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# ANALYSIS OF LYCOPENE CONTENT IN RED GUAVA JUICE (PSIDIUM GUAJAVA L.) BY VISIBLE SPECTROPHOTOMETRY

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**ABSTRACT**

Red guava (*Psidium guajava* L.) is a fruit that is rich in phytochemicals that are beneficial for health and is widely consumed in the form of juice. Lycopene belongs to the carotenoid group which has antioxidant potential and has an important role in the immune system to deal with homeostatic changes caused by oxidative stress. Lycopene is contained in a lot of red fruit. The purpose of this study was to analyze the lycopene content in red guava juice by visible spectrophotometry. Red guava juice extracted using n-hexane:acetone:methanol 1:2:1. Qualitative analysis was carried out by observing the spectrum, then measuring the maximum wavelength, which was supported by observing the spots by thin layer chromatography on silica gel 60 $GF_{254}$ plate using petroleum ether:acetone 9:1. Quantitative analysis was carried out by measuring absorbance by visible spectrophotometry at 470 nm. The results of qualitative analysis by visible spectrophotometry obtained 3 typical peaks of the lycopene spectrum at 400-550 nm, with a typical maximum wavelength of lycopene at 470 nm. Qualitative analysis by thin layer chromatography obtained spot with Rf 0.64. The results of quantitative analysis obtained lycopene levels of 2.91 ± 0.465 mg/100 grams of juice.

**Keywords:** Lycopene; Red Guava Juice; Visible Spectrophotometry

## 1. INTRODUCTION

In general, people tend to be reluctant to consume red guava (*Psidium guajava* L.) directly because of the very high seed content in the fruit flesh. Red guava is more popular when consumed in the form of juice because it can present seedless fruit in a soft texture without reducing the taste and freshness of the fruit when consumed. In addition, fruit juice can be made in a very easy and fast way. Fruit juice is also one form of snack that is often found. One of the fruit juices that is in great demand by the public is red guava juice.

Various benefits of red guava juice have been studied. Consuming 100 grams of red guava juice per day can reduce tracheal damage due to exposure to cigarette smoke (Febrianti & Suryati, 2014). Giving 250 ml of red guava juice for 21 days significantly reduces the risk of heart disease (Anugrah et al., 2017). The effect of red guava juice on increasing immunity has also been studied by Aldi et al. (2012). These various benefits are related to the antioxidant content in red guava juice.

One of the very strong antioxidants in red guava juice is lycopene. Lycopene is a bright red pigment of the carotenoid group that is potentially contained in red-fleshed fruits, such as red guava. Lycopene is able to bind single oxygen 2 times more than β-carotene (Monica & Rollando, 2019) and ward off free radicals 100 times more effectively than vitamin E (Alfa et al., 2019; Fadilah, 2012; Hamsina et al., 2019). Supplementation with lycopene-rich intake for 1 week can increase serum lycopene levels, thereby suppressing lipid, protein, lipoprotein, and DNA oxidation. Thus, lycopene can eliminate or reduce oxidative stress which has a key role in

reducing the risk of various chronic and degenerative diseases (Kumar et al., 2017; Mehta et al., 2018).

Determination of lycopene levels has been carried out on various red-fleshed fruits using visible spectrophotometry (Arifulloh et al., 2016; Hamsina et al., 2019; Sima et al., 2019; Tristiyanti et al., 2018). Extraction of lycopene from watermelon juice (Hamsina et al., 2019) showed optimum results using n-hexane:acetone:ethanol 2:1:1 with a juice and solvent ratio of 1:2. Based on the research results Arifulloh et al. (2016) it is known that the optimal solvent for extracting lycopene from tomatoes is a mixture of n-hexane:acetone:methanol 1:2:1 with a material:solvent ratio of 1:5. While Maulida & Zulkarnaen (2010) states that the amount of lycopene in tomato juice can be up to 5 times more than fresh tomatoes. In the study Tristiyanti et al. (2018), red guava fruit lycopene was extracted by reflux method using n-hexane solvent. However, no research has been conducted to extract lycopene from red guava juice using liquid-liquid extraction method. Therefore, this study aims to analyze the lycopene content in red guava juice by visible spectrophotometry, which was extracted by liquid-liquid extraction method using n-hexane:acetone:methanol 1:2:1 at a juice:solvent ratio of 1:5.

