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THE EFFECT OF EXTRACTION METHODS ON PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY, AND COMPOUND IDENTIFICATION OF *Spirulina platensis*

Aatikah Dewi Ghaisani, Iriani Setyaningsih, Safrina Dyah Hardiningtyas*

Department of Aquatic Product Technology, Faculty of Fisheries and Marine Sciences, IPB University
Lingkar Akademik st Dramaga, Bogor Indonesia 16680

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*Correspondence: safrina_dyah@apps.ipb.ac.id

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Abstract

Spirulina platensis is a spiral-shaped filamentous microalga classified as a cyanobacterium, known for its diverse bioactive properties, including antioxidant, antimalarial, antidiabetic, anticancer, and antibacterial activities. This study aimed to determine the best extraction method based on the total phenolic content and antioxidant activity of *S. platensis* and identify antioxidant-related phytochemicals. The biomass of *S. platensis* was obtained after 14 days of cultivation in the laboratory. Two extraction methods were evaluated: maceration and ultrasound-assisted extraction (UAE), using ethanol as the solvent at a 1:20 (b/v) ratio. The results showed that the UAE method produced significantly higher extract yield, total phenolic content, and antioxidant activity (IC_{50}) than maceration ($p<0.05$). The values obtained using UAE were 14.70±1.97%, 128.657±2.67 mg GAE/g, and 148.652±7.78 ppm, respectively. Thin-layer chromatography (TLC) revealed six spots in the extracts. Two of these, with Rf values of 0.78 and 0.98 (exhibited antioxidant activity, as indicated by the yellow color development). Further phytochemical analysis using $FeCl_3$ confirmed that the spots contained polyphenols. In conclusion, UAE is an effective method for extracting phenolic compounds from *S. platensis*, enhancing both the phenolic yield and antioxidant activity. These findings support the potential use of *S. platensis* extracts as natural antioxidants in functional foods and pharmaceutical applications.

Keywords: bioactivity, maceration, microalgae, ultrasound-assisted extraction

Pengaruh Metode Ekstraksi Terhadap Kandungan Total Fenol, Aktivitas Antioksidan dan Identifikasi Senyawa pada *Spirulina platensis*

Abstrak

Spirulina platensis adalah mikroalga berbentuk spiral dan berfilamen yang diklasifikasikan sebagai sianobakteri, dikenal karena beragam sifat bioaktifnya, termasuk aktivitas antioksidan, antimalaria, antidiabetes, antikanker, dan antibakteri. Penelitian ini bertujuan untuk menentukan metode ekstraksi terbaik berdasarkan kandungan total fenolik dan aktivitas antioksidan *S. platensis*, serta mengidentifikasi fitokimia yang berkaitan dengan aktivitas antioksidan. Biomassa *S. platensis* diperoleh setelah 14 hari kultivasi. Metode ekstraksi yang dievaluasi adalah maserasi dan ekstraksi berbantuan ultrasonik (*ultrasound-assisted extraction*/UAE), menggunakan etanol sebagai pelarut dalam rasio 1:20 (b/v). Hasil menunjukkan bahwa metode UAE menghasilkan rendemen ekstrak, kandungan total fenolik, dan aktivitas antioksidan (IC_{50}) yang secara signifikan lebih tinggi dibandingkan metode maserasi ($p<0,05$). Nilai yang diperoleh dengan metode UAE berturut-turut adalah 14,70±1,97%, 128,657±2,67 mg GAE/g, dan 148,652±7,78 ppm. Kromatografi lapis tipis (KLT) menunjukkan enam bercak pada ekstrak, dan dua di antaranya memiliki nilai Rf masing-masing 0,78 dan 0,98 (menunjukkan aktivitas antioksidan yang ditandai dengan munculnya

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warna kuning). Analisis fitokimia lebih lanjut menggunakan $FeCl_3$ mengonfirmasi bahwa bercak tersebut mengandung senyawa polifenol. Metode UAE efektif dalam mengekstraksi senyawa fenolik dari *S. platensis*, serta meningkatkan hasil fenolik dan aktivitas antioksidan. Temuan ini mendukung potensi penggunaan ekstrak *S. platensis* sebagai antioksidan alami untuk aplikasi pangan fungsional dan farmasi.

