

## Determination and Characterization of Lycopene Compounds from N-Hexane Fraction of Potato-Leaved Tomato (*Solanum Lycopersicum Grandifolium*) by using Fourier Transform Infrared and UV-Vis Spectrophotometry

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### Abstract

Determination and characterization of the lycopene content of the n-hexane fraction from potato-leaved tomato (*Solanum lycopersicum grandifolium*) using Fourier transform infrared (FTIR) spectrophotometry and UV-Vis spectrophotometry. This study aims to determine the levels and characterization of lycopene compounds from potato-leaved tomato fruit using FTIR and UV-Vis spectrophotometers. The methods in this research include maceration, liquid-liquid extraction, crystallization with antisolvent, chromatography with three eluent systems, identification of lycopene compounds, and characterization of lycopene compounds using FTIR and UV-Vis. The results showed the presence of lycopene compounds based on the spectrum with absorption peaks at wave numbers 2924, 18  $\text{cm}^{-1}$  and 2863, 66  $\text{cm}^{-1}$  indicating C-H (stretching); 1637, 43  $\text{cm}^{-1}$  indicating C=C alkene; and the wave numbers of 1461, 63  $\text{cm}^{-1}$  and 1422, 46  $\text{cm}^{-1}$  indicating the presence of a  $\text{CH}_2$  (bending) functional group. The results of UV-Vis spectrophotometric characterization showed the presence of lycopene compounds at three main wavelengths, namely at 445, 467, and 500 nm. The level of lycopene compounds produced from the n-hexane extract of potato-leaved tomato fruit was 7.25 mg/gram.

**Keywords:** characterization; maceration; lycopene; three eluent systems

### Introduction

One of the challenges that the community faces in the surrounding environment is the decline in human immune function, which makes them more vulnerable to free radicals. Consuming antioxidant-rich fruits can help prevent the formation of free radicals. One of

these fruits is tomatoes. Tomato fruit plants contain 60-64 % w/w lycopene compounds (Susanti, Dewi, and Widjaja, 2014). Lycopene compounds have antioxidant activity, which means they can protect human cells from free radicals.

Lycopene has bioactivity as an antioxidant and can prevent or reduce the risk of various chronic diseases, including cancer, cardiovascular disease, and osteoporosis (Dewi, Hakim, and Savalas, 2019). Tomatoes contain lycopene, which can help prevent diseases like cholesterol buildup in blood vessels (Noviyandari Dini, 2019). Lycopene was discovered to absorb single oxygen twice as well as beta-carotene and ten times better than tocopherol (Imran *et al.*, 2020). The extraction method is one method for isolating lycopene compounds in potato-leaved tomato fruit.

The solvent used depends on the state of the sample and the carotenoid composition. Research by Dewi (2018) was successful in extracting lycopene from red tomatoes (*L. Esculentum* Mill) using an n-hexane solvent and a maceration method, resulting in a lycopene content of 2.25 mg/100 g of dried tomato powder. In contrast to Setyawati (2019), the liquid-liquid extraction method was used with different types of fruit tomatoes and a solvent mixture (hexane, acetone, and ethanol) at a concentration of 0.04 mg/g. Similarly, Roh *et al.*, (2015) discovered a lycopene content of 3.50 mg/g in tomato fruit (*L. Esculentum*) extracted using the soxhletation extraction method with n-hexane as the solvent. Though, in this research, the solvent n-hexane was used because it was the most widely used in previous studies.

Some researchers have researched the characterization of lycopene in tomatoes. But, this research utilizes potato-leaved tomatoes because, according to previous literature searches, few have investigated the levels of lycopene in potato-leaved tomatoes. Potato-leaved tomatoes have a unique feature in that they have thick flesh and reddish fruit color, resulting in higher levels of lycopene. The following are some lycopene characterizations using various methods. Susanti, Dewi, and Widjaja, (2014), conducted a liquid-liquid extraction method and characterization using UV-Vis instruments on vegetable tomatoes, producing 0.42 mg/100 g. Dewi, Hakim, and Savalas, (2019) isolated

lycopene from vegetable tomatoes using reflux extraction and antisolvent crystallization, producing a lycopene content of 5.12 mg/100 g. Some of these researches appear to indicate that more research on tomato varieties, particularly potato-leaved tomatoes, is required. Furthermore, researchers who studied the determination of lycopene levels used a variety of instruments and simple methods. As a result, it is necessary to develop a more efficient method for compound characterization, one of which is the combination of FTIR and UV-Vis.