## 2. METHODS

### 2.1. Research Materials and Tools

The materials used include: red guava (*Psidium guajava* L.) from 3 different supermarkets in Surakarta, silica gel GF254 (Merck), petroleum ether p.a (Merck), acetone p.a (Merck), n-hexane (Merck), acetone (Merck), methanol (Merck), distilled water (Brataco).

The tools used include: a set of UV-Vis spectrophotometers (Shimadzu UV-1280), cuvettes (Hellma Analytics 100.600-QG light path 10 mm), analytical balance (Ohaus Pioneer with a sensitivity of 0.0001 g), rotary evaporator (RV 10 basic), shaker (Rotator HSR-200), blender (Cosmos), and glassware commonly used in analytical chemistry.

### 2.2. Research Procedures

#### *2.2.1. Making Red Guava Juice*

Red guava from 3 supermarkets were washed and cleaned. The red guava whose skin surface was clean and dry was cut into several pieces, then homogenized and pureed with a blender until it became juice.

#### *2.2.2. Lycopene Extraction*

Red guava juice was weighed 200.0 mg, then added 50 ml of methanol and stirred for 5 minutes. Separate the seeds from the juice mixture by filtering. The juice mixture that had been freed from seeds was put into a closed Erlenmeyer flask and coated with carbon paper on the outside, then extracted using a mixture of 250 ml of n-hexane, 500 ml of acetone, and 250 ml of methanol. During the extraction process, it was shaken with a shaker at a speed of 150 rpm for 30 minutes. The mixture was put into a separating funnel, added with 10 ml of distilled water and shaken again, then left to stand until two phases were formed. The top layer was taken and then evaporated using a rotary evaporator at a temperature of 40 °C at a speed of 75 rpm. The concentrated extract obtained was then weighed and put into a brown glass bottle (Arifulloh et al., 2016; Monica & Rollando, 2019).

#### *2.2.3. Preparation of sample solution*

The concentrated extract was weighed 10.0 mg then dissolved in petroleum ether p.a to 10.0 ml (Monica & Rollando, 2019). The solution was pipetted 3.0 ml then diluted with petroleum ether p.a to 10.0 ml to obtain a concentration of 3 µg/ml, which was then used as a sample solution. Qualitative Analysis by Visible Spectrophotometry The sample solution was scanned at a wavelength of 400-550 nm. The spectrum shape and maximum wavelength produced by the sample solution were compared with standard lycopene in the reference (Arifulloh et al., 2016).

#### *2.2.4. Qualitative Analysis by Thin Layer Chromatography*

The sample solution was spotted on a silica gel 60 GF254 plate and then eluted with petroleum ether: acetone 9:1. The Retention Factor (Rf) value of the spots produced by the sample solution was compared with the standard lycopene in the reference (Harini & Sumathy, 2016).

#### *2.2.5. Quantitative Analysis by Visible Spectrophotometry*

The absorbance of the sample solution was measured at a maximum wavelength of 470 nm. The lycopene content was calculated using the equation C = A/($\sum_{cm}^{1\%} \times B$), with C the concentration of the sample solution (%), A the absorbance of the sample solution, $\sum_{cm}^{1\%}$ lycopene absorptivity, B the thickness of the solution in a 1 cm cuvette (Hamsina et al., 2019).

## 3. RESULTS AND DISCUSSION

### 3.1. Making Red Guava Juice

In this study, red guava (*Psidium guajava* L.) from supermarkets was used to ensure a more guaranteed level of fruit freshness, because the storage temperature in supermarkets is better maintained compared to traditional markets. The red guava used has the characteristics of ripe fruit with a texture that is not hard, yellowish skin, no defects, and fresh red flesh. The red guava was washed first to clean dirt from the surface of the skin. The clean red guava was cut into several pieces to facilitate the juice making process. Before being made into juice, all pieces of red guava from 3 supermarkets were homogenized to ensure sample homogeneity without comparing the origin of the sample.

