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DETERMINATION OF PERCENTAGE YIELD AND TOTAL PHENOLIC CONTENT OF ETHANOL EXTRACT FROM PURPLE PASSION (*Passiflora edulis* f. edulis Sims) FRUIT PEEL

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Abstract: Free radicals can trigger various diseases, such as cancer, cardiovascular, arthritis, diabetes, neurological disorders, and aging. Thus, antioxidants are needed to reduce these free radicals. The peel of the purple passion fruit (*Passiflora edulis* f. edulis Sims) has many phenolic compounds that indicate antioxidant capacity. This study aims to determine the percentage yield and total phenolic contents in the ethanol extract of purple passion fruit peel. Extraction of purple passion fruit peel using the method of maceration with ethanol solvent. Measurement of total phenolic levels in ethanol extract from purple passion fruit peel using the UV–Vis Spectrophototometry method with Folin-Ciocalteu reagent. The principle of this method is based on the reagent between gallic acid and the Folin-Ciocalteu reagent that will produce a molybdenum-tungsten complex. Furthermore, the addition of Na_2CO_3 solution will change the color of the solution to blue, and its absorbance can be measured at a wavelength of 765 nm. The results showed that the ethanol extract from purple passion fruit peel yielded 1.363% and total phenolic levels of 30.758 mg of gallic acid equivalent per the gram of extract. The existence of the total phenolic content proves that the ethanol extract from purple passion fruit peel has the potential to be used as an alternative source of natural antioxidants.

Keywords: *Ethanol Extract, Purple Passion Fruit Peel, % Yield, Total Phenolic Content, Folin-Ciocalteu Reagent*

INTRODUCTION

Excessive amounts of reactive oxygen group free radicals (ROS) or other free radicals in the human body can cause severe damage to cells and trigger various diseases. Examples: cataract and retinal disease, arteriosclerosis, hypertension, ischemia, cardiomyopathy, heart failure, cancer, aging, diabetes, inflammation, infection, Alzheimer's, Parkinson's, memory loss, depression, stroke, rheumatic, inflammation, asthma, bronchitis, glomerulonephritis, kidney failure, and fetal disorders [1]. The activity of these free radicals can be suppressed using antioxidants or electron-giving compounds that inhibit oxidation reactions by binding to free radicals and highly reactive molecules that prevent cell damage. Prevention efforts against increased oxidative stress in the body are to consume foods that contain antioxidants [2].

One natural antioxidant alternative is passion fruit peel (*Passiflora edulis* f. edulis Sims). Half of the mass of passion fruit is part of the peel. Increased volume of juice production causes a buildup of passion fruit peel waste so that it can pollute the environment [3]. Passion fruit peel has a high content of active substances, such as flavonoids, alkaloids, pectin, and polysaccharides [4]. The peel of passion fruit contains many phenolic compounds that indicate antioxidant capacity.

Phenolic compounds make up the largest group of secondary metabolites in plants. This compound is included in aromatic alcohols because its hydroxyl group is permanently attached to the benzene ring [5]. Phenolic compounds are antioxidants in preventing and treating degenerative diseases, cancer, premature aging, and immune system disorders in the body [6] and help treat diabetes mellitus [2].

This study aims to determine the total phenolic levels in the peel of purple passion fruit extracted using the method of maceration with ethanol solvents. The total phenolic content is expressed as grams of gallic acid equivalent per gram of extract. This research is expected to increase the utilization of purple passion fruit peel to be more maximal as an alternative source of natural antioxidants in herbal medicine for healing various diseases.

RESEARCH METHOD

Tools and Materials

Tools used in this study include glassware, a rotary vacuum evaporator, and a Genesys 10S UV-Vis spectrophotometer. The sample was purple passion fruit peel obtained from passion fruit plantations on Jalan Raya Cigugur RT 05 RW 02 Kuningan Regency 4511 West Java Province. The materials used in this study include ethanol, Folin-Ciocalteu reagents, Na_2CO_3 7%, and aquadest.

Determination of Purple Passion fruit

The determination of purple passion fruit samples was carried out by looking at or observing the morphological characteristics of the purple passion fruit. Plant determination was carried out in the Life Service Unit of the Faculty of Science and Technology, Universitas Airlangga.

Sample Preparation

The peel of passion fruit that has been separated from the flesh of the fruit was weighed, washed using clean water, and cut into small sizes. The sample that had been cut was then dried by aerating–winded. The dried sample was then blended and sifted into a powder with a size of 60 mesh.

Sample Extraction

60 g of passion fruit peel powder was soaked with 180 mL of ethanol solvent in a sealed container and stored at room temperature for 24 hours. Then, it was filtered until obtained filtrate and dregs. Next, the filtrate was collected into a sealed container while the dregs were macerated again using a new solvent for four days. Filtrate results were concentrated using a *Rotary vacuum evaporator* until concentrated extract was obtained. The percentage yield of the extract was calculated using the formula:

$$\% \text{ yield} = (\text{extract weight})/(\text{sample weight}) \times 100\%$$

Determination of Total Phenolic Contents

Manufacture of standard solution

25 mg of gallic acid was weighed and dissolved marked with ethanol in the 25 mL volumetric flask. 5 mL was diluted with ethanol until a volume of 50 mL to produce a concentration of 100 mg/L.

