

Chlorophyll and Magnesium Content of Papaya Leaves Extract (*Carica Papaya* L.) at Various Extraction Times

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ABSTRACT

Purpose: This research is to determine the suitable extraction time required to obtain extracts with the highest contents of chlorophyll and magnesium.

Research Method: Papaya leaves extracted by maceration method with acetone as a solvent, 4 groups with 6 levels of extraction time treatment. Chlorophyll contents were analyzed using the Arnon equation, magnesium contents were analyzed using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), and the energy gap was analyzed for the highest chlorophyll contents by touch plot method.

Findings: 48 hours extraction treatment showed the highest chlorophyll and magnesium content, resulting in an average content of 13.272 mg/L for total chlorophyll, 11.380 mg/L for chlorophyll a, 1.881 mg/L for chlorophyll b and 934.771 mg/kg of magnesium content. The direct energy gap and indirect energy gap of the highest extract are respectively obtained at 2.428 eV and 2.442 eV.

Originality/Value: Based on the obtained results, it was concluded that papaya leaf extraction for 48 hours produced the highest chlorophyll and magnesium contents, which can be used as a natural dye sensitizer for renewable energy Dye-Sensitized Solar cells.

Keywords: *Carica papaya* L. leaves, Chlorophyll, Dye-sensitized solar cell, Extraction, Magnesium.

INTRODUCTION

Renewable energy is a research topic that is often carried out today. Renewable energy has been developed in various sectors, including biomass, water, wind and solar. One of the renewable energies that are currently being widely developed is solar cells or photovoltaics. In photovoltaics, light particles hit the cells and hit the electrons of the atoms that make up the photovoltaic, causing an electric current. One of the photovoltaic systems is Dye-Sensitized Solar Cells (DSSC) as a result of the exploitation of several new concepts and materials based on chemical concepts where the function of light absorption and movement is separated by the use of a light absorbing sensitizer which is chemically bonded to the surface of wide band gap titanium dioxide semiconductor nanoparticles titanium dioxide (TiO₂). Photoelectrochemical solar cells using dye sensitizers have advantages including low production costs and are relatively safe for the environment.

The use of synthetic sensitizers has experienced a shift from the use of synthetic dyes to natural dyes. Ruthenium (Ru) Complex is a synthetic dye that is commonly used for DSSC, because this type of dye is almost pure, resulting in an efficiency of 11% (Shahid *et al.*, 2013). The use of synthetic dye Ruthenium Complex as a sensitizer has provided high DSSC efficiency and long shelf life but has disadvantages such as higher cost, tendency to degrade, and the use of materials that are toxic and difficult to synthesize (Hao *et al.*, 2006; Shalini *et al.*, 2015).

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The weaknesses of the ruthenium complex must be overcome, by replacing it with natural dyes from natural ingredients such as leaves, fruit, and plants. This offers cost efficiency, non-toxicity, and better biodegradability. Natural dyes have an important role in absorbing sunlight and transferring solar energy into electrical energy (Polo and Iha, 2006; Sinha *et al.*, 2012; Al Ba'thi *et al.*, 2013; Abdel Latief *et al.*, 2013). Ruthenium dye extract (N719), shows absorption in the range 300 – 600 nm (Hardani *et al.*, 2016), and natural dyes with absorption in the range 300 – 600 nm can replace ruthenium dye (N719). This deficiency inspired an alternative sensitizer, namely a natural bio-sensitizer. Natural dyes are very attractive for DSSC applications because they are cheap, widely available, have simple manufacturing techniques, are environmentally friendly and sustainable (Hao *et al.*, 2006; Lee and Mengjin, 2011; Zhou *et al.*, 2011; Narayan, 2012; Oluwaseun *et al.*, 2016).