Based on this background, researchers used a Fourier Transform Infrared (FTIR) and UV-Vis spectrophotometer to determine and characterize the n-hexane fraction of lycopene compounds from potato-leaved tomato (*Solanum lycopersicum grandyfolium*).

## Research methods

### Tools and Materials

The tools used were: Fourier Transform Infrared (FTIR) Spectrophotometer (Thermo Scientific Nicolet iS10), UV-VIS Spectrophotometer (Varian Cary 50), rotary evaporator (IKA RV 10 Digital V), thin layer chromatography plate (TLC Silica Gel 60 F254), analytical balance ABJ 220-4M (Kern, Germany) and analytical balance (Precisa, Swaziland), separating funnel (Schot Duran, Germany) and measuring flask (Pyrex).

The materials used were distilled water/aquades, aluminum foil, potato-leaved tomatoes (*Solanum lycopersicum grandyfolium*), ethyl acetate p.a ( $\text{CH}_3\text{COOC}_2\text{H}_5$ ), Whatman 42 filter paper (Ashles filter papers), ordinary filter paper, chloroform p.a ( $\text{CHCl}_3$ ), methanol p.a ( $\text{CH}_3\text{OH}$ ), anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), n-hexane p.a ( $\text{C}_6\text{H}_{12}$ ), petroleum ether p.a, lycopene standard (Macklin L812281-20 mg; CAS: 502-65-8), silica gel 60 (Desiccant), stibium trichloride p.a ( $\text{SbCl}_3$ ) and tissues.

### Work procedures

Potato-leaved tomatoes (*Solanum lycopersicum grandyfolium*) were washed,

then cut, and seeds removed from the tomatoes, then dried at room temperature 75 °C. After drying, they were mashed by using a blender and stored in a dark place.

Then 100 grams of dried tomato powder were weighed. After that, it was put in a maceration container. Maceration was carried out by immersing the sample in a solvent of n-hexane p.a. The process was carried out three times, and every 24 hours the solvent was filtered, and the solvent was changed. Then, it was accommodated into the resulting maceration container for evaporation on a rotary evaporator to obtain a thick extract.

First, the macerated viscous extract was weighed to determine the initial weight. Then, the extract was dissolved with n-hexane p.a as much as 50 mL, put into a separatory funnel with a 50 mL (1:1) distilled water partition, and then shaken to separate the impurity layer. Let it stand until the fraction in the separating funnel is clear. All non-polar layers are accommodated in a beaker glass when two layers are formed. Next, dry it with anhydrous sodium sulfate.

The fraction of the liquid-liquid extraction was dripped little by little with methanol as an anti-solvent. Then, let it stand in a container containing ice cubes until crystals are formed. Filter the formed crystals using Whatman filter paper No. 42. The crystals obtained were weighed to determine their weight, then stored in vials as lycopene crystals.

Three chambers measuring 5 mL each are provided. Three thin layer chromatography (TLC) plates were measured with a length of 7 cm and a width of 2 cm, then heated in an oven at 110°C for 15 minutes. Then the mobile phase was made using three types of n-hexane eluent: PE (9:1); Ethyl acetate: methanol (4:6); and n-Hexane: Ethyl acetate (1:9). The crystals and lycopene standard were dissolved with n-hexane. Spot solutions were made on the three baselines of the TLC plate. Wait for the spot to reach the top line limit of the TLC plate, then let it stand

for a while. Spots were observed at 366 nm UV light and the Rf value was calculated.

Dissolve lycopene crystals, fractions, and extracts with n-hexane. Then 2-3 drops of Carr Price reagent ( $\text{SbCl}_3$  in chloroform) were added, and the changes were observed. Positive results when an orange or brownish-red color is formed in the solution after the addition of  $\text{SbCl}_3$ .

