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Facile Extraction of Spirulina for Ultra-Protection Sunscreen Activity

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Abstract

Spirulina is a cyanobacterium rich in bioactive compounds, including flavonoids, phenolics, and phycocyanins, which contribute to its antioxidant and photoprotective properties. This study aimed to evaluate the bioactive composition, antioxidant capacity, and sun protection factor (SPF) of ethanolic spirulina extracts. Extraction was performed using maceration with 70% ethanol, followed by filtration and evaporation. The antioxidant activity was determined using the DPPH assay, while SPF values were assessed through spectrophotometric analysis at 290–320 nm. The extraction yield was 35.345±0.050%, with a residual solvent content of 0.586±0.059%, indicating efficient extraction. Phytochemical screening confirmed the presence of flavonoids, saponins, phenols, and steroids. The IC_{50} value of 784.914±0.299 ppm classified the extract as having weak antioxidant activity. SPF analysis showed that at 5000 ppm, the extract achieved an SPF value of 20.378±0.084, placing it in the ultra-protection category. These findings suggest that spirulina extract has potential applications in cosmetic formulations as a natural UV-protective agent in maximum-to-ultra protection category. Further research is needed to assess its stability in formulations and its effectiveness in in vivo applications.

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Introduction

The increasing awareness of the potential risks associated with synthetic ultraviolet (UV) filters has driven interest in natural bioactive compounds for sunscreen applications. Many plant- and algae-derived compounds, particularly polyphenols, flavonoids, and pigments, exhibit UV-absorbing properties while providing antioxidant and anti-inflammatory benefits to the skin (Michalak, 2022). These bioactive compounds not only help reduce oxidative damage but also offer additional photoprotective effects, making them promising candidates for incorporation into sunscreen formulations. Recent studies have explored various natural sources, such as marine algae, seed extracts, and cyanobacteria, for their potential in UV protection (Permatasari *et al.* (2024) and Ramdhiany & Tsurayya (2025)). However, research on optimizing extraction methods to maximize bioactive compound yield and photoprotective efficacy remains ongoing.

Spirulina is a filamentous cyanobacterium rich in antioxidants and pigments, has gained attention for its potential photoprotective properties. It contains high levels of phycocyanin, flavonoids, and phenolic compounds, which contribute to its ability to neutralize free radicals and absorb UV radiation included UV-A and UV-B radiation (Dianursanti *et al.*, 2020). Studies have shown that phycocyanin, the primary pigment in spirulina, exhibits photoprotective effects by acting as a natural UV-absorbing agent and reducing oxidative stress in skin cells (Nowruzi *et al.*, 2020). Furthermore, methanol and aqueous extracts of spirulina have demonstrated significant antioxidant capacity, suggesting their potential role in sunscreen formulations (Ragusa *et al.*, 2021). However, despite these promising findings, variations in solvent extraction techniques may affect the stability, concentration, and bioactivity of spirulina-derived compounds.

While previous research has explored the antioxidant and UV-protective effects of spirulina, most studies have focused on aqueous and methanol-based extractions, with limited evaluation of ethanol extraction. Dewantoro *et al.* (2022) reported ethanol as a safe and efficient solvent for extracting phenolic compounds while maintaining their bioactivity, making it a suitable choice for cosmetic applications. However, little is known about how ethanol extraction influences the sun protection factor (SPF) of spirulina extracts. Additionally, while SPF evaluation has been widely conducted for plant-based extracts, research specifically assessing the photoprotective potential of spirulina extracts under ethanol extraction conditions remains scarce. Further investigation is needed to determine the effects of ethanol extraction on the phytochemical profile, antioxidant capacity, and SPF value of spirulina as previous studies have shown that ethanol extraction significantly enhance to its parameters mentioned based on the report from Ghaisani *et al.* (2025) and Milia *et al.* (2025).

The objective of this study was to investigates the bioactive composition, antioxidant capacity, and sun protection factor (SPF) of ethanolic extracts of spirulina. The results are expected to provide scientific evidence supporting the potential of spirulina as a natural sunscreen agent. Additionally, this study would be contributes to the development of alternative bioactive ingredients for cosmetic and pharmaceutical applications.

Method

Materials

The main material used in this study was *Arthospira platensis* spirulina powder, produced by PT. Alga Bioteknologi Indonesia. The chemicals used included ethanol (≥99.5%), DPPH (2,2-diphenyl-1-picylhydrazyl), Magnesium powder, Ferric chloride, and Mayer's reagent obtained from Sigma-Aldrich (Missouri, USA); methanol (≥99.8%), vitamin C, hydrochloric acid, sulfuric acid, acetic acid obtained from Merck (Singapore), and distilled water.