Dyes are one of the keys in the development of high-performance of DSSC. Dyes for natural dye sensitizers must have the following requirements, such as having strong absorption in the visible light spectrum, having chemical group bonds that are suitable for bonding with semiconductors and being able to inject electrons into the semiconductor surface (Rosana *et al.*, 2014). There has been a lot of research regarding natural dyes for DSSC which are based on anthocyanins, but the presence of anthocyanins in nature still competes to fulfill human needs, so researchers use chlorophyll for research. Chlorophyll is not only a safe natural coloring substance but is also abundant in nature, so it will not compete with human needs.

Chlorophyll is a natural dye that is abundant on earth and can be used as a dye sensitizer for DSSC. The physical property of chlorophyll is to receive and reflect light with different waves (fluorescence or fluorescence). Chlorophyll absorbs a lot of light with a wavelength between 400-700 nm, especially red and blue light. Basri's research (Basri *et al.*, 2017) on the use of natural ingredients, shows that the natural ingredients of katuk leaves, kale and spinach with the natural dye chlorophyll have potential as dyes in DSSC. The chlorophyll content of each plant is different, this is because the ability of chlorophyll biosynthesis between one plant and another is also different (Abdilah *et al.*, 2014). Chlorophyll consists of chlorophyll a and chlorophyll b. In the formation of DSSC, the type of chlorophyll that is more widely used is chlorophyll a, because chlorophyll a functions to absorb photons and convert them into energy. Meanwhile, chlorophyll b functions to absorb photons and channel the absorbed photons to chlorophyll a to be processed into energy.

Based on research by Abdilah *et al.*, (2014), among papaya, cassava and peanut plants, papaya plants have the highest chlorophyll content, so in this study papaya leaves chlorophyll extract was used as a sensitizer for DSSC.

Chlorophyll is an important molecule in plants that makes leaves green. In the middle of the chlorophyll molecule, there is a magnesium (Mg) atom which helps plants convert sunlight into energy. Chlorophyll is a complex compound between porphyrin and Mg which contains a V-ring (cyclopentanone ring). The four N atoms are connected by Mg^{2+} ion bonds. Chlorophyll has the chemical formula $C_{55}H_{72}O_5N_4Mg$. Meanwhile, chlorophyll b has the chemical formula $C_{55}H_{70}O_6N_4Mg$. Mg in chlorophyll functions as a regulator of light absorption.

As described by Ludin *et al.* (2014), Mg atoms have the ability to inject electrons into the conduction band of semiconductors and have atoms that can bond to the TiO_2 surface which can increase the rate of electron transfer reactions, so it can be interpreted that chlorophyll is suitable when used as a dye sensitizer in DSSC. Chlorophyll is labile and easily undergoes a degradation process into its derivative molecules. Based on Indrasti *et al.* (2019), heat processing or process conditions at low pH cause changes in chlorophyll, namely the loss of Mg atoms and the formation of pheophytin derivative compounds.

To obtain extracts with high levels of Mg and chlorophyll, simple methods are used at room temperature to minimize chlorophyll damage. There has been no detailed research regarding the extraction time that can produce extracts with the highest Mg content, so in this study the researchers examined the extraction time that could produce the highest chlorophyll a and Mg using the maceration method at room temperature.

MATERIALS AND METHODS

Materials and Instruments

The materials used in this study included papaya leaves, acetone 96%, equates, and $NaHCO_3$. The instruments used were a water heater, container, cutter, blender, drying oven, 177 microns (80 mesh) sieve, vacuum rotary evaporator, pH meter, spectrophotometer, color reader, sample bottle, thermometer, filter paper, volume pipette, analytical balance, dropper pipette, beaker glass, erlenmeyer, measuring cup, volumetric flask and label paper.