Lycopene crystals produced from antisolvent crystallization were analyzed using FTIR with samples in the form of KBr pellets. Lycopene crystals weighing up to 20 mg were weighed and then dissolved in as much as 10 mL n-hexane. Meanwhile, 2.5 mg of lycopene standard was prepared and then dissolved with 25 mL of n-hexane, making a series of 20, 30, 40, 50, and 60 ppm. Next, the absorbance of the solution was measured at a maximum wavelength of 469 nm with a UV-Vis spectrophotometer. The cuvette was cleaned with a solvent suitable for the nature of the tomato extract.

## Results and Discussion

### *Extraction and Separation*

The tomatoes used in this study were potato-leaved tomatoes. The first thing done before dried is washing them with water until they clean to remove impurities that are on the skin of the fruit. Then, cut the tomatoes and remove the seeds. The process of cutting tomatoes needs to be considered. According to Daniel, et al. (2017), 80–90% of the lycopene content in tomatoes is located on the outside of the skin.

The next process is drying tomatoes using an oven at a temperature of 75 °C for 24 hours. Previously, heating was carried out at temperatures of 60 and 70 °C, but the results obtained were not optimal because the tomatoes had rotted after storage. Therefore, the temperature was increased to 75 °C. According to Ma'sum, et al. (2014), heating tomatoes at a temperature of +70 °C makes their components more stable. But at 80 °C, it will be degraded of tomato fruit content such as lycopene.

After drying, maceration was carried out by weighing 100 grams of dried tomato powder. Then, it was macerated using n-hexane as a solvent, which served as an attractant for lycopene compounds. The immersion of the sample in this research was carried out for 3 repetitions of replacement. The results obtained from maceration were as much as 900 mL, continued by evaporation on a rotary evaporator using low pressure at a temperature of 45 °C to speed up the solvent evaporation process (Halim, 2014).

Table 1. the yield of potato-leaved tomato extract.

No.	Sample	Yield (%)	Color
1.	Potato-leaved tomato	1.23	Orange
2.	Lycopene Crystal	0.28	Brownish red

In table 1, the thick extract obtained was 1.2362 grams or a yield of 1.23%. After being compared with several researchers such as Zuorro, (2020), which obtained a thick extract of 0.028 grams of lycopene or a yield of 0.28% using n-hexane as a solvent, research by Aghel and Ramezani, (2007) which obtained a yield of 2.313 mg/100 grams using methanol as a solvent, it can be concluded that this research obtained a higher concentration and the solvent was also a factor affecting the yield.

Liquid-liquid extraction aims to separate non-polar and polar compounds in the macerated viscous extract. This process was carried out using the solvent n-hexane: distilled water with a composition of 1:1 to obtain lycopene content from purer tomatoes. This method was carried out three times or three times with the addition of distilled water, which aims to make the polar components separate. The result of the liquid-liquid partition is 3 layers: the top layer is the polar component, the middle layer is the precipitate formed, and the bottom layer is the non-polar component.

#### **Purification of lycopene compounds**

The lycopene purity test was carried out using crystallization with antisolvent and TLC with three eluent systems. The extract was purified using methanol as an antisolvent, which functions to dissolve the  $\beta$ -carotene compound and triglycerides so that the lycopene compound is insoluble in methanol and forms a separated phase from the solution. The results of this research can be seen in the following figure:

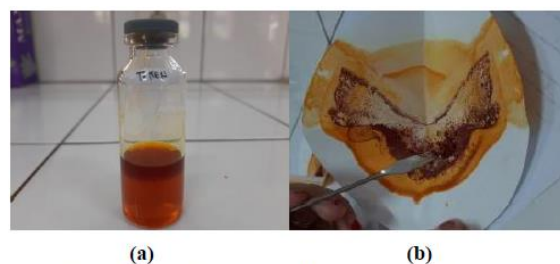


Figure 1. Crystallization of lycopene compounds using methanol. (a) Vial lycopene yield (b) Lycopene crystals on filter paper

In Figure 1 part (a), there was a two-phase formation between the solvent and a precipitate indicated as a lycopene compound, but the precipitate formed was in the upper layer, indicating that the yield in each vial was very light. Figure 1 part (b) is a lycopene crystal formed on filter paper with a brownish-red color, indicating a lycopene compound. The resulting crystals are in powder form with a concentration of 0.2856 grams. The results obtained in this research were higher than the research (Tarigan, Sinaga, and Masyithah, 2016), which obtained levels of antisolvent crystallization of methanol of 3.2 mg/150 mL and ethanol of 2.8 mg/150 mL.