### 3.2. Lycopene Extraction

At the start of extraction, red guava juice was first added with methanol and stirred for 5 minutes, as in Arifulloh et al. (2016). This aims to attract compounds in red guava juice that have polar properties so that in the extraction process more non-polar compounds will be extracted. After being filtered, the red guava juice residue is extracted using the liquid extraction method because lycopene is unstable at high temperatures (Fadilah, 2012) using n-hexane:acetone:methanol 1:2:1 with a juice:solvent ratio of 1:5. Arifulloh et al. (2016) stated that the use of n-hexane:acetone:methanol 1:2:1 was able to extract more lycopene than other solvent variations.

The extraction process is assisted by shaking using a shaker at a speed of 150 rpm for 30 minutes, then filtered to separate the filtrate from the sediment. During the extraction process, the solvent liquid will penetrate the cell wall and then enter the cell cavity containing the active substance. The solvent that has extracted the active substance will be pushed out due to the difference in concentration between the solution and the active substance in the cell and outside the cell. This event is repeated until there is a concentration equilibrium between the solution outside the cell and inside the cell. The results obtained are orange filtrate. The extraction process is continued by adding distilled water in a separating funnel to form two layers of polar and non-polar phases. The nhexane phase in the upper layer containing lycopene is taken and then concentrated with a rotary evaporator until a reddish concentrated extract is obtained.

### 3.3. Qualitative Analysis by Visible Spectrophotometry

The bright red color produced by lycopene is a result of the many double bonds in the chemical structure of lycopene, so that it is able to absorb electromagnetic radiation energy in the visible region (visible light) with a high wavelength (Maulida & Zulkarnaen, 2010). Compounds with more conjugated double bonds have higher maximum wavelength values. Based on the chemical structure presented in Figure 1, lycopene consists of 8 isoprene units with 11 unsaturated double bonds so that it will absorb at the highest wavelength compared to other carotenoids. This is what makes lycopene measurable by visible spectrophotometry.

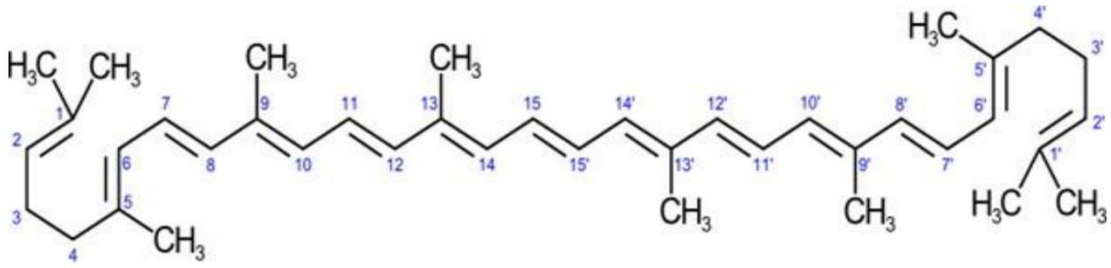

**Figure 1**. Chemical structure of lycopene (Monica & Rollando, 2019)

Identification of lycopene by visible spectrophotometry is done by comparing the spectrum shape and maximum wavelength of standard lycopene in the reference with the measurement results of lycopene in the sample solution. The absorption spectrum of lycopene is typical in the 400-550 nm region, with maximum absorption at 3 main wavelengths depicted in the form of 3 peaks around 444, 470, 502 nm as presented in Figure 2.

