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The Effect of Drying Methods and Solvent Exctraction on the Quality of Phycoerythrin Extracted from *Kappaphycus alvarezii*

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Abstract

Kappaphycus alvarezii is a type of red seaweed (Rhodophyta) that is extensively cultivated in Indonesia. *K. alvarezii* contains phycobiliproteins, particularly the pigment phycoerythrin. This research aims to determine the optimal seaweed drying method and solvent usage for obtaining the best quality of phycoerythrin from *K. alvarezii.* The drying process used the freeze dryer, sun drying and oven methods.The extraction process was carried out in stages using phosphate buffer and DES solvents. The research results indicate that the freeze-drying method with the use of phosphate buffer solvent is the best method, producing a more red-colored pigment with a pigment concentration of 0.1 mg/ml, a purity index of 0.16, and strong antioxidant activity. The use of DES solvent, specifically chcl-urea, resulted in a pigment concentration of 0.038 mg/ml with a purity index of 0.08. Chcl-glucose exhibited a pigment concentration of 0.09 mg/ml with a purity index of 0.11. The protein profile with SDS-Page shows that phycoerythrin has molecular weights of 20, 21.06, and 31.04 kDa, suspected to be phycoerythrin pigments in the α, β, and ɣ subunits.

Keywords: DES, Phycoerythrin, *K. alvarezii*, Drying methods.

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1. Introduction

Phycoerythrin is the dominant pigment found in seaweed belonging to the red seaweed group. According to Osório et al., (2020), phycoerythrin is a polar pigment associated with proteins [1]. Phycoerythrin is widely utilized due to its various biological activities, including antiviral, antioxidant, anti-inflammatory, antidiabetic, antitumor, antihypertensive, immunosuppressive, and neuroprotective properties [2]. The drying process of simplicia is the first step taken to prepare simplicia so that it lasts long. The drying process is an important factor to avoid cell degradation [3]. Therefore, fresh simplicia is rarely used in retail because it can spoil quickly after harvest, making transportation and storage difficult. various drying processes that are commonly carried out such as; sun drying, oven drying, and freeze drying [4]. The process of extracting phycoerythrin pigments can utilize phosphate buffer solvent or water, as well as other polar solvents [5]. Sinaga et al., (2019) reported that the highest concentration of phycoerythrin from fresh raw material was obtained using a phosphate buffer solvent with a pH of 7, amounting to 0.933 mg/mL [6].

The extraction of phycoerythrin can also be carried out using recently developed solvents, namely Deep Eutectic Solvent (DES) [7]. DES solvents exhibit characteristics such as biodegradability, recyclability, and flexible combinations [8]. This research was conducted in two stages of the extraction process. The first stage utilized a phosphate buffer solvent, while the second stage employed a Deep Eutectic Solvent (DES). Two types of DES were used: a mixture of choline chloride with glucose and a mixture of choline chloride with urea. According to Liu et al., (2019), curcuminoid-type pigments are stable when extracted using choline chloride and D-glucose as DES solvents [9]. According to Ghazali et al., (2019), choline chloride and urea are among the most used substances in synthesizing DES [10]. The study aimed to investigate the impact on the quality of *K. alvarezii* phycoerythrin concerning the use of dried seaweed raw materials and the choice of solvent.

2. Materials and methods

2.1. Material

K. alvarezii was obtained from Lontar Village, Serang Regency, Banten. Other materials included choline chloride-urea (CHCL-U), choline chloride-D-glucose (D-CHCL-GL), distilled water with pH 7, phosphate buffer (NaH_2PO_4 and Na_2HPO_4) 0.1 M with pH 7, dialysis membrane bags with a molecular weight cutoff of 14 kDa, 60% ammonium sulfate (Merck), analytical grade methanol, 1,1-diphenyl-1-picrylhydrazyl (DPPH) from Sigma, [PM2700] ExcelBand™ 3-color Broad Range Protein Marker (3.5-245 kDa), 250 µL x2, pyrogen-free water for injection (IKAPHARMINDO), 30% ethanol, 1% acetic acid, 10% acetic acid, 0.1% $AgNO_3$, 2.5% $NaCO_3$, 0.02% formaldehyde, 30% acrylamide (Sigma), 10% sodium dodecyl sulfate (SDS), 10% ammonium persulfate (APS), 1.4 M Tris-Cl pH 6.8, 1.4 M Tris-Cl pH 8.8, glycerol, mercaptoethanol, and bromophenol blue.

2.2. Method

The research involved sample preparation and carrying out the drying process using a freeze drying, oven drying, and sun drying. The dried samples were blended with a blender, and the resulting samples were tested for their moisture content and analyzed for shrinkage yield. The finely ground seaweed was extracted using a phosphate buffer solvent. The best-extracted sample determined based on absorbance spectrum tests, phycoerythrin concentration, purity index, and antioxidant analysis from the extraction process using a phosphate buffer solvent, underwent another extraction process using a DES solvent. The extracted results using DES were subsequently analyzed for the phycoerythrin protein profile using SDS-Page. Additionally, the absorbance spectrum of phycoerythrin was analyzed using a UV-VIS spectrophotometer to calculate the concentration and purity index of phycoerythrin.

2.2.1. Sample and Solvent Preparation

Seaweed preparation involved separating materials from various impurities, followed by drying proses. The drying methods were using a freeze dryer at -50℃ and a pressure of 0.100 bar for 2x24 hours, an oven at 50 °C for 6 hours, and sun drying for 5 hours [11-13]. Subsequently, the dried seaweed was cut into pieces 0.5 cm in size. The seaweed that had been dried underwent moisture content analysis. The solvent preparation began with weighing the DES composition with an accuracy of ±0.0001 g. Two types of Deep Eutectic Solvents (DES) were synthesized by stirring the eutectic mixture at 80°C for 1 hour until a homogeneous liquid was formed. The ratio of choline chloride with urea (chcl-u) and choline chloride with glucose (D-chcl-G) eutectic solutions used in this study followed the reference from Abbott et al., (2005), which is 1:2 [14]. The required concentration of DES solvent in this study was 30%. For a 30% concentration of DES, distilled water was added in a ratio of 70%.

2.2.2. Phycoerythrin Extraction with Modifications

Phycoerythrin from *K. alvarezii* was extracted using a 0.1 M pH 7 phosphate buffer solvent, following a modified method by Sudhakar et al., (2014) [15]. Ten milligrams of dried seaweed were weighed and soaked in the solvent for 1 hour, then blended using a blender. The sample-to-solvent ratio was maintained at 1:20 (w/v). Subsequently, the sample was extracted using ultrasonic waves at a frequency of 40 kHz and a temperature of 4°C for 30 minutes. Ice was gradually added to maintain a constant temperature during extraction. The sample was then centrifuged at a speed of 9,000 g at 4°C for 10 minutes [9]. Purification of crude phycoerythrin pigments was obtained from the supernatant fraction. The supernatant fraction was characterized using UV spectroscopy to determine the pigment composition, concentration, and purity of phycoerythrin. The optimal drying treatment, which resulted in phycoerythrin pigments using a phosphate buffer solvent, was further continued with the extraction process using a DES solvent. The extraction conditions for samples using DES solvent and their characterization were conducted similarly to the extraction process using a phosphate buffer [16]. The DES used was a mixture of choline chloride with urea (chcl-u) and a mixture of choline chloride with glucose (chcl-g).

