^{-1} measurements at 4 cm^{-1} resolution shows that each measuring distance is 4 cm^{-1} there is one intensity measurement point, the selection of a small resolution aims to make the peak clearly visible, because the smaller the resolution the more visible the peak. To avoid variations in spectra between one sample to another, the base spectrum (background) is measured each time before the measurement begins. The basic spectrum reading was done because FTIR spectroscopy was a single beam so that the sample readings were carried out one by one.

The infrared spectrum in *Curcuma aeruginosa* Roxb. rhizome extract taken from three regions **(Figure 1)** that are geographically different, namely Lampung, West Java, and Central Java in the wave number area of 4000-650 cm^{-1} and the fingerprint area in wave number 1400 -800

cm^{-1} shows a similar absorption pattern, there is only a difference in each absorbance value, it is caused by the content or concentration of compounds in each standard extract Curcuma float, the standard extract of Javanese *Curcuma aeruginosa* Roxb. West, and the Central Javanese *Curcuma aeruginosa* Roxb. extract has different levels. The absorption bands raised by the standard extract of Curcuma aeruginosa Roxb. Lampung samples, West Java *Curcuma aeruginosa* Roxb. extracts, and Central Java *Curcuma aeruginosa* Roxb. extracts were produced: band 1 (3450-3300 cm^{-1}) which was wide enough indicated the O-H stretching vibration; band 2 (2975-2840 cm^{-1}) indicates C-H stretching vibration from methyl and methylene; band 3 (1475-1445 cm^{-1}) shows C-H bending vibration; band 4 (1400-1300 cm^{-1}) indicates vibration deformation; and band 5 (1070-1020 cm^{-1}) shows the presence of stretch vibration from C-O.

The turmeric spectrum pattern **(Figure 2)** taken from three different regions, namely Lampung, West Java, and Central Java at wave numbers 4000-650 cm^{-1} and the fingerprint area of the raw turmeric extract at wave numbers 1400-800 cm^{-1} which shows a spectrum pattern that is similar to each other only lies in the difference in each absorptive value. This shows that the content of the chemical compounds contained is the same but the difference is the levels contained in each extract. The peak of the band on the turmeric spectrum is more than the peak of the band in the Curcuma spectrum. Absorption bands raised by the raw extract of turmeric in Lampung samples, the Central Java sample turmeric extract, and the turmeric raw extract of Karawang samples were produced: band 1 (3420-3280 cm^{-1}) indicates O-H stretching vibration; band 2 (3090-3015 cm^{-1}) indicates C-H stretching vibration; band 3 and band 4 (1625-1470 cm^{-1}) indicates that there is a stretching C-C vibration; band 5 and band 6 (1300-1130) presence of C-O stretch vibration; band 7, ribbon 8, and band 9 (880-660) show the presence of a C-H buckling vibration.

The overlay spectra pattern of the *Curcuma aeruginosa* Roxb. raw extract and the turmeric extract at wave numbers 4000-650 cm^{-1} produces a different spectrum pattern. Where can be seen in the spectrum pattern of turmeric raw extract has many distinctive ribbon peaks compared to the Curcuma extract standard spectrum pattern which has fewer ribbon peaks.

FTIR spectrum contains quantitative information that can describe the characteristics of a sample. This information cannot be observed by only looking at

spectrum absorption patterns, but requires tools such as data extraction methods or patterns called chemo metrics to show a more meaningful interpretation.