Spirulina Extract Preparation

Spirulina powder was soaked in 70% ethanol at a solid-to-solvent ratio of 1:20 (b/v). Maceration was carried out for 72 h with stirring for 5 min every 24 h of storage (Sirait *et al.* (2019) and Pannindrya *et al.* (2020)). Filtration was then performed to separate the residual solid from the macerate, which was subsequently evaporated at 37–40°C to obtain a thick extract. The extraction yield, residual solvent content, and pH value were determined using equations (1) and (2).

Yield calculation:

$$\text{Yields} = \frac{\text{Extracts weight (g)}}{\text{Materials weight (g)}} \times 100\% \qquad \ldots(1)$$

Residual solvent calculation:

$$\text{RS}_{\text{content}} = \frac{w_i - w_f}{w_i} \times 100\% \qquad \ldots(2)$$

Note: w_i is initial extracts weight and wf is final extracts weight after evaporation.

Phytochemical Screening

The qualitative analysis of pythochemical compounds in the spirulina extract combined the procedures of Cahyaningsih *et al.* (2019) and Yani *et al.* (2023). **Alkaloid test**: A 2 mL mixture of the extract and hydrochloric acid (1:10, w/v) was prepared and treated with Mayer's reagent. The presence of alkaloids was indicated by the formation of a cloudy white or yellow precipitate. **Flavonoid test**: A mixture of the extract and magnesium powder (2:1, w/w) was dissolved in 5 mL of ethanol, followed by the addition of 1 mL of hydrochloric acid and vigorous shaking. The presence of flavonoids was indicated by a color change in the solution to red, orange, or yellow. **Saponin test**: A mixture of the extract and distilled water (1:20, b/v) was vigorously shaken until foam was formed, followed by the addition of one drop of hydrochloric acid. The presence of saponins was indicated by the stability of the foam after the addition of hydrochloric acid. **Steroid and Terpenoid test**: A test sample mixture was prepared by adding 10 drops of acetic acid and 2 drops of sulfuric acid to 50 mg of extract. The solution was the gently shaken and left to stand for a few minutes. The presence of steroids was indicated by a green color change, while the presence of terpenoids was indicated by a red color change. **Phenol test**: A 50 mg extract was treated with 3 to 4 drops of ferric chloride. The presence of phenols was indicated by a color change to green, red, purple, blue, or dark black.

DPPH Scavenging Assay

The determination of antioxidant activity in the spirulina extract followed the procedures of 'Aisy *et al.* (2022) and Husnani (2024), as detalied below. First, the DPPH and blank solution prepared by dissolving DPPH powder in methanol (1:10, w/v) to prepare a stock solution with a concentration of 100 ppm. A 20 mL aliquot of the stock solution was pipetted and diluted with methanol to obtain a final concentration of 40 ppm. The diluted solution was homogenized for 30 s and stored in a light-protected storage. Then, a stock solution of vitamin C was prepared at a concentration of 100 ppm by dissolving it in methanol at a solid-to-solvent ratio of 1:10 (w/v). The solution was the diluted to obtain final concentrations of 2, 4, 6, 8, and 10 ppm. For each concentration, 4 mL of the vitamin C solution was pipetted and mixed with 1 mL of DPPH solution, followed by incubation for 30 min. After incubation, the samples were measured using a UV-Vis spectrophotometer at a wavelength of 517 nm.

Test sample from spirulina extract was prepared by dissolving a 40 mg of spirulina extract in 20 mL of methanol to obtain stock solution with a concentration of 2000 ppm. The solution was the diluted to final concentrations of 125, 250, 500, 1000, and 2000 ppm. For each concentration, 2 mL of the extract solutuon was mixed with 2 mL of 40 ppm DPPH solution. The mixture was homogenized and incubated for 30 min before measureing its absorbance at a wave-length of 517 nm. Then, the absorbance measurement included the blank sample, vitamin C, and spirulina extract solutions was measured at a wavelength of 517 n,, with each measurement performed in triplicate. The recorded absorbance values were used to calculate the percentage of inhibition (%inhibition) using equation (3). The results were then plotted as a linear regression equation to determine the IC50 (inhibition concentration 50) value, which represents the antioxidant capacity required to inhibit 50% of free radical.

$$\%\text{Inhibition} = \frac{\text{A}_{\text{blank}} - \text{A}_{\text{sample}}}{\text{A}_{\text{blank}}} \times 100\% \qquad ...(3)$$

Note: A_{blank} is absorbance value of blank sample and A_{sample} is absorbance value of test sample (vitamin C and/or spirulina extracts).