Kata kunci: bioaktivitas, maserasi, mikroalga, *ultrasound-assisted extraction*

INTRODUCTION

Spirulina platensis is a microalga approved by the Food and Drug Administration (FDA) as a functional food ingredient and is listed in the International Nomenclature of Cosmetic Ingredients (INCI). This microalga belongs to the *Cyanobacteria* group, is microscopic in size and blue-green in color, and possesses a multicellular, filamentous, helical (spiral) structure with a length of 200–300 µm and a width of 5–10 µm (Benelhadj *et al*., 2016). In addition, *S. platensis* is relatively easy to cultivate. The chemical composition of *S. platensis* includes proteins (55–70 %), fats (6–6.5%), carbohydrates (17–25%), and various other bioactive compounds (Christwardana *et al*., 2013). Rahim *et al*. (2021) reported that the dry weight of *S. platensis* contains 1.69 mg/g carotenoids, 7.44 mg/g chlorophyll-a, 6.41 mg/g chlorophyll-b, 18.25% phycocyanin, 5.34% allophycocyanin, and 3.47% phycoerythrin, with a total phycobiliprotein content of 27.06%. These compounds have the potential to act as free radical-scavenging agents (Gad *et al*., 2011).

In general, the extraction of active compounds from *S. platensis* can be carried out using either polar or nonpolar solvents through various techniques, such as maceration, Soxhlet extraction, reflux, supercritical CO_2 extraction, and Ultrasound-Assisted Extraction (UAE). Ethanol is one of the most widely used polar solvents because of its effectiveness in dissolving bioactive compounds and its relatively low boiling point (78°C), which helps minimize the degradation of active compounds. However, the maceration method has several limitations, including long extraction times and low yields. Therefore, UAE is a promising alternative as it can shorten the extraction time and improve the extract yield. Purdi *et al*. (2023) showed that protein extract from *S. platensis* using UAE (at 4°C for 30 min with a 60% cycle) resulted in a yield of 76.83%, whereas maceration yielded only 32.48%. Sudirman *et al*. (2024) showed that polysaccharide extract from *Pistia stratiotes* using the UAE method resulted in a yield of 9.16 ± 2.14%, while maceration produced only 4.41 ± 0.36%.

Several studies have reported the total phenolic content and antioxidant activity of extracts. Safithri *et al*. (2020) demonstrated that maceration of *S. platensis* yielded a total phenolic content of 21.93±1.79 mg GAE/g and an IC50 value of 324.92±4.06 ppm. Martins *et al*. (2023) showed that ultrasound-assisted extraction (UAE) of *S. platensis* (at 60°C for 20 min with a solvent-to-biomass ratio of 70 mL/mg) using a Natural Deep Eutectic Solvent (NADES) resulted in a total phenolic content of 36.50±0.98 mg GAE/g. A study conducted by Pyne *et al*. (2020) reported that ultrasound-assisted extraction (UAE) of *S. platensis* resulted in a yield of 30.89 mg GAE/g. However, comparative studies on extraction methods aimed at maximizing phenolic compound yield and antioxidant activity have not been conducted yet. Therefore, this study was conducted to compare both extraction methods to determine the most efficient and optimal technique for improving the extract yield while preserving the desired phenolic compounds.

Spirulina platensis also exhibits significant biological activity with potential applications in cosmetics and pharmaceuticals. Hardiningtyas *et al*. (2022) reported that the ethanol extract of *S. platensis* contains bioactive compounds such as saponins, tannins, phenols, and steroids. Additional biological activities of the crude extract of *S. platensis* include antioxidant activity, with an IC_{50} value of 65.89 ppm (Firdiyani *et al*., 2015), antimalarial activity, with an IC_{50} value of 19.11 µg/mL (Wulandari *et al*., 2016), and anticancer activity against MCF-7 breast cancer cells, with an IC_{50} value of 36.23 ppm

(Sirait *et al*., 2019). Furthermore, the extract exhibited potential anticancer activity against epithelial cells, with an inhibition percentage of 32.5% at a concentration of 25 mg/mL (Fayyad *et al*., 2019) and demonstrated antibacterial properties (Setyaningsih *et al*., 2020). However, the specific compounds responsible for these inhibitory activities have not yet been identified, indicating the need for compound separation. Thin-layer chromatography (TLC) is one method for identifying active compounds. TLC separates chemical compounds based on their migration rate or distribution ratio between stationary and mobile phases (Hancu *et al*., 2011). Wulandari *et al*. (2016) demonstrated that the TLC separation of *S. platensis* successfully isolated alkaloid compounds in fraction 08 with an Rf value of 0.78, which exhibited antimalarial activity against *Plasmodium falciparum*. Based on these findings, the separation of active compounds in *S. platensis* is necessary to optimize its application as an antioxidant agent in the nutraceutical, pharmaceutical, and cosmeceutical fields. This study aimed to determine the best extraction method based on the total phenolic content and antioxidant activity of *S. platensis* and identify antioxidant-related phytochemicals.

