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The Polarity Identification of Secondary Metabolite Compounds from Ethanol Extracts of Akar Bulu (*Merremia fitovilia*) Leaf through Thin Layer Chromatography (TLC) Analysis

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Abstract

This study aims to determine the solubility of secondary metabolites from ethanol extract of *akar bulu* (*Merremiafitovilia*) leaf. The research method through the stages of sample preparation in the form of sample collection, drying, and sieving; extraction using ethanol solvent and qualitative testing of solubility of secondary metabolites using thin layer chromatography (TLC). The results showed that the secondary metabolites from ethanol extract of *Merremiafitovilia* leaf were notinsoluble in n-hexane solvents, dissolved in chloroform and ethyl acetate solvents.Separation of secondary metabolites is most effective by using chloroform solvents.

Keywords: *extraction; etilacetat; cloroform; cromatografi; merremiafitovilia*

1. Background

Indonesia is a tropical country with the second largest source of biodiversity after Brazil. Indonesia is thought to have around 30.000 medicinal plants from 40.000 types of medicinal plants in the World (Salim, Z., and Munadi E., 2017). Utilization of Indonesian tropical plants which has medicinal properties has been done since long time ago and has become a national cultural heritage that must be maintained. The use of plants as medicine in general is based on traditional experience for generations without being accompanied by scientific research. Therefore, proof through scientific research about the efficacy of plants as medicine needs to be done. Utilization of medicinal plants consists of pure bioactive compounds or mixtures in extract form. These compounds are obtained from various parts of plants such as leaves, bark, wood, roots, fruit, or parts of fruit or herbs (Saifudin et al., 2011).

One of the efforts to develop herbal medicine towards *phytofarmaca* that has been done is to isolate bioactive compounds from medicinal plants through extraction and chromatography methods. Extraction method is carried out to obtain medicinal plant extracts, where the extract is still in the form of a mixture of compounds while the chromatography method is carried out to separate the components of secondary metabolites from an extract.

One of the simplest chromatographic methods is thin layer chromatography (TLC). Thin Layer Chromatography is the separation of a mixture into pure compounds and the quantity can be known. Displacement of components of a compound separated by this

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chromatography depends on the type of solvent, absorbent and its absorption properties for each dissolved component that will be carried by the mobile phase (solvent) through the absorbent stationary phase with different transfer rates (Susanti, 2001). The use of TLC has several advantages, namely separation can be done quickly, substances that are acidic or strong bases can be used, the analysis can be done more sensitive with simple tools so that its use is easy. In addition, this method is simple, fast in separation, sensitive, and easy to recover separated compounds (Khopkar, 2002). The TLC results are Rf values that describe the physical characterization of compounds contained in an extract or sample.

In 2017, Sukarti conducted phytochemical tests on *Merremiafitovilia* plants. *Merremiafitovilia* plant is believed by the Tana Luwu community as a drug that can accelerate wound drying in diabetics. Theresults are known that these plants contain flavonoids compounds, alkaloids, phenolic, terpenes and steroids. Further research was conducted to test the characterization of secondary metabolite compounds in *Merremiafitovilia* plants through TLC to determine solubility, selection of effective solvents for the isolation stage and the chances of types of secondary metabolite compounds that can be isolated based on the color of the stain that appears on the TLC plate.

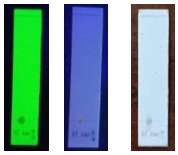
2. Methods

Silica gel plates measuring 20x20 cm are cut to the size of 5x1 cm. The plates were marked with a bottling line using a pencil at the bottom with a distance of 1 cm and an elution line of 0.5 cm at the top. Next the chamber and its cover are prepared and the eluent is inserted into the chamber then saturated. After that, bottling is done by taking a little extract using a capillary tube and then bottled on a TLC plate that has been given a lower limit of 1 cm and an upper limit of 0.5 cm. The plate is inserted into a chamber that already contains eluents. If the eluent has reached the upper limit of the silica gel plate, then the plate can be removed, then the plate is observed under UV lamps of 254 nm and 366 nm. In this study three types of eluents were used, namely 100% ethyl acetate, 100% chlorophyll and 100% n-hexane.

3. Results and Discussion

Hexane 100% Eluent

The TLC test results using 100% hexane eluent at UV λ 254 nm and λ 366 nm showed the presence of a spot of brown fluorescent stains and an Rf value of 0. The same thing happened on plates that had been sprayed with serum sulfate and heated, still showing the presence of one point of fluorescent stain chocolate with an Rf value of 0. The presence of an Rf value of 0, both at UV λ 254 nm and at λ 366 nm indicates that the secondary metabolite compound of *Merremiafitovilia* extract does not have a soluble compound in n-hexane. Thus the compound components in the extract have higher polarity than n-hexane. Further research for the separation of compound components in extracts cannot be carried out using 100% n-hexane, but mixing with other polar solvents such as alcohol, ethyl acetate and chloroform must be carried out.

