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The determination of vitamin C in guava (*Myrtaceae species*) using spectrophotometric approach

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Abstract
Vitamin C is one of the essential vitamins for body health. However, vitamin C cannot be produced by the body itself. Thus, to fulfill the vitamin C requirement, it can be obtained by consuming fruits and vegetables. The purpose of this study was to find out and analyze the levels of vitamin C in guava (*Psidium guajava* L.), crystal guava (*Psidium guajava* (L.) Merr.), and water guava (*Syzygium aqueum*). This study used UV-Vis spectrophotometry to analyse the vitamin C content. The results of vitamin C level in samples such as red guava, crystal guava, and water guava were 21.16 mg/5 g ± 0.005, 20.79 mg/5 g ± 0.029, and 19.16 mg/5 g ± 0.089, respectively. Based on this outcomes, it can be concluded that the high levels of vitamin C can be acquired in red guava (*Psidium guajava* L.)

Keywords: *Guava, Vitamin C, UV-Vis Spectrophotometry*

Introduction

Vitamin C (ascorbic acid) is a type of water-soluble vitamin and has an important role in the body, as a coenzyme or cofactor. The function of vitamin C has a lot to do with the formation of collagen which is a protein compound that affects the integrity of cell structures in all connective tissues, such as cartilage, teeth, capillary membranes, skin and muscle tendons. Thus, vitamin C plays a role in healing wounds, broken bones, maintaining healthy teeth and gums [1]. Guava fruit also has a fairly complete nutritional content, especially high levels of vitamin C, and has a pleasant taste. Guava can be processed in the form of juice, fruit juice and also consumed fresh [2].

Several detections have been applied to determine the vitamin C levels, such as UV-Vis spectrophotometry method, which is a method that has a working principle based on the absorption of light or radiation energy of a solution. This method is faster and the results are more accurate [3-4]. UV spectrophotometry has a wavelength of 200-400 nm, and visible light has a wavelength of 400-750 nm [5-6].

The purpose of this study was to determine the levels of vitamin C in guava (*Myrtaceae Sp.*) using the UV-Vis spectrophotometry method.

Materials and Methods

The tools used were UV-Vis spectrophotometry, cuvette, rotary evaporator, aluminum foil, glass beaker, Erlenmeyer, volumetric flask, measuring pipette, dropping pipette, pro pipette, blender, flannel cloth, funnel, test tube, test tube rack, watch glass , stir bar, knife and analytical balance.

The materials used were samples of red guava (*Psidium guajava* L.), crystal guava (*Psidium guajava* (L) Merr), and water guava (*Syzygium aqueum*), 70%

ethanol, $KMnO_4$, ammonium molybdate, H_2SO_4 and distilled water.

Procedure

Collection of Materials and Preparation of Simplicia

There were 3 types of samples of guava fruit extract, namely red guava, crystal guava, and red guava which were obtained from the Gamping Market in Yogyakarta.

Extraction Process of Guava Fruit (*Myrtaceae Sp*.)

The samples collected were cut into small pieces using and weighed as much as 50 g using an analytical balance. The sample was mashed using a blender until it became mush, then the sample was macerated using 100 ml of 70% ethanol then stirred using a stir bar for 2 hours. The marinade was left for 2×24 hours protected from light and wrapped in aluminum foil, while stirring occasionally. The maceration solvent was filtered using filter paper to separate the dregs and filtrate and then re-macerated using the similiar solvent. The maserate was evaporated using a rotary evaporator and concentrated in waterbath until a compact extract was formed, then the yield was calculated using the following formula:

$$\text{Chemical rendition} = \frac{\text{Mass of extract}}{\text{Mass of simplisia}} \text{x } 100\ \%$$

Preparation of standard solution of vitamin C (100 ppm)

Approximately, 10 mg of vitamin C powder solution was weighed, then 10 ml of 70% ethanol was added to the mark and homogenized.

Oxidation-reduction reaction of vitamin C with $KMnO_4$

2 ml of sample solution was taken and then 0.1% (w/v), $KMnO_4$ was added, then a brown color formed and then slowly disappeared.

Maximum wavelength determination

The absorbance of a stock solution with a concentration of 100 ppm was measured using UV-Vis spectrophotometry, then the absorbance of the solution was measured in the wavelength range between 400-800 nm using 70% ethanol as a blnak solution. Then 1 ml of the sample solution was pipetted, added up to 10 ml of 70% ethanol, then the sample was transferred to a test tube, and then added 1.0 ml of 0.1% $KMnO_4$. This solution was left for 10 min and measured at the maximum wavelength of 400-800 nm.

