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Effect of Solvent Type on Pigment Concentrations in Extracts of *Spirulina* sp.

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ABSTRACT

Spirulina sp. is a photosynthetic cyanobacterium with potential as a source of natural pigments, including chlorophyll and phycobiliproteins (phycocyanin, phycoerythrin, and allophycocyanin). This study aimed to determine the pigment content of *Spirulina* sp. extracted using five different solvents: ethanol, distilled water, $NaHCO_3$, KCl, and NaCl. Extraction was performed using the freeze-and-thawing method at -4°C for 24 hours, followed by centrifugation to obtain the supernatant for spectrophotometric analysis. The results showed that the highest phycocyanin and total phycobiliprotein contents were obtained using NaCl (5.98 mg/mL and 8.08 mg/mL, respectively), while the lowest was obtained using ethanol (0.28 mg/mL phycocyanin). Conversely, ethanol produced the highest chlorophyll-a (553.09 mg/L) and allophycocyanin (5.70 mg/mL) contents, far exceeding the other water-based solvents. Phycoerythrin content was relatively uniform across all solvents (0.28–0.34 mg/mL), Visual color differences among the extracts corroborated the quantitative results, with ethanol extracts appearing green (chlorophyll-dominated) and water/salt-based extracts appearing blue (phycocyanin-dominated). These findings indicate that solvent type strongly influences the type and amount of pigment extracted from *Spirulina* sp., suggesting that solvent selection should be tailored to the target pigment salt solutions such as NaCl are recommended for optimal phycocyanin recovery, while ethanol is more suitable for chlorophyll-a recovery.

INTRODUCTION

Various types of pigments occur naturally and are widely distributed across plants, animals, and microbes. The presence of these pigments gives rise to the diverse natural colors found in these organisms. These pigments contain numerous bioactive compounds, making them effective antimicrobial, anti-inflammatory, and anticancer agents. Natural pigments include flavonoids, carotenoids, chlorophylls, anthocyanins, and phycobiliproteins (George & John, 2023). *Spirulina* sp. is a photosynthetic cyanobacterium known to contain several groups of pigments with distinct characteristics regarding color, solubility, and stability; these include chlorophyll (the primary pigment) and phycobiliproteins (accessory pigments) such as phycocyanin, phycoerythrin, and allophycocyanin which play a crucial role in broadening the light absorption spectrum to facilitate photosynthesis (Amrulloh & Dewi, 2026; Widawati *et al.,* 2022; Nuryadin *et al.,* 2023). Due to the diversity of these pigment characteristics, extraction yields from *Spirulina* sp. biomass can vary consequently, the resulting pigment content is influenced by the extraction process and the factors involved therein.

The extraction process for *Spirulina* sp. is influenced by numerous factors, including solvent type, polarity, pH, ionic strength, temperature, extraction time, and cell disruption

methods (Buliga *et al.,* 2024). Among these, the choice of solvent plays a crucial role as each solvent possesses distinct properties and characteristics, their respective capacities to extract pigments from *Spirulina* sp. biomass vary. These factors ultimately determine the yield of chlorophyll and phycobiliproteins in the microalga *Spirulina* sp. (Agustina *et al.,* 2018). Previous studies have mainly focused on aqueous based extraction of phycobiliproteins, while direct comparisons among ethanol, distilled water, $NaHCO_3$, KCl, and NaCl for the simultaneous recovery of phycocyanin, allophycocyanin, phycoerythrin, total phycobiliprotein, and chlorophyll a remain limited. These five solvents were selected to represent different solvent polarities and ionic conditions, with the concentrations used in this study chosen based on their suitability for pigment extraction and comparison under consistent experimental conditions. Therefore, this study provides a comparative assessment of how these extraction media influence the pigment profile of *Spirulina* sp.

METHOD

This study was conducted in April 2026 at the Fishery Product Technology (THP) Laboratory, Department of Fisheries Science, Sultan Ageng Tirtayasa University (UNTIRTA). The materials used in the study included wet *Spirulina* sp. biomass, ethanol, distilled water, sodium bicarbonate ($NaHCO_3$) 1%, potassium chloride (KCl) 1% 1%, and sodium chloride (NaCl) 1%. The equipment used included an analytical balance, graduated cylinders, beakers, test tubes, volumetric pipettes or micropipettes, a vortex mixer, a centrifuge, filter paper, and a UV-Vis spectrophotometer for pigment content measurement.