2.3. Analysis Procedure

2.3.1. Material Characterization

2.3.1.1. Moisture Content Analysis

Moisture content analysis was conducted following the AOAC (2005) standard [17]. A porcelain dish used for analysis was dried at 105°C for 3 hours and then cooled in a desiccator. Three grams of seaweed were placed in the porcelain dish and dried using an oven at 105°C for 3 hours. The dried sample was cooled in a desiccator and then weighed. The moisture content was calculated using the following formula:

$$Weight\ Loss\ (\%) = \frac{weight\ of\ wet\ sample - weigh\ t\ of\ dried\ sample}{weight\ of\ wet\ sample}\ x\ 100$$

2.3.1.2. Analysis of Solid Seaweed Waste Residue Yield

The analysis of solid seaweed residue yield was conducted by weighing the initial weight of the dried sample, which was used as the raw material for the pigment extraction process. After the extraction process, the obtained residue was dried in an oven at 50℃, and then its weight was measured. The calculation of the residue yield can be performed using the following formula:

$$Residue\ yield\ (\%) = \frac{weight\ of\ wet\ sample - weigh\ t\ of\ dried\ residue}{weight\ of\ dried\ residue}\ x\ 100$$

2.3.2. Pigment Characterization

2.3.2.1. Analysis of Concentration and Purity Index of Phycoerythrin Pigments

The analysis of the concentration and purity index of phycoerythrin pigments was conducted using a Shimadzu UV-Vis Spectrophotometer UV-1700 at a wavelength range of 200 to 700 nm [18]. The purity index of phycoerythrin was determined by the ratio A565/A280.

The concentration of phycobiliproteins can be calculated using the following equations:

Allophycocyanin (µg/mL) = 181.3 A_{651} – 22.3 A_{614}

Phycocyanin (µg/mL) = 151.1 A_{614} – 99.1 A_{651}

Phycoerythrin (µg/mL) = 155.8 $A_{498.5}$– 40.0 A_{614} – 10.5 A_{651}

2.3.2.2. Analysis of Antioxidant Activity

The analysis of antioxidant activity can be performed using various methods, one of which is the DPPH method [19]. A DPPH reagent of 0.15 mM was prepared by dissolving 0.0006 g of DPPH powder in methanol to a volume of 10 mL. Stock samples were prepared at concentrations of 50 and 80 ppm and dissolved in distilled water. The sample-to-DPPH volume ratio was 3:1. The mixture was vortexed for 30 seconds and then incubated for 1 hour in the dark. Absorbance measurements were taken at a wavelength of 517 nm. A blank was prepared by mixing distilled water and DPPH reagent in a 3:1 volume ratio. The same process was then carried out with the sample. Antioxidant activity can be calculated using the following formula:

$$Inhibition\ (\%) = \frac{Absorbance\ blank - Absorbance\ sample}{Absorbance\ blank}\ x\ 100$$

2.3.2.3. Analysis of Protein Profile with SDS-PAGE

The analysis of the protein profile in phycoerythrin pigments was conducted using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [20]. The analysis involved several steps, including the preparation of the SDS-PAGE gel, sample preparation, and electrophoresis. Identification and analysis of the SDS-PAGE pattern were performed by comparing the protein bands in the sample with standard proteins. The molecular weight of each protein was determined using Photocapt software. The determination of molecular weight and protein arrangement was analyzed using SDS-PAGE. The SDS-PAGE test was conducted using a 3% acrylamide stacking gel and a 12.5% acrylamide separating gel. A total of 10 µL of the sample was mixed with 10 µL of sample buffer and denatured at 95°C for 10 minutes. The denatured samples were then loaded into the acrylamide gel wells. A protein marker with molecular weights ranging from 10 to 250 kDa was used, and the target molecular weight for size comparison was 16 kDa. According to Putri et al., (2021), the protein profile obtained using SDS-PAGE showed that phycoerythrin has a molecular weight of 16 kDa for the α subunit, 17 kDa for the β subunit, and 30 kDa for the γ subunit [21]. Electrophoresis was conducted at a voltage of 170 V for 60 minutes. Subsequently, the gel was stained using Coomassie Brilliant Blue (CBB), and excess stain not bound to proteins was removed, allowing the protein bands to become visible.

2.4. Experimental Design and Data Analysis

The experimental design used in this study consists of two stages: determining the optimal drying method and identifying the best solvent for extracting phycoerythrin pigments. Each stage of the research utilized a Completely Randomized Design (CRD) with different drying method treatments and the determination of the best solvent for phycoerythrin pigment extraction. Each treatment was repeated twice. Data were analyzed using analysis of variance (ANOVA) followed by Duncan's post hoc test with a confidence interval of 5%.

3. Results and Discussions

3.1. Drying of K. alvarezii

The drying process in this study involved three drying methods: freeze drying at -5 °C for 24 hours, sun drying at temperatures ranging from 35-38°C for 7 hours, and oven drying at 55°C for 6 hours. The results of the dried seaweed using freeze drying, oven drying, and sun drying methods are presented in Figure 1. The results indicate that dried seaweed using the freeze-drying method has a more intense color appearance compared to seaweed dried using sun-drying and oven-drying methods. The seaweed dried using the sun drying method exhibited a somewhat faded color, while seaweed dried using the oven drying method had a dark brown color due to direct heat contact inside the oven heating medium. Higher airflow rates during drying result in a faster transfer of water vapor from the material to the atmosphere. The ability of the material to release moisture from the surface increases with the rising drying air temperature. An increase in air temperature also leads to a decrease in the amount of heat required to evaporate water in the material [22].

3.1.1. Moisture Content of Dried Kappaphycus alvarezii

Moisture content represents the amount of water contained in a material. Determining the moisture content of a food material is crucial to ensure appropriate handling during processing and distribution [23]. The higher the drying temperature of a material, the lower its moisture content will be. The results of the analysis of variance (ANOVA) indicate that the drying method applied to *K. alvarezii* significantly affects ($P < 0.05$) its moisture content (Figure 2). The moisture content of *K. alvarezii* dried using the sun drying method is 13.73%, which is higher compared to the moisture content of *K. alvarezii* dried using the oven drying method (9%), and the lowest moisture content is observed in the freeze-dried *K. alvarezii*, with a moisture content of 7.6%. Higher drying temperatures result in an increased amount of absorbed water [24].

3.1.2. Weight Loss in Drying Process

The drying process aims to reduce the moisture content in seaweed. During drying, weight loss of the dried material occurs. The weight loss results for *K. alvarezii* are presented in Table 1. The analysis of variance (ANOVA) indicates a significant difference ($P < 0.05$) in the weight loss of *K. alvarezii* among the drying methods applied. Freeze-drying in this study resulted in the highest weight loss of *K. alvarezii*, approximately 85%. The freeze-drying process operates through a solid-to-gas phase transition under vacuum conditions using freezing temperatures [25]. Drying using the sun drying method in this study resulted in a shrinkage of *K. alvarezii* by 73.6%. The advantage of sun drying is that it doesn't require specific handling and is the most economical drying method [22]. Drying with an oven in this research led to a shrinkage of *K. alvarezii* by 80%. Oven drying is considered more advantageous compared to sun drying as it can significantly reduce moisture content in a shorter duration [26].