The production and determination of various concentrations of vitamin C

Vitamin C powder was weighed approximately 10 mg then the powder was put into a 10 ml volumetric flask, dissolved using distilled water up to the mark, then homogenized. Dilute the 100 ppm of vitamin C solution into various concentrations such as 20 ppm, 25 ppm, 30 ppm, 35 ppm, 40 ppm, and 45 ppm into a 10 ml volumetric flask then added 0.1 N H_2SO_4 4 ml, and 5% ammonium molybdate 3 ml.

Results and Discussion

Determination of Guava (*Myrtaceae Sp.)*

Determination was done to identify the plants used and to avoid mistakes during the research process. Determination of the guava plant (*Myrtaceae Sp*.) was carried out at the Biology Laboratory of the Faculty of Applied Science and Technology, University of Ahmad Dahlan Yogyakarta with the results obtained in accordance with the Flora Of Java book that the plant identified was a guava plant (*Myrtaceae Sp*.). The determination that has been carried out contains numbers and letters in the form of a determination code indicating that all the characteristics and anatomical shape of the plant are guava fruit so that it can be ascertained that the plant used is actually guava fruit with the scientific name *Myrtaceae Family L., Myrtaceae Family L. Meer, Syzygium aqueum.*

Material storage

The material obtained from the sample to be studied was the ethanol extract of guava (*Myrtaceae Family*). Guava fruit (*Myrtaceae Sp.*) obtained from Gamping Market, Yogyakarta. Guava extract (*Myrtaceae Sp.*) was used by dissolving 50 g of each sample in 100 ml of 70% ethanol.

The process of extract

Guava fruit was weighed approximately 50 g and mashed using a blender, then extracted using the maceration approach. The solvent performed in this study was 70% ethanol with a ratio of sample slurry and solvent of 1:2 and remaceration was carried out 2 times then evaporated using a rotary evaporator.

Chemical rendition

Guava ethanol extract (*Myrtaceae Sp.*) using 70% ethanol solvent obtained yields of 6.334%, 10.254%, 8.773% and colored pink, light green and dark red.

Determination of the Vitamin C Concentration Series Calibration Curve

Vitamin C powder was weighed as much as 10 mg then the powder was put into a 10 ml volumetric flask, dissolved using distilled water up to the mark, then shaken until homogeneous. Dilute the vitamin C solution into a 10 ml volumetric flask, then add 3 ml of vitamin C series solution with concentrations of 20 ppm, 25 ppm, 30 ppm, 35 ppm, 40 ppm, and 45 ppm into a 10 ml volumetric flask containing 0.1 N H_2SO_4 4 ml, then add 5% ammonium molybdate up to 10 ml. Then this solution was allowed to stand for 1 hour and the series concentrations were measured using UV-Vis spectrophotometry at a predetermined maximum wavelength. The results of concentration series measurements are used to produce calibration curves and linear regression [7]. Furthermore, the data can be seen in Table 1 and Figure 1.

Table 1. The concentration vs. absorbance of Vitamin C

Conc.	Abs.
20	0,214
25	0,328
30	0,451
35	0,583
40	0,688
45	0,748

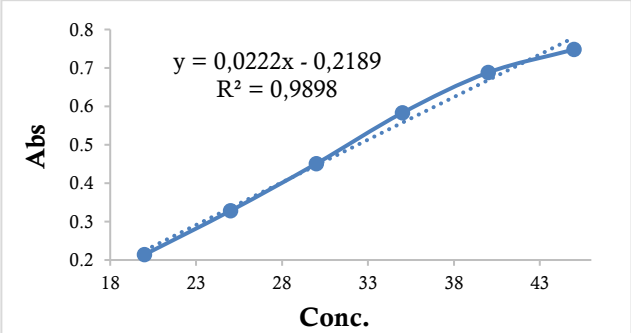

Figure 1. The calibration curve of vitamin C

Vitamin C Levels in Guava Using UV-Vis Spectrophotometry

Sample extract was weighed as much as 5 mg, put the sample into a 10 ml volumetric flask, add distilled water to the mark, then each sample was taken as much as 3 ml, put into a 10 ml volumetric flask, added 4 ml of 0.1 N H_2SO_4, and then added 5% ammonium molybdate up to 10 ml. Then the levels of vitamin C in the sample were calculated using the obtained linear regression equation. The results of vitamin C levels in guava samples (*Myrtaceae Family*) can be seen in Table 2 and Figure 2.