Making calibration curve

It was taken at 1, 2, 3, 4, and 5 mL from a 100 mg/L standard solution. It was diluted in a 10 mL volumetric flask to produce 10, 20, 30, 40, and 50 mg/L concentrations. Furthermore, each concentration was taken by 1 mL, was added with 0.4 mL of Folin-Ciocalteu reagents, shaken, and left for 4 – 8 minutes. Then, it was added with a 7% Na_2CO_3 solution, homogenized, added with aquadest up to 10 mL, and let stand for 2 hours. After 2 hours, the absorbance of each concentration was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm. Next, the calibration curve was the relationship between the concentration of gallic acid and absorbance [7].

Determination of phenolic contents

Purple passion fruit peel ethanol extract was weighed at 25 mg and then dissolved with 25 mL of ethanol. From the solution, 1 mL was inserted into a 10 mL volumetric flask and added by 0.4 mL of Folin-Ciocalteu reagent, shaken, and left for 4-8 minutes. Then, added a 7% Na_2CO_3 solution of 4 mL and then added by aquadest to the boundary mark, homogenized, and let stand for 2 hours. Blanko consists of aquadest and Folin-Ciocalteu reagents. The total phenolic content was expressed as grams of gallic acid equivalent per gram of extract. The sample absorbance was measured at a wavelength of 765 nm [7].

RESULT AND DISCUSSION

The percentage yield of Ethanol Extract of Purple Passion Fruit Peel

The extraction method used in this study is the maceration method. The selection of the maceration method due to the necessary procedures and equipment is very simple, and the operational costs are relatively low. In addition, there is no need to heat up in the maceration method so that natural materials do not decompose [8]. It prevents damage to active compounds that are thermolabile or not heat resistant [9]. The maceration process is carried out using a comparison between purple passion fruit peel powder and ethanol solvent (1: 12). It takes 60 g of fruit peel powder in 180 mL of ethanol in one day and is continued by re-maceration four times. The collected filtrate is then evaporated using a Rotary vacuum evaporator until a concentrated extract is obtained. The yield of purple passion fruit peel ethanol extract is shown in Table 1.

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

Extract Types	Color	Extract weight (g)	Sample weight (g)	% Yield
Ethanol extract	Blackish brown	0.818	60	1.363

In this study, the yield of purple passion fruit peel ethanol extract obtained from passion fruit plantations on Jalan Raya Cigugur RT 05 RW 02 Kuningan Regency 4511 West Java Province amounted to 1.363%. In another study, purple passion fruit peels were extracted using a maceration method with 96% ethanol solvent and tested against *Staphylococcus aureus* [10]. In addition, the peel of the purple passion fruit was extracted using ethanol solvent and tested for toxicity to the mice's liver [11]. Furthermore, the purple passion fruit peel has also been boiled and tested for antidiabetic activity against the histopathological picture of the streptozotocin-induced mouse pancreas. However, from the three studies, no determination of % of the yield is not done. Based on literature studies, the yield of extracts from the type of hyphenyl (*Passiflora lingularis* f. lobalata) is 3.27%. The percentage yield of extracts was influenced by the concentration of solvents, the type of plant species, temperature differences, types of solvents, length of extraction time, sample particle size, conditions, and storage time, as well as comparison of sample amount to the amount of solvent, used [12-13].

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Total Phenolic Levels of Purple Passion fruit Peel Ethanol Extract

Phenolic levels are determined using gallic acid as a standard solution. Gallic acid is one of the natural and stable phenols and is relatively cheaper than others [14]. The use of gallic acid as a standard solution because this compound is very effective for forming complex compounds with Folin-Ciocalteu reagents so that the reactions that occur are more sensitive and intensive [15]. The reaction between gallic acid and Folin–Ciocalteu reagents is shown in Figure 1.

Gallic acid reacts with the Folin-Ciocalteu reagent to produce a yellow color, meaning phenol content in the purple passion fruit peel [14]. A reaction is a phenol-hydroxy group reducing heteropoly acid (phosphomolybdate-phosphotungstate) contained in the Folin–Ciocalteau reagent into a molybdenum-tungsten complex [16]. Furthermore, a solution of Na_2CO_3 is added, and the color of the solution turns blue. It was then measured by absorbance at a wavelength of 765 nm. Phenolic compounds that react with Folin-Ciocalteu reagents only in an alkaline atmosphere for proton dissociation of phenolic compounds into phenolic ions so that Na_2CO_3 solution is added, which is a base solution [14]. Based on the calibration curve in Figure 2, linear regression requirements for gallic acid absorbance are obtained at concentrations of 10, 20, 30, 40, and 50 mg/L of y = 0.0372x + 0.9508 with a correlation coefficient value (r) of 0.991. The linear regression equation was used to determine the total phenolic contents in ethanol extract of purple passion fruit peel, shown in Table 2.