MATERIAL AND METHOD

Cultivation of *Spirulina platensis*

Spirulina platensis was cultivated at Aquatic Product Biotechnology Laboratory 2, Department of Aquatic Product Technology, Faculty of Fisheries and Marine Sciences, IPB University using Walne medium (technical grade), seawater with a salinity of 15 ppt, a light intensity of 3,000 lx (40 W), and continuous aeration for 24 h. Cultivation was performed at an inoculum ratio of 20% of the total volume of the culture. *S. platensis* growth was monitored daily by measuring optical density (OD) using a UV-Vis spectrophotometer (Genesys, USA) at λ 670 nm until the OD value reached ≥ 0.5 (Afriani *et al*., 2018). Harvesting was performed after 14 days of cultivation using a 400 mesh nylon sieve. The collected wet biomass was then dried using a chiller (Sharp, Japan) at 5°C for 48 h.

Extraction Biomass *Spirulina platensis*

The dried biomass of *S. platensis* was extracted using two methods. For maceration, the biomass was soaked in 96% ethanol (Merck, Germany) (1:20 w/v) for 3×24 h at room temperature, following the method described by Setyaningsih *et al*. (2020). For *Ultrasound-Assisted Extraction* (UAE), a modified method from Purdi *et al*. (2023) was used, with a probe sonicator (Branson, USA) at 26 kHz and 60% cycle for 20 min in 96% ethanol (Merck, Germany) (1:20 w/v) at room temperature. In both methods, the resulting filtrates were concentrated using a vacuum evaporator (Buchi, Switzerland) at 40°C to obtain a crude extract. The crude extracts were weighed to determine the extraction yield and subjected to phytochemical screening (Harborne 1987), total phenolic content analysis, antioxidant activity assay, and thin-layer chromatography (TLC)-based identification of antioxidant-active spots.

Total Phenolic Content Analysis

The total phenolic content of the *Spirulina platensis* extract was analyzed according to the method described by Chatatikun *et al*. (2013). The extract was dissolved in distilled water (aquades) to a concentration of 10,000 ppm. A 50 µL volume of the extract was mixed with 50 µL of distilled water. Subsequently, 50 µL of 10% Folin's reagent (Merck, Germany) and 50 µL of sodium bicarbonate solution (60 g/L) were added to the mixture. Microplates (LabSelect, Canada) were incubated at room temperature for 60 min. The absorbance was measured at 750 nm using a spectrophotometer (Genesys, USA). A gallic acid standard curve was constructed by dissolving gallic acid (Merck, Germany) in distilled water at defined concentrations. Both the standards and blanks were treated in the same manner as the samples. The total phenolic content was calibrated against a gallic acid standard and expressed as mg gallic acid equivalent (GAE) per gram dry weight (g^{-1}).

$$\text{Total phenolic contents} = \frac{\text{y-b}}{\text{A}} \times \frac{\text{Volume extract (mL)}}{\text{Sample weight (mg)}}$$

y: slope

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b: intercept
A: absorbance value

Antioxidant Activity

Antioxidant analysis was performed using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA) method, based on Molyneux (2004). This analysis was conducted to determine the potential antioxidant activity of the substances in reducing free radicals. The antioxidant activity test began with the preparation of a stock solution of *S. platensis* extract by dissolving 0.02 g of the sample in 20 mL absolute ethanol. The solutions were then prepared at concentrations of 25, 50, 100, 200 ppm, and 400 ppm. A total of 2 mL of the test sample solution from each concentration was mixed with 0.5 mL of 0.4 mM DPPH solution and homogenized. A blank was prepared by mixing 0.5 mL of DPPH solution with 2 mL of absolute ethanol in a test tube. The homogenized solution was then incubated in a dark room at 37°C for 30 min. Absorbance was measured at a maximum wavelength of 517 nm using a spectrophotometer (Genesys, USA), and the percentage of inhibition was calculated using the following formula:

$$\text{Antioxidant Activity} = \frac{\text{Blank abs} - \text{Sample abs}}{\text{Blank abs}} \times 100\%$$