The results of spectrum measurements obtained X and Y values that are stored in format (CSV) so that they can be converted to Microsoft Excel which can then be analyzed chemo metrically using the Principal Component Analysis (PCA) method

Principal Component Analysis (PCA) Analysis

The results of FTIR spectrum measurements were further analyzed using chemo metrics. This method was done using Unscramble X 10.4 software. The chemo metric method used is Principal Component Analysis (PCA). PCA is data interpretation that is done by reducing data, where in the number of variables the matrix is reduced to produce new variables while maintaining the information held by the data. Validation used in PCA is cross validation. The results of the PCA analysis are scores and loading where each of these 3 PCs is obtained. But the data used is only PC-1 data on PC-2 because the results of the grouping are very good compared to PC-1 and PC-3. Based on PC-1 results on PC-2, a plot score curve can be made. Score plots using the first two PCs are usually most useful because these two PCs represent the greatest variance of data **[11]**. The plot score curve is used to estimate the data structure, which is the basis of the difference between the *Curcuma aeruginosa* Roxb. and turmeric extract based on geographical regional differences. The distance between samples shows the similarity between samples. The farther the distance, the less similarity is shared between the samples **[12]** if the closer between the samples on the score plot, the greater the similarity between the samples.

Based on the results of the plot score curve above PC-1 to PC-2 representing a variance of 99% (PC-1 = 83% and PC-2 = 16%) **(Figure 4)** the extract of *Curcuma aeruginosa* Roxb. with turmeric extract spread separately and formed a separate grouping on Different smells, where the *Curcuma aeruginosa* Roxb. extract was gathered together with the Curcuma extract group while the turmeric raw extract was gathered together with other turmeric extracts, it showed the characteristics between the *Curcuma aeruginosa* Roxb. extract and the different turmeric extract.

Based on the PC-1 plot score curve on PC-2, it represents a variance of 97% (PC-1 = 73% and PC-2 = 19%) **(Figure 4)**, a combination of *Curcuma aeruginosa* Roxb. extract, turmeric extract and instant Curcuma extract shows separation. different where extract.