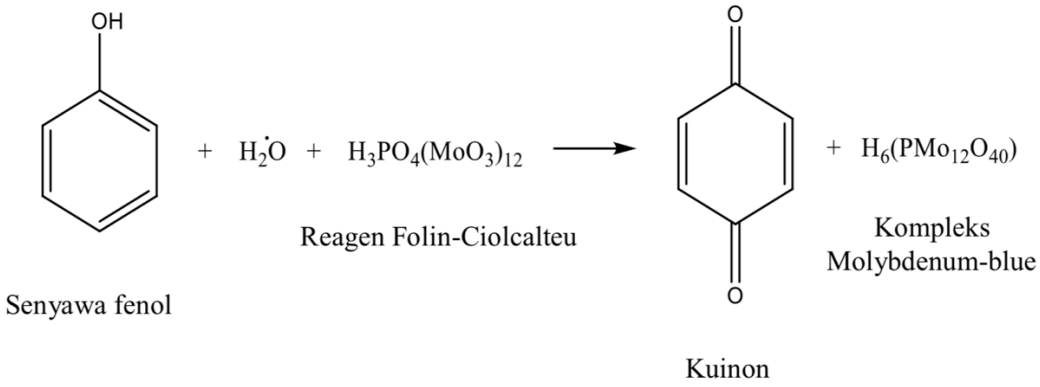

Figure 1. The reaction between Gallic Acid and Folin–Ciocalteu reagent [9]

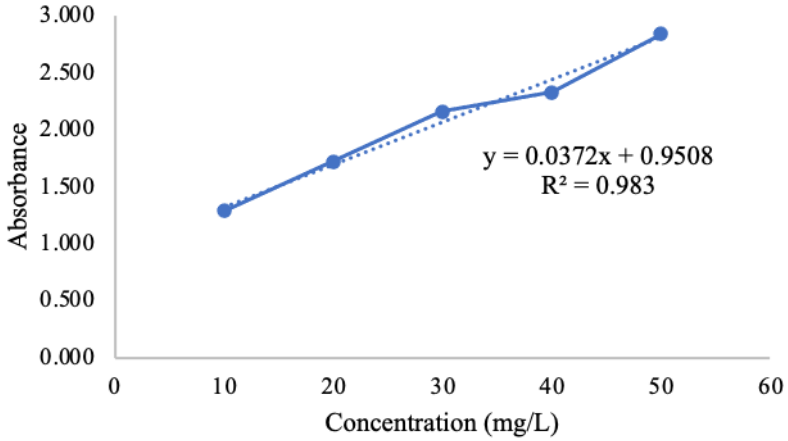

Figure 2. Calibration curve of gallic acid standard solution

Table 2. Results of determining total phenolic levels in ethanol extract of purple passion fruit peel

Extract sample	Concentration (µg/mL)	Concentration (mg/mL)	Total phenolic levels (mg GAE/g extract)
Ethanol extract of purple passion fruit peel	30.76	0.030758	30.758

Table 2 shows the results of test sample measurements to determine total phenolic levels in purple passion fruit peel ethanol extract. The absorbance value (y) of the test sample is substituted into the regression equation y = ax + b that has been obtained from the gallic acid calibration curve so that the concentration (x) is obtained. The obtained x value is substituted in the formula for calculating the total phenolic rate. The total phenolic content in purple passion fruit peel ethanol extract amounted to 30,785 mg GAE/g. Every gram of purple passion fruit peel ethanol extract has a phenolic content equivalent to 30,785 mg of gallic acid. This study showed that the total phenolic content was higher than in other studies where purple passion fruit peel was extracted using 40% ethanol solvent [17]. Factors that affect phenol levels in a plant include temperature, UV rays, nutrients available to plants, and genetic factors. [18]. In other reports, some phenolic compounds show antimicrobial, anti-atherosclerotic, cardiovascular protective activity, anti-carcinogenic [19], Prevent heart disease, reduce inflammation, lower the incidence of cancer and diabetes, and reduce the rate of mutagenesis in human cells. The protection obtained from consuming plant products such as fruits, vegetables, and nuts is mostly related to the presence of phenolic compounds in these plants [20]. The type of solvent used affects total phenolic levels. Phenols are polar compounds, so their solubility is highest in polar solvents. Polar solvents can dissolve phenols better so that the levels in the extract become high [21-23]. Solvents such as methanol and ethanol are widely used and effective solvents for extracting phenolic components from natural materials [15].

CONCLUSION

Purple passion fruit peel ethanol extract yields 1.363% and phenolic levels of 30.758 mg of gallic acid equivalent per gram of purple passion fruit extract. The presence of total phenolic content demonstrates that the ethanol extract from purple passion fruit peel has the potential to be used as a natural antioxidant alternative.

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