Figure 1: The process of extracting chlorophyll pigments from papaya leaves.

a = papaya leaves, b = papaya leaves powdered, c = extraction process, d = extract

Preparation of Extracts

Papaya leaves are washed first, then drained and cut into pieces of $5\text{ cm} \pm 0.5\text{ cm}$, dried at 50°C to a moisture content of $6\% \pm 0.5\%$, and powdered and sieved with a size of 80 mesh (177 microns). In the chlorophyll extraction process, papaya leaf powder was added with NaHCO_3 as a stabilizer as much as 3 %, then acetone was added with a ratio of 1:10 by weight of material and solvent. Chlorophyll was extracted by maceration method at room temperature (27°C) with the specified extraction time (12, 24, 36, 48, 60 and 72 hours). Then the filtrate obtained was filtered using Whatman filter paper no.1 and evaporated in a rotary vacuum evaporator at a pressure of 0.18 atm and a temperature of 50°C .

Figure 1 shows the stages of the extraction process. Starting from fresh papaya leaves, then papaya leaves powder, followed by the maceration extraction process and the sludge extracted from papaya leaves. Observations made in this study were total chlorophyll contents, chlorophyll content, chlorophyll b contents, Mg contents, pH levels, yields extract and energy gap.

Chlorophyll Contents

The chlorophyll extract obtained was centrifuged for 10 minutes at 1000 rpm, then 1 mL of the supernatants diluted in a 10 mL volumetric flask. Chlorophyll concentration was obtained from extract absorbance measurements with a UV-Vis spectrophotometer. Chlorophyll concentration calculation was carried out using the Arnon equation for extraction using acetone (Sumiati, 2021):

$$\text{Total chlorophyll (mg/L)} = (20.2 \times A_{645}) + (8.02 \times A_{663})$$

$$\text{Chlorophyll a (mg/L)} = (12.7 \times A_{663}) - (2.69 \times A_{645})$$

$$\text{Chlorophyll b (mg/L)} = (22.9 \times A_{645}) - (4.68 \times A_{663})$$

Where A_{663} is the solution absorbance at 663 nm and A_{645} is the absorption at 645 nm of wavelengths.

Magnesium (Mg) Contents

Analysis of Mg levels in chlorophyll using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). In a test tube, 0.5 grams of extracted paste was added to 10 mL of concentrated HNO_3 solution, then destructed by microwave digestion at 180°C for 2 hours. The extract was cooled and added with distilled water until it reached 50 mL. Then filtered using Whatman filter paper. The obtained extracts were read with the ICP-OES tool.

Ph Levels

Extract pH level was measured before extraction and after the extract was filtrated, measurements were carried out using a digital pH meter.

Yields Extract

The yield extract was analyzed using the formula (Health Department, 2000):

$$\text{Yield Extract (\%)} = \frac{\text{Extract Weight (gram)}}{\text{Leaves Powder Weight (gram)}} \times 100$$

Energy Gap

Determination of optical band gap energy values using the Tauc plot method. This method is determined by using transition values produced from a UV-Vis spectrophotometer by extrapolating the linear region of the $h\nu$ and $(h\nu\alpha)^{1/2}$ relationship graph until it cuts the energy axis (Tauc and Abeles, 1972). Where the equation is used:

$$(h\nu\alpha)^{1/n} = A(h\nu - E_g)$$

With h the Planck constant, ν the frequency with $\nu = \frac{c}{\lambda}$, α the absorption coefficient, E_g the band gap energy, and A a proportional constant. The exponent value n shows the nature of the sample transition (Suprasetyo and Setiarso, 2016). For direct transitions allowed $n = \frac{1}{2}$, indirect transitions allowed $n = \frac{3}{2}$.

