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Effect of Different Solvent Concentrations on *Spirulina Platensis* Extract using Sonication Method as Antioxidant

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A B S T R A C T

Spirulina platensis was a species of cyanobacterium known as blue-green algae that contains bioactive compounds. Antioxidant content in Spirulina platensis has the potential to be utilized in health and food. Extracting antioxidants using sonication method enhances efficacy. Methanol and water solvents at varying concentrations effectively influence extract characteristics based on polarity principles. Thus, the present study has focused on evaluating different concentrations of methanol solvent using sonication method to obtain extracts of Spirulina platensis. The extracts were evaluated regarding yield, antioxidant activity and digital image processing. The result showed that Sample B had the best treatment that got an inhibition percentage of 68.088% which higher than control. The yield results of sample B was 8.99%, then got 39.33±8.23b for L* (Lightness), 4.92±4.24b for a* (redness), and 37.3±8.17b for b* (yellowness).

Keywords: *Spirulina platensis, Methanol, Antioxidant, Sonication*

1. Introduction

Spirulina platensis, a species of cyanobacterium known as blue-green algae (Ismaiel & Piercey-Normore, 2024). Although classified as a blue-green algae, *Spirulina* is dark green due to the presence of chlorophyll, whose pigments cover the bluish color of phycocyanin and the yellow color of carotenoids (Mallamaci et al., 2023). *Spirulina platensis* is commonly used in the health and food industries. The utilization is very wide as it is rich in nutrition and has potential benefits for health due to its high protein content and strong antioxidant content (Altyar et al., 2024).

Antioxidants are substances that have ability to delay, control, or prevent the oxidation process caused by free radicals, which can lead to food deterioration and the spread of degenerative diseases in the body (Shahidi & Zhong, 2015). Antioxidants exert their inhibitory through mechanism of transferring electrons to free radicals or accepting free radicals to become stable thus protecting cells from free radical damage (Andarina & Djauhari, 2017).

Antioxidant compounds in *Spirulina platensis* can be obtained through ultrasonic extraction, by using sonication method. Ultrasonic waves optimize the efficiency and rapidity of the extraction process of bioactive compounds, including antioxidants. The cavitation formed by ultrasonic waves will destroy the plant cell walls, which allows the compounds to be released into the solvent (Gallo et al., 2018).

The extraction procedure was carried out using methanol and water solvents with different concentrations. Both solvents are effective in extracting bioactive compounds, especially antioxidant activity and phenolic content. Research conducted by Riyadi et al., (2023) showed that extract of *Spirulina platensis* from methanol and water solvents contained 91.81% antioxidant activity of DPPH and positive for phenols. Water dissolves the antioxidants that produce phenolic compounds in *Spirulina platensis* extracts.

Various levels of solvent concentration are thought to produce different characteristic of extracts. The principle of extraction using solvents is like dissolves like, where the solvent only extracts compounds that have similar polarity. The same solvent with different concentrations also has different polarities (Widarta & Arnata, 2017). The more similar polarity of solvent with the polarity of compounds contained in the material, the more components of compounds that can be extracted.

This study aimed to determine content of *Spirulina platensis* extract with different concentrations of methanol solvent using yield, antioxidant activity of DPPH, and color analysis methods.

2. Materials and methods

2.1 Materials and tools

The materials used were *Spirulina platensis* powder from PT Alga Biotechnology Indonesia. The tools used in this study were sonicator (Branson 1800), centrifuge (Gemmy PLC-05), vacuum rotary evaporator (Buchi Rotavapor® R-100), UV/Vis Spectrophotometer (Hitachi U-2900), analytical balance (Ohaus Adventurer Pro), vortex (Corning LSE), micropipette (scilogex), blue tip and glassware.

2.2 Extraction

The extraction method carried out by Monteiro *et al*., (2020) with modifications of ratio solvent and using multiple extraction. 10 g *Spirulina platensis* powder dissolved into 100 ml solvent with a ratio 1:10 (b/v). Different solvent concentrations were used,

- A: (1) 50 ml methanol 96%, (2) 50 ml aquadest
- B: (1) 30 ml methanol 96% + 20 ml aquadest, (2) 50 ml methanol 96%
- Control: (1) 50 ml aquadest, (2) 50 ml aquadest

Sample with the first solvent was extracted using a sonicator with a frequency of 50 kHz for 15 minutes at room temperature, then centrifuged at 4000 rpm for 10 minutes. The supernatant was stored in a vial, while the precipitate (natan) was re-extracted using the second solvent. The collected extracts were concentrated using a vacuum rotary evaporator at 40°C and 327 mbar of pressure to remove the solvent.

