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Fingerprint Analysis of Adulterant in Traditional Medicine of *Orthosiphon aristatus* Leaves Using FTIR Method

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***Abstract* -- Orthosiphon aristatus is a medicinal plant that used as traditional medicine raw material. These leaves have similar morphology with Eupatorium riparium so that it is possible to adulterate the raw material of Orthosiphon aristatus. The purpose of this research was to identify the existence of Eupatorium riparium leaves as an adulterant in the traditional medicine of Orthosiphon aristatus leaves using FTIR fingerprint analysis. The method of fingerprint analysis determined by measure dried extract that macerated using ethanol 96% with FTIR at wave number 4000 – 650 cm^{-1}, resolution at 4 cm^{-1} with reflectance sample handling. The dried extract that measured consists of Orthosiphon aristatus and Eupatorium riparium leaves from three different region and 3 samples of traditional medicine of Orthosiphon aristatus leaves. FTIR spectra were analysis by Principal Component Analysis (PCA). The result shows that PC1 and PC2 can be used to detect Eupatorium riparium adulterant in Orthosiphon aristatus leaves signed by different quadrant between Orthosiphon aristatus and Eupatorium riparium leaves with score plot as 97 %. One of three samples believed containing Eupatorium riparium leaves as an adulterant.**

Keywords: adulterant, fingerprint analysis,* Orthosipon aristatus*, Eupatorium riparium, FTIR, PCA

I. INTRODUCTION

Orthosiphon aristatus is one of the plants that used as traditional medicines raw material which can treat and prevent various diseases. *Orthosiphon aristatus* has long been used by people in several countries in Southeast Asia such as Indonesia, Malaysia, Thailand, and the Philippines as medicines for high blood pressure, jaundice, diabetes, kidney disease, and rheumatism. It has various pharmacological effects, such as antioxidant [1], anti-inflammatory [2], diuretic and hypouricemic [3].

Orthosiphon aristatus contain a variety of active compositions from the monoterpene, diterpene, triterpene, saponin, flavonoid, volatile oil, and organic acid groups, and contain bioactive components, including minerals which are mostly potassium minerals, around lipophilic flavones (sinensetin and isosinensetin), glycosides flavonols, caffeic acid (rosmarinic acid), essential oils, diterpene [4] orthosiphol d and orthosiphol E [5]

The adulteration of *O.aristatus* using *Eupatorium riparium* has been supported by Van Eijk (1980) [6] and Asmanizar and Katrin (1995) [7]. Besides, tackle leaves are easily obtained from a cat's whiskers.

Adulteration of *O.aristatus* needs to be detected to avoid losses to consumers. Several methods can be used to detect them such as chromatography (TLC, HPLC, and KG) and spectroscopy (UV-Vis, FT-IR, NMR, and mass). Among these techniques, FT-IR spectroscopy can be an option because it can meet the criteria of efficient analysis such as easy to use, fast, and inexpensive [8].

FT-IR spectroscopy can measure samples quickly and can analyze several components simultaneously. The use of FT-IR in plant analysis is still limited because the matrix and the resulting variations are quite complex. The FT-IR fingerprint analysis produced is very complex data information so that it can be made into a chemical example. Changes that occur in the position of the band and its intensity in the FT-IR spectrum will be related to changes in chemical composition in a sample. Therefore, the FT-IR spectrum can be used to distinguish one plant from another including its chemical composition is not yet fully understood [9]

This analysis method was developed using typical fingerprint pattern information, as variables that affect chemical samples such as biological activity and concentration [10]. From this background, then in this study fingerprint analysis was carried out on the raw material of *O. aristatus* herbal medicine using FT-IR

II. METHOD

This research was done by experimental methods. The study began with the preparation of extract, FTIR spectra analysis, and data analysis using Principal Component Analysis (PCA).

III. MATERIALS AND TOOLS

Materials: Ethanol and silica gel were purchased from Merck, 3 samples ashitaba herbal medicine products were from 3 different suppliers, Plant Materials: *Ortosiphon aristatus* and *Eupatorium riparium* leaves were collected from (Indonesia region): Bandung, Lampung, and Yogyakarta,

2019. The plant was identified by a botanist in the herbarium of Institute Technology Bandung of SITH, Bandung, Indonesia (Plant Identification Certificate, Number: 5638 / I1.CO2.2 / PL / 2018).

Tools: pipettes, beaker glasses, measuring flasks, rotary evaporator, Fourier Transform Infrared (FTIR) spectrometer (Agilent Cary 630)

IV. PROCEDURE

- Preparation of Extract

O. aristatus and *E. riparium* leaves were separated from the plant, washed and dried. The dried leaves were powdered and weighed 100 grams to be extracted by maceration method using 1 L ethanol solvent, soaked for 6 hours while occasionally stirring, then let stand for 24 hours. The solvent replacement was carried out 2 times with the same maceration procedure. The macerate obtained from the extraction was collected then concentrated with a rotary evaporator until viscous extract was obtained, then dried by evaporating it in a vaporizer cup with a water bath, then stored in a desiccator that contained silica gel.