Sunscreen Activity

Spirulina extract was dissolved in the ethanol to prepare a stock solution with a concentration of 5000 ppm. The solution was the diluted to obtain final concentratios of 1000, 2000, 3000, 4000, and 5000 ppm. The absorbance of each solution was measured at 5 nm intervals within the wavelength range of 290–320 nm (Dewantoro *et al.*, 2022). The measured absorbance values were used in equation (4), multiplied by the $EE \times I$ constant, and further adjusted using a correction factor (CF) of 10 to determine the SPF value (Yulianti et al., 2015).

$$\text{SPF} = \text{CF} \times \sum_{290}^{320} (EE \times I) \times \text{Abs} \qquad ...(4)$$

Note: CF is correction factor and the value is 10, $EE \times I$ is constant value for each wavenumber, and Abs is absorbance measured.

Results and Discussion

Extract Characteristics

The spirulina extract obtained in this study exhibited a deep green color, as shown in Figure 1. The extraction yield was 35.345±0.050%, with a residual solvent content of 0.586± 0.059% indicating the effectivenes of the applied extraction technique (Hasanah *et al.*, 2019). Additionally, the low residual solvent content suggests high extract quality, as residual solvents can cause irritation and allergic reactions if present in significant amounts (Evania (2019) and Prasad *et al.* (2022)). The pH of the spirulina extract was recored at 5.870±0.008, whic is associated with the presence of phycocyanin pigments (Adjali *et al.*, 2022).

Figure 1. Spirulina extracts.

Phytochemicals Composition

Phytochemical screening aims to qualitative identifyication for secondary metabolites such as alkaloids, flavonoids, saponins, phenols, and steroids in natural materials. Table 1 presents the screening results for the spirulina extract used in this study. Both fresh materials and extract tested negative for alkaloids, while flavonoids, saponins, phenols, and steroids were positively identified. According to Firdiyani *et al.* (2015) alkaloids are commonly found in higher plants and are therefore absent in spirulina which belongs to lower plant groups. Nevertheless, the succesful identification of other secondary metabolites confirms the presence of bioactive compounds in *A. platensis* spirulina.

Table 1. Phytochemicals screening results.

Compounds	Sample test	
	Fresh materials	Extracts
Alkaloid	n.a.	n.a.
Flavonoid	+	++
Saponin	+++	+++
Phenolic	++	+++
Steroid	++	+++

Note: "n.a." mean not appeared and "+" mean the intensity of color change appeared.

The screening results showed an increase in intensity from fresh materials to the extract. Flavonoids in the fresh materials exhibited a pale yellow color (+), while other phytochemicals, including saponins, steroids, and phenols, displayed stronger color intensifies (++). This phenomenon occurs due to changes in concentration and compound conditions, as explained by Handoyo (2020). Additionally, Notonegoro *et al.* (2018) stated that variations in extraction methods applied to fresh materials can influence the detected phytochemical content. The choice of solvent also play critical role, as polarity differences determine the solubility and recovery of specific phytochemicals. According to Agustini *et al.* (2015), polar solvents such as ethanol are more effective in extracting phenolics and flavonoids, while less polar solvents may favor terpenoids or steroids.

Antioxidant Activity

Figure 2 illustrates an increasing inhibition trend with rising sample concentrations for both vitamin C and spirulina extract. However, the antioxidant activity of spirulina extract was classified as very weak, with an IC_{50} value of 784.914±0.299 ppm. These findings confirm that the spirulina extract produced in this study could only inhibit 50% of DPPH free radicals at concentrations exceeding 700 ppm (Husnani, 2024). In contrast, vitamin C, a naturally abundant antioxidant, exhibited a significantly stronger antioxidant

capacity, with an IC_{50} value of 6.019±4.311 ppm, placing it in the category of very strong antioxidant activity.

