



DETERMINATION OF FLAVONOID CONTENT IN TAPAK DARA ROOT DECOCTION (*CATHARANTHUS ROSEUS*)

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ABSTRACT

Catharanthus roseus is a plant that grows wild and is widely used by people as an ornamental plant. This plant is known to have antibacterial, antioxidant, antifungal, anthelmintic, antineoplastic, antihyperglycemic, anticancer, antidiarrheal and antiviral activities so it is used to treat various diseases such as malaria, constipation, cancer, diuretics, diabetes mellitus, cholesterol, and hypotension. This research aims to determine the levels of flavonoids in the roots of the tapak dara plant (*Catharanthus roseus*) with varying concentrations of 5%, 10% and 15%. The method used in this research is the dekokta method. Determination of decok flavonoid levels in the roots of the tapak dara plant (*Catharanthus roseus*) done by adding AlCl₃ reagent, then analyzed using a UV-vis spectrophotometer with a wavelength of 431 nm. The average yield of tapak dara plant root flavonoids obtained with a concentration of 5% was 0.18±0.0015 mg/L, 10% was 0.76±0.0020 mg/L and 15% was 2.31±0.0015 mg/L. The conclusion of this study shows that the greater the sample concentration, the greater the flavonoid content.

Keywords: *Catharanthus roseus*, Roots, Flavonoids, UV-vis Spectrophotometry.

1. INTRODUCTION

Indonesia is one of the countries located in the tropics with enormous biodiversity, rich in plant raw materials that are efficacious for health. Along with the development, there has been a lot of research and development of plants that are efficacious for health. WHO (*World Health Organization*) data shows 70-80% of the world's population uses herbal medicine as an alternative treatment, found in research which states that medicinal plants contain flavonoids (Karimi et al., 2013).

Flavonoids are one of the natural compounds found in many plants and foods that promise to treat various diseases such as cancer, inflammation, and cardio-vascular dysfunction. Flavonoids have a very important activity, namely as an antioxidant in preventing wounds caused by free radicals (Bustanul & Sanusi, 2018).

One plant that has the potential to be used for the utilization of natural antioxidants is the tapak dara plant (*Catharanthus roseus*). Tapak dara (*Catharanthus roseus*), which has been considered a wild and cheap flower, is now starting to be looked at and favored by many people (Noer et al., 2018) because tapak dara (*Catharanthus roseus*) is one of the natural ingredients that has been widely researched and reported to have many properties in curing various diseases, including as an anticancer, urinating, lowering blood pressure and stopping bleeding, while tapak dara roots contain alkaloids, saponins, flavonoids and tannins (Verrananda et al., 2016).

Tapak dara is a shrub that has a reddish-colored stem that is round, woody at the base, short-haired, and has a lot of branching. Leaves are single, the leaf surface is shiny and hairy, with pinnate leaf bones. Compound trumpet-shaped flowers emerge from the tips of stalks and leaf axils. The flower crowns are five in number and the periwinkle flowers are red, easy red, light purple, and white (Figure 1) (Andalia et al., 2019).



Figure 1. Tapak dara Plant (*Catharanthus roseus*)

According to (Hadriyani, 2022) tapak dara is known to contain flavonoids and tannins that can have analgesic effects. Flavonoids have analgesic activity because flavonoid compounds have activity similar to aspirin, which inhibits the formation of inflammatory mediators through inhibition of the cyclooxygenase enzyme so that it will reduce the production of prostaglandins by arachidonic acid, thereby reducing pain.

According to (Nayak & Pinto Pereira, 2006) in various regions people also use tapak dara (*Catharanthus roseus*) to treat headaches, burns, and traditional medicine for diabetics. In addition, empirically tapak dara is also known to have properties as analgesic (anti-pain) and anti-inflammatory (anti-inflammatory). This is also based on the many studies on the utilization of tapak dara leaf extract as anti-inflammatory (Putri et al., 2019) wound healing and antipyretic drugs whose effectiveness has been tested in mice and rats (Puspita Dewi et al., 2013) Some people have also used tapak dara as a muscle pain reliever, nosebleed medicine, and swelling relief due to wasp stings (Koul et al., 2013).

The roots of the tapak dara plant are often used by the community as a treatment by drinking the boiled water, but it is not yet known exactly how much flavonoid content is contained in the roots of the tapak dara plant (*Catharanthus roseus*). Therefore, the decoction of the roots of the tapak dara plant is used as a medicine, it is necessary to determine the right dose for its use (Kabesh et al., 2015).

Therefore, it is necessary to conduct a study that aims to determine the flavonoid content of decoction on the roots of tapak dara (*Catharanthus roseus*). It is hoped that the results of this study can provide information on the ability of antioxidants as an antidote to free radicals.