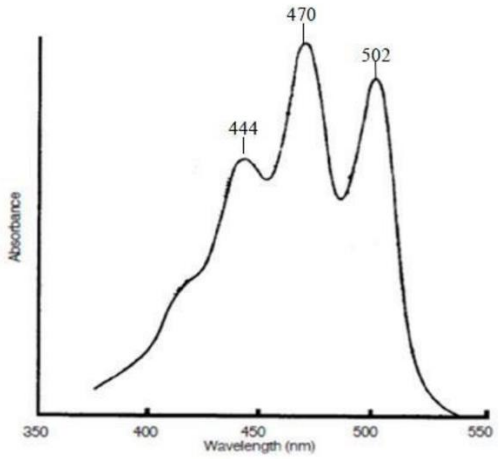


**Figure 2**. Standard spectrum of lycopene in petroleum ether (Arifulloh et al., 2016)

In the measurement of the absorbance of the sample solution, an absorption spectrum with 3 main peaks located between 400-550 nm was also produced, as shown in Figure 3. The absorbance spectrum of the sample solution indicates the presence of lycopene in red guava juice with the presence of three main wavelengths typical of lycopene, which are similar to the standard lycopene spectrum in the reference, with the results presented in Table 1. This is in accordance with the characteristics of unsaturated double bonds in the chemical structure of lycopene.

The conformity of the spectrum shape is strengthened by the conformity of the maximum wavelength value produced by the sample solution to the maximum wavelength of the standard lycopene in the reference. Based on the data in Figure 3 and Table 1, it is known that the highest absorbance of the sample solution was obtained at a wavelength of 470 nm. This is the same as the maximum wavelength of the standard lycopene in Figure 2, which is 470 nm. The maximum wavelength is determined by the chemical structure of the compound containing the chromophore (Gandjar & Rohman, 2018), namely the unsaturated group in the covalent bond which is responsible for the occurrence of electronic absorption (Dachriyanus, 2004). Therefore, the suitability of the maximum wavelength value of the analyte and standard is used as a parameter for identifying the analyte in the sample solution. Based on the suitability of the spectrum shape, the peak wavelength of the spectrum and the maximum wavelength produced by the absorbance of the sample solution to the standard lycopene in the reference, it proves that red guava juice contains lycopene.

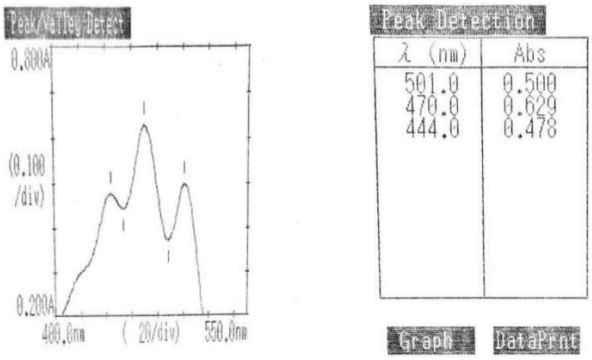


**Figure 3**. Lycopene spectrum of sample solution in petroleum ether

**Table 1**. Peak wavelength of the sample solution spectrum in petroleum ether

| Sample Solution | Peak 1 | | Peak 2 | | Peak 3 | |
|---|---|---|---|---|---|---|
| | λ (nm) | Abs | λ (nm) | Abs | λ (nm) | Abs |
| 1 | 444.0 | 0.478 | 470.0 | 0.629 | 501.0 | 0.500 |
| 2 | 443.5 | 0.495 | 470.0 | 0.638 | 501.0 | 0.510 |
| 3 | 443.5 | 0.473 | 470.0 | 0.625 | 501.0 | 0.496 |

### 3.4. Qualitative Analysis by Thin Layer Chromatography

Thin Layer Chromatography (TLC) is a method used to separate a compound based on the difference in distribution of two phases, namely the stationary phase and the mobile phase. Qualitative analysis using the TLC method aims to identify the presence of lycopene compounds in sample solutions by comparing the Rf value of the lycopene standard in reference to the Rf value of the spots produced by the sample solution. The elution process is carried out on a stationary phase of silica gel 60 GF254 with a mobile phase of petroleum ether: acetone 9:1 (Harini & Sumathy, 2016). The elution results as presented in Figure 4, are in the form of a single reddish orange spot with an Rf value of 0.64. These results are the same as the Rf value of standard lycopene in the research study Harini & Sumathy (2016). Thus, red guava juice is proven to contain lycopene.