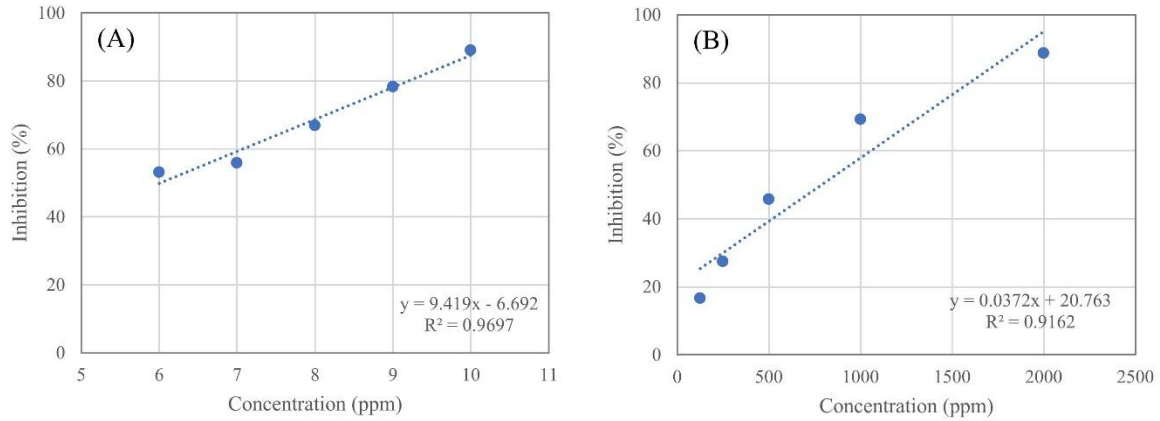

Figure 2. Inhibition plot of (A) vitamin C and (B) spirulina extracts for determined antioxidant activity.

According to AlFadhly *et al.* (2022), the antioxidant activity of spirulina can be influenced by cultivation environmental factors such as light exposure, pH, temperature, nutrients, and culture media type. This was demonstrated by Sirait *et al.* (2019), who compared spirulina cultivated in Walne and organic media, resulting in IC_{50} values of 36.23 ppm and 117.78 ppm, respectively. Additionally, Marjanović *et al.* (2024) reported that spirulina cultivated in fertilizer-based media exhibited stronger antioxidant activity than commercially available spirulina. Another factor affecting antioxidant performance is the presence of phenolic and flavonoid compounds, which act through different mechanism. Phenolics generally react more slowly with DPPH free radicals, requiring approximately 1–6 h to reach a stable equilibrium (Gulcin, 2020), while flavonoids contribute by donating hydrogen atoms or electrons and by chelating transition, thereby enhancing radical scavenging activity. The effectiveness of these compounds is also influenced by solvent extraction, since solvent polarity determines the recovery and concentration of phenolics and flavonoids, which in turn affects the measured antioxidant capacity.

SPF Value

The SPF value increased proportionally with the concentration of spirulina extract, as presented in Table 2. At a concentrationof 5000 ppm, the extract achieved an SPF value of 20,378±0.084, categorizing it as providing ultra protection. Widyawati *et al.* (2019) stated that higher SPF values indicate greater effectiveness as a UV protector. Additionally, the lowest tested concentration (1000 ppm) met the minimum SPF standard of 4, as required by SNI 16-4399-1996, for application as a sunscreen additive. These findings suggest that spirulina extract has strong potential for further application in cosmetic formulations, particularly in photoprotective and sunscreen products.

The sunscreen activity of spirulina extract is attributed to its secondary metabolites, particularly phenolic compounds. One of the key phenolics present in spirulina extract is flavonoids, which serve as photoprotectors through three main mechanisms according to Nunes *et al.* (2018) were: (a) UV absorption, (b) antioxidant activity; and (c) modulation of DNA signaling pathways. Dewantoro *et al.* (2022) further explained that flavonoids can absorb UV-A and UV-B radiation due to the presence of chromophore groups or conjugated aromatic systems in their benzene structure, functioning similarly to chemical sunscreen agents. When exposed to UV radiation, these chromophore groups undergo electron transfer resonance, thereby reducing UV intensity on the skin (Lisnawati *et al.*, 2019).

Table 2. Sunscreen activities of spirulina extracts based on measured SPF value.

Consentration	SPF value	Protection Category
1000 ppm	4.150±0.089	Minimum
2000 ppm	9.612±0.075	Maximum
3000 ppm	13.016±0.054	Maximum
4000 ppm	16.672±0.155	Maximum
5000 ppm	20.378±0.084	Ultra

Conclusion

This study examined the bioactive composition, antioxidant capacity, and sun protection factor (SPF) of spirulina (*Arthrospira platensis*) extracts. The results showed that spirulina extract contain flavonoid, saponin, phenol, and steroid with extraction yield was 35.345±0.050%, with a residual solvent content of 0.586±0.059%. The antioxidant activity, expressed as IC_{50}, was 784.914±0.299 ppm, indicating very weak free radical scavenging. SPF analysis revealed that at a concentration of 5000 ppm, spirulina extract achieved an SPF value of 20.378±0.084, classifying it under the ultra-protection category. These findings suggest that spirulina extract has potential applications in cosmetic and pharmaceutical formulations, particularly for sun protection. However, further research is needed to refine extraction parameters, evaluate formulation stability, and assess in vivo photoprotective efficacy to support its commercial application.

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