DETERMINATION OF FLAVONOID CONTENT OF ETHANOL AND ETHYL ACETATE EXTRACT FROM PURPLE PASSION FRUIT PEEL

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Abstract: Purple passion fruit is a fruit that is widely cultivated in Indonesia for syrup and juice making. Purple passion fruit has a high nutritional value, such as antioxidants. Passion fruit contains vitamin A and C, β-carotene, flavonoid, and fiber components. However, passion fruit peel is a waste that can not be appropriately facilitated, so it becomes a waste that can pollute the environment. In addition, the passion fruit peel contains flavonoids that can be used as antidiabetic drugs. This study aimed to determine the difference in levels of flavonoid in the peel extract of purple passion fruit by using ethanol and ethyl acetate solvents. The test of total flavonoid using the AlCl₃ method and the absorbance was measured using a UV-Vis spectrophotometer. The results showed that the total flavonoid levels of passion fruit peel extract with ethanol and ethyl acetate solvents of 15.01 mg QE/g samples and 1.27 mg QE/g extract. The highest total flavonoid contents were obtained in ethanol extract of 15.01 mg QE/g extract.

Keywords: Purple Passion Fruit Extract, Total Flavonoids, UV-Vis Spectrophotometer

INTRODUCTION
Passion fruit is a plant that lives long and is included in vines. Passion fruit plants have slightly woody, posting stems and many overlapping branches. In young plants, the branches are green and, after old, become brownish-green. The shape of the leaves of this plant is ting, jagged, green, and shiny, with a stalk length of 2-3 centimeters, leaf length of 9-12 centimeters, and width of 7-9 centimeters [1]. Purple passion fruit is a fruit that is widely cultivated in Indonesia for syrup and juice making. Purple passion fruit has a high nutritional value, such as antioxidants. Passion fruit contains vitamin A and C, β-carotene, fiber, and flavonoid [2].

Passion fruit peel is a waste that can not be appropriately facilitated, so it becomes a waste that can pollute the environment. In North Sumatra, a factory produces drinks from passion fruit. In a day, the factory can produce 10-15 tons of waste in seeds and fruit peels, as much as 2-3 tons per day [3]. The waste needs to be used properly because the passion fruit peel contains highly active substances, including alkaloids, pectin, polysaccharides, and flavonoids [4].

Flavonoids are the most abundant phenolic compounds in all plants [5]. All plant parts contain flavonoids, including fruits, peels, roots, and stems. All plants that contain flavonoids can be used as antiviral, antibacterial, anti-inflammatory, anticancer, and antiallergic [6]. On the other hand, flavonoid compounds also have potential as antioxidants. The antioxidants can prevent free radicals that cause damage to the system of body's immune and the oxidation of lipids and proteins [7].

Before testing flavonoids, a sample of passion fruit peel must be extracted first. In this study, researchers used a maceration extraction method. Extraction is done to get extracts that contain highly active substances. The extraction effectiveness depends on the solvent, solvent sample, and extraction time [8]. The effectiveness of extraction of a compound by a solvent depends on the solvent solubility, like dissolve principle. A compound will be dissolved in a solvent with the same properties [9]. Researchers need to determine the highest flavonoid levels on the peel of passion fruit by using ethanol and ethyl acetate solvents based on the description. Flavonoid tests using the UV-Vis Spectrophotometry method.

RESEARCH METHODS

The tools used in this research are a rotary evaporator vacuum, UV-Vis spectrophotometer, and glassware. The ingredients used in this study are purple passion fruit peel, ethanol, methanol, acetyl acetate, quercetin solution, AlCl₃ 10%, sodium acetate 1 M, and aquadest.

Sample Preparation
The purple passion fruit peel was weighed. Next, the sample was washed with clean water and cut into small pieces. After that, the sample was dried by wind. The dried fruit peel was mashed with a blender and sold until a powder size of 60 mesh was obtained.

Extraction
Sixty grams of the powder was soaked using 120 mL ethanol solvent at room temperature for twenty-four hours. Then, it was filtered until it separated between filtrate and dreg. Next, Fitrat is
put in a container, and the dregs are re-macerated using ethanol as much as 120 mL. The maceration process is carried out for three days. Filtrate has been collected. After that, filtrate was concentrated using a rotary evaporator at 60°C until concentrated extract was obtained [10]. The same treatment is also applied to maceration with ethyl acetate solvents. The yield calculation uses the formula:

$$\% \text{Yield} = \frac{\text{Extract weight (g)}}{\text{Powder weight (g)}} \times 100\%$$

**Determination of Total Flavonoid Levels**

25 mg standard quercetin was weighed and dissolved with 25 mL of ethanol. Then, the standard quercetin solution was made in the concentration of 10 – 50 mg/L. Then, it was added with 1 mL of each concentration and 0.1 mL of aluminum chloride 10%, 0.1 mL of CH₃COONa 1 M, and 2.8 mL of aquades. The incubating process of each solution was carried out for 22 minutes. The absorbance was read at UV-Vis spectrophotometry with a wavelength of 444 nm [10].

25 mg of ethanol and ethyl acetate extract was weighed. Each extract was dissolved with 25 mL of ethanol and ethyl acetate to obtain a concentration of 1000 mg/L [10]. Then, each 2.5 mL test solution of ethanol extract and ethyl acetate concentrated 1000 mg/L was pipetted and added with 0.1 mL of aluminum chloride 10%, 0.1 mL of CH₃COONa 1 M, and 2.8 mL of aquades. Then, the solution was incubated for 22 minutes. Finally, the absorbance was read at wavelength of 444 nm using UV-Vis spectrophotometry [10].