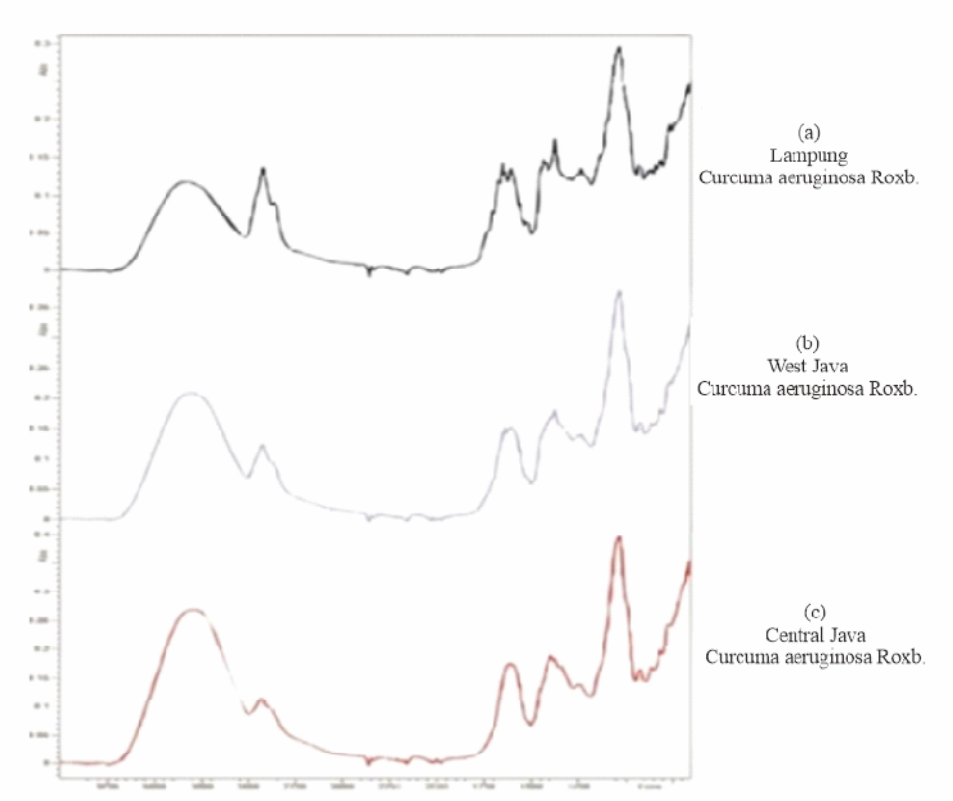

Figure 1: Overlay of the FT-IR *Curcuma aeruginosa* Roxb.spectra

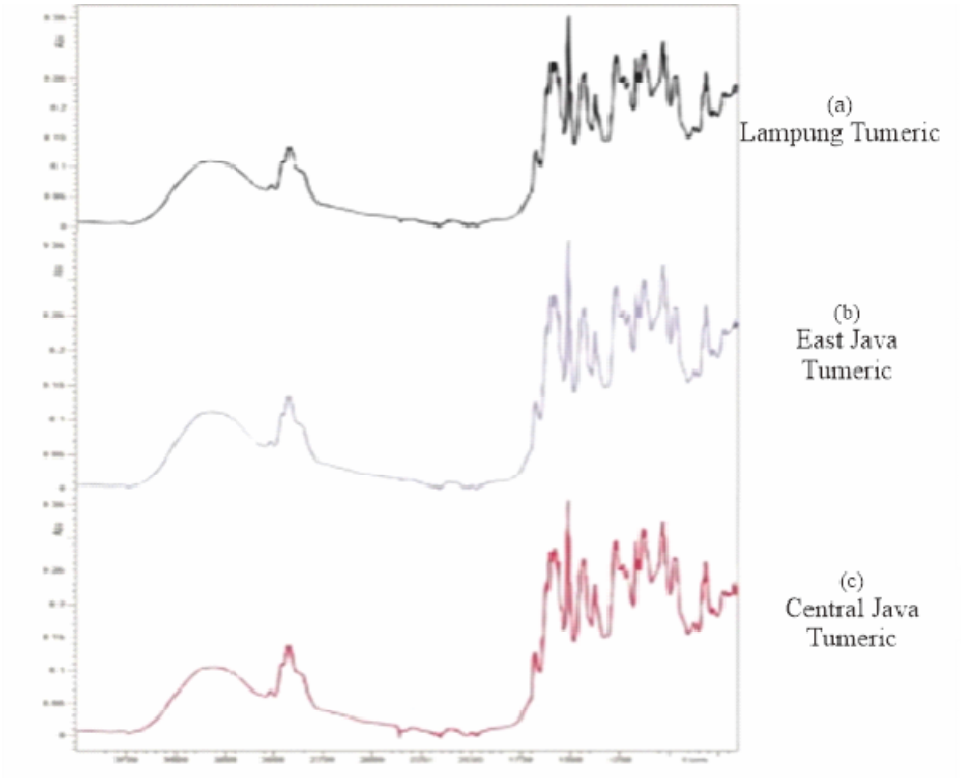

Figure 2: Overlay of the FTIR Tumeric Spectra

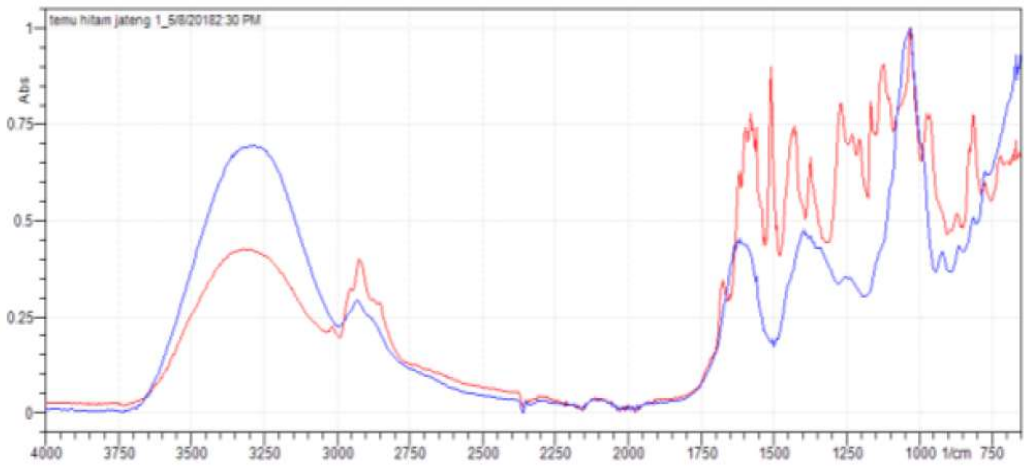

Figure 3: Overlay of the FTIR Curcuma aeruginosa Roxb.extract (blue) and raw turmeric extract (red) spectra