The pigment extraction procedure for *Spirulina* sp. in this study was based on methods developed by (Rahmawati et al. 2017; Nuryadin et al. 2023), modified to suit the study's requirements. Wet *Spirulina* sp. biomass was mixed with the respective solvents at a 1:6 (v/v) ratio and subjected to extraction via a freeze-thaw technique at -4°C for 24 hours. Following extraction, the mixture was centrifuged at 3,000 rpm for 30 minutes to separate the supernatant, which was subsequently used for analysis. The analytical steps involved determining the concentrations of phycobiliproteins—specifically phycocyanin (PC), phycoerythrin (PE), and allophycocyanin (APC)—as well as chlorophyll-a. Measurements were taken using a UV-Vis spectrophotometer at wavelengths of 620 nm, 652 nm, 615 nm, 562 nm, 663 nm, and 645 nm, followed by calculations using the equations of Bennett and Bogorad (1973) and Khazi et al. (2018), as follows:

- CPC (mg/mL) = $\frac{(A\ 620) - 0{,}474\ (A652)}{5{,}34}$
- APC (mg/mL) = $\frac{(A\ 652) - 0{,}208\ (A615)}{5{,}09}$
- PE (mg/mL) = $\frac{(A\ 562) - 2{,}41\ (PC) - 0{,}849\ (APC)}{9{,}62}$
- Total PBP = CPC + APC + PE
- Chlorophyll a (mg/L) = 12.7 x OD663 – 2.69 x OD645

Information: CPC = konsentrasi C-fikosianin (mg/mL); OD = optical density; APC = allofikosianin (mg/mL); PE = fikoeritrin (mg/mL); PBP = Phycobiliprotein (Fikobiliprotein)

RESULT AND DISCUSSION

Phycocyanin Content (C-Phycocyanin, CPC)

Phycocyanin is a water-soluble, non-toxic, blue photosynthetic pigment widely utilized in the food, cosmetic, and pharmaceutical industries. It is found in *Spirulina* sp. at a content of 14–20% and consists of an alpha (α) polypeptide with a molecular weight of 10–19 kDa and a beta (β) polypeptide with a molecular weight of 14–21 kDa, which together form a monomer (Fernandes *et al.,* 2023). Phycocyanin can be extracted from *Spirulina* sp. by disrupting the thylakoid membrane using various physical or chemical methods, such as the use of suitable solvents (Morya *et al.,* 2023; Fernandes *et al.,* 2023). This study employed ethanol, distilled water, $NaHCO_3$, KCl, and NaCl as solvents to extract the phycocyanin pigment from *Spirulina* sp.

The extraction results (Figure 1) indicate that the highest phycocyanin (CPC) content was obtained using an NaCl solvent (5.98 mg/mL), followed by KCl (4.83 mg/mL), distilled water (4.20 mg/mL), and $NaHCO_3$ (2.48 mg/mL), with the lowest yield observed in ethanol (0.28 mg/mL). Water-based solvents containing specific ions tend to be more effective at dissolving phycocyanin from *Spirulina* sp. compared to volatile organic solvents (Hidhayati *et al.,* 2020). Ethanol, aqueous solvents, and salt solutions can create different chemical environments that affect protein solubility and denaturation, ionic interactions, and the stability of phycobiliproteins and chlorophyll a during extraction. This is supported by Purnamayati et al. (2016), whose research demonstrated that phycocyanin is readily soluble in polar compounds but poorly soluble in nonpolar solvents consequently, the use of saline aqueous solvents like NaCl and KCl in this study offered an advantage in solubilizing the pigment. The salt solutions used during extraction are thought to increase the ionic strength of the extraction medium, thereby facilitating cell membrane disruption and weakening electrostatic interactions between phycocyanin and other cellular components, which allows the pigment to be released more easily into the solvent. Fernandes et al. (2023) explained that the release of phycocyanin results from the rupture or damage of cell membranes during the extraction process. This is further corroborated by Li et al. (2020), whose research showed that the minimum ionic strength required for the extraction process is >5 g/L NaCl. Solvents with a limited ability to lyse the *Spirulina* sp. cell wall such as pure distilled water without added salt or buffer yielded lower phycocyanin levels compared to solvents with different polarity and ionic strength can affect pigment extraction. These quantitative findings are supported by the visual evidence in Table 1, where the phycocyanin solutions obtained via NaCl and KCl extraction exhibited a blue color, contrasting sharply with the ethanol extract (Table 1), which appeared dark green. Phycocyanin extracted using various solvents has been shown to reduce microsomal lipid oxidation and scavenge free radicals (hydroxyl and peroxyl), thereby acting as an antioxidant pigment (İsar *et al.,* 2022).