Table 2. TLC results with three eluent systems

Sample	Value of Rf		
	n-Hex: PE (9:1)	EtOAc: MetOH (4:6)	n-Hex: EtOAc (1:9)
Potato-leaved tomato	0.1	0.6	0.9
Lycopene Standard	0.1	0.6	0.9

Furthermore, the purity test of lycopene compounds was carried out by chromatography. There are several chromatographic methods, including thin-layer chromatography and column chromatography. Research by (Aghel and Ramezani, 2007) Furthermore, the purity test of lycopene compounds was carried out by chromatography. There are several chromatographic methods, including thin-layer chromatography and column chromatography. Research by (Aghel and Ramezani, 2007) explained that column chromatography is effective in lycopene purification, but this method is not suitable for this research because it has drawbacks such as requiring large samples and only providing color-visible results. The TLC method was chosen because the shape of the spot and its color will be observed. In addition, the sample required is also small.

This research used the thin layer chromatography method with three eluent systems. The mobile phase is very important to perform TLC because it must be adjusted to the level of the polarity of a compound to be identified. This method uses several eluents, including n-hexane: petroleum ether (9:1), ethyl acetate: methanol (4:6), and n-hexane: ethyl acetate (1:9). These eluents are the best of several experiments that have been carried out.

The eluent of n-hexane: petroleum ether (9:1), the spot formed between the sample and the standard formed the same spot with Rf value of 0.1. The second plate using ethyl acetate eluent: methanol (4: 6) produces the same spot between sample and standard with Rf value of 0.6. And the last plate used n-hexane eluent: ethyl acetate (1:9) with Rf value of 0.9 (Table 2). The resulting stains are all orange in color.

### Identification of lycopene compounds

Identification of lycopene compounds in potato-leaved tomatoes was carried out by adding Carr Price reagent to determine the color changes that occurred. The results of the

color changes can be seen in Table 3 and Figure 2.

Table 3. Phytochemical test using  $SbCl_3$  (in chloroform)

Sample	Color Change	
	Before	After
Lycopene Extract	Orange	Blue
Lycopene Fraction	Orange	Orange
Lycopene Crystal	Orange	Brownish Red

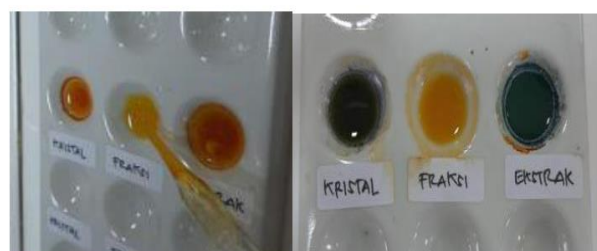


Figure 2. Phytochemical test using  $SbCl_3$  (a) Before adding Carr Price reagent (b) After adding Carr Price reagent

The color changes that occurred in the crystals after the addition of the Carr Price reagent are brown, the fraction is orange and the extract is almost bluish brown. In line with the research (Sumarlin *et al.* 2015). It explains that the presence of carotenoids is indicated by the formation of a red-orange or brown color after the addition of the Carr Price reagent. The extract formed has a brown color because the carotenoids form complexes with Sb metal ions as shown in the reaction in Figure 3.