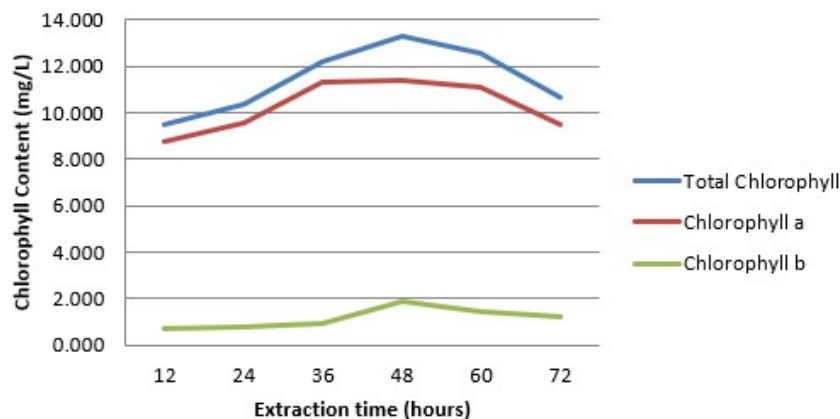


Figure 2: The average chlorophyll contents with extraction time

Statistical Analysis

This experiment was a 1-factor experiment using a simple randomized block design (RBD). The experimental factors given were 6 levels of chlorophyll extraction time, namely 12 hours, 24 hours, 36 hours, 48 hours, 60 hours and 72 hours. 4 groups with 6 treatment levels each, thus obtaining 24 experimental units.

RESULTS AND DISCUSSION

Total Chlorophyll, Chlorophyll a and Chlorophyll b Content

Figure 2 shows the treatment for 48 hours of extraction time, resulting in the highest chlorophyll content of papaya leaves. The total chlorophyll obtained was 13.272 mg/L, chlorophyll a was 11.380 mg/L and chlorophyll b was 1.881 mg/L. At the extraction time of 48 hours, the solvent had reached its saturation point with the extracted compound. So at 60 and 72 hours the chlorophyll content is damaged and oxidized which causes the chlorophyll content to decrease. The effective time for maceration can affect the length of contact of the solvent with the sample so that a high pigment content is obtained (Sudarmi *et al.*, 2015). The longer the maceration occurs the longer the contact between the solvent and the material so it will increase the number of broken cells and dissolved active ingredients (Wahyuni and Widjanarko, 2015). An extract with optimum chlorophyll contents means it has the maximum number of photon-absorbing molecules. The photon energy can be converted into electrical energy in dye-sensitized solar cell applications. Statistically, the length of extraction treatment made a significant difference to total chlorophyll. In chlorophyll a, the 48-hour treatment was not significantly different from the 36 and 60-hour treatment but was significantly different from the 12, 24 and 72-hour treatment. Chlorophyll b also showed that the 48-hour

treatment was not significantly different from the 60-hour treatment, but there was a significant difference to the other treatments, namely 12 hours, 24 hours, 36 hours and 72 hours of extraction.

Magnesium (Mg) Content

Chlorophyll is a natural dye that is easily degraded into its molecular derivatives, this is caused by the influence of light, temperature, and oxygen, which makes chlorophyll unstable. The loss of magnesium (Mg) from the central molecule is the beginning of the degradation of chlorophyll. A number of derived molecules such as pheophytins, chlorophyllides and pheophorbides which depend on the main molecule will be formed if the chlorophyll molecule is degraded. Molecules resulting from the degradation of magnesium (Mg) chlorophyll atoms are pheophyllins and pheophorbides (Carlson and Simpson, 1996).

The extraction time treatment had a significant interaction effect based on analysis of variance (ANNOVA) on magnesium (Mg) contents in papaya leaves chlorophyll extract solution, but there was no significant difference between treatments on magnesium (Mg) contents in papaya leaves chlorophyll extract. Figure 3 shows that the R^2 value is 0.9954, which indicates that the extraction time treatment has a significant effect on the magnesium content in papaya leaves extract as much as 99.54%. Magnesium is an element that affects chlorophyll in plants, produces chlorophyll perfectly, determines the quality and quantity of chlorophyll (Carlson and Simpson, 1996). Thus, the magnesium content in the extract is directly proportional to the chlorophyll content produced. From the diagram above, it can be seen that magnesium (Mg) contents in papaya leaf extract increased from 12 hours, 24 hours, 36 hours to 48 hours. Then it decreased from 48 hours to 72 hours. The highest average magnesium (Mg) content was obtained in the chlorophyll extraction process of papaya leaves for 48 hours, which was 934,771 mg/kg.