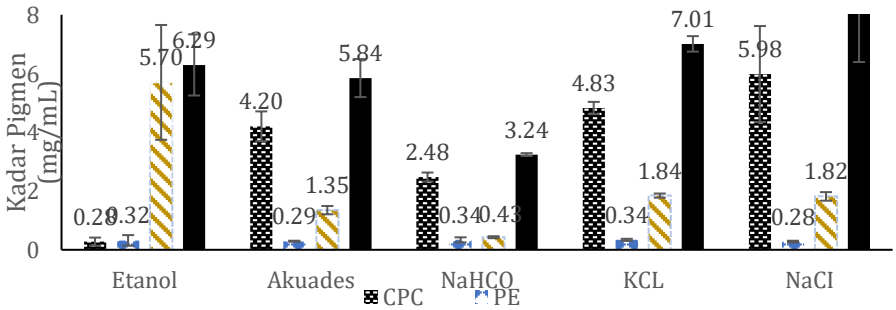


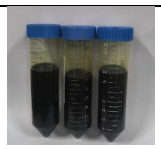
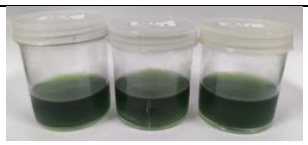
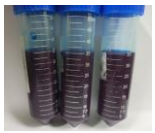
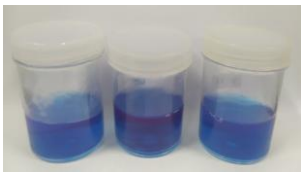
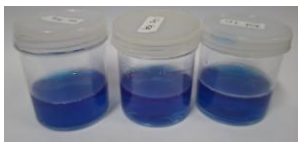
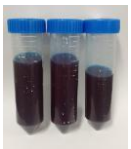
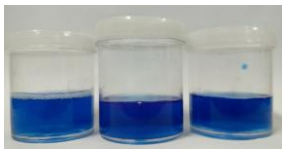
Figure 1. Content of phycocyanin (CPC), phycoerythrin (PE), allophycocyanin (APC), and total phycobiliproteins (PBP) in *Spirulina* sp. extracted using five types of solvents (ethanol, distilled water, $NaHCO_3$, KCl, and NaCl).

Phycoerythrin (PE) Content

Phycoerythrin (PE) is a pink/red natural pigment found in blue-green algae (cyanobacteria) such as *Spirulina* sp., with an absorption peak in the 540–565 nm wavelength range (George & John, 2023). Phycoerythrin generally exists in a hexameric form, specifically $(\alpha\beta)_6$. The molecular masses of the α and β subunits range from 17 kDa to 19 kDa, depending on their origin (Kamble *et al.,* 2018). Based on its absorption spectrum, phycoerythrin is classified into three main types: (a) B-phycoerythrin (B-PE), derived from red algae of the order Bangiales, with peaks at 545 and 565 nm and a shoulder at 499 nm; (b) C-phycoerythrin (C-PE), derived from blue-green algae (Cyanophyta/Cyanobacteria), which exhibits a single maximum absorption peak at a visible wavelength of 565 nm; and (c) R-phycoerythrin (R-PE), derived from red algae (Rhodophyta), with peaks at 499 and 565 nm and a shoulder at 545 nm (George & John, 2023). Extraction is a crucial step for disrupting cell membranes to release the

phycoerythrin pigment from microalgae, a process that requires a suitable solvent. The solvents used in this study were capable of extracting the phycoerythrin pigment from the cell membranes of *Spirulina* sp. The study results showed that the C-PE content across the five types of solvents was relatively uniform, ranging from 0.28 to 0.34 mg/mL; the highest values were observed with $NaHCO_3$ and KCl (± 0.338 mg/mL), while the lowest was with NaCl (0.279 mg/mL). The minimal variation observed in phycoerythrin (PE) concentrations may be partly explained by the relatively low abundance of PE in *Spirulina* sp. compared with phycocyanin and allophycocyanin. However, this represents only one possible explanation, as the small differences may also be influenced by the analytical sensitivity of the method, particularly when PE concentrations are close to the lower quantification range of the assay. Among the three types of phycobiliproteins in *Spirulina* sp., phycocyanin is the most dominant component, whereas phycoerythrin is present in small quantities (Fithria *et al.,* 2022; Widawati *et al.,* 2022). The phycoerythrin content across the five solvents was relatively uniform and low (0.28–0.34 mg/mL) making it difficult to distinguish visually in Table 1 because phycoerythrin is pink and exists in only a very small proportion compared to the other pigments in *Spirulina* sp. The absence of a visible pink hue (as seen in Table 1) further reinforces the conclusion that phycoerythrin is not a dominant pigment component in *Spirulina* sp. biomass, regardless of the solvent used.