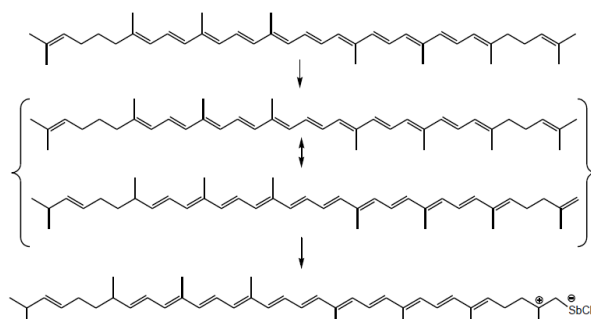


Figure 3. The reaction of lycopene compounds after the addition of  $SbCl_3$ .

**Determination and characterization of lycopene compounds**

Crystal characteristics of lycopene compounds from potato-leaved tomato fruit using FTIR were used to identify the functional groups of lycopene compounds. This research used KBr as a pellet. The results of the lycopene crystal test can be seen in table 4 and figure 4.

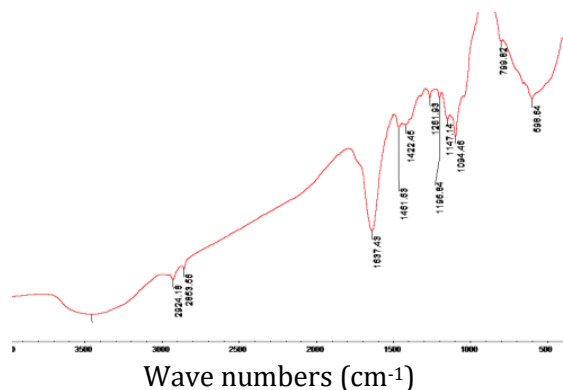


Figure 4. the FTIR results of potato-leaved tomato fruit

Table 4. The FTIR results of potato-leaved tomato fruit

Wave number	Absorption area (Dachriyanus. 2004)	Functional group forecast (Vibration type)
2924. 18-2862. 66	3000-2700	C-H (stretch)
1637. 43	1675-1500	C=C
1461. 63-1422. 46	1475-1300	CH (bending)

The table and figure above show the spectrum of lycopene compounds contained in potato-leaved tomato fruit samples. The absorption peak is at wave number 2924, 18 cm<sup>-1</sup>; 2862, 66 cm<sup>-1</sup>; 1637, 43 cm<sup>-1</sup>; 1461, 63 cm<sup>-1</sup>; 1422, 46 cm<sup>-1</sup>; 1261, 93 cm<sup>-1</sup>; 1147, 14 cm<sup>-1</sup>; 1094, 46 cm<sup>-1</sup>; 799,82 cm<sup>-1</sup>, and 598,64 cm<sup>-1</sup>. Absorption of 2924, 18 cm<sup>-1</sup> and 2862, 66 cm<sup>-1</sup> indicate the presence of a C-H group (stretching) with low intensity, which can be seen in the band that does not form a straight line. Absorption of 1637, 43 cm<sup>-1</sup> shows a C=C

group (aromatic ring). Absorption of 1461, 63 cm<sup>-1</sup> and 1422, 46 cm<sup>-1</sup> indicate the presence of a CH<sub>2</sub> group (bending). There is absorption in the fingerprint area at wave number 799,82 cm<sup>-1</sup>, which is CH<sub>2</sub> with rocking vibrations, and 598, 64 cm<sup>-1</sup> indicates the presence of a long chain.

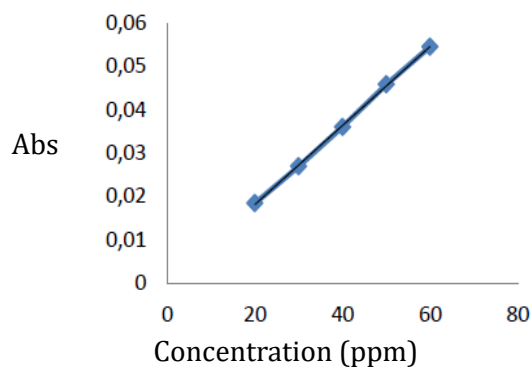
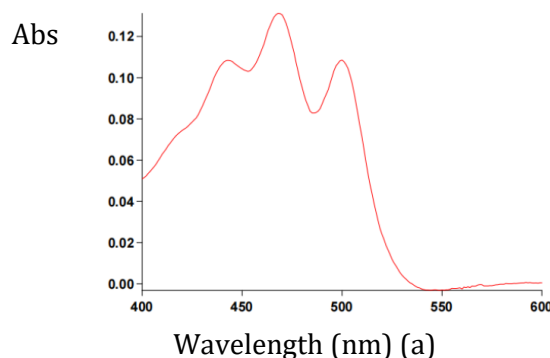