2. METHODS

This study uses the decoction method to extract the active content that is soluble in water. Meanwhile, to determine the flavonoid levels contained in the roots of tapak dara (*Catharanthus roseus*) with different sample concentrations, the UV-Vis spectrophotometric method was used. The results obtained after extraction with the dekokta method are brownish samples, sample extracts are not too thick and also not too liquid and have a distinctive smell like herbal medicine.

2.1. Tools and Materials

The equipment used in this study are blender, sieve, sample storage container, erlenmeyer, 1 unit of decoction pot, 100 ml volumetric flask, 25 ml volumetric flask, 10 ml volumetric flask, test tube, test tube rack, water bath, thermometer, filter paper, drop pipette, 5 ml measuring pipette, 10 ml measuring pipette, suction rubber, funnel, 100 ml beaker, stirring rod, spatula, analytical balance, digital balance, UV-Vis spectrophotometer, cuvette, aluminum foil and stopwatch (Ahmad et al., 2015).



The materials used in this study were powdered simplisia of tapak dara root (*Catharanthus roseus*), water/aquades, quercetin solution, ethanol 96%, aluminum (III) chloride 10%, potassium acetate 1 M (Ahmad et al., 2015).

2.2. Work Procedure

Preparation of root simplification of tapak dara plant (Catharanthus roseus)

Root samples of tapak dara (*Catharanthus roseus*) were taken in Lasoani Village. Next, wet sorting was done by washing with clean running water, then chopping, then drying for 3 days without direct sunlight. After that the sample was mashed using a blender, then sifted using a sieve. Furthermore, the sample is stored in a container (Ahmad et al., 2015).

Root preparation of tapak dara plant (Catharanthus roseus)

The dried powder samples were extracted using the decoction method. The decoction concentration variations tested were 5%, 10%, and 15%. A total of 5 grams of sample and 95 grams of water were put into erlenmeyer 1, 10 grams of sample and 90 grams of water in erlenmeyer 2, and 15 grams of sample and 85 grams of water in erlenmeyer 3. (Furthermore, to anticipate the decoction to remain 100 grams, each erlenmeyer was added 20% of the sample and 20% of the water from each erlenmeyer. For example, in erlenmeyer 1, 20% of 5 grams of sample and 20% of 95 grams of water are added and so on). Then the sample is put into the top pot. Next, put the pot and its contents into the bottom pot that already contains water. After that, the bottom pot is heated over direct heat and allowed to boil (meaning the temperature reaches 100°C). Heating is done for 30 minutes from the time the water in the lower pot boils (the temperature of the upper pot reaches 90°C), while stirring occasionally. Then the decoction extract is filtered with filter paper to take the filtrate. The filtrate obtained will then be analyzed for flavonoid content (Ahmad et al., 2015).

2.3. Quantitative Analysis of Flavonoids

Preparation of Quercetin Standard Solution

Standard solution was prepared by weighing 10 mg of quercetin, then dissolved with ethanol up to 10 mL in a volumetric flask as a standard solution of quercetin concentration of 1000 µg/mL. Then 10 mL was taken and dissolved in 100 mL of ethanol as 100 µg/mL quercetin standard solution. Then a series of quercetin standard solution of 0, 1, 2, 3, 4 and 5 µg/mL was made. How to make a series of 1 µg/mL solution is using the dilution formula, the results obtained as much as 0.25 mL of quercetin standard solution in 100 µg/mL added with ethanol up to 25 mL (so on). From each concentration of standard solution, 0.5 mL was pipetted and put into a test tube, then added with 1.5 mL of 96% ethanol, 0.1 mL of 10% aluminum (III) chloride, 0.1 mL of potassium acetate 1 M and 2.8 mL of distilled water. After that, the solution was shaken and then allowed to stand for 30 minutes, then measured the absorbance using a UV-Vis spectrophotometer at a wavelength of 431 nm. Then a calibration curve was made by connecting the absorption value as the coordinate (Y) and the concentration of the standard solution as the abscissa (X) (Ahmad et al., 2015).

Preparation of Blank Solution

Blank solution was made by taking 0.5 mL of distilled water, then added with 1.5 mL of 96% ethanol, 0.1 mL of 10% aluminum (III) chloride, 0.1 mL of potassium acetate 1 M and 2.8 mL of distilled water. Then allowed to stand for 30 minutes and measured the absorbance at a wavelength of 431 nm (Ahmad et al., 2015).

Determination of Total Flavonoid Level in Decoction

Each decoction sample was pipetted as much as 25 mg and then dissolved in 25 mL of 96% ethanol to obtain a concentration of 1000 µg/mL. Taking as much as 0.5 mL of test sample was added with 1.5 mL of 96% ethanol, 0.1 mL of 10% aluminum (III) chloride, 0.1 mL of potassium acetate 1 M and 2.8 mL of distilled water.



Then incubated for 30 minutes. The absorbance of the quercetin standard solution was measured using a UV-Visible spectrophotometer at a wavelength of 431 nm. The average of three measurements was calculated and the flavonoid content was expressed with the equivalence of quercetin standard comparison (Ahmad et al., 2015).