Table 1. Extraction results using different solvents.

Extraction Solvent	Photo of Extraction Results	
Etanol		
Akuades		
NaHCO		
KCL		
NaCI		

Allophycocyanin (APC) Content

Spirulina contains C-phycocyanin and allophycocyanin, which serve as light-harvesting accessory pigments within phycobilisomes (İsar *et al.,* 2022). These pigments exhibit good solubility and stable physical properties. Their primary role within the phycobilisome is to mediate energy transfer. Isolated and purified natural allophycocyanin consists of α and β

subunits, typically organized as (αβ)3 trimers. Allophycocyanin also exhibits optical activity through its association with phycocyanobilin, which serves as its chromophore. Conventional extraction methods recover only 50–60% of the total allophycocyanin present in the algae (Shang *et al.,* 2023).

Extraction results using different solvents revealed a pattern for allophycocyanin that differed from that of phycocyanin; the highest allophycocyanin content was obtained using ethanol (5.70 mg/mL), far exceeding the yields from KCl (1.84 mg/mL), NaCl (1.82 mg/mL), distilled water (1.35 mg/mL), and $NaHCO_3$ (0.43 mg/mL), which yielded the lowest amount. The differing allophycocyanin response to ethanol compared to phycocyanin can be attributed to structural differences and the respective locations of these two proteins within the phycobilisome complex. Allophycocyanin is situated in the phycobilisome core positioned beneath the phycocyanin pigment where it is in direct contact with the thylakoid membrane (Dagnino-Leone *et al.,* 2022; George & John, 2023). The extraction yield with ethanol is thought to be primarily influenced by the solvent's ability to disrupt the phycobilisome structure within the *Spirulina* sp. biomass.

Total Phycobiliprotein (PBP) Content

Phycobiliproteins are brightly colored, water-soluble fluorescent proteins commonly found in cyanobacteria and rhodophytes (red algae) that function as light-harvesting antenna pigments. These proteins are components of subcellular structures known as phycobilisomes; they are covalently linked via cysteine amino acid chromophores and attached to the chloroplast thylakoid membrane. Generally, phycobiliproteins are categorized based on their absorption spectra into phycoerythrin (PE)—a red pigment; phycocyanin (PC)—a blue pigment; and allophycocyanin (APC)—a light blue pigment (George & John, 2023). The highest total phycobiliprotein content (the combined total of phycocyanin, phycoerythrin, and allophycocyanin) was obtained using NaCl solvent (8.08 mg/mL), followed by KCl (7.01 mg/mL), ethanol (6.29 mg/mL), and distilled water (5.84 mg/mL), with the lowest yield observed in $NaHCO_3$ (3.24 mg/mL). The high total values in NaCl and KCl were primarily driven by the dominant contribution of phycocyanin, whereas the relatively high total value in ethanol was largely influenced by the significant contribution of allophycocyanin, despite the phycocyanin yield itself being very low. A solvent's effectiveness in extracting total phycobiliproteins cannot be assessed based on a single pigment type; rather, it results from complex interactions between the physicochemical characteristics of each pigment component and the chemical properties of the solvent employed. Research by Li et al. (2020) on the extraction of the pigment phycocyanin using NaCl indicates that the ionic strength of the salt solution can help optimize the extraction process for *Spirulina* sp. pigments. Photographs of the crude extracts from all water-based treatments (Table 1) consistently showing a deep purple to blackish hue—indicate that the crude extracts contain a mixture of phycobiliproteins (blue-red) and chlorophyll (green), which optically blend to form a dark purple color.

Chlorophyll Content

Chlorophyll is a green pigment capable of absorbing light at various wavelengths, thereby enabling the process of photosynthesis (assimilation) in microalgae (Dharmadewi, 2020). There are several types of chlorophyll with varying distributions among photosynthetic organisms: chlorophyll *a*, found generally in almost all green plants; chlorophyll *b*, in higher plants and green algae; chlorophyll *c*, in diatoms, brown algae, and flagellates such as *Euglena*; and chlorophyll *d*, in red algae. The chlorophyll *a* content in *Spirulina* sp. ranges from 0.8% to 1.5% of its dry weight (İsar *et al.,* 2022).

