The Determination of Total Flavonoid Content and Test for Glucose Level Reduction in Parijoto Fruits Extract from Bandungan

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ABSTRACT

The variety of flora in Indonesia can be used as herbal medicine, one of which is the Parijoto fruit (*Medinilla speciosa* Blume). Parijoto fruit prospers in highland areas and looks like purple grapes. The purpose of this study was to determine the activity of reducing glucose levels and to determine the total flavonoid content of parijoto fruit extract from Bandungan. Extraction of secondary metabolites of parijoto fruits using maceration method, with 96% ethanol solvent. The activity of reducing glucose levels was tested in vitro using the Nelson-Somogyi method. The total flavonoid content was determined spectrophotometrically using $AlCl_3$ reagent with the results expressed in quercetin equivalents (µg QE/g extract). The results showed that parijoto fruits extract at concentrations of 10,20, 30, 40, 50, and 60 ppm was able to reduce glucose levels by 68.6419%, 53.2158%, 45.7885%, 52.3996%, 55.2563%, and 39.5854%. The total flavonoid content of parijoto fruit extract was 15.98 µg QE/g extract. These results mean that flavonoids in parijoto fruits extracted using 96% solvent have the potential to reduce glucose levels.

Keywords: *Parijoto, fruit, glucose levels, total flavonoids*

INTRODUCTION

Diabetes mellitus (DM) is a disease indicated by metabolic disorders that cause an increase in blood sugar levels above normal (1). The results of Riskesdas 2018 show that the prevalence of diabetics in Indonesia based on a doctor's diagnosis at the age of 15 years is 2%. This figure shows the results of an increase in diabetics in Indonesia, and only about 25% of diabetics know that they have diabetes. So it is threatened to develop progressively into complications without realizing it and without prevention (2). Risk factors that can influence the incidence of diabetes prevalence are genetics, obesity, and dyslipidemia (3).

Complementary therapy using herbal medicine is one of the efforts to treat and prevent diabetes mellitus. Indonesia has a diversity of flora that is useful as an alternative for people with diabetes mellitus, such as cinnamon, bitter melon, wuluh starfruit, mulberry leaves, tapak dara leaves, guava fruit, bitter leaves and cat whiskers (4,5). One of the natural ingredients that can also be an alternative to complementary therapy is parijoto fruit (*Medinilla speciosa Blume*)

This plant can grow on mountain slopes or in forests and is also cultivated as an ornamental plant. Parijoto grows well on high humus and moist soil at an altitude of 800 to 2,300 meters above sea level (6). The activity of reducing glucose levels in the extract of Parijoto fruit from Kudus has been investigated (7). At concentrations of 10, 20, 30,40,50 and 60 ppm parijoto fruit extract was able to reduce glucose levels by 38.707%, 41.055%, 44.736%, 47.211%, 50.637%, and 52.922%. It is suspected that secondary metabolites of flavonoids are involved in the activity of lowering glucose levels (8).

In this study, an analysis of flavonoid levels and determination of glucose-lowering activity was carried out in vitro from the ethanolic extract of parijoto fruit from Bandungan, Semarang. Analysis of flavonoids using spectrophotometric techniques, then to test the decrease in glucose levels using the Nelson-Somogyi method and detection using spectrophotometry.

So that it can be known flavonoid levels and activity of reducing glucose levels of ethanol extract of Parijoto fruit from Bandungan. Differences in where plants grow can affect the levels of active compounds in a plant and their biological activity. And the potential of parijoto fruit as a medicinal raw material for the prevention and treatment of various diseases, especially degenerative diseases can be developed to the fullest.

MATERIAL AND METHODS

1. **Tools**

   Maceration vessel, Oven (Memmert), Rotary Evaporator (RE 100-Pro), TLC Plate (Merck), 5, 10, 50, and 100 mL volumetric flasks (Iwaki), 1, 5, and 10 mL Gauge Pipettes (Iwaki), Volume 1 and 2 mL Pipettes (Iwaki), Micropipette 10-100 L (Socorex), Micropipette 1000 L (Socorex), Dropper Pipette, Bulb (D&N), Beaker glass 100, 200, and 500 mL (Iwaki), Erlenmayer 250 mL (Iwaki), 250 mL Cap Erlenmayer (Iwaki), Glass Funnel (Iwaki), Waterbath (Memmert), Porcelain Cup, Chamber, Filter Paper, Analytical Balance (Ohaus), Test Tube (Iwaki), Tube Rack, Tube Clamp, Spatula, Stirring Rod, Aluminum Foil (Klin Pak), Cotton (Lydia), Thermometer, Blender (Philips), Electric Stove (Maspion), UV254 Lamp and UV-Vis Spectrophotometer (Shimadzu UV-1800).