3.2. Extraction of Phycoerythrin pigments with different drying Methods

3.2.1. Phycoerythrin Absorbancphe Spectrum

Phycoerythrin is a protein containing a tetrapyrrole ring and belongs to the chromophore group. All phycobilin chromophores bind specifically to cysteine in the polypeptide chain through thioether bonds [27]. Mayasari et al., (2019) stated that the darker the red colour obtained in phycoerythrin extract, the higher the phycoerythrin content [2]. According to Dumay et al., (2014), phycoerythrin can be measured in the wavelength range of 400-650 nm [28]. The maximum absorbance of the phycoerythrin pigment spectrum can be seen in Figure 3b. As seen in Figure 3a, the extract from raw material dried with the freeze dryer method shows a redder colour compared to the extract from sun drying and oven drying. This occurs because the freeze-dryer method tends to preserve the colour during the drying process. Research by Sudaryati et al., (2014) states that the freeze-dryer drying method tends to preserve the colour of the dried material [29]. Sun drying and oven drying are suspected to reduce the colour of a material. This is in line with the statement of De-Fretes et al., (2012) that the instability of pigments to high light intensity and prolonged radiation exposure can cause pigment degradation in algae. Pigments in algae degrade to form CIS-Isomers as a degradation form due to stereo isomerization [30]. The absorbance spectrum at various wavelengths in phycoerythrin can be seen in Figure 3b. Absorbance peaks were identified at wavelengths of 497, 532, and 565. These peaks indicate the characteristic features of phycoerythriphycoerythrinn. The peaks are clearly identified in the pigment obtained from raw material dried with the freeze-drying method. The absorbance spectrum results show a similarity to the research reported by Shudakar et al., (2015), which stated that the maximum absorbance of phycoerythrin extract was found at wavelengths of 497, 543, and 563 nm [15].

3.2.2. Concentration and Purity Index of Phycoerythrin

The analysis of variance (ANOVA) results indicates that the drying method on *K. alvarezii* and the use of phosphate buffer solvent significantly affects ($P<0.05$) the concentration of crude phycoerythrin extract from *K. alvarezii* (Figure 4). The results of phycoerythrin pigment extraction show the highest concentration in the extract from raw material dried with the freeze dryer method, with a concentration value of 0.1. The extract from raw material dried with the sun-drying method has a concentration value of 0.036, while the extract from raw material dried with the oven method has a concentration value of 0.038. Hidhayati et al., (2020) reported that crude phycobiliprotein extract (phycoerythrin type) from the cyanobacterium Chroococcus turgidus showed a concentration of 0.032 mg/mL [31]. The purity index of crude phycoerythrin extract from *K. alvarezii* (Figure 5) indicates that the highest purity index is obtained in the extract from samples dried with the freeze dryer method, with a value of 0.16. Meanwhile, the sun-drying method has a purity index value of 0.8, and the oven-drying method has a purity index value of 0.81. The analysis of variance (ANOVA) results shows that the drying method and the use of phosphate buffer solvent significantly affect the purity index of crude phycoerythrin extract. The purity index of phycoerythrin is considered food-grade if it is above 0.7, and for pharmaceuticals, a minimum purity index of 2.0 is recommended [32]. Rito-Palmares et al., (2001) reported that phycoerythrin can be utilized for laboratory analysis with a minimum purity index of 4 [33].

3.3. Waste Residue Weight

The yield of residue is a byproduct of the extraction process, indicating the solvent's ability to extract compounds or active components in a sample being extracted. This study focuses on the extraction of phycobiliprotein pigments, particularly phycoerythrin, from *Kappaphycus alvarezii*. Several factors affecting the yield include the extraction temperature, solvent concentration, and extraction time [34]. The waste residue from the extraction process is depicted in Figure 6. The extraction process results in the yield of residues, which is related to the percentage of absorption from the extracted material (Figure 7). Each residue from the extraction process still shows some retained color, possibly due to the extraction process using a low temperature of 4°C. Additionally, the use of dried seaweed samples may hinder the diffusion of color substances into the solvent, rendering it suboptimal. According Budiyanto et al., (2008), a higher extraction temperature can increase the kinetic energy of the solution, thereby increasing solvent diffusion into the cells [35]. Dirhaninggrum et al., (2018) stated that the extraction temperature affects the yield of sodium alginate, as a higher extraction temperature leads to a higher yield of sodium alginate [36]. This results in the release of alginate from brown seaweed cells, leading to an increased production of alginate. Samples dried with a freeze-dryer absorb more water through the sample pores because the freeze-drying method does not cause the sample to shrink. Therefore, when soaked, the sample absorbs water more quickly than samples dried using oven and sun-drying methods. According to Hariyadi (2013), the freeze-drying method has the advantage of maintaining product stability, structural stability, and improving rehydration ability [25]. Samples dried using sun-drying and oven-drying methods result in a wrinkled and tough texture, causing the samples to take longer for water to penetrate into their pores. The analysis of variance (ANOVA) results shows that the drying method and the use of phosphate buffer solvent in the process of extracting phycoerythrin from *K. alvarezii* significantly affect ($P<0.05$) the yield of *K. alvarezii* residue. The percentage of solvent absorption into the sample using the freeze-drying method is higher, at 90%, with an extract yield of 10%. This absorption is higher compared to the sun-drying method, with a solvent absorption percentage of 78% and an extract yield of 22%. Meanwhile, the oven-drying method has a solvent absorption percentage of 74%, with a residue percentage of 26%.

3.4. Antioxidant activity of Phycoerythrin pigments

Antioxidants are compounds that donate electrons to free radicals. Free radicals are highly reactive molecules with unpaired electrons produced as a result of radiation or from by-products of the body's metabolic [37]. Phycoerythrin pigments not only serve as natural colorants but also exhibit potential antioxidant properties [38]. Antioxidants play a crucial role in neutralizing attacks or exposure to free radicals by donating electrons to these reactive compounds. Sangeetha et al., (2017) state that antioxidant activity testing on phycoerythrin pigments will demonstrate a significant scavenging effect, particularly using the DPPH method [38].

The treatment of *K. alvarezii* drying methods significantly affects ($P < 0.05$) the IC50 values of antioxidant activity in crude phycoerythrin pigment extracts (Figure 8). The crude phycoerythrin pigment extract from freeze dryer drying has antioxidant activity with an IC_{50} value of 96 µg/ml. The antioxidant activity of phycoerythrin from seaweed dried with the sun-drying method has an IC_{50} value of 164 µg/ml, while the phycoerythrin extract from seaweed dried with the oven method shows antioxidant activity with an IC_{50} value of 242 µg/ml. Molyneux (2004) categorized antioxidant activity into four groups based on IC_{50} values: antioxidants with IC_{50} < 50 µg/mL are classified as very strong antioxidants, IC_{50} 50-100 µg/mL falls into the category of strong antioxidants, IC_{50} in the range of 101-150 µg/mL is considered as moderate antioxidants, and IC_{50} >150 µg/mL is categorized as weak [39]. The antioxidant activity test in this study indicates that the freeze dryer drying method exhibits strong antioxidant activity with an IC_{50} value of 96 µg/mL. This is caused by the freeze-dryer drying method, which is a freeze-drying process that does not directly contact the heating medium, preserving the antioxidant activity of the seaweed. Yabuta et al., (2010) reported antioxidant activity in phycoerythrobilin extracted from *Porphyra sp*., tested in vitro using the 2,2-diphenyl-1-picrylhydrazyl method, demonstrating strong antioxidant activity [40].

