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Isolation and Identification of Ethyl Acetate Fractions in Flour Banana Fruit (*Musa troglodytarum* L.)

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***Abstract*— The use of traditional medicine is recommended by WHO to maintain of peoples health, preventing and treatment of diseases. Indonesia as a country that has biodiversity of banana, one of them is a tongka langit banana. *Musa troglodytarum* L. better known as banana tongka langit is one of the banana plants used by the community around Tasikmalaya to overcome various diseases such as diabetes mellitus, immune enhancers and overcome anemia. Previous research stated that the administration of ethyl acetate fraction from tongka langit banana can reduce blood glucose levels in mice induced by alloxan. Based on that, further study is needed to isolate and identify the compounds in the ethyl acetate fraction of ethanol extract of banana fruit tongka Langit (*Musa troglodytarum* L.). Simplicia maserated using ethanol 70%, then fractionated with LLE method using n-hexane, ethyl acetate and water, ethyl acetate fraction was isolated by using classical column chromatography and preparative thin layer method, and ensuring the isolation purity using two-way thin layer chromatography. Compound identification methods include organoleptic, thin layer chromatography (TLC) with specific spotting appearance, infrared spectrophotometry and mass spectroscopy. From the study four isolates were produced, but the MT-II-2 isolates had more results and were possible to be identified. The results of the identification group compound showed that MT-II-2 isolates were thought to be steroid. Infrared spectrophotometry results showed that isolates had functional groups C-O (Aldehyde), C-O (Carboxylate / Alcohol), C-H (Alkana), C-H Bend, C = C, C = O (Aldehyde), C-H and O-H. and mass spectroscopy results that the isolate has a molecular weight of 507,1436 m / z with predictions of the molecular formulas $C_{31}H_{23}O_7$, $C_{38}H_{19}O_2$, $C_{24}H_{27}O_{12}$.**

Keywords:* Musa troglodytarum L., *isolation, steroid

I. INTRODUCTION

Plants that are widely used as a treatment are bananas, in Indonesia alone more than 230 types of bananas are spread in various regions. In general, the content of vitamins, minerals, carbohydrates, fats and fiber is often used as a medicinal ingredient and raw material for the health industry. One of the bananas which is quite often used for treatment is Banana Tongka Langit with the Latin name *Musa troglodytarum* L. Based on previous studies, banana tongka sky contains carotenoid compounds. The benefits of carotenoids themselves can be used as a source of vitamin A, food coloring, food additives, enhancement of red blood cells, antibacterials, antidiabetic, antioxidants, immune enhancers, and can replace damaged cells in the body [1]. The antioxidant compound that is dominant in the banana tongka sky is β-carotene, besides being a precursor of vitamin A it is also categorized as a secondary antioxidant. Secondary antioxidants have a function in capturing free radicals and prevent lipid peroxidation which lead to atherosclerosis where vascular complications occur in diabetics. Based on research, consuming vegetables and fruits containing high carotenoids (lutein and β-carotene) can maintain blood glucose levels in healthy respondents, thus protecting against hyperglycemia [2]. In addition, previous research suggests that long-term administration of low-dose beta carotene can reduce blood glucose levels in diabetic induced mice [3]. Research on bananas in the sky is the presence of carotenoid content in crude banana extract conducted by Samson, et al., 2013. And testing of anti-diabetic activity in mice that have been induced using extracts and fractions of n-hexane, ethyl acetate and water, with the results of ethyl fraction significant acetate in reducing blood glucose levels in mice. Therefore, further research is needed to identify the compounds of ethyl acetate in *Musa troglodytarum* L.

II. MATERIAL AND METHOD

A. Plant Material

Plant material is the fruit of the plant Tongka langit banana (*Musa troglodytarum* L.) collected from the Galunggung Tasikmalaya mountain area.

B. Chemicals

The chemicals used were aquadest, acetone, hydrochloric acid (Agung Menara Abadi), iron (III) chloride (Merck), 70% ethanol (Merck), ether (Merck), ethyl acetate (Merck), 5% potassium hydroxide, gelatin 1%, methanol, KLT plate, dry potassium bromide, chloroform (Merck), standard beta carotene (Sigma Aldrich), n-hexane, sodium acetate, Dragendroff reagent (bismuth (III) nitrate in 10M hydrochloric acid), Liebermann-Buchard reagent.

C. Chemicals

This research was conducted experimentally in the Laboratory with the following research lines:

1) Material Collection and Determination

a) Material Processing

The banana plants that have been obtained are then processed into simplicia through several stages, namely, pedaling, wet sorting, drying, and dry sorting [4].

