Determination of Total Flavonoid Levels of Ethanol Extract of Sweet Potato Leaves (*Ipomoea batatas* L.)

Elisma, Yorida Febry Maakh, Falentinus Duly
Health Polytechnic of Kupang
Email: elismasinulingga@gmail.com

Abstract

**Introduction:** Dengue hemorrhagic fever (DHF) caused by the dengue virus carried by Aedes aegypti and Aedes albopictus mosquitoes as a vector to the human body through the bite of the mosquito. **Method:** Sweet potato leaves taken were made into the dried material and extracted using the percolation method using 70% ethanol until a thick extract is obtained. Tests are carried out microscopically on the dried material. The qualitative identification, testing extracts specific gravity and measurement of total flavonoid levels on the extract. **Results:** Testing of Specific Parameters observed is organoleptic identity of the extract produced is Color: blackish green, Smell: No smell and Shape: Thick. Qualitative Test with Thin Layer Chromatography, the sample contained flavonoid compounds in flavone and flavonol groups. Total flavonoid levels were measured as equivalent to standard quercetin based on the standard quercetin curve. The result of total flavonoid content from ethanol extract of sweet potato leaves was 31630 mg QE / 100 g extract. **Suggestion:** Total flavonoid content of 3163mg QE / 100g or 31.63% b / b.

**Keywords:** Determination of Total Flavonoid Levels Sweet Potato Leaves

*Correspondence: elismasinulingga@gmail.com
Present Address: Penfui, East Nusa Tenggara-Indonesia

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INTRODUCTION

Dengue hemorrhagic fever (DHF) is a disease that can affect children and adults. Data from all over the world shows Asia ranks first in the number of sufferers (DHF). In Indonesia, Dengue Fever was first discovered in the city of Surabaya in 1968, where as many as 58 people were infected and 24 people died (Mortality Rate (AK): 41.3%) and since then, the disease has spread throughout Indonesia (Hastuti 2008).

DHF is a disease that can affect children and adults with the main symptoms of fever, muscle and joint pain, which usually worsen in the first two days (Mansjoer et al, 1999). DHF is caused by the dengue virus carried by Aedes aegypti and Aedes albopictus mosquitoes as a vector to the human body through the bite of the mosquito. The dengue virus that is transmitted by Aedes aegypti enters the human body attacks monocytes, one type of white blood cell that plays a role in building the body’s immunity. As a result, platelet levels in the blood will decrease. If the platelet count is reduced, it will cause bleeding, even death. Not infrequently dengue fever sufferers also experience shock or often called dengue shock syndrome (Suharmiati, 2007).

Thrombocytes are one of the blood cell elements that play a role in the body’s homeostatic process by forming platelet plugs when blood vessel injuries occur. In situations where platelets below normal values can lead to severe bleeding, blood flow to the tissues decreases and cause shock. The shock that is not treated seriously will cause acidosis and anoxia which can end in death.

Corticosteroid drugs are initially the main treatment of treating thrombocytopenia in acute and chronic cases of Idiopathic Thrombocytopenia Purpura (ITP). However, epidemiological studies conducted by McMillan (2002) show that 25-30% of the study population did not experience a significant increase in platelet count after corticosteroid administration. In addition, WHO also no longer recommends the use of this drug as a therapeutic option for dengue and there are no drugs that can paralyze the dengue virus, which can be done by giving an infusion as early as possible to prevent
blood circulation failure that can lead to death. Seeing this, research to find new compounds that are effective in increasing platelet counts still needs to be done, including through research on medicinal plants that are traditionally used by the community to increase platelet counts. Research on “Effect of Giving Guava Leaf Extract on Increased Thrombocyte Amount in DHF Cases in Children” in RSUD dr. Soetomo Surabaya showed that the group given guava leaf extract increased the platelet count faster than the control group (Nasiruddin, 2005). In addition to guava, the plants commonly used are sweet potato leaves, sweet potato leaves have long been used traditionally in the Philippines to increase platelet counts in DHF sufferers. In Indonesia itself, especially in East Nusa Tenggara, sweet potato leaves are used as vegetables and animal feed because people do not yet know the benefits of sweet potato leaves in handling DHF.