2.3 Yield

The calculation of yield is performed by calculating the weight of *Spirulina platensis* extract obtained with the weight of the extracted sample. The following is the yield formula (Sijabat *et al.*, 2023):

$$\%Yield = \frac{Weight\ of\ extraction\ (g)}{Sample\ Weight\ (g)} x\ 100\%$$

2.4 Antioxidant Activity

Antioxidant activity testing on Spirulina platensis extract using the DPPH method refers to the research of Arifin *et al.* (2023) which has been modified. DPPH solution was prepared by dissolving 0.002 g of DPPH powder into 6 mL of methanol pro analysis. The sample solution was prepared by mixing 4.5 mL of sample extract and 0.5 mL of 1 mM DPPH, then vortexed for 30 seconds. The blank measurement used a mixture of 4.5 mL of methanol pro analysis and 0.5 mL of DPPH solution. The mixture was incubated for 30 minutes under closed and dark conditions at room temperature. The color change from purple to yellow indicates the efficiency of free radical scavenging. The absorbance was measured at a wavelength of 517 nm using a spectrophotometer. The percentage of inhibition was calculated by the formula:

$$\%Inhibition = \frac{(Blank\ absorbance - Sample\ absorbance)}{Blank\ absorbance} x\ 100\%$$

2.5 Digital Image Processing

Color testing or digital image processing on *Spirulina platensis* samples with the sonication extraction method was carried out using a MATLAB application based on Pramudya *et al*. (2020) with minor modifications. Existing data results were processed again using SPSS version 16. Samples that have been dried using an oven are placed in a studio box for image data collection. The studio box was designed to be opaque with a milky white color and waterproof material. Taking photos of the samples was done using a cellphone camera and using the help of 2 white 5 watt LED lights as a light source placed at the top of the studio box. Photos of samples that have been taken are then cropped so that the photos will only focus on samples with intense colors. Color analysis was carried out to determine the difference in color intensity with the L*a*b component.

2.6 Data Analysis

Data analysis of this study was carried out using statistical tests with the SPSS 16 application, including tests of normality, homogeneity, and ANOVA (Analysis of Variance). Completely Randomized Design was used for homogeneous treatment. The data obtained showed normal and homogeneous data distribution so that it was possible to further analyze using ANOVA.

3. Result and Discussions

3.1. Yield

The extract yield is calculated based on the ratio of the final weight (weight of extract produced) to the initial weight (weight of cell biomass used) multiplied by 100%. The sample used for the extraction process uses 5 grams of *Spirulina platensis*.

Table 1 - Yield Results

Sample	Yield (%)
Control	26.44 ± 3.05^{a}
A	7.89 ± 0.76^{b}
B	8.99 ± 3.10^{b}

Different superscript in the same column indicates significantly different ($p<0.05$)

From (table 1) it can be shown that there is a significant effect ($p<0.05$) on the use of different types of solutions on the yield of *Spirulina platensis* extract. Post-hoc test using LSD showed a significant difference between the use of different types of solvents on the yield of *Spirulina platensis* extract. *The* data in (table 1) shows that the control treatment has the highest percentage yield of 26.44%.

These results are due to differences in the ratio and type of solvent used in extracting *Spirulina platensis* microalgae. Solvents also has an effect on the high yield value, because the solvents used have different polarity properties, where distilled water has a higher level of polarity compared to methanol. The polarity of the solvent will increase along with the decrease in concentration when dissolved in water (Tan *et al*., 2013). The more similar the solvent's polarity to the polarity of the substance contained in the extracted material, the more components of the substance that can be extracted so that there can be an increase in the yield obtained (Asworo *et al*., 2022).

3.2. Antioxidant Activity

Different types and concentrations of solvents in *Spirulina platensis* extracts have a significant effect ($p<0.05$) on antioxidant activity according to the results of analysis of variance (ANOVA). Post-hoc test using LSD (Least Significance Different) showed significant differences between the treatment types and solvent concentrations of Spirulina platensis extract. The highest value was obtained from treatment B of *Spirulina platensis* extract with a mixture of methanol and water solvents with a final ratio of 80:20 which showed a percentage inhibition value of 68.088% (Table 2). The inhibition percentage was calculated by measuring the absorbance difference between the extract and DPPH. The presence of antioxidant compounds in an extract result in a change in DPPH color from purple to yellow brown. This process occurs because antioxidant compounds react with DPPH free radicals, which begins with antioxidant compounds giving protons or hydrogen to DPPH. As a result, new free radicals are formed that are more stable or less reactive, causing the color change. This is an indicator of antioxidant activity in the extract, where a faster color change indicates higher antioxidant activity (Melati, 2021). The content of spirulina platensis, namely carotenoids, phycocyanins, chlorophyll, phenolics, and vitamins C and E are ingredients to counteract free radicals so that they can be called the main antioxidant components (Nouri and Abbasi, 2018).