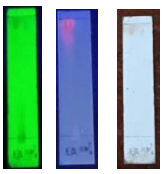


(a) (b) (c)

Figure 1. Chromatogram (n-hexane 100%
(a) UV λ 254 nm (Short wave); (b) UV λ 366 nm (Long wave); c. After heated

Ethyl Acetate 100% Eluent

The results of TLC analysis using 100% ethyl acetate eluent showed the presence of two spots of brown fluorescent stains on UV lamps λ 254 nm with Rf values of 0.93 and 0. Whereas on UV lamps λ366 nm showed the presence of one spot with red fluorescent stains and an Rf values of 0.93. On a heated plate, it shows a spot of brown fluorescent stain with an Rf value of 0.93. The existence of a very high Rf value indicates that the compound component in the ethanol extract of *M. fitovilia* is very soluble in ethyl acetate. However, this eluent is not effectively used in chromatographic methods for the isolation of compounds because it does not provide a good separation system. This is indicated by the presence of an Rf value on the TLC plate. Chromatograms from TLC results of an extract should have various Rf values that represent the solubility of the compound components in the extract. A glowing red stain indicates that the extract contains flavonoids compounds, while a brownish color after burning indicates that the extract contains compounds that are easily oxidized.



(a) (b) (c)

Figure 2. Chromatogram (100% Ethyl Acetate)
(a) UV λ 254 nm (Short wave); (b) UV λ 366 nm (Long wave); c. After heated

Chloroform 100% Eluent

The results of the analysis of TLC by using 100% chloroform eluents showed a brown spot fluorescence spot at UV λ 254 nm with an Rf value of 0, whereas in UV lightsλ 366 nm showed the presence of five red spot blemishes and Rf value of 0.8 respectively. ; 0.5; 0.4; 0.3 and 0. The heated plate shows a spot of brown fluorescent stain with an Rf value of 0. The presence of five Rf values that appear on the TLC plate under UV UV lamps λ 366 nm indicates that the chloroform solvent has the ability to separate component compounds from extracts ethanol *M. fitovilia*. Separation of the compound components from the extract can be obtained at least 5 classes of compounds based on the stain on the TLC plate. The Rf value of each stain on the chromatogram is very specific to the component's polarity. The luminescence of colors that only appear under UV light λ366 nm indicates that the compound components of the extract have a conjugated group. The electron conjugation of a compound that causes it to absorb dyes at certain wavelengths. This conjugation can be found in phenolic compounds, flavonoids compounds and other compounds that have aromatic groups. In addition, on UV lamp λ366 nm the stain will fluoresce, and the plate will be dark in color. The appearance of the stain on the UV lamp λ366 nm is due to the interaction between the UV rays and the chromophore group which is bound by the auxochromes present in the stain. Visible light fluorescence is the emission of light emitted by these components when electrons that are excited from the base energy level to a higher energy level then return to their original state while releasing energy. So that the stain that appears on the UV lamp λ366 nm looks bright because the silica gel used does not fluent to UV lampλ366 nm (Gibbons, 2006). Based on the results of TLC analysis using 3 kinds of eluents, it can be seen that the eluent that is best used for the process of separating the components of secondary metabolites from the ethanol extract of *M. fitovilia* is a chloroform solvent. This is in accordance with the theory that a good eluent is an eluent that can separate compounds in large quantities is marked by the appearance of a clear stain. The stains that form are tailless and the distance between one stain is clear (Harborne, 1996). The

characteristics of secondary metabolites from the ethanol extract of *M. fitovilia* based on TLC analysis are shown in Table 1.

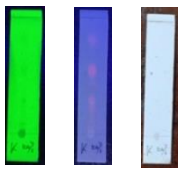


Figure 3. Chromatogram (100% CHCl3)
(a) UV λ 254 nm (Short wave); (b) UV λ 366 nm (Long wave); c. Serum sulfate + heating

Table 1. Characteristics of compounds from *M. fitovilia* leaf ethanol extract based on TLC analysis

UV / **Eluen**	Number of Stains and Rf Value (cm)		
	Ethyl acetate 100%	Chloroform 100%	Hexane 100%
UV λ 254 nm *(Short wave*	1 stain; Rf 0 (brown)	1 stain; Rf 0 (brown)	1 stain; Rf 0 (brown)
UV λ 366 nm *(Long wave);*	1 stain; Rf 0,93 (red, tailed	5 stain; Rf0,8; 0,5; 0,4; 0,3 (red) dan 0(brown)	1 stain; Rf 0 (brown)
Serum sulfate + heating	1 stain; Rf 0,93; (brown, tailed)	1 stain; Rf 0 (brown)	1 stain; Rf 0 (brown)

4. Conclusion

Based on the results of TLC analysis of the ethanol extract of *akar bulu* leaf (*M. fitovilia*) it can be revealed that the compound components can be dissolved in ethyl acetate and chloroform but not soluble in n-hexane solvent. The most effective solvent used as a reference in selecting eluents in the process of separating components through chromatography is chloroform solvents.

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