Antioxidant Bioautography Assay

Antioxidant activity was evaluated using a TLC-based bioautography assay (Merck, Germany) based on the method described by Samirana *et al.* (2018). A total of 20 µL of the extract was photographed on a silica gel 60 GF_{254} plate and developed using dichloromethane (Merck, Germany): chloroform (Merck, Germany) (3:1) as the mobile phase. After drying, the plate was sprayed with a 0.4 mM DPPH solution and observed for ~10 min at room temperature. Yellowish-white spots on a purple background indicate antioxidant activity. The Rf values of the active spots were calculated as follows:

$$\text{Rf} = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the eluent}}$$

TLC-Based Phytochemical Analysis

Phytochemical profiling of antioxidant-active extracts was performed using TLC (Merck, Germany) based on the method described by Samirana *et al.* (2018). The extract was dissolved in its original solvent and applied to a silica gel 60 GF_{254} plate using capillary tubes. The plate was developed in a pre-saturated chamber using a dichloromethane:chloroform (3:1) mixture as the mobile phase. After elution and drying, the spots were visualized under UV light at 254 and 366 nm and sprayed with $FeCl_3$ reagent to detect phenolic compounds. The Rf values were recorded using the same formula as above.

Data Analysis

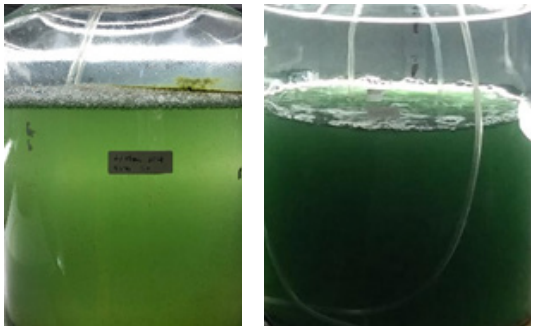
All tests were performed in triplicate to ensure the accuracy and reliability of the experimental results. The data were subjected to statistical analysis using the SPSS software. All data were initially checked for variance, normality, and homogeneity. To compare the mean values across the experimental groups, an independent sample t-test with Duncan's multiple range test post-hoc was used. *Statistical* significance was set at $p<0.05$.

RESULTS AND DISCUSSION

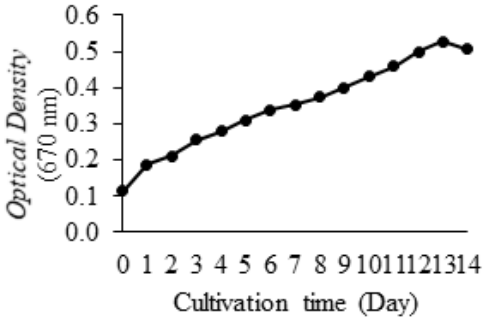
Growth and Biomass of *Spirulina platensis*

The growth of *Spirulina platensis* was carried out over a 14-day period and was directly observed through the change in color intensity from light green to deep green during the cultivation period, as shown in Figure 1a. The cell density during cultivation was measured based on optical density (OD) values using a spectrophotometer at a wavelength of 670 nm. The cell density of *S. platensis* during cultivation is depicted in Figure 1b.

As shown in Figure 1a, a color change from light green to dark green was observed, indicating an increase in cell density. This observation is consistent with the increase in OD values shown in Figure 1b. At the beginning of cultivation, the OD value was 0.11, which increased to 0.5 at the time of biomass harvesting. The growth of *S. platensis*



(Day-0) (Day-14))



(A) (B)

Figure 1 (A) Cultivation of *S. platensis*; (B) Optical density value of *S. platensis*

can be influenced by various factors, such as salinity, temperature, light intensity, aeration, pH, and the availability of nutrients or type of cultivation medium (Jung *et al*., 2019). *Spirulina platensis* was harvested on day 14, and the wet biomass yield reached 0.77 ± 0.18 g/L. After drying the biomass using a chiller at 5–8°C for 48 h, the dry biomass yield was 0.12 ± 0.03 g/L with a water content of 7.51 ± 0.15%