3.5. Extraction of Phycoerythrin pigments using Deep Eutectic Solvent (DES)

3.5.1. Phycoerythrin absorbance spectrum

The appearance of crude phycoerythrin extract can be seen in Figure 9. The extract with the DES solvent does not significantly extract pigments. The use of chcl-urea (A) type DES shows results that do not release red coloration, which is due to the very low efficiency of the chcl-urea solvent in extracting pigments from dried seaweed samples. The spectrum results (Figure 9b) indicate that the chcl-urea solvent extract does not show absorption at the wavelength characteristic of phycoerythrin pigments. According to Ghazali et al., (2019), urea with inappropriate thermal stability will decompose first, followed by the decomposition of chcl [10]. However, the decomposition time of chcl is slightly higher than pure chcl, which occurs due to the formation of hydrogen bonds on the -OH from the silanol group of Cl- in chcl. The use of a decomposed urea solvent is suspected to cause the extracted pigments to undergo degradation during the extraction process. Ghazali et al., (2019) found that 5-15% of chcl-urea remains stable at 100°C, ensuring that chcl-urea remains stable and does not decompose [10]. The extract with chcl-glucose solvent shows a colorless result, but when compared to the extract using chcl-urea solvent, the chcl-glucose extract tends to exhibit a slightly reddish-brown color. The spectral results of the extract from the chcl-glucose solvent indicate the presence of insignificant high waves, with a wave height of only 0.01 and 0.001 at wavelengths of 506 nm and 577 nm. According to Liu et al., (2019), Deep Eutectic Solvents (DES) consist of two or more components in a specific molar ratio [16]. Different molar ratios can affect its stability. The citric acid-glucose mixture is stable when the molar ratio is between 4/1 and 1/1. When the molar ratio reaches 1/2 and 1/3, a clear liquid can be formed by mixing, but a solid white precipitate appears in the solvent after 12-24 hours. This may be due to the hydroxyl groups (-OH) of glucose requiring association with the carboxyl groups (-COOH) or hydroxyl groups (-OH) of citric acid. With a molar ratio of 1/2 and 1/3, there are too many hydroxyl groups from glucose that cannot associate with specific groups in citric acid, leading to the deposition of glucose [41]. Therefore, the formation of a white solid precipitate at ratios of 1/2 and 1/3 M results in the suboptimal performance of chcl-glucose in extracting phycoerythrin. According to Liu et al. (2019), the addition of 15% (v/v) water with a component ratio of 1/1 M citric acid/glucose can form efficient viscosity in extracting pigments, resulting in an optimal extraction medium [16].

3.5.2. Concentration and Purity Index of Phycoerythrin

The crude extract of phycoerythrin from *K. alvarezii* using choline chloride-glucose (chcl-g) as a DES solvent showed the highest concentration at 0.09 mg/mL. Chcl-urea had a pigment concentration of 0.038 mg/mL (Figure 10). Putri et al., (2021) reported that the crude extract of phycobiliprotein (phycoerythrin type) from *K. alvarezii* exhibited a concentration of crude phycoerythrin pigment at 0.315 mg/mL [21]. The purity index of the crude phycoerythrin extract from *K. alvarezii* obtained using chcl-glucose solvent had a purity index value of 0.11. Meanwhile, the extract using chcl-urea had a purity index value of 0.08. The low concentration and purity index values of phycoerythrin extracted with DES solvents in this study are suspected to be due to the unstable conditions of the DES solvent affected by the concentration ratio of the DES solvent composition used. DES solvents with a combination of choline chloride-urea, having inadequate thermal stability, will decompose first, followed by the breakdown of choline chloride [10]. Furthermore, DES solvents with a molar ratio of 1/2 and 1/3 contain an excess of hydroxyl groups from glucose that cannot effectively combine with specific groups in citric acid, leading to glucose precipitation. The formation of white solid precipitates at ratios of 1/2 and 1/3 M causes choline chloride-glucose to be suboptimal for extracting phycoerythrin [41].

3.6. Protein Profile of Phycoerythrin Pigment

Phycoerythrin is a globular protein, and its solution is a multi-component solution [27]. The visualization of the SDS-PAGE analysis results for phycoerythrin can be seen in Figure 11. The visualization results of the phycoerythrin protein profile with SDS-PAGE show the formation of 8 protein bands with varying thicknesses. The thickness of the protein bands is affected by the type of sample and different protein concentrations [42]. The variation in band thickness can also be caused by differences in the number of migrating molecules [43]. The visualization results of the phycoerythrin protein profile with SDS-PAGE indicate the presence of several protein bands, indicating that a precipitation process is needed in the crude phycoerythrin extract to purify the phycoerythrin pigment. The estimation results suggest that the protein bands at molecular weights of 20, 21.06, and 31.04 kDa are presumed to be phycoerythrin pigments in the α, β, and ɣ subunits. Similarly, Dumay et al., (2014) reported that the phycoerythrin subunits α, β, and ɣ have molecular weights of 20 kDa, 21 kDa, and 31 kDa, respectively [28].

Table 1: Weight loss of *K.alvarezii* during Drying Process.

Drying Method	Weight Loss (%)
Freeze drying	*83 ± 0.00*
Sun drying	*73.6 ± 0.28*
Oven drying	*80 ± 0.28*

Description: Results % Depreciation of *K. alvarezii* showed significantly different results (<0.05).

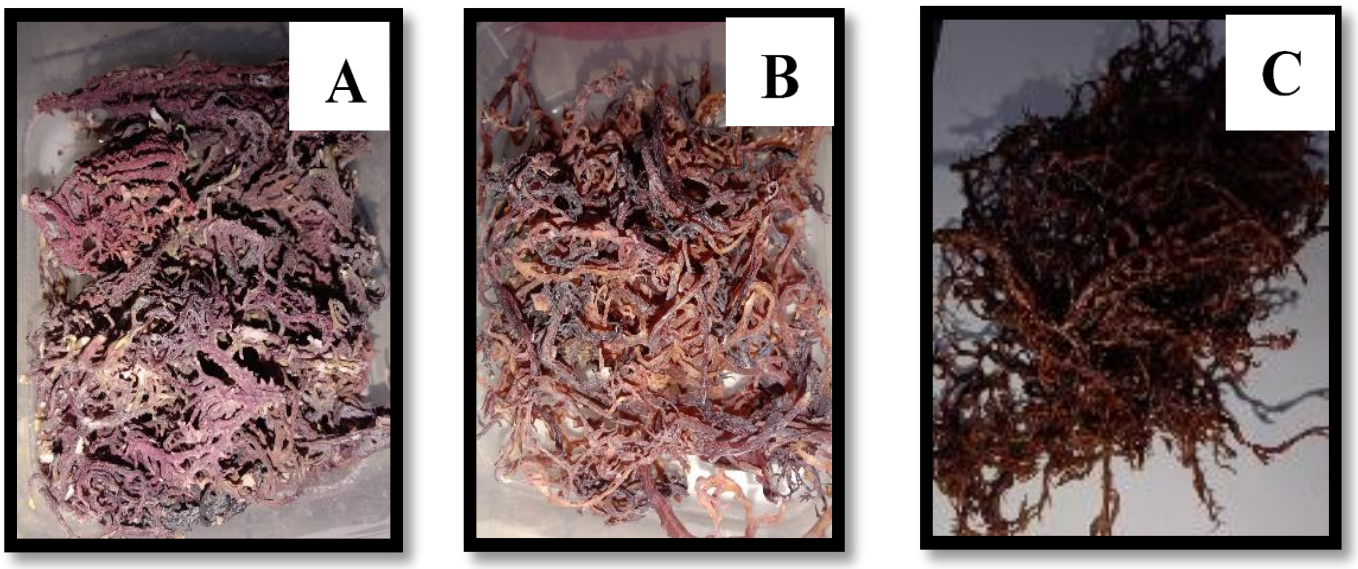

Figure 1. Dried *K. alvarezii* apperence obtained by 3 drying methods A) Freeze drying, B) Sun drying, C) Oven drying.