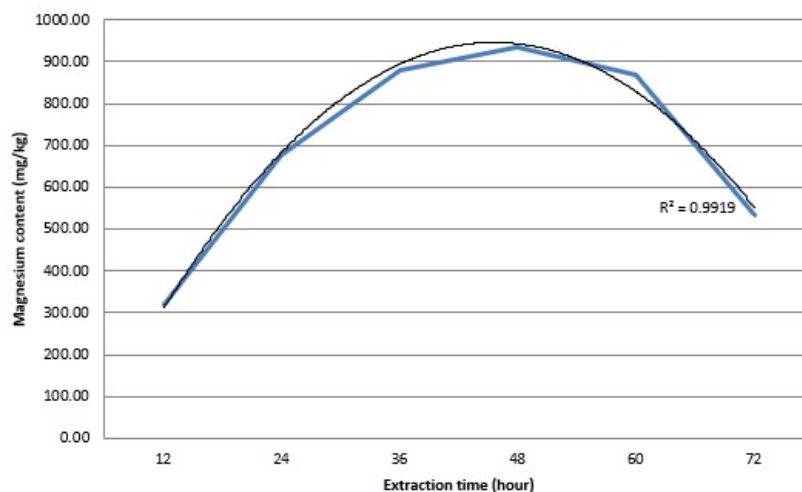


Figure 3: The average magnesium (Mg) content with extraction time

The increase and decrease in the levels of magnesium (Mg) in the chlorophyll extract was in line with the increase and decrease in the chlorophyll contents value.

Treatment Time Effect on The Extract pH

Extraction time treatment gave a significant interaction effect based on analysis of variance (ANNOVA) on the pH of the papaya leaves chlorophyll extract solution as much as 97.49%. From the data generated and shown in Figure 4, it was found that the pH of the solution increased as the extraction time was longer. The results of these observations are in line with research conducted by Andi *et al.*, (2016), where the pH of the extract solution in each treatment was close to neutral pH. According to Putri *et al.*, (2012), the pH value of chlorophyll is influenced by the pH of the extracting solution. This shows that the higher the pH value of the extracting solution, the higher the pH value of the chlorophyll extract will be. The pH value with a high concentration can cause the degradation of chlorophyll pigment into its derivatives so that the green pigment undergoes a color blanching known as blanching (Koca *et al.*, 2007).

Treatment Time Effect on Extract Yield

Extraction time treatment had a significant interaction effect based on analysis of variance (ANNOVA) on the yield of papaya leaves chlorophyll extract. Figure 5 shows that the extract yield increased with increasing extraction time. At the extraction time of 12, 24, 36, 48, 60 and 72 hours, the average extract yields were 6.433%, 6.497%, 6.759%, 7.363%, 7.444% and 7.506%. The yield of papaya leaf extract is directly proportional to the longer the extraction time. The longer the extraction time, the higher the

yield obtained; this is because more compounds are dissolved into the solvent. The longer the extraction time, the longer the contact time between the sample and the solvent so that the number of compounds extracted is increasing. This condition will continue until an equilibrium condition is reached between the concentration of the compound in the raw material and the concentration of the compound in the solvent. This has been emphasized by Mandal *et al.*, (2007), the longer the extraction time, the quantity of material that can be extracted will increase because the opportunity between the material and the solvent for contact will be longer so that the yield produced will also be higher. This is also in line with the statement of Mardiana and Ratnasari (2011), where the longer the extraction time, the higher the yield produced because the opportunity for the solvent to penetrate the cell material will be better which causes more and more compounds to diffuse out of the cell. Appropriate extraction time will produce a large amount of yield.