The extraction results regarding chlorophyll-a content revealed a pattern starkly different from that of phycobiliprotein pigments: the highest chlorophyll-a yield was obtained using ethanol (553.09 mg/L), far exceeding the yields from distilled water (48.04 mg/L), $NaHCO_3$ (59.55 mg/L), KCl (60.30 mg/L), and NaCl (58.75 mg/L). The values for these latter solvents were relatively uniform and significantly lower; indeed, the chlorophyll-a content in ethanol was approximately 9–11 times higher than in the four water-based solvents.

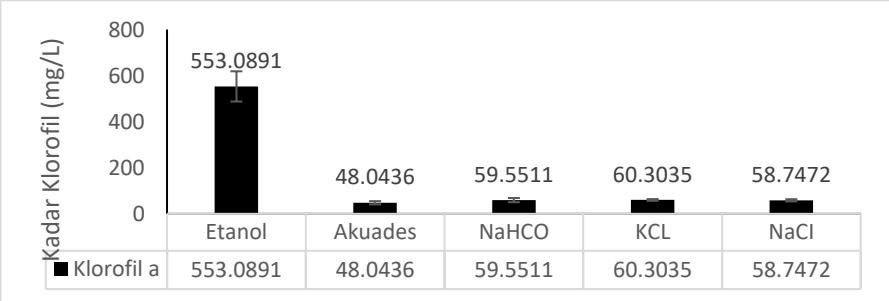


Figure 2. Chlorophyll-a content of *Spirulina* sp. extracted using five types of solvents (ethanol, distilled water, $NaHCO_3$, KCl, and NaCl).

The superior chlorophyll-a yield obtained with ethanol aligns with findings by Nikolova et al. (2024), who reported a maximum chlorophyll-a content of 5.46 mg g^{-1} using ethanol under conditions of a 2-hour extraction time, 40°C temperature, and 45 kHz frequency. Chlorophyll molecules consist of two parts: a hydrophilic region (macrocycle) and a hydrophobic region (phytol chain); in plants, chlorophyll exists in two forms, chlorophyll-a and chlorophyll-b (Agustina *et al.,* 2019). Chlorophyll-a features a methyl group (CH_3), is less polar, and exhibits a blue-green color (Indrasti *et al.,* 2019). The varying properties of the solvents used in this study influence their ability to penetrate the biomass and release chlorophyll from *Spirulina* sp. cells. Solvents such as distilled water, $NaHCO_3$, KCl, and NaCl possess distinct characteristics, resulting in differing chlorophyll yields following the extraction process.

CONCLUSION

Based on research findings regarding the pigment content of *Spirulina* sp. extracted using various solvents, it can be concluded that the type of solvent significantly and differentially affects the yield of specific pigment groups namely phycobiliproteins (phycocyanin, phycoerythrin, and allophycocyanin) and chlorophyll-a. The NaCl solvent yielded the highest phycocyanin content (5.98 mg/mL) and total phycobiliprotein content (8.08 mg/mL), followed by KCl; thus, neutral salt solutions proved most effective for extracting phycocyanin, the natural blue pigment that is the primary target of *Spirulina* sp. utilization. Conversely, the ethanol extract exhibited the lowest phycocyanin (CPC) concentration (0.28 mg/mL) but the highest calculated concentrations of chlorophyll a (553.09 mg/L) and allophycocyanin (APC) (5.70 mg/mL) among the tested solvents. The relatively high APC value obtained for the ethanol extract should, however, be interpreted with caution because the APC concentration was estimated using spectrophotometric measurements, which may be affected by overlapping absorbance from other pigments or matrix components, indicating that ethanol is more suitable when the extraction objective is to obtain lipophilic pigments like chlorophyll rather than phycobiliproteins. Phycoerythrin content showed no significant variation across solvents (0.28–0.34 mg/mL), confirming that it is not a dominant pigment component in *Spirulina* sp.. Visual documentation of the extracts reinforces the validity of the quantitative data: the intensity of the blue color in the solutions correlated with phycocyanin levels, while the deep green color of the ethanol extract confirmed the dominance of chlorophyll in that solvent. Overall, these results confirm that no single solvent is universally optimal; therefore, the choice of solvent for *Spirulina* sp. extraction must be tailored to the specific pigment targeted for utilization.

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