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Analysis of Total Flavonoid Levels In Young Leaves and Old Soursop Leaves (*Annona muricata* L.) Using UV-Vis Sepctrofotometry Methods

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Abstract

Soursop (Annona muricata L.) is a type of fruit plant that contains nutrients and is a traditional medicinal ingredient that has multiple properties. This study aims to determine total flavonoid levels in young and old soursop leaf extracts using a UV-Vis spectrophotometer. Extraction of chemical content in young and soursop (Annona muricata L.) leaf extracts was carried out by maceration method using 96% ethanol solvent. Determination of total flavonoid levels of sample extracts was carried out through absorption measurements at a wavelength of 432 nm. The results of the quantitative test were obtained from this study, namely total flavonoid levels in the samples of young soursop leaf extract as much as 410,833 mg /100g, whereas in large soursop leaf extract samples as many as 505,208 mg /100g.

Keywords: Flavonoids, young leaf and old soursop (Annona muricata L.) leaves, UV-Vis spectrophotometry.

1. Introduction

One of nature's medicinal plants is Soursop (Annona muricata L). Soursop (Annona muricata L.) is one of the plants of the Annonaceae family that has great benefits for human life, namely as a fruit plant with nutritional requirements and is a traditional medicinal ingredient that has multiple benefits. Besides fruit, the most useful part of the soursop plant is the leaf [1]. Soursop leaves have been used by some Indonesian people as a cure for lumbago, reduce pain, itching, rheumatism, boils, reduce high blood pressure, diabetes and reduce fever [2]. Chemical compounds in soursop leaves that play an important role for use as a drug are flavonoids [1]. Flavonoids are one of the largest natural phenol compounds found in all green plants [3]. Many studies have shown that flavonoids have biological activities, namely hypo-allergenic, antiviral, anti-inflammatory, and visodilation (enlargement of blood vessels) actions. However, the most interesting thing is that flavonoids have been devoted to have antioxidant activity, because they are able to reduce the formation of free radicals [4]. Analysis of flavonoid levels can be done using UV-Vis spectrophotometry methods, because flavonoids contain a conjugated aromatic system so that it shows strong absorption bands in the ultraviolet (UV) and visible light spectrum [5]. In addition, using the UV-Vis

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spectrophotometric method has many advantages, namely younger, faster and specific for the analysis of test substances [6].

2. Methods

The stages of this research are explained as follows:

2.1.Sample Preparation

Samples of young soursop leaves (Annona muricata L.) that have been mashed, weighed as much as 2 grams using a digital balance, then put into erlenmeyer, then added as much as 50 mL of ethanol solvent. Next, the erlenmeyer containing the sample is closed using aluminum foil, then stirred using a stirring machine with speed of 150 rpm for 1 hour. Furthermore, the sample is filtered using filter paper. The filtrate obtained was concentrated using a rotary evaporator at a temperature of 25 oC - 30 oC, so that a thick extract of young soursop leaves was obtained. Repeat the same treatment for old soursop leaf samples. The thick extract of young soursop leaves and old soursop leaves is ready for use for further analysis [8].

2.2.Making Parent / Stock Solution

A total of 10 mg of quartzite was weighed, then dissolved with 10 mL of 96% ethanol to produce a mother liquor / stock with a concentration of 1000 ppm. Then the 1000 ppm mother stock / stock is diluted to 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm as a comparison solution [9].

2.3.Making of Quaretin Standard Solution

As much as 0.5 mL of standard solution is put into a test tube, then added 1.5 mL of 96% ethanol, 0.1 mL of 10% aluminum chloride (AlCl3), 0.1 mL of potassium acetate and 2.8 mL of distilled water. After that, the mixture is allowed to stand for 30 minutes at room temperature. Absorbance from quaretin solution (comparison) was measured using a UV-Vis spectrophotometer at a wavelength of 432 nm [9].

2.4.Making of Quaretin Standard Curve

The standard curve is created by connecting the absorbance value as coordinates (y) and the concentration of the standard solution as abscissa (x) to obtain the regression equation and correlation coefficient. Based on the value of the correlation coefficient can be known either linearity or not. The correlation coefficient is said to be good if r = 0.998 [10]. Prepare tools and materials that will be used in research. Young leaves and soursop old leaves that will be used as samples are washed thoroughly, then dried by aerated for approximately 3 days. After drying, young leaves and soursop old leaves are then cut into small pieces, then blended to become fine powder. Next, it is sieved using a sieve to produce finer powder. Fine young leaf powder and old soursop leaves are ready for further use [7].

2.5.Sample Extraction

2.6.Determination of Sample Flavonoid Levels

Thick extract of young soursop leaves weighed as much as 10 mg, put into 100 mL volumetric flask, then added 96% ethanol solution as much as 10 mL, then added distilled water to mark the mark. The sample extract was diluted, taken as much as 0.5 mL, then put into a test tube. Next, 1.5 mL of 96% ethanol were added, 0.1 mL of 10% aluminum chloride (AlCl3), 0.1 mL of potassium acetate and 2.8 mL of distilled water. After that, the mixture is shaken and then allowed to stand for 30 minutes at room temperature. The change in color of the mixture to yellow indicates the presence of flavonoids. Next, measure the absorbance of the sample using a UV-Vis spectrophotometer wavelength of 432 nm. Total flavonoid levels were calculated using a linear regression equation from the previously measured quateretin calibration curve [11]. This treatment is done with 2 repetitions. The same treatment was carried out for samples of old soursop leaves. Based on absorbance measurements, the flavonoid concentration is read in (μg / mL) calibration line, then the flavonoid content in the extract is expressed in quarsetin equivalent (mgQE / g extract) [12].