Previous research conducted by researchers with the title Test of Ethanol Extract of Sweet Potato Leaves (Ipomoea batatas L) on Increasing the Number of Platelet Cells in the Swiss female mice in 2013 showed that sweet potato leaf extracts had an activity of increasing platelet count in swiss female mice with Maximum concentration of Sweet Potato Ethanol extract (Ipomoea batatas L) 20% b / v with platelet count 142.8 x 10³ / mm³-185.2 x10³ / mm³ calculated against the decrease in platelet count by inducing sodium phenytoin. The 2015 study entitled Test of Ethanol Extract Ethyl acetate Fraction, chloroform fraction of Ipomoea batatas L Leaves L) on Increased Thrombocyte Amount in Swiss Strain Mice with results having an increase in platelet counts with optimal concentration of 20% b / v with cell count platelets 332.8 x 10³ / mm³ 248 x10³ / mm³- 339.6 x10³ / mm³ was calculated against the decrease in platelet count using sodium phenytoin induction. The study in 2016 entitled Anti-Oxidant Activity Test of sweet potato leaf tea (Ipomoea batatas L.) showed leaf tea sweet potato has weak intensity antioxidant activity with IC50 value of 446-1271 ppm because> 150 ppm, powder size affects the antioxidant activity of sweet potato leaf tea bags.
(Ipomoea batatas L.), the smaller the powder size the lower antioxidant activity, addition of Sariwangi tea powder affect the antioxidant activity of tea bags of sweet potato leaves. Based on the explanation above in 2017 the researchers were interested in examining the Determination of Total Flavonoid Levels of Ethanol Extract of Sweet Potato Leaves (Ipomoea batatas L.) to determine and calculate the total flavonoid content of ethanol extract of sweet potato leaves (Ipomoea batatas L.) using AlCl3 colorimetric spectrophotometry.

**RESEARCH METHODS**

The type of research conducted is descriptive. The research was conducted at the Kupang Ministry of Health Polytechnic Pharmacy Study Program in June-October 2017. The variable in this study is a single variable which only measures the total flavonoid content of ethanol extract of sweet potato leaves (Ipomoea batatas L ...). The population in this study was the sweet potato leaves taken in Baumata village, Taebenu district. The sample used in this study is sweet potato leaves that are not old and too young. The sampling technique used in this study is Purposive sampling, which is sampling based on certain criteria by taking sweet potato leaves that are not old and not too young.

The tools used in this study are percolator, blender, glassware, analytic balance (EW 220-3 NM type), volumetric pipette, filer, aluminum foil, UV-Vis spectrophotometer (Shimadzu type W-1700), 60 mesh sieve, a rotary evaporator (Eyela), water bath (type 1042). The materials used in this study were sweet potato leaves, silica gel GF254, quercetin, ethyl acetate, methanol, water, 70% ethanol, 96% ethanol, AlCl3, NaOH pa, FeCl3, concentrated H2SO4, Mg pa powder, concentrated HCl, distilled water, NaNO2. Chloroform ammoniacal and Mayer reagent.

Sweet potato leaves are taken from Baumata village in Taebenu sub-district with purple bulbs. Sweet potato leaves taken were made into the dried material and extracted using the percolation method using 70% ethanol. Maserati obtained by applying concentrated using a rotary evaporator and above the water bath until a thick extract is obtained.
Tests are carried out microscopically on the dried material. The qualitative identification of ethanol extract of sweet potato leaves was identified by identification of flavonoids, terpenoids, saponins, and phenolics.

Testing extracts specific gravity. The process is the clean, dry pycnometer weighed. Then calibrated by setting the pycnometer weight and the weight of the water at 250°C then weighing (W1). The liquid extract is set to about 200°C and then put into a blank pycnometer, discarding the excess extract, adjust the temperature of the pycnometer which has been filled to a temperature of 250°C then weighed (W2) (Anonim, 2000).

Measurement of total flavonoid levels was carried out by making quercetin mother solution, determining the maximum wavelength, making a calibration curve, making blank solutions. Making quercetin mother solution was carried out by weighing 20 mg quercetin dissolved in a 50 mL volumetric flask with 96% ethanol to the mark (400 ppm). Determination of the maximum wavelength by piping 10 mL from the mother liquor quercetin 400 ppm and put it in a 50 mL volumetric flask then add 2 mL distilled water and 0.15 mL 5% NaNO2, then let stand for 6 minutes. Add 0.15 mL AlCl3 10% to the solution, then let stand for 6 minutes. The solution was reacted with 2 mL of 4% NaOH and then diluted with distilled water until the total volume was 50 mL and allowed to stand for 15 minutes. Measured maximum absorption at wavelength 380-560 nm. Making a calibration curve is done by piping a 400 ppm quercetin into a 50 mL volumetric flask with a concentration of 50 ppm, 60 ppm, 70 ppm, 80 ppm, 90 ppm, and 100 ppm. 0.5 mL of each solution concentration was reacted with 2 mL distilled water and 0.15 mL 5% NaNO2, then let stand for 6 minutes. Add as much as 0.15 mL AlCl3 10% to the solution, then leave it back for 6 minutes. The solution was reacted with 2 mL of 4% NaOH and then diluted with distilled water until the total volume was 5 mL and let stand 15 minutes. Then the absorbance was measured at a maximum wavelength of 418.80 nm.