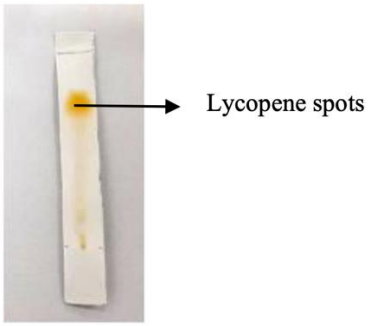


**Figure 4**. Results of Elution of Sample Solution on Silica Gel 60 GF254 with Petroleum Ether: Acetone 9:1 as Mobile Phase

### 3.5. Quantitative Analysis by Visible Spectrophotometry

Quantitative analysis was carried out by measuring the absorbance of the sample solution using visible spectrophotometry at 470 nm. Based on Sima et al. (2019), standard lycopene in the reference is used as a comparative standard in quantitative analysis, so that the calculation of lycopene levels is carried out using the Lambert-Beer equation with a value of $\sum_{cm}^{1\%}$ 3.450. Absorptivity expressed in $\sum_{cm}^{1\%}$ value describes the absorption power or ability of the analyte to absorb electromagnetic radiation at the measurement wavelength. Absorptivity does not depend on the concentration and intensity of radiation but is determined by the chemical structure of the compound, solvent, and wavelength of radiation. Thus, the $\sum_{cm}^{1\%}$ value becomes a setting or

constant that is specific to each compound molecule in a certain solvent and wavelength (Gandjar & Rohman, 2007), so that the $\sum_{cm}^{1\%}$ value is also called the specific extinction coefficient (Dachriyanus, 2004).

**Table 2**. Lycopene content in red guava juice

| Sample | Content (mg/100 mg juice) |
|---|---|
| 1 | 2.38 |
| 2 | 3.25 |
| 3 | 3.10 |
| Average | 2.91 |
| Standard Deviation | 0.465 |

Based on the data in Table 2, in this study, the lycopene content in red guava juice was obtained with an average of 2.91 ± 0.465 mg/100 grams of juice. These results were obtained using the liquid-liquid extraction method because lycopene is unstable at high temperatures (Fadilah, 2012) using n-hexane:acetone:methanol 1:2:1 with a juice:solvent ratio of 1:5 (Arifulloh et al., 2016). Acetone is soluble in both n-hexane and methanol, so acetone is expected to act as a polarity bridge in the mixed solvent. Therefore, in a ratio of 1:2:1, it is expected to optimize the withdrawal of lycopene from polar solvents (watermethanol) into non-polar solvents (n-hexane). Arifulloh et al. (2016) stated that the use of n-hexane:acetone:methanol 1:2:1 was able to extract more lycopene compared to other solvent variations, such as petroleum ether:acetone (3:1), n-hexane:acetone:methanol 2:1:1, and n-hexane:acetone:methanol 1:1:1. In the research study Tristiyanti et al. (2018), the lycopene content in guava fruit was obtained at 7.5 mg per 100 grams of fruit. The higher content in the study was thought to be due to the use of the reflux method with n-hexane as the sole solvent in the extraction process, so that it was better able to draw non-polar lycopene.

## 4. CONCLUSION

Red guava juice extracted by liquid-liquid extraction method using nhexane:acetone:methanol 1:2:1 at a juice:solvent ratio of 1:5, can obtain lycopene with an average content of 2.91 ± 0.465 mg/100 grams of juice. The use of n-hexane as a single solvent is recommended to obtain more lycopene.

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## 6. AUTHOR DECLARATION

### Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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### Availability of Data and Materials

All data are available from the authors.

**Competing Interests**
The authors declare no competing interest.

**Additional Information**
No additional information from the authors.

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