The energy gap of papaya leaf extract

In this study, the energy gap was measured based on the extract with the highest magnesium content. The energy gap has been measured both directly and indirectly. In direct band gap semiconductors, the end of the valence band and the end of the conduction band occur at the same momentum value. In indirect band gap semiconductors, the maximum energy of the valence band occurs at a different momentum value than the minimum energy of the conduction band. Papaya leaf extract was analyzed using a spectrophotometer, and the data obtained was calculated using the tauc plot method. The energy gap of papaya leaf extract was obtained directly at 2.428 eV as shown in Figure 6 and indirectly at 2.442 eV as shown in Figure 7.

The difference between direct energy gap and indirect

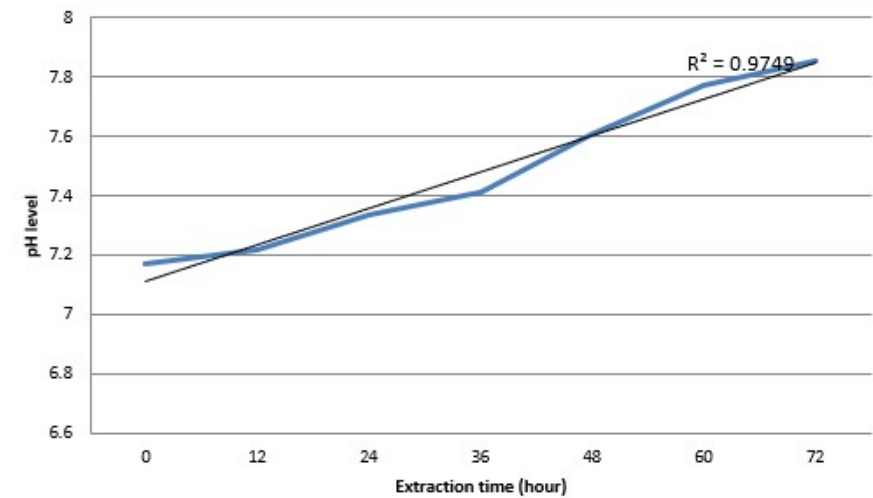


Figure 4: The average extract pH with extraction time

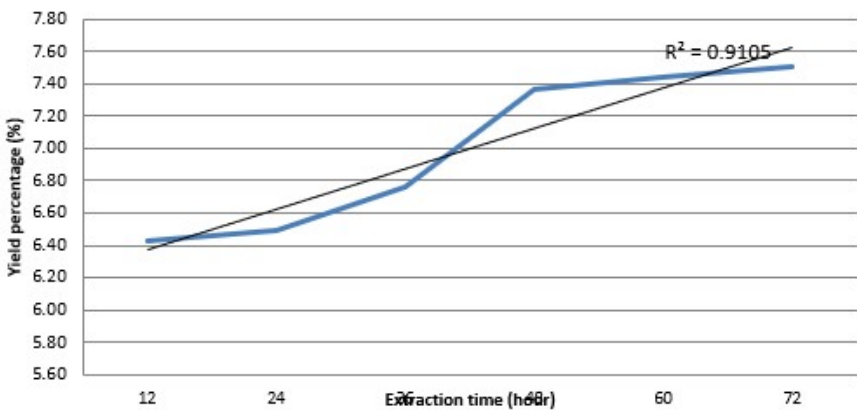


Figure 5: Diagram of The average extraction yield with extraction time

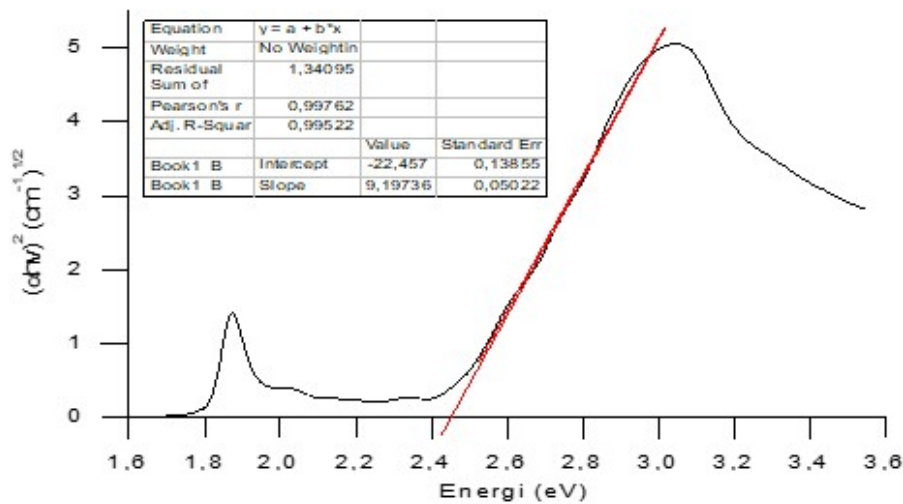


Figure 6: Indirect energy gap of papaya leaves extract

energy gap is very important in carrying carrier charges in semiconductors, protons can produce energy to produce electron-hole pairs. Referring to research conducted by Aryanti *et al.*, (2016) and Ganta *et al.*, (2017) which states that the gap energy in the anatase