2. **Ingredient**

   Parijoto Fruit, Ethanol 70% (Technical), Ethanol 96% (Technical), Ethanol pa (Merck), Quercetin (Sigma), Rutin (Chemcruz), Ammonia (Merck), n - Butanol (Merck), Glacial Acetic Acid (Merck)), Aquadest, Glucose Anhydrous (Merck), Nelson's Reagent A, Nelson's Reagent B, Arsenomolybdate Reagent, Sodium Nitrate (Merck), Aluminum Chloride (Merck), Sodium Hydroxide (Merck), Ion-free aqua dest (SWCU FSM Chemistry Lab), Quercetin dihydrate (HWI pharma service GmbH Germany)

3. **Research methods**

   a. **Plant determination**

   Parijoto fruit was obtained from Bandungan in May 2021. The determination of parijoto fruit was carried out at the Ecology and Biosystematics Laboratory, Faculty of Science and Mathematics, Department of Biology, Diponegoro University, Semarang.

   b. **Extraction**

   Fresh parijoto fruit samples were washed with running water and cleaned of dirt contained in the test material and then air-dried for several days until the fruit had shriveled and then in an oven at 40°C for several days until the fruit was dry. The parijoto fruit was ground using a blender into a fine simplicia powder.

   Parijoto fruit Simplicia powder as much as 200 grams was macerated with 2 L 96% ethanol (1:10). Maceration was carried out with 1.5 L of 96% ethanol in a container protected from light for 2 days, stirred 1 x 24 hours, then the obtained macerate was filtered and macerated with 500 mL of 96% ethanol for 1 day. The obtained macerate was then evaporated

using a rotary evaporator at 70°C, then followed by a water bath at 70°C until the weight was constant and the yield was calculated.

c. Determination of flavonoid levels

Sample preparation

As much as 1 g of the sample was weighed in a glass container and added 25 ml of 60% v/v ethanol. Extraction was taken out for 15 minutes in an ultrasonicator set at 40°C. The extract solution was filtered and the filtrate was collected, while the residue was re-extracted with 25 ml of 60% v/v ethanol. This stage was repeated three times and the filtrate was combined into one. All of the filtrates were accommodated in a 100 ml volumetric flask and added with 60% v/v ethanol solvent to the top line. The obtained extract solution concentrates 10 mg/ml

Analysis of flavonoid levels

A total of 1 ml of the sample was added with 4 ml of distilled water and then homogenized with a vortex mixer. The mixture was added 0.3 ml of 0.5% NaNO 3 then homogenized with a vortex mixer and left for 5 minutes. In the next step, 0.3 ml of 10% AlCl 3 was added and then homogenized with a vortex mixer and left for 5 minutes. Then 2 ml of 1 M NaOH was added and homogenized with a vortex mixer. The solution was added to 10 mL of distilled water and then homogenized with a vortex mixer, and left at room temperature for 15 minutes, before measuring. Absorbance measurements were carried out at a wavelength (l) of 510 nm. Conversion of absorbance value to total flavonoid concentration based on the quercetin calibration curve. Calibration curves were made based on the quercetin concentration series of 20, 40, 60, 80, and 100 mg/ml

***d.* In vitro glucose lowering activity test**

The ethanol extract of parijoto fruit was made in series with concentrations of 10, 20, 30, 40, 50, and 60 ppm. 100, 200, 300, 400, 500, and 600 L extract solutions were pipetted, and each was put into a 10 mL volumetric flask and then diluted with distilled water to the mark. Each series of 2 mL extract solution was taken plus 2 mL of standard glucose solution with a concentration of 30 ppm into a test tube. A total of 1 mL of the solution mixture was put into a test tube, plus 1 mL of Nelson's reagent covered with cotton, heated over boiling water using an iron container and wrapped with aluminum foil for 10 minutes at 96°C, cooled for 5 minutes (soaked in water). Then transferred to a 10 mL volumetric flask, added 1 mL of arsenomolybdate reagent was into the flask, diluted with distilled water to the mark, shaken, and allowed to stand for incubation time. Incubate the solution at room temperature for 16 minutes. The absorbance was read with a UV-Vis spectrophotometer at a wavelength (l) of 748.20 nm. Measurement of the activity of reducing glucose levels of parijoto fruit extract was observed with a UV-Vis spectrophotometer, where first a series of glucose standard curves were measured and the absorbance value was obtained to make linear regression and obtain the equation y = bx + a. Calculation of the percentage