Sirait *et al.* (2019) reported that *Spirulina platensis* cultivated using Walne medium yielded a dry biomass of 0.33 ± 0.02 g/L, whereas cultivation with an organic medium resulted in a lower yield of 0.17 ± 0.01 g/L. Similarly, Notonegoro *et al.* (2020) demonstrated a dry biomass yield of 0.34 ± 0.049 g/L using Walne medium modified with 80 g of $NaNO_3$. The variation in dry biomass yield may be attributed to differences in the nitrogen sources provided by the cultivation media. The choice of growth medium plays a critical role in the proliferation of *S. platensis*, with Walne medium containing $NaNO_3$ presumed to be the key factor in promoting cell division. Nitrogen is essential for amino acid synthesis, which forms the primary protein constituent of *S. platensis* cells (Wibowo *et al*., 2024).

Yield Extract of *Spirulina platensis*

An appropriate extraction process yields both high extract recovery and strong biological activity. This outcome was influenced by the solvent type, temperature, and extraction duration (Tavakoli *et al*., 2021). In the present study, *S. platensis* was extracted using ethanol as the solvent. According to Wang *et al.* (2010), ethanol exhibits excellent capability to penetrate cell membranes and extract intracellular compounds because of its semi-polar nature. Ethanol is also more selective in dissolving and extracting phenolic compounds than other solvents because phenolic compounds contain polar hydroxyl (-OH) groups and slightly nonpolar aromatic rings. Furthermore, ethanol was chosen because it is classified as a Generally Recognized as Safe (GRAS) solvent (Rodrigues *et al*., 2015). The extraction methods applied in this study included *ultrasound-assisted extraction* (sonication) for 20 min at a frequency of 26 kHz and maceration for three days. The yield of S. platensis extract is shown in Figure 2.

Based on the results shown in Figure 2, there was a significant difference ($p<0.05$) in the extract yield between the sonication and maceration extraction methods, with values of 14.70±1.97% and 7.93±0.47%, respectively. The physical characteristics of the extracts were dark green and paste-like. These findings are consistent with those of Sudirman *et al.* (2024), who reported that ultrasonic-assisted extraction of *Pistia stratiotes* resulted in a yield of 9.16±2.14%, compared to 4.41±0.36% using maceration. Another study by Yucetepe *et al.* (2018) demonstrated that the ultrasound-assisted extraction of *S. platensis* protein achieved a yield of 29.05%. This can be attributed to the disruption of the cell walls caused by the ultrasonic cavitation generated

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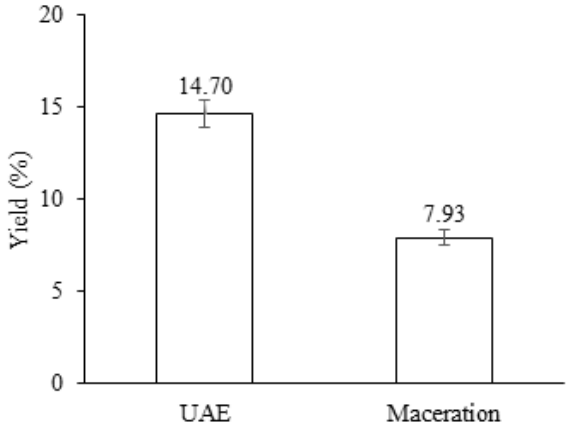

Figure 2 Yield extract of *S. platensis*

by wave propagation (Lu *et al*., 2013). Microbubbles produced during cavitation disrupt microalgal cell walls, facilitating solvent penetration into intracellular extract components.

Ultrasound-assisted extraction offers advantages such as shorter extraction times, lower extraction temperatures, greater selectivity for target compounds, and reduced undesirable reactions (Cikoš *et al*., 2018). Several factors influence the ultrasonic extraction efficiency, including the amplitude, frequency, and duty cycle of the equipment (Kumar *et al*., 2021). Kohestani *et al*. (2023) also reported the amount of ethanol solvent and the extraction method significantly affect the active compound content in *S. platensis* extracts. A higher extraction yield indicates a higher presence of active compounds in the extract (Hasnaeni, 2019).

Phytochemical Compounds of *Spirulina platensis*

Phytochemical analysis is a preliminary test conducted to identify the classes of secondary metabolite compounds present in *S. platensis* extracts. The results of the phytochemical analysis of *S. platensis* extract are presented in Table 1.