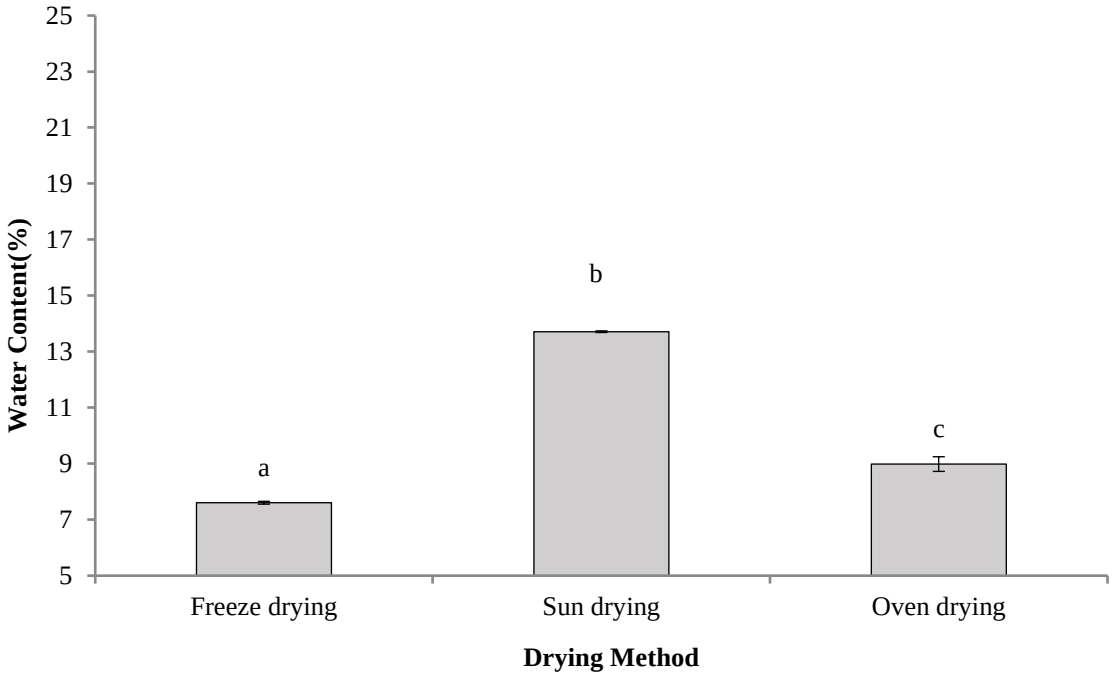

Figure 2. Moisture content values of dried *K. alvarezii*.

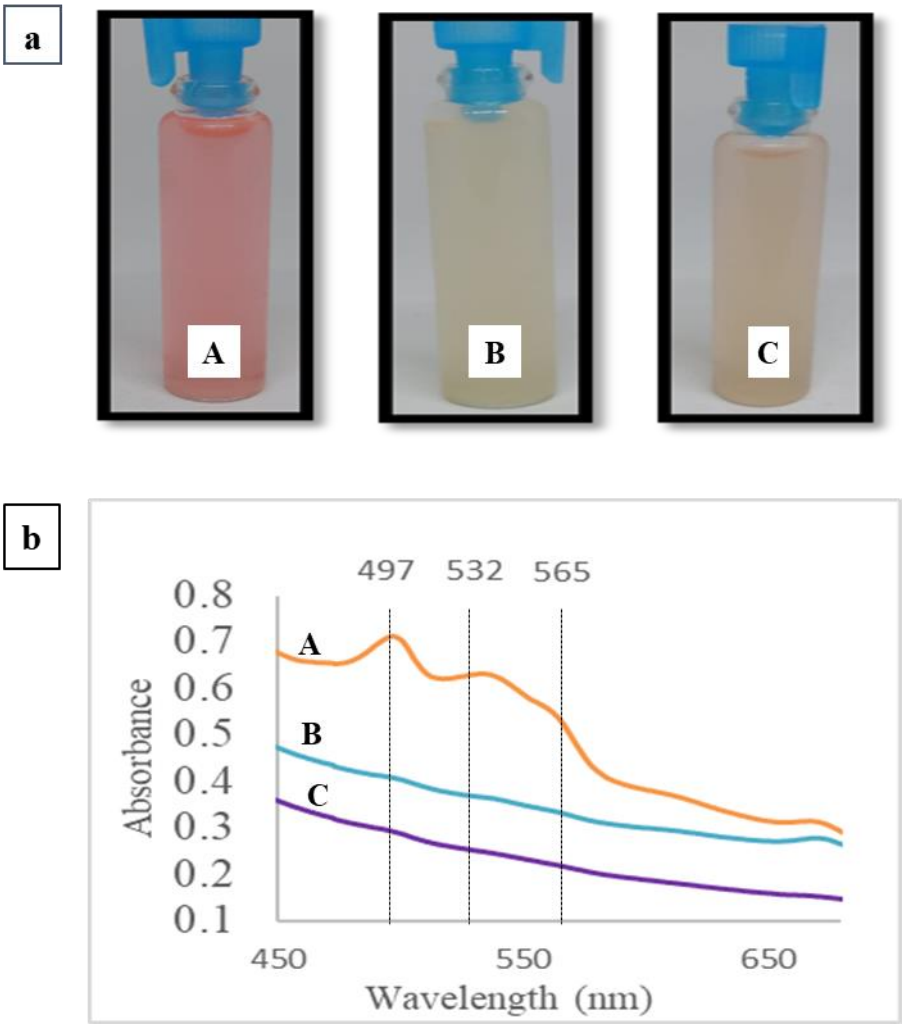

Figure 3. a) Visual extracs of phycoerythrin pigment, using (A) Freeze drying, (B) Sun drying, (C) Oven drying, b) Spectra of phycoeryhrin pigment using (A) Freeze drying, (B) Sun drying, (C) Oven drying.

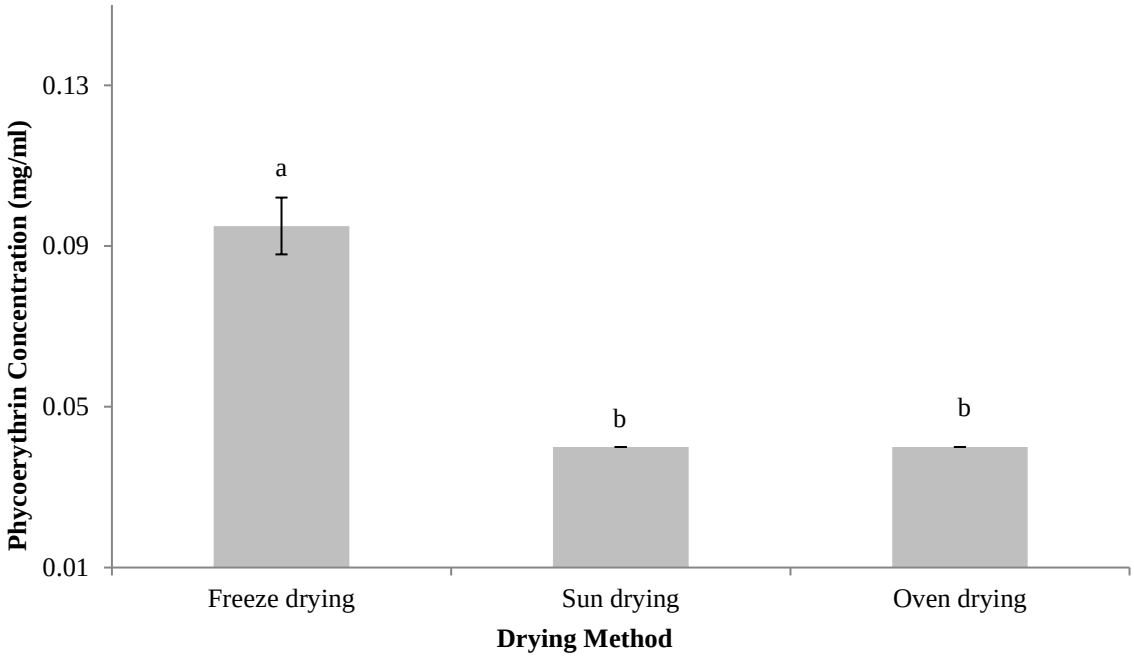

Figure 4. Concentration of *K. alvarezii* phycoerythrin.