phase TiO₂ semiconductor is 3.2 eV, thus it can be interpreted that the dye gap energy is smaller than the energy anatase phase TiO₂ semiconductor gap. So as the band gap value decreases, the effectiveness of the photocatalyst used in DSSC will increase.

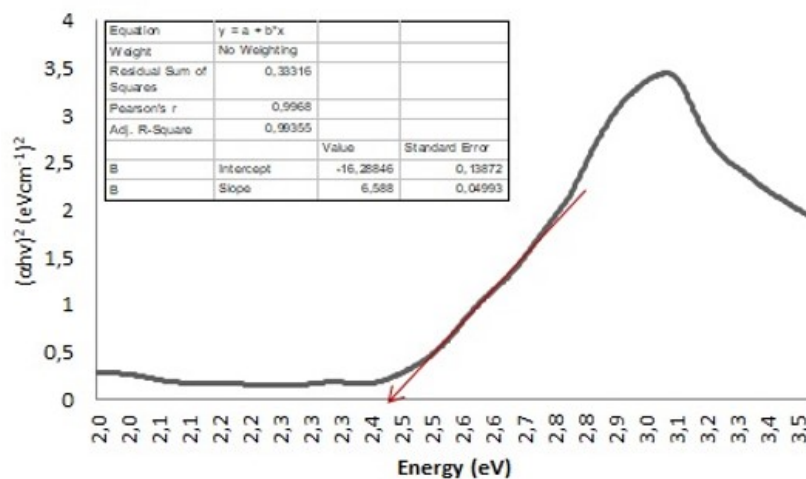


Figure 7: Direct energy gap of papaya leaves extract

The Smallest energy gap obtained in this research is 2.428 eV, which has better results compared to research conducted by Palupi *et al.* (2019) with the best treatment at 2.5% chlorophyll of papaya leaves doped BST produced an energy gap of 2.98 eV.

CONCLUSION

This research shows that extracting papaya leaves using the maceration method and 96% acetone for 48 hours produces an extract with the highest total chlorophyll,

chlorophyll a, chlorophyll b, and magnesium content. Total chlorophyll was 13,272 mg/L, chlorophyll a was 11,380 mg/L, chlorophyll b was 1,881 mg/L and magnesium levels were 934,771 mg/kg. The resulting papaya leaf extract has a direct gap energy of 2.428 eV and an indirect gap energy of 2.442 eV. With the gap energy of papaya leaves being smaller than the TiO₂ semiconductor and with high chlorophyll and magnesium content, papaya leaf extract meets the requirements for use as a natural dye sensitizer in renewable energy dye-sensitizing solar cells.

The authors have no conflict of interest in this article

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