2.7.Data analysis technique

The data analysis technique used refers to Puspitasari (2016). Total flavonoid levels of young and old soursop leaf extracts can be calculated using the following formula:

$$F = \frac{c\ x\ V}{m}$$

Where: F = amount of flavonoid AlCl3 method; m = sample weight (grams); C = Equivalence quartetin (mL); and V = total extract volume.

3. Results and Discussion

3.1.Measurement of absorbance of standard solution of quartz

Measurement Results Absorbance of Quaretin Standard Solution (comparison) with concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm using UV-Vis spectrophotometry at 432 nm wavelength can be seen in Table 1.

Table 1. Results of absorbance measurements of standard solution of quartzite at a wavelength of 432 nm

quartz concentration (ppm)	Absorbance
20	0,313
40	0,519
60	0,737
80	0,991
100	1,301

3.2.Making the standard solution curbetin curves

The standard curve is created by connecting the absorbance value of the standard solution as coordinates (y) and the concentration of the standard solution as abscissa (x) listed in Table 1 to obtain the regression equation and correlation coefficient as shown in Figure 1.

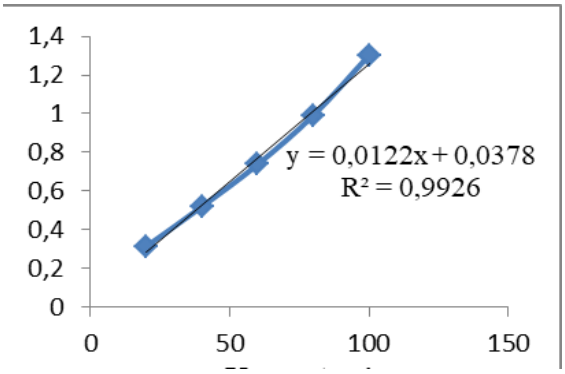

Figure 1. Standard curve standard solution and linear regression equation

3.3.Qualitative test results of total flavonoid levels in young leaf extract and soursop old leaves

Qualitative analysis is seen from the color changes that occur in the extract of young leaves and soursop old leaves. The results obtained can be seen in Table 2.

Table 2. The results of the qualitative analysis of flavonoid extracts of young leaves and soursop old leaves

The Sample	Class test	Reactor	Color	Information
Young Soursop Leaves	Flavonoids	Alumunium Chloride Solution	Yellow	Positive
Old soursop Leaves	Flavonoids	Alumunium Chloride Solution	Yellow	Positive

3.4.Quantitative test results of total flavonoid levels in young leaf extract and old soursop leaves

The results of quantitative tests of total flavonoid levels in young leaf extract and old soursop leaves obtained can be seen in Table 3.

Table 3. Results of quantitative analysis of flavonoid extracts of young leaves and soursop old leaves

The Sample	Absorbance	Concentration Flavonoids (ppm)	Content Flavonoids (mg/100g)
Young Leaves 1	1,002	80,417	402,083
Young Leaves 2	1,044	83,917	419,583
Old Leaves 1	1,225	99,000	495,000
Old Leaves 2	1,274	103,083	515,417

Plant samples used in this study were young soursop leaves and old soursop leaves. Soursop leaf types used, namely young leaves which are in the order of the 2nd to 4th of the soursop plant shoots, and the old leaves which are in the 5th to 7th order of the soursop shoots. Young soursop leaves and old soursop leaves samples that will be used are dried by aerated without direct sunlight, then the sample is mashed to powder and then extracted [13]. The purpose of drying by aerating, namely that secondary metabolite compounds contained in soursop leaves are not damaged, and the drying process aims to eliminate water content, and increase the durability of the sample so as not to be damaged [14]. Then the sample is blended using a blender. The purpose of the sample is smoothed, namely to expand the surface so as to facilitate contact between the sample and the solvent at the time of extraction [15].

Extraction method is the process by which a desired compound is withdrawn using a solvent in accordance with the nature of the compound to be separated. The purpose of the extraction process is to attract the chemical components contained in the sample [16]. The extraction process has many types. The type of extraction used in this study was maceration extraction [17]. The selection of this extraction method is because it is a simple, easy, and without a heating process, so that the possibility of damage to components of chemical compounds can be minimized [18]. Maceration is also an easy way to do it and uses simple equipment [12]. In addition, maceration is also best used in determining flavonoids [1].

