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PROFILE COMPOUNDS OF CENTELLA ASIATICA L. BASED ON GEOGRAPHIC LOCATION AND VARIATION OF SOLVENTS USING FTIR

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Abstract

Centella asiatica L. is a plant that has long been used as herbal medicine. The plant's geographical location will affect the levels of bioactive compounds, which are the general criteria in selecting quality herbal medicinal raw materials to ensure the consistency of their efficacy. This study uses FTIR to compare the component profiles of Centella asiatica *L.* leaves based on geographic location and solvent variation. Extraction was carried out by the maceration method using three types of solvents with different polarity levels, namely ethanol (polar), ethyl acetate (semi-polar), and n- hexane (nonpolar). Based on the results of the study, which obtained three different groups of spot stains from the appearance of TLC, the spectral results were relatively the same in each variation of the filtrate with different absorption intensity values, while for FTIR analysis combined with chemometrics, Bengo and Tombolopao locations had a similarity of 98.95% were classified in group I, the Cenrana location had a similarity of 93.85% classified in group II. In contrast, the Bungaya location had 84.85% similarity in group III. The ethyl acetate extract at the Bengo and Tombolopao locations had a 99.71% similarity in group I, and the Cenrana location had a 99.17% similarity in group II. In comparison, the Bungaya location had a similarity of 93.98% in group III, and the n-hexane extract in the Cenrana and Tombolopao locations had 99 similarities, 84% were classified in group I, the Bengo location had a similarity of 99.44%, and was classified into group II. The Bungaya location had a similarity of 97.18% in group III. The research concludes that there are differences in the profiles of *Centella asiatica L.* leaf compounds from the four sampling locations, with variations in the solvents.

Keywords: *Centella asiatica* L., variety of solvents, FT-IR and chemometrics

Introduction

Centella asiatica L. is a wild plant often found in Indonesia and has long been used as herbal medicine. Centella asiatica has many benefits and properties related to antimicrobial, antioxidant, wound healing, anti- inflammatory, and anticancer activities (1). Centella asiatica *L.* contains alkaloids, flavonoids, saponins, tannins, steroids, triterpenoids, and glycosides (2).

The geographical origin of the plant will affect the levels of bioactive compounds, which are the general criteria in selecting quality herbal medicinal raw materials to ensure the consistency of their efficacy (3). The extraction process of bioactive components is strongly influenced by several things, one of which is the type of solvent (4). Differences in solvent extraction can affect the total content of bioactive compounds (5). The chemical composition contained in plant extracts is complex. The FTIR spectroscopy technique is one of the analytical techniques that can thoroughly describe the chemical characteristics (6).

FTIR spectroscopy can be an attractive option because it can meet efficient analytical criteria such as being easy to use, fast, and inexpensive (7). Changes in the position and intensity of a band in the FTIR spectrum will be related to changes in the composition of the chemical

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compounds present in the sample.

Distinguishing the FTIR spectrum of a species with different growth locations will be very difficult because the difference is only in the intensity of the signal produced, so chemometric assistance is needed to make it easier to distinguish (8). Chemometric support expands the potential of FTIR spectroscopy as an alternative method for analyzing plant components.

This study aims to compare the component profiles of *Centella asiatica* *L.* leaf compounds using FTIR based on the geographical location of the sampling location extracted with variations in the solvent.

RESEARCH METHODS

Tools and Materials

The tools used in this research include aluminum foil, UV lamp, TLC plate, pyrex, capillary tube, dropper pipette, iron horn spoon, silica gel, tissue, glass jar, FTIR Thermo Fisher Scientific, device engineering tools software, namely Acer with the following specifications: *Intel® Core™* i3-6006U CPU @ 2.00GHz (4 CPUs), ~2.0GHz, Memory 4 GB DDR3L, system type Acer One 14 Z476 and the software used is *Metaboanalyst 5.0,* minitab version 16 and origin 2019b.

The materials include Pegagan (Centella asiatica L.) leaves, 70% ethanol, ethyl acetate, N-hexane, and KBr powder.

Sample Preparation

Then, items obtained from several places are sorted wet to separate dirt or foreign material. Then it was washed with running water to remove soil and other impurities attached to the sample and dried using the simplicia oven at 50 °C. Finally, dry sorting was done to separate the dirt and Simplicia damaged by the previous process to obtain good quality dry Simplicia (9).