As shown in Table 1, *the S. platensis* extracts obtained using the two different extraction methods contained alkaloids, phenols, and steroids. These findings are consistent with those of previous studies reporting that the ethanol extracts of *S. platensis* contain phenolic compounds (Fithriani *et al*., 2015), alkaloids, and steroids (Mane & Chakraborty, 2019). Compounds such as alkaloids, phenols, and steroids are known to exhibit free radical-scavenging activity (Ali *et al*., 2014) and are presumed to possess antibacterial properties. The mechanism of free radical inhibition by the active compounds in the extract involves hydrogen atom transfer, single-electron transfer, and sequential proton loss electron transfer.

Alkaloid compounds were considered positive when a white to yellowish precipitate formed with Mayer's reagent due to the interaction with tetraiodomercurate (II) ions, and an orange to brown precipitate with Dragendorff's reagent due to the interaction with tetraiodobismuthate (III) ions (Sangi *et al*., 2013). Steroid compounds were identified by a color change to green, as reported by Agustini *et al*. (2015). This color change occurs because of the oxidation of triterpenoid/steroid compounds through the formation of conjugated double bonds (Sulistyarini *et al*., 2016). The presence of hydroquinone-type phenolic compounds was indicated by the formation of a green color. This color change occurs upon the addition of $FeCl_3$ due to the presence of hydroxyl groups (Sangi *et al*., 2013; Artini *et al*., 2013). Phenolic compounds range from simple phenolics (phenolic acids) to complex, high-molecular-weight polymers (tannins). Biosynthetically, phenolics are derived from the shikimate/phenylpropanoid or acetate/malonate (polyketide) pathways, the convergence of which leads to the formation of flavonoid compounds (Lin *et al*., 2016).

Table 1 Phytochemical compounds in *Spirulina platensis* extract

Active compound	*S. platensis* crude extract		Description
	Ultrasound-assisted extraction	Maceration	
Alkaloid			
Dragendorff	+	+	Orange presipitate
Wagner	+	+	Brown presipitate
Meyer	-	-	-
Phenol hydroquinone	+	+	Green
Flavonoid	-	-	-
Tannin	-	-	-
Saponin	-	-	-
Steroid	+	+	Green
Triterpenoid	-	-	-

(-) not detected; (+) detected

Total Phenolic Content of *Spirulina platensis*

The total phenolic content was determined using Folin–Ciocalteu reagent. Phenols contain one or more aromatic rings with one or more hydroxyl groups, which play a role in prevent oxidative processes and possess the ability to scavenge free radicals (Singla *et al.,* 2019). Phenolic compounds can function as antioxidants, antiaging, anti-inflammatory, and antibacterial agents (Bouarab-Chibane *et al*., 2019; Tungmunnithum *et al*., 2018).

Based on the results shown in Figure 3, the t-test showed a significant difference (p<0.05) in total phenolic content between the sonication and maceration extraction methods, with values of 128.657 ± 2.67 mg GAE/g and 60.981 ± 4.82 mg GAE/g, respectively. The total phenolic content obtained in this study was higher than that of the aqueous extracts of *S. platensis*, which reported values of 26.64 ± 0.16 mg GAE/g (Hidayati *et al*., 2020). Kumar *et al.* (2022) reported that *S. platensis* extract using aqueous yielded 9.919 ± 0.449 mg GAE/g, and ethanol 3.476 ± 0.362 mg GAE/g. The parameters contributing to the differences in the total phenolic content in *S. platensis* are influenced by factors such as the type of solvent, temperature, duration, and extraction method used (Farahani 2021).

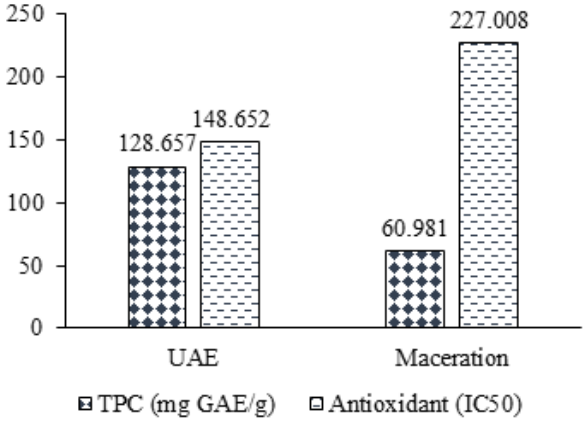

Figure 3 Total phenolic content ( ) and antioxidant activity ( ) of *Spirulina platensis*

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Martins *et al.* (2023), *S. platensis* extracted using UAE for 20 min at 60°C was considered optimal, yielding the highest total phenolic content (36.50±0.98 mg GAE/g). The use of UAE extraction temperatures above 30 min can cause degradation of heat-sensitive compounds, such as polyphenols (Guitescu *et al. 2015*; Guo *et al*. 2013). Extraction of *S. platensis* using sonication can enhance the active compound content because ultrasonic waves generate pressure and cavitation, which disrupt the cell walls of bioactive compounds, such as phenols, and maximize the release of active components.