EXTRACTION:

By maceration of 200 grams dried simplicia using ethanol 70%

FRACTIONATION:

By Liquid-liquid extraction of 10 grams thick extract.

PHYTOCHEMICAL SCREENING:

The screening method used is a modification of the Farnsworth method, 1966 [5].

b) Column Chromatography

Column preparation is done by passing the mobile phase in the column and then let it stand for one day. The eluent used for the column consists of two gradients namely gradient 1 (n-hexane: ethyl acetate) and gradient 2 (ethyl acetate: methanol). Column chromatography results are monitored using thin layer chromatography with the developer that has been optimized first. Furthermore, the plate is inserted into a chromatographic vessel which is saturated with the mixture the developer reaches the development deadline on the chromatographic plate that has been previously marked. The chromatographic plate is then removed from the vessel and dried. Then, the chromatogram was observed in visible light, UV light 254 nm and 366 nm. The subfraction results with the same TLC pattern are combined into a subfraction combination (S.C).

c) Identification of Isolates

The isolates produced from the isolation process will then be identified by several methods, namely using Infrared spectrophotometry, and mass spectrometry [6,7].

III. RESULTS AND DISCUSSION

TABLE 1: RESULTS OF THE TLC PATTERN OF N-HEXANE, ETHYL ACETATE, WATER AND BANANA EXTRACT

Sample	Rf	UV 254 nm	UV 366 nm	Visible Rays
N-Hexane fraction	1. 0.66 2. 0.76 3. 0.98	-	1. faint blue 2. faint blue 3. bright blue	-
Ethyl Acetate fraction	1. 0.26 2. 0.36 3. 0.66 4. 0.76 5. 0.98	-	1. Faint blue 2. faint blue 3. bright greenish 4. faint blue 5. bright blue	-
Water Fraction Extract	1. 0.36 2. 0.66	-	1. faint blue 2. blue is bright enough	-

The spectrum obtained is presented in the form of a curve between the relative index and the molecular weight of the fragment. The following picture of the resulting spectrum:

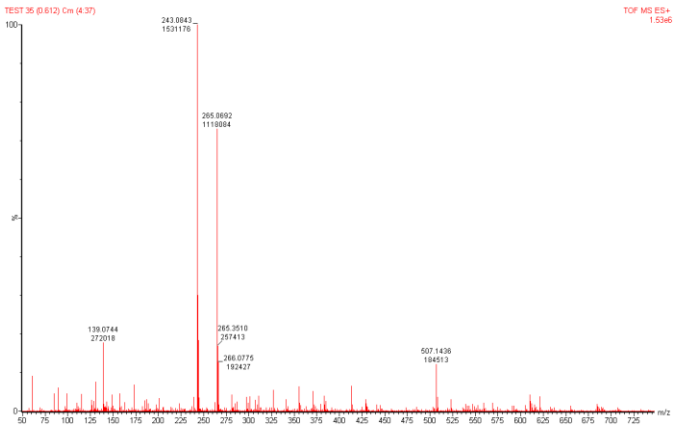

Figure 1: MS Spectrum of isolate

At the base of the ion peak with a molecular weight of 243.0843 m/z and molecular ion peaks weighing 507.1436 m/z. Based on the fragmentation pattern found in the results of the spectrum shows that the presumption compound may have a molecular formula $C_{31}H_{23}O_7$, $C_{24}H_{27}O_{12}$ or $C_{38}H_{19}O_2$.

IV. CONCLUSION

From the results of the study it can be concluded that the process of compound isolation carried out through extraction, fractionation, purification methods by classical column chromatography, preparative thin layer chromatography and purity testing. Obtained 4 relatively pure isolates namely MT-II-1, MT-II-2, MT-II-3, MT-II-4. The results of identification of MT-II-2 isolate compounds have green powder/crystal characteristics, odorless and weighing 2.5 mg. Identification of groups is assumed that isolates are steroid groups supported by the results of infrared spectrophotometry showing that compounds have CO (Aldehyde) groups, CO (Carboxylate/Alcohol), CH (Alkane), CH Bend, C = C (Aromatic), C = O (Aldehyde), CH and OH. As well, mass spectroscopy produced a compound isolate having a molecular weight of 507.1436 m / z with the estimated molecular formula $C_{31}H_{23}O_7$, $C_{38}H_{19}O_2$, or $C_{24}H_{27}O_{12}$.

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