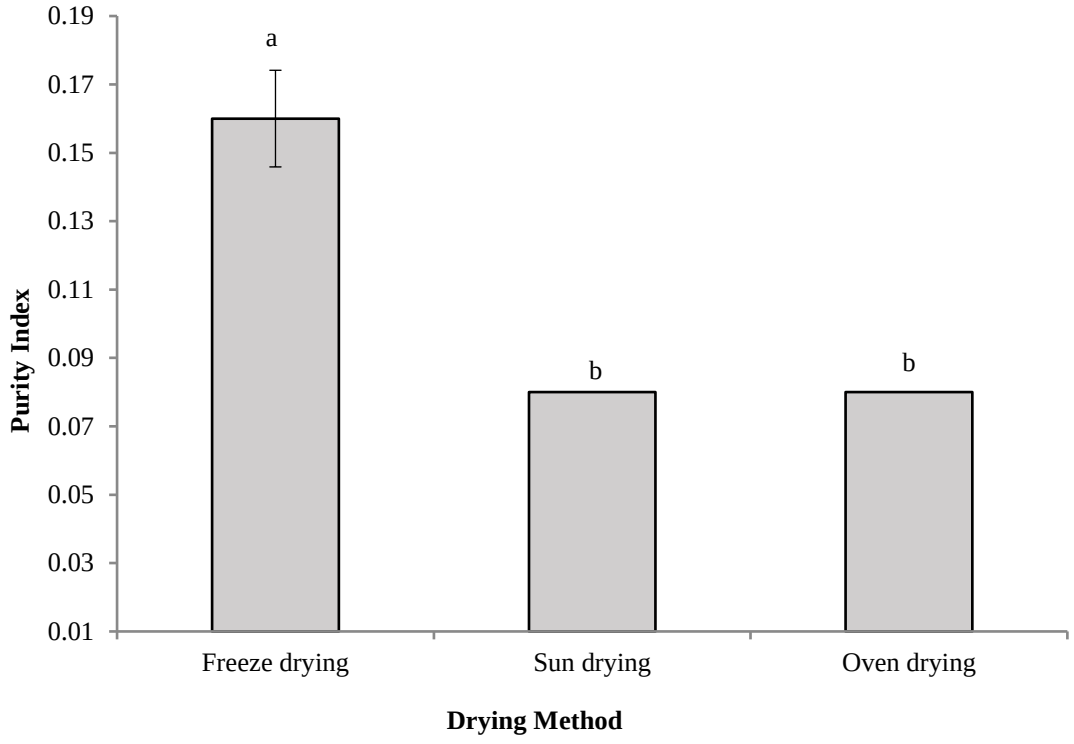

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Figure 5. Purity index of *K. alvarezii* phycoerythrin.

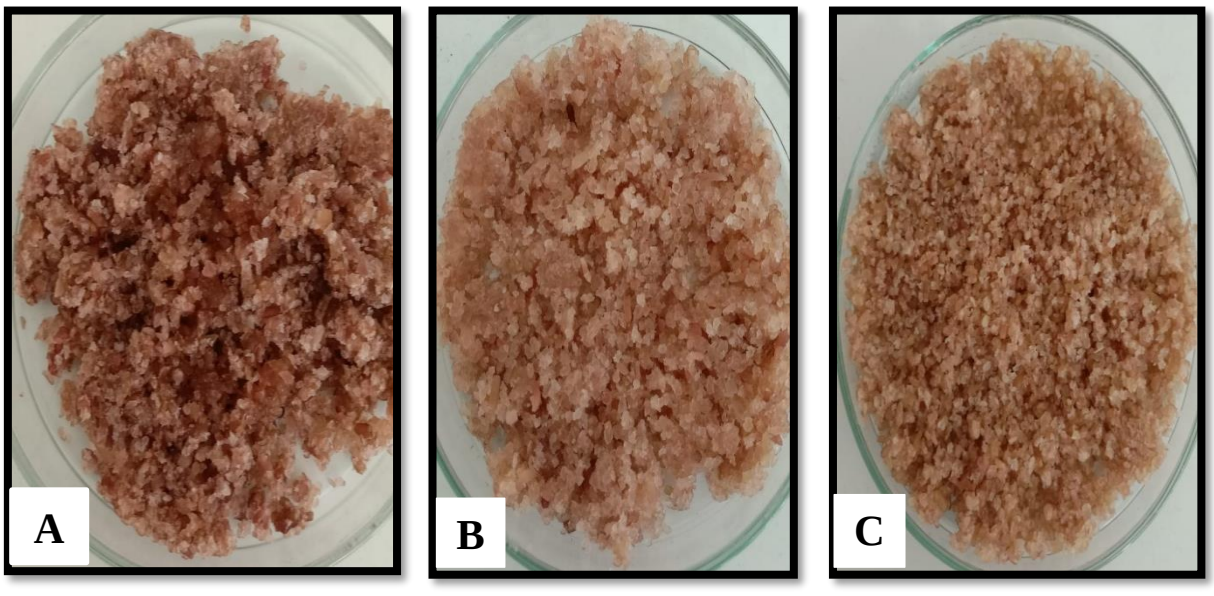

Figure 6. Solid waste residue of *K. alvarezii* obtained from: A)Freeze-drying B) Sun drying C) *Oven drying*