Table 2. The vitamin C levels in guava (*Myrtaceae Sp.*)

Sample	Absorbance (s.d.)	Vit. C level (ppm)
Red guava	0.213 ± 0.005	21,16
Crystal guava	0.209 ± 0.029	20,79
Water guava	0.235 ± 0.089	19,16

This study used guava fruit (*Myrtaceae Family*) where guava fruit is found from Gamping Market, Bantul, Yogyakarta. The determination that can be made is numbers and letters in the form of a determination code indicating that all the characteristics and anatomical shape of the plant are guava fruit so that it can be ascertained that the plant used in the research is actually guava fruit with the scientific name *Mytaceae family L., Mytaceae family L. Meer, Syzyqium aquenum.*

Figure 2. The outcomes of coloration process of guava (*Myrtaceae Family*)

Guava fruit is soaked using 70% ethanol solvent using the maceration method. The maceration method has the aim of separating chemical compounds contained in guava fruit which is soaked using 70% ethanol solvent to prevent decomposition of compounds due to heating [8]. The solvent used by the maceration method was 70% ethanol. Ethanol is a polar solvent [9]. 70% ethanol still contains quite a lot of water (30%) which helps the extraction process so that some of these compounds are extracted in the ethanol and some are attracted to the water [10-11].

The reason for choosing 70% ethanol solvent is because ethanol can attract polar active compounds. Ethanol has a low boiling point of 79°C so it requires a little heat for the extract concentration process [12].

Extraction was carried out by remaceration 2 times filtering using a flannel cloth to produce filtrate and residue of guava samples. The filtered filtrate was

concentrated using a vacuum rotary evaporator for 2 hours at a temperature of 80℃ and a speed of 90 rpm to separate the ethanol solvent and then condensed again using a water bath at 85℃ for 6 hours to form a thick extract. The yield of a sample is used to determine the amount of extract obtained. The extracts obtained were in the form of semi-solid colors of pink, light green and dark red.

Identification by adding potassium permanganate or 0.1% $KMnO_4$, where the original color disappears at room temperature and then turns brown. This means that the sample and standard standard solution positively contain vitamin C. Based on the results of the identification with the color reaction that has been carried out according to what is stated in the literature [13]. The results can be seen in Figure 3.

Figure 3. The alteration of vitamin C after reacted with $KMnO_4$

The reaction between 2 ml of potassium permanganate or $KMnO_4$ with vitamin C, which functions as a reducing agent is vitamin C, meaning that vitamin C in the reaction is a substance that undergoes oxidation. Meanwhile, potassium permanganate or $KMnO_4$ is a substance that undergoes reduction, meaning that $KMnO_4$ in this reaction functions as an oxidizing agent and functions as a reducing agent is vitamin C. Potassium permanganate or $KMnO_4$ can be reduced by vitamin C so that the permanganate ion (MnO_4-) which has a purple color turns into a precipitate brown indicates the formation of manganese ion (Mn^{2+}). Meanwhile, ascorbic acid or vitamin C is oxidized by permanganate to dehydroascorbic acid [14-15].

Preparation of vitamin C calibration curve

The 100 ppm standard solution of vitamin C showed the results of absorbance measurements of several standard vitamin C concentration solutions, namely 20 ppm, 25 ppm, 30 ppm, 35 ppm, 40 ppm, and 45 ppm which will be measured for the calibration curve using the linear regression equation. based on the vitamin C calibration curve in Figure 1. The greater the concentration of the vitamin C standard solution, the greater the absorbance that will be produced. This also shows that the relationship between concentration and absorbance produced at the Visible wavelength is linear with the R2 value of the linear regression equation of 0.9898 [7].

Based on the data obtained from the standard solution linear regression values, the equation Y = 0.0222x + -0.2189 was obtained with a correlation coefficient R^2 of 0.9898 which showed the linearity curve of the equation. The same if the R^2 value obtained is appropriate to show linear results because it meets the requirements where the R^2 value is in the range $0.9 \leq r \leq 1$. Then this calibration curve is quite good, and the regression line equation can be used to calculate the vitamin C content in sample [7]. The sensitivity of an analytical method can be expressed in the limit of detection (LOD). Limit of detection (LOD) is the smallest analyte content in the sample that can be detected and giving a significantly different response from blank or noise. The limit of detection is the level of analyte that responds to three times the standard deviation of the blank measurement. LOD is measured by the equation 3.3 SD/b, whereas the limit of quantition (LOQ) is calculated by the equation 10 SD/b. SD is the standard deviation of the absorbance value of the measurement results, and b is the slope and calibration curve equation [16]. The LOD formula = 3.3 (STEYX/SLOPE) obtained 3,496 ppm indicating that this method is capable of detecting an analyte around level of 3,496 ppm. If the sample contains vitamin C less than or below 3,496 then the selectivity and sensitivity of the method is not suggested. LOQ is the smallest amount of analyte in a sample that can still be measured accurately and precisely by a tool or instrument [17]. The LOQ formula = 10*(STYEX/SLOPE) The value obtained was 10.594 ppm. This indicated that the value of the analyte that can still be quantified with precision is above 10.59 ppm. The LOQ value is said to be good because the concentration value tested is above the LOQ value, so it can be accepted in terms of accuracy and precision [16].