The extraction process is done by immersing the sample powder simplicia with a solution of the finder [8]. The solvent solution will penetrate the cell wall and enter the cell cavity which contains the active substance, the active substance will dissolve and because of the difference in concentration between the solution of the active substance and that is outside the cell, the concentrated solution is pushed out [20]. The solvent solution used to search for flavonoid compounds contained in the study sample, namely ethanol 96%. This solvent selection, because flavonoid compounds are generally contained in the form of polar glycosides that must be dissolved with polar solvents [10]. The ethanol extract obtained was then concentrated using a rotary evaporator until a thick ethanol extract was obtained. Evaporation aims to vaporize the solvent used at the time of maceration so as to obtain a thick ethanol extract from the sample [21].

For the analysis of total flavonoid levels of young soursop and old soursop (*Annona muricata* L.) leaf extracts, absorbance measurements were first performed on a standard solution to be used as a comparison solution. Concentration of standard solutions used, namely 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. Measurement of

absorbance of standard solutions was carried out using UV-Vis spectrophotometry with a wavelength of 432 nm [22]. The color produced from the standard solution of quercetin is yellow. The formation of a yellow color in a standard solution of quaretin indicates the presence of flavonoid compounds in a standard solution [23]. The higher the concentration used the more concentrated the yellow color produced. Quercetin is chosen as a standard solution (comparison) because it is one of the most effective flavonoid compounds capturing free radicals (hydroxyl radicals, superoxide and peroxyl) and inhibits various oxidation reactions because it can produce phenolic radicals that are stabilized by the resonant effect of the aromatic ring [24].

In absorbance measurement for quartetin standard solution to determine the quateretin calibration curve at a wavelength of 432 nm, a standard curve for quaretin solution is made by connecting the absorbance value of standard solution and concentration of standard solution, the regression equation is obtained, which is y = 0.0122x + 0.0378 . From the regression equation obtained from the standard solution obtained a linear relationship between absorbance and concentration in the absorbance measurement with a correlation coefficient of 0.9926. A value (r) close to one indicates that the regression equation is linear [25]. The calibration curve equation above can be used to determine the total flavonoid levels in the extract of young leaves and soursop old leaves [26].

Total flavonoid levels in this study were determined using the calorimetry method. The principle of this method, namely the addition of AlCl3 which will form a stable acid complex with C-4 ketone groups, as well as in C-3 or C-5 hydroxyl groups of flavones and flavonoids as in Figure 2. AlCl3 also forms stable acid complexes with The orthodihydroxyl group in ring A or B of the flavonoid compounds will have a maximum absorption at a wavelength of 432 nm [27].

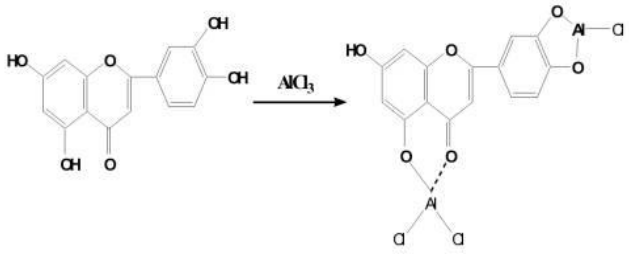

Figure 2. Reaction of flavonoid-Aluminium Chloride ($AlCl_3$) complex formation [23].

Before analyzing the total flavonoid levels contained in the sample, absorbance of each sample was first calculated using a UV-Vis spectrophotometer at a wavelength of 432 nm [28]. The results obtained from the measurement of absorbance of young and old soursop leaf samples, namely for young soursop (1) uptake of 1,002, young soursop leaf (2) uptake of 1,044, old soursop leaf (1) obtained uptake of 1.225 and leaves of absorption old soursop (2) obtained uptake of 1,274. The results obtained indicate that the absorbance of samples and young soursop is greater, compared to the absorbance of young soursop leaf samples. Next, calculate the concentration of each sample of young leaves and old soursop leaves, and the results obtained, namely for young soursop leaves (1) obtained a concentration of 80,417 ppm, young soursop leaves (2) obtained a concentration of 83,917 ppm, old soursop leaves (1) a concentration of 99,000 ppm was obtained and soursop leaves (2) obtained a concentration of 103,083 ppm. After that, calculate the total flavonoid levels from each sample and the results obtained, namely for young soursop leaf samples (1) obtained total flavonoid levels of 402,083 mg / 100g, for young soursop leaf samples (2) the total flavonoid levels were obtained 419,583 mg / 100g , for samples of old soursop leaves (1) total flavonoid levels were obtained 495,000 mg / 100g, and for samples of old soursop leaves (2) total flavonoid levels were obtained 515,417 mg / 100g.

4. Conclusion

Based on the results of research that has been done, the data obtained total flavonoid levels in each sample of soursop young leaf extract and soursop old leaf extract using a UV-Vis spectrophotometer, namely for young soursop leaf extract (1) of 402,083 mg / 100g, young soursop leaf extract (2) at 419,583 mg / 100g, old soursop leaf extract (1) at 495,000 mg / 100g, and old soursop leaf extract (2) at 515,417 mg / 100g. Based on data from total flavonoid levels obtained, it can be concluded that the extract of old soursop leaves has a large total flavonoid levels, when compared

with the levels of flavonoids in young soursop leaf extracts.

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