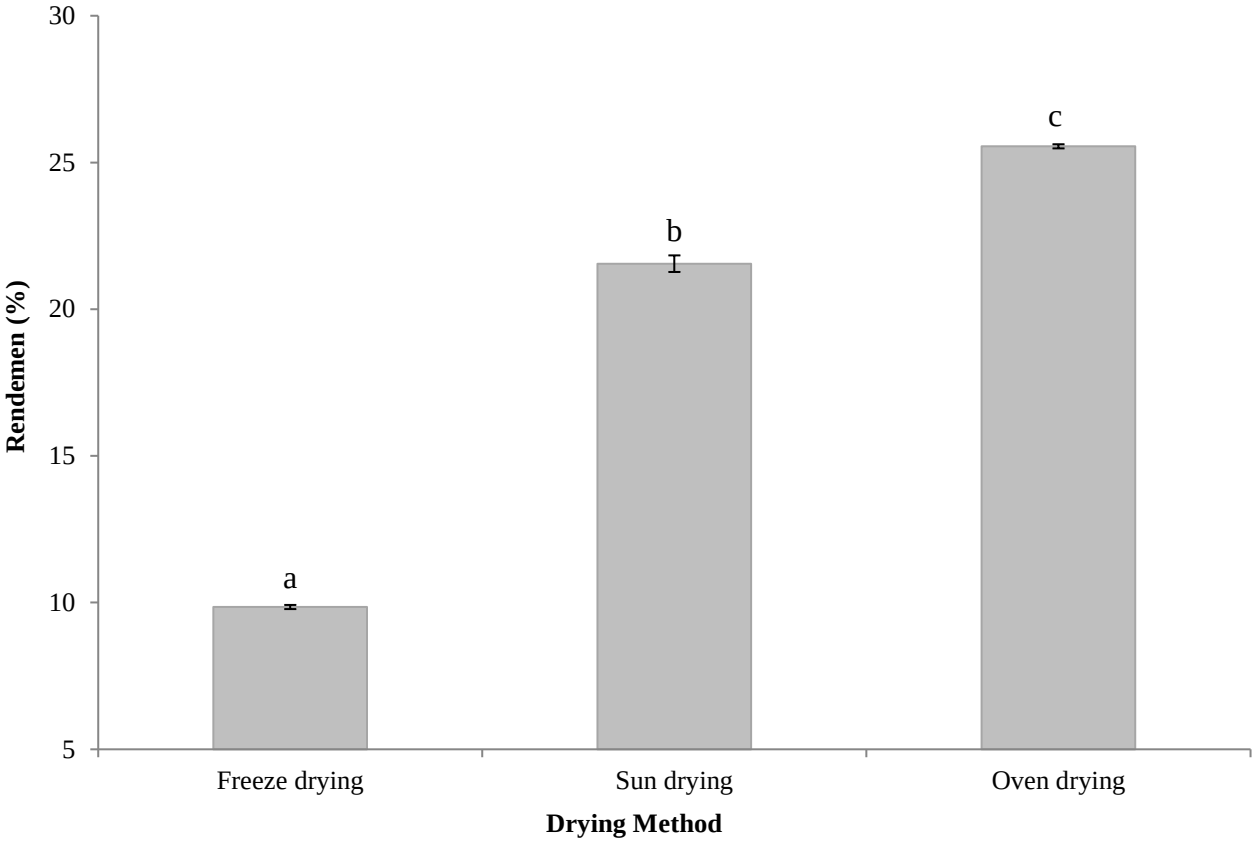

Figure 7. Yield of solid waste residue obtained from *K. alvarezii* extraction.

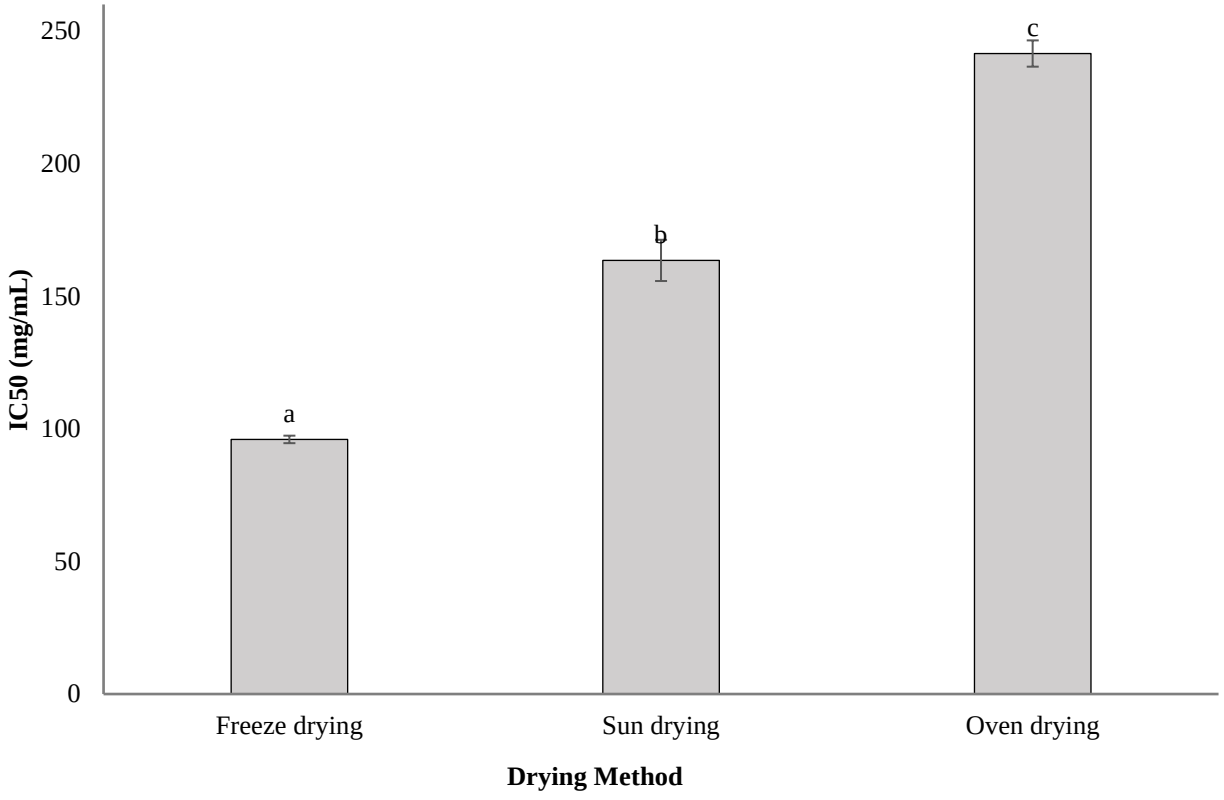

Figure 8. IC_{50} values of phycoerythrin Antioxidants.

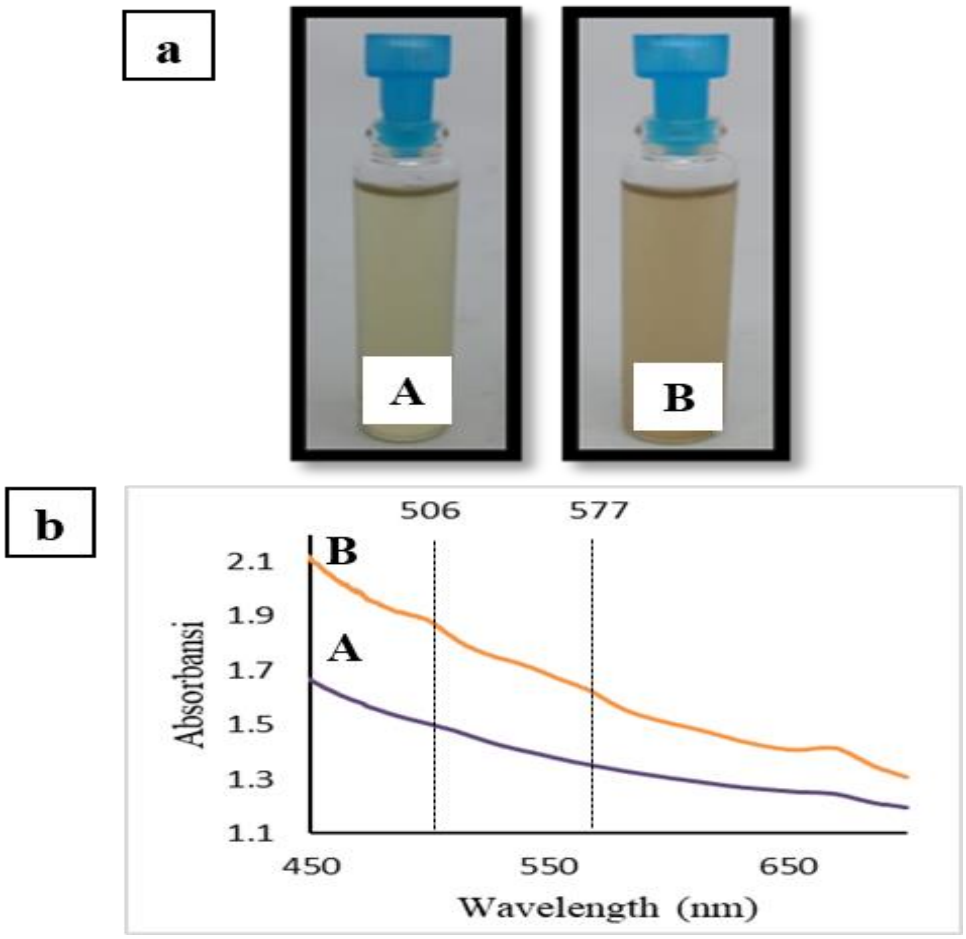

Figure 9. a) Extraction of phycoerythrin pigment, b) Spectra of phycoerythrin pigment using (A) chcl-Urea solvent, (B) chcl-glucose solvent.