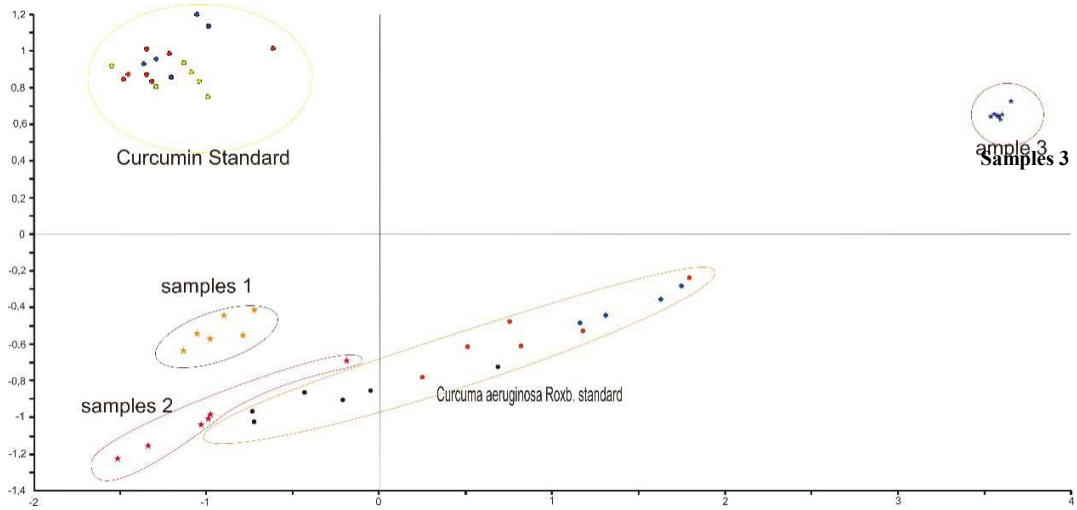

Figure 4: Plot PCA of Curcuma aeruginosa Roxb. Extract, turmeric extract and samples

Curcuma aeruginosa Roxb. standard with instant Curcuma extract samples 1 and 2 were in the same grouping area and close together while the raw turmeric extract formed its own grouping whereas the sample Curcuma instant extract plots sample 3 did not form a close group extract score Curcuma standard score or score plot of raw turmeric extract. The results of the PCA analysis showed that the three instant Curcuma extract samples from three different producers showed that sample 1 and sample 2 were close together in the grouping area of the *Curcuma aeruginosa* Roxb. extract and had a distance apart from the turmeric extract indicating that sample 1 and sample 2 were negative. contains turmeric adulterant, while sample 3 forms its own group association and is not adjacent to the score group of the Curcuma plotted extract plot and the score

plot of the raw turmeric extract so that in sample 3 it is negative to contain turmeric adulterant presumably in sample 3 there is another adulterant content.

4. CONCLUSIONS

1. Fingerprint analysis using FTIR combined with chemo metric analysis using PCA method is able to detect adulterant in instant Curcuma aeruginosa Roxb. preparation raw materials and is able to detect the characteristic of *Curcuma aeruginosa* Roxb. extract and turmeric raw extract from three different regions based on geographical location.
2. three Herbal preparation Curcuma samples from three different manufacturers showed that sample 1 and sample 2 were negative as well as sample 3 containing turmeric adulterant but in sample 3 it was thought that there were other adulterants.

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