Table 2 - Antioxidant Activity

Extraction Method	Antioxidant Activity (%Inhibition)
Control	16.3590 ± 5.85217^{a}
A	$33.7643 \pm 26.35977^{ab}$
B	68.0880 ± 4.47046^{b}

Different superscript in the same column indicates significantly different ($p<0.05$)

The results of antioxidant activity analysis showed that treatment A got an inhibition percentage of 33.764% which was higher than the control treatment which was 16.359%. This shows that the use of type and concentration has a real effect because in treatments A and B using a mixture of methanol, while the control only uses water solvents. Microalgae antioxidant compounds have different polarities (Fithriani et al., 2015). The difference in polarity between methanol and distilled water (aquadest) affects the compounds dissolved in an extract. Alcohol solvents are solvents that have a wide scope to attract compounds during the extraction process. Water solvents are the most polar compounds compared to other solvents, so the ability to extract in the extraction process is limited to polar compounds (Rizki et al., 2022). Based on the polarity index, methanol and water have a high polarity index, where the polarity index value of methanol is 5.1 and water is 9, so methanol and water are more chosen for the extraction of medicinal plants containing metabolite compounds (Holil and Griana, 2020).

3.3. Digital Image Processing

Table 3 – L*a*b Results

Sample	L*	a*	b*
Control	28.52±4.51^{ab}	-1.91±0.90^{ab}	-6.45±2.42^{a}
A	14.04±5.82^{a}	-4.01±1.91^{a}	2.50±1.22^{a}
B	39.33±8.23^{a}	4.92±4.24^{b}	37.36±8.17^{b}

Different superscript in the same column indicates significantly different ($p<0.05$)

a b c

Fig. 1 - (a) control; (b) A; (c) B

a. Lightness (L*)

The L* or Lightness value shows the brightness level of an object. According to Widiastutik *et al*. (2018), this value ranges from 0 to 100, where the higher the L* value will indicate the brighter the color of a sample. brightness intensity of the sample after drying using an oven. The highest L* value in *Spirulina platensis* was found in sample B and the lowest L* value was in sample A. In the table above, it is shown that the ratio has a significantly different effect on the L* value of the sample. This is because water, one of the solvents used, is a polar solution. Phycocyanin, one of the substances contained in *Spirulina platensis*, dissolves well in polar solutions so that its L* value can be higher. Ariyanto *et al*. (2022) stated that the more Phycocyanin that dissolves in a polar solution, the higher the brightness of the sample. The brighter the color of a sample, the higher the L* value.

b. Redness (a*)

The a* value indicates the degree of red-green color in a sample. The a* value in the results shows a negative value (-). Negative values indicate that the green color in the sample dominates. According to Ariyanto *et al*. (2022), the more negative value indicates that the sample tends towards green, while the more positive value indicates that the sample tends towards red. The table above shows that the lowest a* value is found in sample A, which indicates that samples A tend to be green in color. Sample B tends to be red in color because the value is positive. Samples with code B are positive because the extraction process uses a vacuum rotary evaporator. The use of these tools using high temperatures will cause chlorophyll degradation.

c. Yellowness (b*)

The notation b* indicates a mixed chromatic color of blue as well as yellow. A positive b* value with a range of 0 to +70 indicates that the yellow color is more dominant in the sample, while a negative b* value with a range of 0 to -70 indicates that the blue color is more dominant in the sample. The b* value in samples A and B is positive, indicating a dominant yellow color, different from the control which is negative with a dominant blue color. According to Farihah *et al*. (2014), the phycobiliprotein group is a soluble polar compound that is taken up by water solvents which are also polar compounds. The use of water solvents in extracting is relatively safe and can attract active substances, but is sensitive to temperature and pH which results in color instability. The use of high temperatures during extraction can affect the color produced.

4. Conclusions

The results of this study showed that sample B had the best treatment. The results of antioxidant activity analysis showed that sample B got an inhibition percentage of 68.088% which was higher than the control treatment which was 16.359%. This shows that the use of type and concentration has a real effect because in treatments A and B using a mixture of methanol, while the control only uses water solvents. Sample B, which has best antioxidant activity, got 8.99% for yield results. Then, sample B got 39.33±8.23^{b} for L* (Lightness), 4.92±4.24^{b} for a* (redness), and 37.3±8.17^{b} for b* (yellowness).

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