The results of the research on vitamin C levels resulted in vitamin C levels from samples of red guava, crystal guava, and red guava, with the calculation results of the formula for vitamin C levels being 0.209% (w/v), 0.176% (w/v), and 0.137% (w/v), and the results of vitamin C levels from samples of red guava, crystal guava, and red guava by calculating the linear regression equation, namely 21.16 mg/5 g ± 0.005, 20.79 mg/5 g ± 0.029, and 19.16 mg/5 g ± 0.089 [18], [19], [20]. Differences in

the content of vitamin C in fruits can be influenced by various factors, namely harvest conditions, temperature, exposure to light, heating process, level of fruit maturity can also affect the amount of vitamin C levels in fruit. The more ripe the fruit, the higher the levels of vitamin C contained in the fruit. This is because the fruit ripening process has increased vitamin C [21]. From the comparison of the data obtained, it can be seen that the highest levels of vitamin C were found in red guava fruit, 21.16 mg/5 g, which is not in accordance with the theory that vitamin C in red guava fruit should be higher than red guava and crystal guava [22].

Vitamin C levels in curly red chilies were obtained by converting the absorbance data into the form of concentration (ppm) which were obtained respectively at 4.478; 4,478; 4.434 ppm and obtained an average yield of 4.463 ppm, which is 0.4463% w/b. Ascorbic acid recommended for consumption by adults is approximately 45 mg/day for 40 g of fresh chili. The need for vitamin C can be fulfilled if you consume 1000 g of red chili peppers [23].

From the calculation of the curve regression equation, it is obtained that the line equation is y = 0.215x + 0.015 with a correlation coefficient (r) of 0.999. From these results it can be said that there is a positive correlation between levels and absorption. That is, with increasing concentration, the absorbance will also increase. This means that there is 99.9% of data that has a linear relationship [24].

LOD is the smallest amount or concentration of analyte in a sample that can be detected, but does not need to be measured according to the actual value. LOQ is the smallest amount of analyte in a sample that can be determined quantitatively at a good level of accuracy and density. The results of the determination of LOD and LOQ. From the calculation results, the LOD value is 2.1546 mg/L, this value indicates the smallest amount of analyte that can still be detected by the UV-Vis spectrophotometry method [25].

The ascorbic acid calibration curve shows that the increase in the measured absorbance value is affected by the increase in the vitamin C concentration. This means that the greater the concentration of the vitamin C standard solution, the greater the absorbance produced. stated that an increase in the concentration of vitamin C would result in a linear increase in the absorbance value read on the UV-Vis spectrophotometry. This is in accordance with the Lambert-Beer law which states that the concentration of a sample is directly proportional to the absorbance value. Based on the absorbance values of the standard solution, the linear regression equation obtained from the calibration curve is y = 0.0813 x - 0.735 with a correlation coefficient (r) of 0.9341 which indicates the linearity of the equation. If the value of r = + (positive), then the relationship between concentration and absorbance is directly proportional, meaning that the value of r obtained is in accordance with the Lambert-Beer law. This is in accordance with study reported which stated that this correlation coefficient shows linear results, because it meets the requirements where the value (r) is in the range $0.9 \leq r \leq 1$. So, calibration curve is satisfactory, and the regression line equation can be used to calculate vitamin C levels in the sample [26].

Vitamin C is a water-soluble vitamin, therefore in this study a sterile aquabides solvent was used with the aim of reducing the risk of the presence of impurities and free of pyrogens. A standard solution of ascorbic acid was made at a concentration of 100 ppm, then diluted to 10 ppm to measure the maximum absorption wavelength and the result was 264 nm using a UV-Vis spectrophotometer. From the 100 ppm mother liquor, a series of concentrations of 4, 6, 8, 10 and 12 ppm was made and the absorbance was measured at a wavelength of 264 nm and then a calibration curve was made which was formed from the concentration and absorbance data. The linear regression equation of the calibration curve is y = 0.005 + 0.0657x with a correlation coefficient (r) of 0.9992 which shows the linearity of the equation [27].

Conclusion

Based on the results of the research that has been done, the following conclusions can be drawn: The level of vitamin C contained in red guava (*psidium guajava L.)* is 21.16 mg/5 g. The level of vitamin C contained in crystal guava (*psidium guajava L. Meer*) is 20.79 mg/5 g. The level of vitamin C contained in the red guava (*Syzygium aqueum*) is 19.16 mg/5 g.

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