Making a blank solution is done by means of 0.5 mL 96% ethanol reacted
with 2 mL distilled water and 0.15 mL NaNO25% then let stand for 6 minutes. Add 0.15 mL AlCl3 10% to the solution, then let stand for 6 minutes. The solution was reacted with 2 mL of 4% NaOH, then diluted with distilled water until the total volume was 5 mL and allowed to stand for 15 minutes. The absorbance of the standard solution is measured at the maximum wavelength obtained.

Making a sample solution of ethanol extract of sweet potato leaves. Weighed 50 mg extract was dissolved in a 100 mL volumetric flask with 96% ethanol to a mark (500 ppm). The 500ppm solution is then replicated 3 times. Each replication was taken as much as 0.5 mL of reaction with 2 mL of distilled water and 0.15 mL of 5% NaNO2 then left for 6 minutes. A total of 0.15 mL of 10% AlCl3 was added to the solution, then allowed to stand back for 6 minutes. The solution was reacted with 2 mL of 4% NaOH, then diluted with distilled water until the total volume was 5 mL and allowed to stand for 15 minutes.

Flavonoid levels were calculated based on the calibration curve of the reading from the UV-Vis spectrophotometer, and the linear regression equation using the Lambert-Beer law: $y = bx + a$. Description: $y$: Absorbance of ethanol extract of sweet potato leaves, $x$: Concentration (ppm), $b$: Slope and $a$: Intercept

And to calculate the total flavonoid content, the total flavonoid content is obtained by entering sample absorbance data into the quercetin standard curve equation, absorbance is used as the $y$ value and the value of $x$ as the quercetin concentration in ppm, the formula is used:

$$C = C_1 \times \frac{V}{m} \times F_p$$

Description: $C$: Total flavonoids (mg QE / g extract), $C_1$: Concentration of quercetin (mg / L), $V$: Volume of extract (L), $m$: Weight of extract (g) and $F_p$: Dilution factor.

**DISCUSSION**

Extract of Sweet Potato Leaves. The thick extract of sweet potato leaves obtained as much as 47.72 grams with a percentage of yield of 23.86% can be seen in table 1.
Table 1. Results of Calculation of the Rendemen of Ethanol Extract Sweet potato leaves.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dried Material (g)</th>
<th>Extract weight (g)</th>
<th>Rendemen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract of sweet potato leaves</td>
<td>200</td>
<td>47.72</td>
<td>23.86</td>
</tr>
</tbody>
</table>

Testing of Specific Parameters observed is the dried material of sweet potato leaves and organoleptic identity of the extract produced. Results can be seen in Table 2.

Table 2. Specific Parameter Test Results

<table>
<thead>
<tr>
<th>Identity and Organoleptic</th>
<th>Extract name: Ethanol extract of sweet potato leaves</th>
<th>Color: blackish</th>
<th>Smell: No smell</th>
<th>Shape: Thick</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latin name: (Ipomoea batatas L)</td>
<td>Part of plant used: leaf</td>
<td>(Source: primary research data, 2016)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Qualitative Test with Thin Layer Chromatography. Samples on silica were sprayed with 3% AlCl3 if yellow stains were seen, this meant the sample contained flavonoid compounds in flavone and flavonol groups (Harborne, 1987). The results can be seen in Figure 1.
Test Results for Total Flavonoids. Ethanol extract of sweet potato leaves was tested for total flavonoid content by a spectrophotometric method with measurements based on the formation of complexes between AlCl3 with ketone groups on C-4 atoms and hydroxy groups on C-3 or C-5 atoms adjacent to flavones and flavonols (Azizah, et al, 2014). The maximum wavelength measurements carried out in this study are wavelengths with a range between 380-560 nm, and a wavelength of 418.80 nm is produced at a concentration of 80 ppm. The maximum wavelength is then used to measure the absorption of the calibration curve and sample ethanol extract of sweet potato leaves.