The phenolic compounds identified in *Spirulina* include catechin, epicatechin, pyrocatechol, pyrogallol, gallic acid, protocatechuic acid, and salicylic acid (Shalaby *et al.* 2013). According to Deng and Chow (2010), the total phenolic content of extracts correlates with antioxidant activity, which may act alone or synergistically. The ability to neutralize free radicals is associated with the hydroxyl groups present in the phenolic compounds (Mehdinezhad *et al*., 2016). These hydroxyl groups act as hydrogen atom donors and react with free radicals through an electron transfer mechanism, thereby reducing the reactive oxygen species that cause oxidative stress (Socrier *et al*., 2019). Phenolic compounds also possess antibacterial (Ullah *et al*., 2019), anti-inflammatory (Liu *et al*., 2018a, b), antidiabetic (Chen *et al*., 2019), and anticancer (Martini *et al*., 2018) properties.

Antioxidant activity of *Spirulina platensis*

The antioxidant activity of *S. platensis* was evaluated using the DPPH assay to determine its free radical scavenging ability. Antioxidants act as electron or hydrogen donors and radical scavengers, and are capable of inactivating the propagation of oxidative reactions by preventing the formation of free radicals (Abdel-Dalim *et al*., 2013). During the antioxidant assay, a color change from purple to yellow occurred, accompanied by a decrease in the absorbance values measured using a UV-VIS spectrophotometer at a wavelength of 517 nm.

Based on the results shown in Figure 3, there was a significant difference ($p<0.05$) in antioxidant activity between the sonication and maceration extraction methods, with IC_{50} values of 148.652 ± 7.78 ppm and 227.008 ± 7.84 ppm, respectively. The antioxidant activity obtained in this study was generally better than that in previous studies using methanolic extracts of *S. platensis* with maceration extraction, which yielded IC_{50} values of 323.70 ppm (Setyaningsih *et al*., 2013), 256.45±20.77 ppm (Wikantyasning *et al*., 2019), and 246.8 ppm (Fithria *et al*., 2022). The antioxidant assay results of the crude extract of *S. platensis* using sonication showed better free radical scavenging activity and fell into the moderate category, whereas the maceration method was classified as very weak activity. According to Mardawati *et al.* (2008), an antioxidant is considered very strong if its IC_{50} value is <50 ppm, strong if between 50 and 100 ppm, moderate if between 100 and 150 ppm, weak if between 150 and 200 ppm, and very weak if >200 ppm. A higher IC_{50} value indicates lower antioxidant activity, whereas a lower IC_{50} value indicates higher antioxidant activity (Sudirman *et al.,* 2022). The weak antioxidant activity observed in the *S. platensis* extract is presumed to be due to the use of crude extracts. Crude extracts of *S. platensis*, along with bioactive compounds such as flavonoids, carotenoids, and pigments, may act synergistically to enhance the antioxidant activity (Abd El-Hack *et al*., 2019; Abdel-Moneim *et al*., 2022).

Extraction of *S. platensis* using sonication can produce higher antioxidant activity, as ultrasonic waves generate pressure and cavitation that disrupt the cell walls of active compounds, thereby maximizing the release of these components. According to Hidayati *et al.* (2020), there is a correlation between the antioxidant activity and total phenolic content of *S. platensis* extracts. Phenolic compounds in *S. platensis* contain a benzene ring with at least one hydroxyl (-OH) group, enabling them to act as electron donors and inhibit free radical formation through radical scavenging mechanisms (Machu *et al*., 2015; Bortolini *et al*., 2022).