decrease in glucose levels using the equation Percent Decrease in Glucose Levels =

$$\frac{standard - sampel}{standard} x\ 100\%$$

RESULT

1. Determination of Parijoto Fruit

The purpose of plant determination is to determine the plant material used, namely parijoto fruit according to the specified morphological characteristics. The habitus parijoto is in the form of a bush, the trunk and old branches are pseudo-segmented, whitish gray. The fruit is pink when young, the old fruit is red, and the ripe fruit is black. The seeds are small and black**.**

The key to plant determination is listed as follows: :

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Super Division	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Sub Class	: Rosidea
Order	: Myrtales
Family	: Melastomataceae
Genus	: Medinilla
Species	: *Medinilla speciosa* Blume (Parijoto)

Figure 1. Parijoto (*Medinilla speciosa* Blume)

The results showed that the plant used was true Medinilla speciosa Blume (Parijoto) .

2. Parijoto Fruit Extract Flavonoid Content

Components of secondary metabolites that are thought to have glucose-lowering activity are flavonoids. Flavonoid compounds are compounds that are generally soluble in solvents and are easily damaged at high temperatures (9). So that the extraction of flavonoid parijoto fruit extract using the maceration method with 96% ethanol solvent. The thick extract obtained was dark brown , with a yield of 6.67% w/w.

Extract of Parijoto fruit was analyzed using the $AlCl_3$ staining method using detection spectrophotometric techniques (10). The first stage is sample preparation by carrying out a hydrolysis process using acid to break the bonds of flavonoids with sugar (11). The flavonoids in the form of aglycones were then separated from the sugar through a liquid-liquid partition and reacted with a complex with $AlCl_3$ (12). The aglycone-$AlCl_3$ complex was measured its absorbance value using a UV-Vis spectrophotometer. Analysis using UV-Vis spectrophotometry because of the conjugated aromatic system in flavonoids so that it shows strong absorption bands in the ultraviolet and visible spectrum regions.

Quercetin was used as a comparison because it is a flavonoid of the flavonol group. The structure of flavonols has a keto group at C-4 and a hydroxy group at C-3 or C-5 atoms, thus forming a color complex with $AlCl_3$ (13). The purpose of adding acetic acid is to keep the C-4 keto and 3 or 5-OH stable (14).

Table 1. The results of determination of flavonoid content of ethanol extract of parijoto fruit

Replication	Total flavonoid content (µg QE/g extract)	Average total flavonoid content (µg QE/g extract)
1	16,24	15,94
2	15,72	
3	15,88	

Next, determine the maximum wavelength and operating time used. The maximum wavelength obtained is 510 nm. The operating time obtained is 15 minutes. This treatment is intended so that the reaction runs perfectly and the resulting color intensity is maximized. Determination of the standard curve of quercetin by making 6 series of quercetin concentration solutions, namely 20, 40, 60, 80, and 100 ppm. The standard results of quercetin obtained are linear regression equations, namely y = 0.00060x + 0.0070, with the value of r being 0.9978. Determination of total flavonoid levels by entering the absorbance value of the parijoto fruit extract sample into the equation of the quercetin standard curve. So from the results of this study, the total flavonoid content of the extract was 15.94 g QE/g extract, which can be seen in Table 1. In 1 gram of ethanol extract of parijoto fruit containing quercetin group flavonoids of 15.94 µg.

3. Parijoto Fruit Extract Glucose Decrease Test

Testing of glucose level-lowering activity was conducted to determine the ability of ethanol extract of parijoto fruit from Bandungan in forming a secondary metabolite-glucose complex. The test method uses the Nelson-somogyi method in *vitro*. The concentrations of the extracts were tested using a concentration series of 10, 20, 30, 40, 50, and 60 ppm.

Operating determination time aims to get a stable measurement time. Colored compounds will produce absorbance values that increase for a certain time until a stable absorbance is obtained (15). Furthermore, the absorbance and color intensity will decrease due to the possibility of the compound being damaged or oxidized. This reason makes the basis that the measurement of colored compounds from chemical reactions is carried out at the optimum time (16). The measurement results obtained the optimum time in the 16th minute.