Preparation of Extracts

Each Centella asiatica L. leaf from several places has been dried, mashed, and sieved with a mesh of 18. Each was weighed and then put into a maceration vessel, after which solvent was added (70% ethanol in the first container at a ratio of 1:10, ethyl acetate in the second container at a ratio of 1:10, and n-hexane in the third container at a ratio of 1:10) sufficient for the wetting process. The samples were allowed to stand for ± 15 minutes. Then, the solvent was added again until all the Centella asiatica L. leaf Simplicia was completely submerged. Maceration was carried out for three days while stirring occasionally. The maceration vessel was tightly closed and stored protected from direct sunlight after maceration was filtered to separate the filtrate and the dregs. Then the dregs are repressed. Finally, the liquid extract was combined and evaporated using a rotary evaporator to obtain a thick extract (10).

Identification of Extracts by TLC Method

Analysis was carried out using the stationary phase of an aluminum TLC plate coated with 60 GF 254 silk gel and the mobile phase of n-hexane: ethyl acetate (5:5). The TLC plate was cut at the bottom edge marked 1 cm, and the top edge 0.5 cm. After activating the silica gel plate in the oven at a temperature of 110 °C for 30 minutes, the chromatography chamber is saturated with the mobile phase using filter paper. After that, the sample is spotted using a capillary tube on the silica gel TLC plate GF254 with a spotting distance of 1 cm. Next, insert the TLC plate into the chamber containing a saturated mobile phase solution and observe the movement of the mobile phase until it reaches the top line. Then the chamber is opened, after which the TLC plate is taken and aerated. The resulting chromatogram was observed for stains under ultraviolet (UV) light at 254 nm and 366 nm (11).

Measurement by FTIR

A certain amount of Centella asiatica L. leaf extract was then mixed uniformly with KBr to form pellets using a press. This process lasted for 10 minutes. Finally, the pellets were put into the sample container and measuredusing FTIR. Then the spectrum obtained from the measurement results is stored using the appropriate name (12).

Data Analysis

The obtained FTIR spectrum results were processed using a chemometric analysis program using the metaboanalyst 5.0 program. The FTIR data is then processed using the Minitab version 16 application and the origin program (13).

Results And Discussion

This study used Centella asiatica L. leaves obtained from 4 locations where it grew: Bengo District, Bone District, District, Gowa District, Cenrana District, Maros District, and Kuncio Pao District, Gowa District. Sampling in this study took into account the geographical origin and altitude, as shown in table 1. The geographical origin of the plant will affect the levels of bioactive compounds, which are the general criteria in selecting quality herbal medicinal raw materials to ensure consistency of efficacy. At the same time, the altitude is 0-2,500 meters above sea level and a slightly humid environment, either exposed to sunlight or protected from it, is an excellent place to grow Centella asiatica L. (14). It is known that differences in soil type and climate in the area where plants are cultivated can affect the content

of bioactive compounds in medicinal plants. These differences can result from discrepancies in quality and efficacy (15). Therefore, knowing the geographical origin of plant growth is very important because it affects the number of bioactive compounds, which has developed into a common standard for selecting high-quality raw materials for herbal medicines to ensure consistency of efficacy (3,16).

Centella asiatica leaf Simplicia was extracted by the maceration method with various solvents. The maceration method was chosen because it is simpler, accessible, and without heating (10). The extraction solvent is one of the most important factors influencing the efficient extraction of bioactive compounds from plant materials and their consequent health benefits (17). Selectivity, capacity to extract, toxicity, ease of evaporation, and solvent cost are some of the variables that must be considered when selecting the type of solvent. In addition, the selected component's polarity is considered when adjusting the extraction solution. According to the principle of like dissolves like, a solvent will usually dissolve substances that have the same degree of polarity, according to the law of equality. For example, polar solvents will dissolve polar compounds and vice versa (18,19). As for the variation of the solvent used with different levels of polarity, namely 70% ethanol, ethyl acetate, and N-hexane, the difference in solvent extraction can affect the totalcontent of bioactive compounds contained in the extraction results (5).