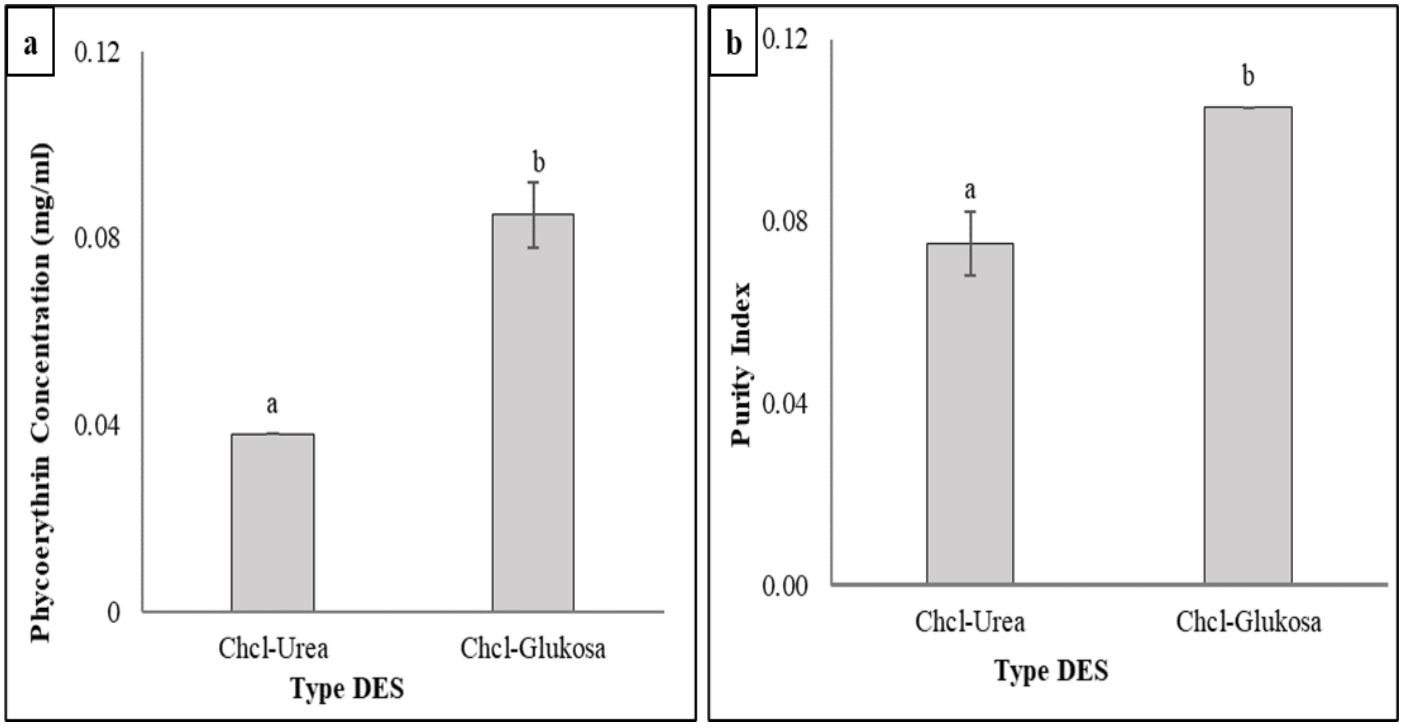

Figure 10. a) Concentration, and b) purity index of phycoerythrin using deep eutectic solvents (DES): chcl-urea and chcl-glucose.

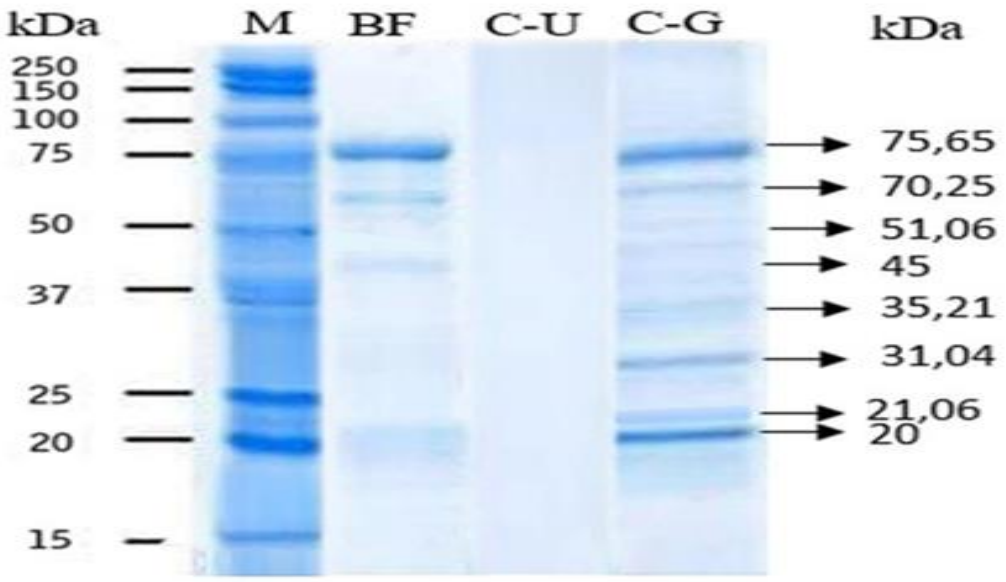

Figure 11. SDS-PAGE profile of phycoerythrin pigment. M: marker, BF: Extract from Phosphate Buffer, C-U: Extract from chcl-urea solvent, C-G: Extract from chcl-glucose solvent.

4. Conclusion

The best drying method for *K. alvarezii* is found in the freeze dryer drying method, which results in a more-red hue of phycoerythrin higher concentration, and purity index, as well as a lower IC_{50} value falling into the strong antioxidant category. The use of two different solvents, namely phosphate buffer and DES, results in different qualities and quantities. Phosphate buffer solvent produces better quality and quantity of phycoerythrin compared to DES solvent, as evidenced by concentration tests, purity index of phycoerythrin, and SDS-Page analysis, which all yielded superior results compared to the use of DES solvent. Further research is needed to enhance the quality and quantity of phycoerythrin extract from dried *K. alvarezii*.

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Authors' Contributions

All authors have contributed to the final manuscript. The individual contributions of each author are as follows: KK conceived and developed the conceptual ideas, prepared, executed, and supervised the research, collected and analyzed data, and drafted the manuscript. US, BS, and NR contributed to the development of conceptual ideas, monitored and supervised the research, and critically revised the article. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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