Determination of the maximum wavelength is done to get the wavelength with the maximum absorbance. Determination of the maximum wavelength at a wavelength between 700 - 800 nm. The maximum wavelength obtained is 748.20 nm, not much different from the research conducted by Luhurningtyas which is 757.4 nm (8).

The standard curve for glucose solution uses a concentration series of 2, 9, 16, 23, and 30 ppm. The absorbance of each solution was measured. The linear regression equation obtained is y = 0.0137x + 0.3496 with a value of r (relational coefficient) = 0.9974 (close to a value of 1). The standard curve that forms a straight line shows that the Lambert-Beer law is fulfilled. There is a correlation between the higher the concentration, the greater the absorbance value.

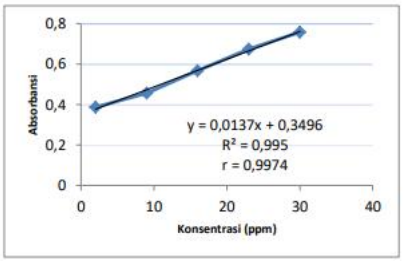

Figure 2. Glucose standard curve

Tests for reducing glucose levels in vitro using the Nelson-Somogyi method. This method is a method of determining reducing sugar content (17). The working principle of reducing sugar will reduce Cu^{2+} ions to Cu^{+} , then Cu^{+} ions reduce arsenomolybdate compounds to form a greenish-blue complex. Based on Table 1. The average percent decrease in glucose levels in the 96% ethanol extract of parijoto fruit was able to reduce glucose levels at the maximum concentration at a concentration of 50 ppm by 55.26%.

The activity of reducing glucose levels in parijoto fruit is due to the presence of flavonoids. The structure of flavonoids that have a hydroxyl group (OH) can react with glucose to form a flavonoid-glucose complex(18). Glucose levels are decreasing, due to complex bonds with flavonoids. The higher the concentration of the extract, the higher the percent decrease in glucose levels. The hydroxyl group (OH) that is freely bound to C-3 flavonoids binds to glucose to form a flavonoid-glucose complex (7). Glucose levels are reduced by binding of glucose to flavonoids and the remaining glucose will react with Nelson's reagent to form a brick red precipitate which is then reacted with arsenomolybdate reagent to form molybdate blue (7).

Table 1. Percent decrease in glucose levels of parijoto extract

Sample	Concentration (ppm)	The average percent decrease in glucose levels (%)
Ethanol extract	10	56,154
	20	37,463
	30	45,054
	40	52,726
	50	35,386
	60	28,485
Quercetin	2	29,954
	4	31,995
	6	34,035
	8	39,667
	10	42,850

In Table 1. the results of the percent decrease in glucose levels of the ethanol extract of parijoto fruit show a non-linear graph. Research that has been carried out by Vifta et al, also shows a non-linear graph, at a concentration of 10-20 ppm the ethyl acetate fraction of parijoto fruit experienced a decrease in activity in lowering glucose levels then decreased maximum glucose levels at a concentration of 40 ppm and subsequently experienced a decrease in glucose levels (7).

Based on the growing area, the parijoto fruit used in this study came from the Bandungan area, Semarang. A study conducted by Luhurningtyas, 2021, regarding the decrease in glucose levels of parijoto fruit from Kudus, The result showed that the higher the concentration of the extract, the greater the percentage decrease in glucose levels, with an EC_{50} of 48.750 ppm (8). Factors that affect the quality of the content of secondary metabolites of flavonoids are the growing area such as geography, temperature, water availability, soil moisture and fertility,

and light intensity(19). The higher the light intensity and the longer the exposure, the shorter the half-life so that the damage to flavonoid compounds is also faster (18).

In the study, it was found that the comparison compound quercetin had greater glucose-lowering activity than the ethanol extract of parijoto fruit. The concentration of quercetin for lowering glucose levels is lower than the concentration of parijoto ethanol extract, but it can produce a higher percentage of lowering glucose levels. Quercetin has been known to act as a lowering of glucose levels by inhibiting the enzyme alpha-glucosidase. This enzyme plays a role in the breakdown of carbohydrates into glucose in the digestive tract (20).

CONCLUSION

The ethanol extract of parijoto fruit from Bandungan contained flavonoids of 15.94 g QE/g extract and concentrations of 10,20, 30, 40, 50, and 60 ppm were able to reduce glucose levels in vitro by 68.6419%, 53, respectively. 2158%, 45.7885%, 52.3996%, 55.2563%, and 39.5854% .

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