TLC Bioautography and Phytochemical Profiling of *Sprilina platensis*

Thin layer chromatography (TLC) is a method of separating mixtures of compounds into pure substances that is relatively simple, rapid, and commonly used to identify pharmaceutical substances. Component separation identification can be performed using color reagents, fluorescence, or radiation with ultraviolet (UV) light (Hadisoebroto & Budiman, 2019). Compounds were identified using various eluents has been conducted, and the best combination was found to be using an eluent of dichloromethane:chloroform (3:1). Based on the combination of eluents, spots appeared, which could be expressed with a Retardation Factor (*Rf*) value that varied, as shown in Figure 4A.

Based on Figure 4A, the eluent dichloromethane:chloroform (3:1) produced six fractions with Rf values of Rf1 (0.42), Rf2 (0.68), Rf3 (0.78), Rf4 (0.86), Rf5 (0.94), and Rf6 (0.98). However, in Figures 4B and 4C, only five Rf fractions were observed. Fractions Rf3, Rf5, and Rf6 were presumed to be polyphenolic compounds, as indicated by the appearance of black spots after spraying with $FeCl_3$, as shown in Figure 4D (Warsi and Sholichah, 2017). The dark spots are due to the reaction between polyphenols and $FeCl_3$. The hydroxyl groups on the phenol rings react with Fe^{3+} ions in $FeCl_3$ to form dark brown complexes (Sulasmi *et al.*, 2018). Bayani (2021) explained that the reaction formed after spraying produces a $Fe(O\text{-}Ph)_3$ complex (iron-phenolate), which appears black, dark green, or dark blue. Polyphenols are bioactive compounds with antioxidant properties. The polyphenol group includes tannins, phlorotannins, and flavonoids (Pawestri *et al.* 2021).

Figure 4E shows the antioxidant activity, indicated by four distinct spots corresponding to fractions that exhibited antioxidant activity, marked by the presence of white or yellow color. The intensity of the yellow or white color was proportional to the antioxidant activity in stabilizing the free radicals from the DPPH solution. Variations in color intensity were attributed to differences in antioxidant potency. The higher the antioxidant activity, the more the purple color of DPPH fades into a yellow hue (Prins *et al.* 2022). This result is consistent with the previous antioxidant activity test, in which the crude extract of *S. platensis* obtained via ultrasonication-assisted extraction (UAE) demonstrated an IC_{50} value of 148.652 ppm, which falls within the moderate-activity category.

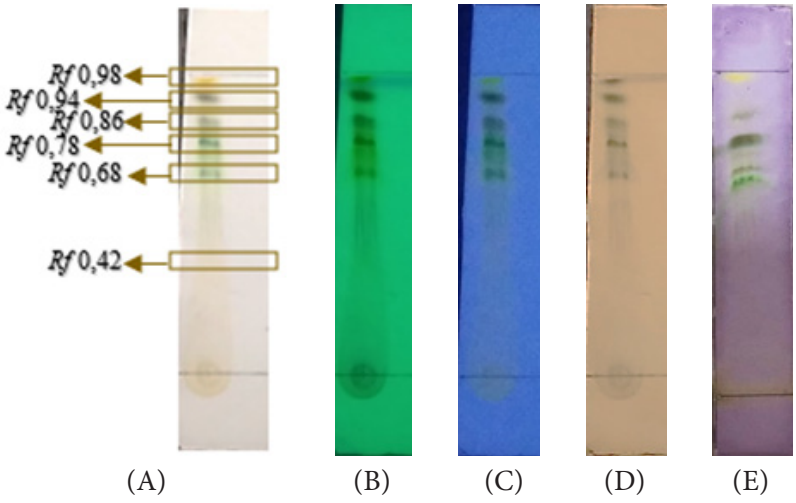

Figure 4 (A) Thin layer chromatography of crude extract of *Spirulina platensis*, (B) under UV light at 254 nm, (C) under UV light at 366 nm, (D) after spraying with $FeCl_3$, (E) after spraying with DPPH

http://dx.doi.org/10.17844/jphpi.v28i7.64276

CONCLUSION

The crude extract of *S. platensis* obtained using ultrasound-assisted extraction yielded the best results, as indicated by the values of extract yield, total phenolics, and antioxidant activity. Phytochemical testing revealed that the *S. platensis* extract contained steroids, phenolics, and alkaloids. Thin layer chromatography analysis showed the separation of compound components into 6 spots, and bioautography results revealed 2 positive spots as antioxidant agents, indicated by the presence of a yellow color change. UAE is an effective approach for extracting phenolic compounds from *S. platensis*.

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