**RESULTS AND DISCUSSIONS**

**Passion Fruit Peel Extraction**

Extraction in this research used the maceration using ethanol and ethyl acetate solvents. Using two solvents with different polarities determines the yield and obtains the active compound based on polarity. The passion fruit peel powder was weighed and soaked with solvent until the powder was ideally submerged. Once the solvent was ideally submerged, it was stored in a dark room and was not exposed to sunlight for 24 hours. After being silenced for 24 hours, the dregs and filtration were obtained. The dreg obtained was re-macerated again using the same solvent. Maceration was done four times to attract the active compound as a whole to obtain more extracts [11-12]. The yield of passion fruit peel extract is shown in Table 1. The percentage of ethyl acetate extract yielded from the purple passion fruit peel is greater than that of ethanol extract from purple passion fruit peel. More active compounds from the purple passion fruit peel were extracted into semi-polar solvents such as ethyl acetate.

### Table 1. The yield of extract of purple passion fruit peel

<table>
<thead>
<tr>
<th>Extracts types</th>
<th>Colour</th>
<th>Extract weight (g)</th>
<th>Sample Weight (g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Dark brown</td>
<td>0.818</td>
<td>60</td>
<td>1.36</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>Brownish-yellow</td>
<td>1.596</td>
<td>100</td>
<td>1.59</td>
</tr>
</tbody>
</table>

**Determination of Total Flavonoid Levels**

First, absorbance measurements were taken in a standard solution used to compare the measurement of total flavonoid compounds in the sample. Quercetin was chosen as the standard solution because it includes flavonoid compounds that effectively counteract free radicals. Quercetin is equivalent to mg QE/g of dry matter and is used as a standard for flavonoids [13-14]. The manufacture of standard curves aims to know the relationship between the concentration and its absorbance value so that the concentration of the sample is known. An R-value close to one indicates a good correlation between quercetin levels and absorbance. The correlation coefficient (r) indicated a linear relationship between the two variables. This study obtained an r-value of 0.9981, which can be interpreted as linear or good because the result is close to 1. Based on Figure 1, the linear regression equation y = 0.0182x - 0.0072 can be used to calculate the total flavonoid level value in the test sample.

The determination of flavonoid levels in this study used a method of colorimetry with the reagent AlCl₃. The aluminum chloride forms a stable acidic complex with the C4 keto group and C3 or C5 hydroxyl groups of flavones and flavonols. The formation of a complex that occurs so that the shift of the wavelength towards the solution marked by the visible (visible) produces a more yellow color [15].

In addition, AlCl₃ also forms a labile acid complex with orthodihydroxyl groups in rings A or B of flavonoids to have maximum absorption at a wavelength of 444 nm [10]. It then measured its uptake using a maximum wavelength ultraviolet-visible (UV-Vis) spectrophotometer. The addition of sodium acetate to flavonoid tests aims to maintain wavelengths in the visible area. The test solution was incubated for 22 minutes before the
measurement to make the reaction run perfectly so that the intensity of the resulting color was maximal. The results of this study obtained the total flavonoid levels of ethanol extract and ethyl acetate as follows table 2.

Table 2. Total flavonoid levels of purple passion fruit peel extract

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Sample Weight (g)</th>
<th>Sample volume (L)</th>
<th>Dilution factor</th>
<th>Absorbance</th>
<th>Concentration (mg/L)</th>
<th>Total Flavonoids (mg QE/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>0.025</td>
<td>0.0025</td>
<td>10</td>
<td>0.266</td>
<td>15.01</td>
<td>15.01</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>0.025</td>
<td>0.0025</td>
<td>10</td>
<td>0.016</td>
<td>1.27</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Based on Table 2, the total flavonoid levels of each test extract had different results. The total flavonoid levels of the ethanol extract was greater (15.01 mg QE/g extract) than the ethyl acetate extract (1.27 mg QE/g extract). The highest total flavonoid levels of flavonoid compounds are found in ethanol extracts because flavonoid compounds found in the passion fruit peel have better levels of polarity and solubility in ethyl acetate solvents. In addition, ethanol also has a polarity close to the polarity of phenols, so it is used as a solvent at extraction. In principle, polar compounds can dissolve in the same compounds, namely polar. Therefore, the polar ethanol solvents attract better flavonoid compounds that are polar. However, flavonoid compounds are divided into several groups with different polarity levels. This is due to the amount and position of each flavonoid group's hydroxyl group (-OH), thus affecting the solubility of flavonoids in the solvents [16]. The greater total flavonoid levels of ethanol extract than ethyl acetate extract agreed with previous studies [17]. In addition, it has also been reported that Limnophila aromatica ethanol extract has the highest total flavonoid levels compared to water extracts, methanol, and acetone [18]. The phenolic and flavonoid content of the ethanol extract of Tagetes erecta L. was the highest followed by the ethyl acetate and n-hexane extracts [19-21]. In another study, it was also reported that the ethanolic extract of P. emblica leaves had the highest total flavonoid content (3.594 g QE/100 g) followed by ethyl acetate and n-hexane extract [22-23]. Some of these reports suggest that ethanol solvent is suited to extract total flavonoids from plants.

CONCLUSION

The results showed total flavonoid levels of passion fruit peel extract with ethanol and ethyl acetate solvents of 15.01 and 1.27 mg QE/g extract, respectively. Based on the research results from both samples, the ethanol extract has higher total flavonoid contents.

REFERENCES