The first step is thin layer chromatography (TLC) analysis to provide an initial description of the compound profile based on the chromatogram pattern (thin layer chromatography). At this analysis stage, each extract was eluted using N-Hexane: ethyl acetate (5:5) as an eluent to observe the separation of compounds from each sample. Based on the results of TLC using N-Hexane: ethyl acetate (5:5) eluent, there is a separation of stains in each extract, which can be seen from the difference in the height of the stain and the color produced in each extract from each sample. The chemical components move up to follow the mobile phase because the adsorbent's absorption of the chemical components is not the same, so that the chemical components can move at different distances based on their level of polarity. Based on the TLC profile in Figure 1, It can be seen that there are three groups of stains shown on the TLC plate, but this cannot be concluded only from the staining pattern shown. To clarify the differences, all samples of Centella asiatica *L.* extract were analyzed using FT-IR to see the spectral pattern indicated by the instrument.

The results of reading the infrared spectrum profile of Centella asiatica *L.* leaf extract with various filters from different locations using FTIR with a wave number of 4000-400 cm^{-} The results of the FTIR spectrum showed relatively similar results based on the spectrum pattern of each filter variation. This statement is shown in Figure 2. different absorption intensities. Therefore, direct observation of the spectrum is not enough to see differences in geographic origin, so we proceed to chemometric analysis to see differences in the profile of the compound components of Centella asiatica *L.* leaf extract with variations in the filter from the four locations.

PCA analysis was carried out because PCA analysis is one of the chemometric techniques that can be used to analyze the data obtained. We can perform pattern recognition for grouping Centella asiatica *L.* leaf extract with the test sample. The distance between the samples in the analysis (PCA) shows the similarity between the samples. The closer the distance between one and the other, the greater the similarity is obtained (12). The principle of PCA is to find the principal component, a linear combination of the original variables. These principal components are chosen so that the first principal component has the next most considerable data uniformity (20). The results of the PCA analysis can provide information that PC1 has the most significant data uniformity. The results of PC1 in Figure 3. (a) can explain 54.2% of data uniformity, while PC2 in Figure 3. (a) can explain 9.6% of data uniformity. Both components PC1 and PC2 in Figure 3. (a) represent 63.8%, which means that two PCs represent 63.8% of all data in Figure 3. (a). the PCA results in Figure 3. (b) show that PC1 results explain 50.2% of data uniformity while PC2(b) explains 17.9% of datauniformity. The results of PC1 and PC2 represent 68.1% of all data. In comparison, in Figure 3. (c), on PC1, 48.9% uniformity of data, while on PC2 explains 16.7% uniformity of data, the results of PC1 and PC2 represent 65.6 % uniformity of all data. It can be seen that the sample of Centella asiatica *L.* extracts using 70% ethanol solvent in Figure 3 (a) clearly shows the grouping based on the sampling location. This shows the characteristics of the 70% ethanol extract of the guava leaves taken from 4 locations. There are differences based on the groups shown in the figure. 3(a). The N-Hexane extract sample of Centella asiatica *L.* in Figure 3 (b) also shows different characteristics among the four sampling locations. In Figure 3 (c), two sampling locations show the same characteristics.

Table 1. Geographical Location of Sampling.

No.	Area	Geographical Location	Altitude area (mdpl)
1.	Bengo (Bone)	4° 37'29"S 119° 59'58"E	87 mdpl
2.	Cenrana (Maros)	5° 23'41"S 119° 50'27"E	376 mdpl
3.	Bungaya (Gowa)	4° 58'34"S 119° 47'05"E	892 mdpl
4	Tombolo Pao (Gowa)	5° 11'51"S 119° 55'31"E	1077 mdpl

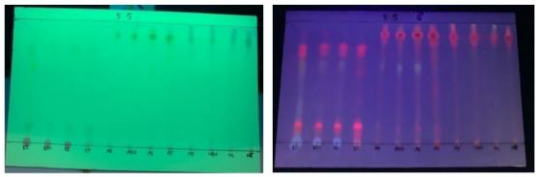

Figure 1. TLC Profile of C. asiatica L. Leaf Extract

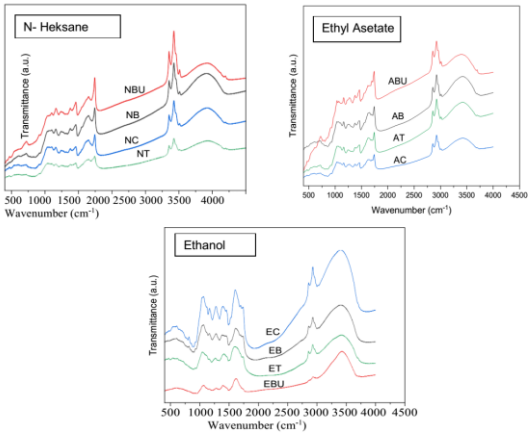

Figure 2. IR spectra of extracts from four growing area, EC, AC, AND NC (Cenrana), ET, AT AND NT (Tombolo Pao), EB, AB AND NB (Bengo) and EBU, ABU and NBU (Bungaya).