The standard solution in this study was quercetin. Quercetin is used as a comparison because it is a flavonoid flavonol group. Quercetin is also in the amount of about 60-75% of flavonoids (Lukman, 2015). The raw solution of quercetin was made from a mother solution with a concentration of 400 ppm, then made 6 series of
concentrations, namely 50 ppm, 60 ppm, 70 ppm, 80 ppm, 90 ppm, and 100 ppm. Making 6 series of concentrations is based on the use of regression methods in making line equations based on absorbance values and standard concentrations in order to provide linear uptake. The test results can be seen in table 3.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Absorbance</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>0.186</td>
<td>y = 0.0036x - 0.0432</td>
</tr>
<tr>
<td>80</td>
<td>0.234</td>
<td>r = 0.991</td>
</tr>
<tr>
<td>100</td>
<td>0.303</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>0.372</td>
<td></td>
</tr>
<tr>
<td>140</td>
<td>0.474</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>1.569</td>
<td></td>
</tr>
</tbody>
</table>

(Source: primary research data, 2017)

From the data above, the results obtained are linear regression equations $y = 0.00187x - 0.0021$ with a correlation coefficient of 0.9934 which is positive and close to 1 which means that the curve is linear and the results show that with increasing concentration the absorbance is greater. This relationship can be seen in Figure 2.

Color Formation Reactions (Source: Kartikasari, 2015)
Total flavonoid levels were measured as equivalent to standard quercetin based on the standard quercetin curve. The result of total flavonoid content from ethanol extract of sweet potato leaves was 31630 mg QE / 100 g extract. Results can be seen in table 4.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Absorbance</th>
<th>Total flavonoids equivalent to quercetin (QE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg QE/100 g</td>
</tr>
<tr>
<td>500 ppm</td>
<td>0.398</td>
<td>30360 mg/100 g</td>
</tr>
<tr>
<td></td>
<td>0.417</td>
<td>31950 mg/100 g</td>
</tr>
<tr>
<td></td>
<td>0.422</td>
<td>32620 mg/100 g</td>
</tr>
</tbody>
</table>

The raw material used in this study is sweet potato leaves from Baumata village, Taebenu sub-district. The sweet potato leaves taken are fresh green leaves that are not too old and not too young. In making sweet potato leaf extract, the powder of sweet potato leaves is done first so that it is easy to macerate. The purpose of maceration is to extract phytochemical compounds contained in the sample. The maceration process is carried out in 70% ethanol for 72 hours and evaporated at 50 ° C to reduce the boiling point of the solvent so that the solvent will evaporate below its normal boiling point and so that the phytochemical compounds contained in the extract do not experience damage due to excessive heating. Extracts obtained from each sample are used as stock for further testing.

The solvent used is 70% ethanol because it has the polarity that is in accordance with the flavonoid compounds that want to be withdrawn. This is also based on Harborne (1987) which states that flavonoid compounds can be extracted well using 70% ethanol. After extracting the extract, it was concentrated to get a thick extract. The extract used was ethanol extract of sweet potato leaves (Ipomoea batatas L.) and the plant parts used were leaves. The organoleptic test showed extract,
odorless and thick in shape. The results obtained from the thin layer chromatography test after being sprayed showed yellow stains, therefore it was concluded that the sample contained flavonoids. Furthermore, it can be tested for determination of total flavonoid levels.

From the results of testing the total flavonoid content shows a linear curve but the absorbance at the standard does not meet the range between 0.2-0.8. Trials have been carried out 4 times but the results obtained are still not fulfilling so the results of the fourth trial are approaching the range. This happens because it is caused by several factors, which are improper weighing, as well as inappropriate transfer of analyte and raw material, extraction of analytes from an inefficient matrix, improper use of burettes, pipettes, and flask, measurement using a tool that is not calibrated, failure to eliminate interference by additional materials in analyte measurements (Gandjar and Rohman, 2007).

In each work process carried out added AlCl₃, NaNO₂, and NaOH reagents. The reagents cause the catechol group of flavonoids in ring B to be oxidized by sodium nitrite to ketone. The formed ketone group will complex with aluminum cations (Al³⁺) from AlCl₃, the compound is then reduced by NaOH which results in the quino structure (Figure 3).
CONCLUSIONS and SUGGESTIONS

Based on the research data, it can be concluded that the ethanol extract of sweet potato leaves using the AlCl3 colorimetric spectrophotometry method has a total flavonoid content of 3163mg QE / 100g or 31.63% b / b. The suggestions for researchers can do the same research but uses fractionation by testing antioxidant activity than for institutions is tools such as: volume pipettes, measuring flasks, etc. are more equipped in the laboratory.

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