Figure 5. Standard curve of lycopene compound on UV-Vis spectrophotometer

Value of the regression equation in Figure 5:

$$Y=0.0009X-0.0001$$

$$R^2=0.9995$$



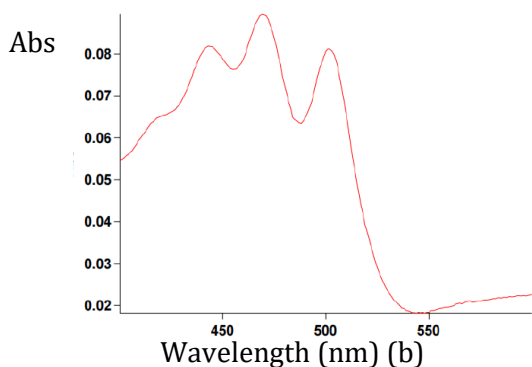


Figure 6. Absorption of lycopene at UV-Vis wavelength (a) Lycopene crystalline absorption (b) Lycopene standard absorption

Table 5. Absorbance and levels of lycopene compounds

Sample	Absorbance	Concentration mg/gram
Potato-leaved tomato	0.1304	7.25

Compounds that have a lot of conjugated double bonds will form the highest wavelength ( $\lambda$  max) or spectrum. Lycopene is a compound with 11 conjugated double bonds. It is higher than other carotenoid compounds in forming wavelength absorption. From Figure 6, part (a) at the absorption of lycopene crystal wavelengths in potato-leaved tomato fruit, three main wavelengths were obtained, namely the first peak with a wavelength of 445 nm, the second peak of 467 nm, and the third peak of 500 nm. This is almost by Lusweti (2017) who produces the main peaks of lycopene compounds with maximum wavelengths of (444, 472, and 502 nm) and (444, 471, and 502 nm).

The lycopene standard wavelength data in Figure 6 part (b) aims to compare the results of lycopene crystal samples. The absorption of lycopene standard wavelengths is 445, 469, and 501 nm, which is almost the same as that obtained in crystals indicated by lycopene. It confirms that lycopene crystals

from potato-leaved tomato fruit have been proven to contain lycopene compounds. After analyzing the wavelength, the lycopene content was determined by using the regression equation formula. The absorbance result is 0.1304 with a concentration of 7.25 mg/gram (Table 5). The concentration result is higher than the Tesalonika (2016) research, which obtained 1117.152  $\mu\text{g/mL}$  from cherry tomato fruit using n-hexane as a solvent. It is proven that the solvent greatly affects the amount produced between the ratio of solvents or not. As mentioned in Tesalonika's research using a solvent ratio, the resulting concentration was smaller than in this research.

## Conclusions and suggestions

### Conclusion

The determination and characterization of n-hexane fraction lycopene compounds from potato-leaved tomato fruit (*Solanum lycopersicum grandyfolium*) by FTIR spectrophotometry showed spectra at absorption peaks such as 2924, 18  $\text{cm}^{-1}$  and 2863, 66  $\text{cm}^{-1}$  indicating C-H (stretch); 1637, 43  $\text{cm}^{-1}$  indicates C=C alkene; 1461, 63  $\text{cm}^{-1}$  and 1422, 46  $\text{cm}^{-1}$  indicates the presence of the CH<sub>2</sub> (bending) functional group. The results of UV-Vis spectrophotometry analysis showed the presence of lycopene compounds at three main wavelengths, namely 445, 467, and 500 nm. The lycopene content produced from the n-hexane fraction of potato-leaved tomato fruit was 7.25 mg/gram.

### Suggestion

For further research, it is necessary to conduct research on tomato fruit by using multiple solvent comparisons and extraction so that it is known which solvent produces the highest lycopene yield.

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