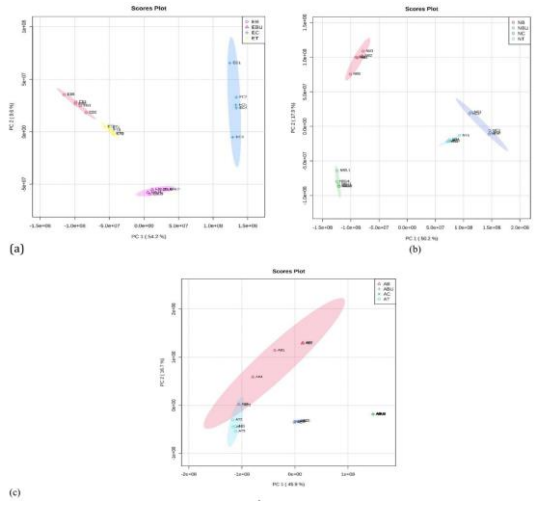

Figure 3. Plot score in the wavenumber range 4000-400 cm-1 from the main two PCs in extract ethanol 70% (a), extract Ethyl Acetate (b) and exctract N-Heksane (c).

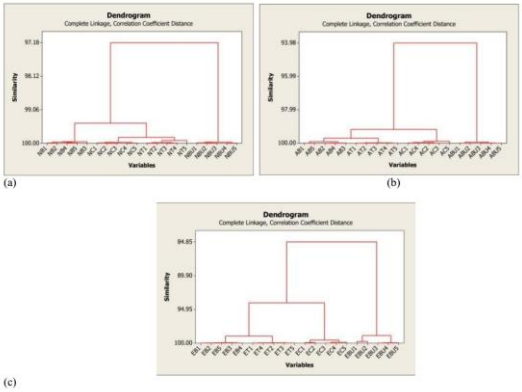

Figure 4. Cluster analysist in extract N-Heksan (a), Ekstract Ethyl Acetate (b), and extract Ethanol 70%

The dendrogram analysis aims to determine the weight of the differentiating value in each separation. The dendrogram results in the cluster analysis were based on the similarity between the variables used. The cluster analysis on the dendrogram diagram shows the greater the value of similarity (similarity) indicated by the lower the line connecting the types of samples (variables), the smaller the difference in chemical characteristics possessed. Based on the results obtained after analyzing the dendrogram of the cluster results, Figure 4 (c) shows the grouping of samples from 4 locations of Centella asiatica *L.* leaf growing against 70% ethanol extract, indicating that the locations of bengo and buttonopao have similarities, namely 98.95%, which is classified into group I. Likewise, the location of cenrana has a similarity. The similarity of 93.85% was classified into group II, while the location of the bungaya had a similarity of 84.85%, classified into group III. While the results obtained after analyzing the dendrogram of the cluster results in Figure 4 (b) show the grouping between samples from 4 locations of Centella asiatica *L.* leaf growing against the ethyl acetate extract of Centella asiatica *L.* leaves show the bengo and buttonopao locations have similarities, namely 99.71%, which is classified into group I, the location of cenrana has a similarity of 99.17%, which is classified into group II. In contrast, the bungaya locations with 93.98% similarity were classified into group III. And in the results obtained after analyzing the dendogram of the cluster results in Figure 4 (c), the grouping between samples from 4 locations of Centella asiatica *L.* leaf growing against the n-hexane extract showed 99.84% of the cenrana and buttonopao locations, which were classified into group I, the bengo location had 99 similarities, 44% were classified into group II, and the location of the bungaya had a similarity

of 97.18% and was classified into group III.

CONCLUSION

From the results of this study, it can be concluded from the results of the TLC profile that there are three groups of spot stains produced. This is in line with the results of the dendrogram analysis, where several clusters are shown. The component profiles of Centella asiatica L. leaf compounds based on geographical location and variations in the filter solution have different shapes with similar groupings, which showed PCA results on 70% ethanol extract, ethyl acetate, and n-hexane. As we can see from the PCA results of Centella asiatica, solvent polarity and geographic location differences affect the compound profile.

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