- FTIR Spectrometry

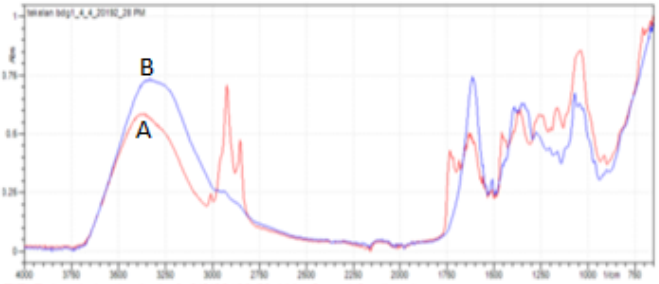
Dried extract of *O. aristatus*, *E. riparium* and samples were measured infrared spectra using FT-IR spectrometer with Spectra Manager Version 2.01.03 as the application of the instrument. The FT-IR spectrum was read at a frequency of 4000-650 cm^{-1} with a resolution of 4 cm^{-1}, with reflectance sample handling techniques.

- Data Analysis using PCA

FTIR spectra were analyzed by the chemometric method using the Principal Component Analysis (PCA) method. PCA was done by using software *Unscrambler 10.4*. In this study, the experiments were performed in triplicates (n = 3).

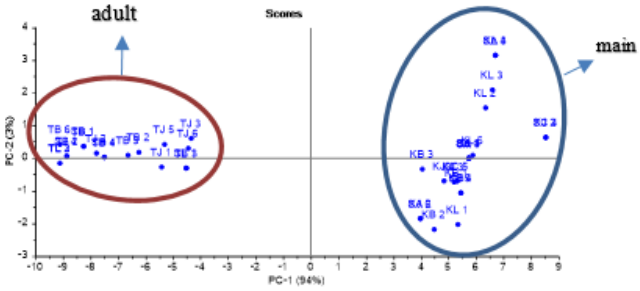
V. RESULTS AND DISCUSSION

Dried extract of *O. aristatus*, *E. riparium* and samples were measured infrared spectra using FT-IRATR spectrometer. FTIR spectra of *O.aristatus* and *E.riparium* are shown in Picture 1. The strong and distinctive spectra for *O.aristatus* and *E.riparium* were measured in the region of 650 – 4000 cm^{-1}, with a resolution of 4 cm^{-1}. The difference spectra between *O.aristatus* and *E.riparium* were observed in the region 2750 – 3600 cm^{-1} and 650 – 1750 cm^{-1}.



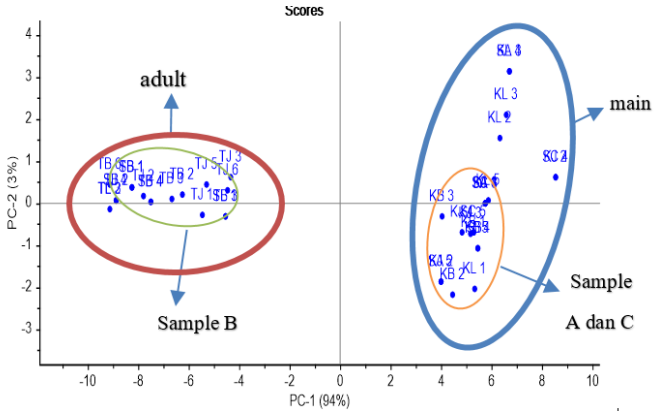
PICTURE 1. FTIR spectra of *O.aristatus* (A) and *E. riparium* (B)

The results of FTIR spectra measurements were further analyzed using chemometrics with Principal Component Analysis (PCA) method. This method was carried out using Unscrambler 10.4 software. PCA is data interpretation done by data reduction, wherein the number of matrix variables 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 where each of them is obtained 3 PCs. However, the data used are only PC-1 data against PC-2 because the grouping results are very good compared to PC-1 and PC-3. Based on the results of PC-1 against PC-2, a score plot curve can be made. A plot score using the first two PCs is usually the most useful because these two PCs represent the greatest variance of the data[16]. The score plot curve is used to estimate the data structure as the basis for the difference between the raw extract of *O.aristatus* and *E.riparium* extract based on geographical differences in regions. The distance between samples shows the similarity between samples. The farther the distance, the less similarity between the sample, the closer the location of the sample is to the score plot, the greater the similarity between the samples[17].



PICTURE 2. PCA Score Plot of FTIR Spectra *O.aristatus* (main) and *E. riparium* (adulterant)

The acquired data were analyzed by the application of the PCA method. The first two Principal Component (PC) were chosen to display the grouping of ashitaba as main substance and celery as an adulterant. While the PC-1 explained 94 % of the total variance, PC-2 explained 97 % of the total variance with eigenvalue at 42,87 and 1,23, respectively. The score plot of the two PC of PC-1 versus PC-2 can be seen in Picture 2.
And next to the FTIR spectra data of three samples were projected to PCA Score Plot of FTIR Spectra *O.aristatus* and *E.riparium* for determining sample position whether in *O.aristatus* and *E.riparium* grouping. The result of samples projection is in Picture 3.



PICTURE 3. Project sample A, B, and C onto PCA Score Plot of FTIR Spectra *O.aristatus* (main) and *E.riparium* (adulterant)

The result of samples projection shows that sample B is in *E.riparium* grouping which indicates positive containing *E.riparium* as an adulterant, while sample A and C are in *O.aristatus* grouping which indicates negative containing *E.riparium* as an adulterant.

VI. CONCLUSION

It can be concluded that fingerprint analysis using FTIR spectroscopy in combination with PCA can be used to detect adulteration in *O.aristatus* herbal medicine with *E.riparium* as an adulterant. The first two PC was used to show the grouping of *O.aristatus* and *E.riparium* with cumulative variance at 97 %. From three analyzed sample, one of them was suspected positive containing *E.riparium* as an adulterant.

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