2.4. Data Analysis Technique

Total Flavonoid Content

Total flavonoid levels are calculated with the linear regression equation of the calibration curve to be measured. The linear regression equation of X against Y. $Y = a + bX$.

3. RESULTS & DISCUSSION

3.1. Research Results

This study uses the decoction method to extract the active content that is soluble in water. Meanwhile, to determine the flavonoid levels contained in the roots of tapak dara (*Catharanthus roseus*) with different sample concentrations, the UV-Vis spectrophotometric method was used. The results obtained after extraction with the dekokta method are brownish samples, sample extracts are not too thick and also not too liquid and have a distinctive smell like herbal medicine.

3.2. Determination of Absorbance of Quercetin Standard Solution

The absorbance measurement results of quercetin standard solution as a comparison using UV-VIS spectrophotometer at a wavelength of 431 nm, with concentrations of 0 µg/mL, 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, and 5 µg/mL are presented in Table 1.

Table 1. Results of absorbance standard solution

Concentration (µg/L)	Absorbance
0	0.000
1	0.019
2	0.036
3	0.051
4	0.065
5	0.078

3.3. Preparation of Standard Curve for Quercetin Standard Solution

The standard curve is made by connecting the absorbance value of quercetin standard solution as the coordinate (y) and the concentration of the standard solution as the abscissa (x) contained in Table 1 so as to obtain the regression equation $Y = 0.0155x + 0.0027$ shown in Figure 2.

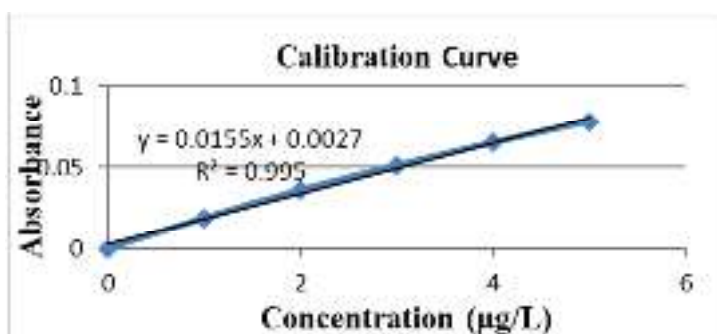


Figure 2. Calibration curve of quercetin at maximum wavelength (431 nm)

3.4. Flavonoid Content in Decoction of Tapak dara (*Catharanthus roseus*) Plant

The results of the analysis of flavonoid levels of tapak dara plant decoction (*Catharanthus roseus*) based on sample concentration can be seen in (Table 2) as a 5% concentration, (Table 3) as a 10% concentration and (Table 4) as a 15% concentration as follows.

Table 2. Results of Flavonoid Level Analysis of Tapak Dara Plant (*Catharanthus roseus*) at 5% concentration

Treatment to	Absorbance (Y)	Average Absorbance	Average flavonoid content $y=ax+b$
1	0.007	0.0056±0.0015	0.18 mg/L
2	0.004		
3	0.006		

Table 3. Results of Flavonoid Level Analysis of Tapak Dara Plant (*Catharanthus roseus*) at 10% concentration

Treatment to	Absorbance (Y)	Average Absorbance	Average flavonoid content $y=ax+b$
1	0.0017	0.0146±0.0020	0.76 mg/L
2	0.013		
3	0.014		

Table 4. Results of Flavonoid Level Analysis of Tapak Dara Plant (*Catharanthus roseus*) at 15% concentration

Treatment to	Absorbance (Y)	Average Absorbance	Average flavonoid content $y=ax+b$
1	0.037	0.0386±0.0015	2.31 mg/L
2	0.039		
3	0.040		

In this study, the average flavonoid results obtained by 5% decoction concentration is 0.18 mg/L, 15% concentration is 0.76 mg/L and 15% concentration is 2.31 mg/L, so it can be concluded that the higher the concentration of a sample extracted, the higher the flavonoid content. This is because the higher the flavonoid content, the more molecules contained in the root extract of tapak dara (*Catharanthus roseus*) as a medicinal plant so that the molecules that will absorb light at a certain wavelength are also more. Thus resulting in higher



absorbance values, absorbance as a quantitative analysis is carried out based on Lambert-Beer Law which states that absorbance with flavonoid levels has a linear relationship, namely the higher the measured absorbance, the higher the flavonoid content contained in a plant (Gusnedi, 2013).

4. CONCLUSION

Based on the results of the research that has been done, it can be concluded that the average flavonoid content of the roots of tapak dara (*Catharanthus roseus*) with the decoction method at a concentration of 5% is 0.18 ± 0.0015 mg/L 10% is 0.76 ± 0.0020 mg/L and 15% is 2.31 ± 0.0015 mg/L.

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