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Potentially Antihyperglycemic from Biomass and Phycocyanin of *Spirulina fusiformis* Voronikhin by in Vivo Test

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**Abstract**

*Spirulina* is one of the microalgae containing nutrients used as functional food and in therapeutics (active compound). Bioactive compound in *Spirulina* has antihyperglycemic activity or antidiabetes activity. Diabetes mellitus is a major health problem in the world. The research about the potential of microalgae as antihyperglycemic is important to do. The cultivation of microalgae was conducted in laboratory using flask and aquarium completed with non-stop aeration. Measurement of the antihyperglycemic activity in vivo using mice that were fed with biomass containing *Spirulina fusiformis* Voronikhin and phycocyanin. Blood glucose levels were measured by methods of oral glucose tolerance test after a fasting period of 18 h. Oral administration of biomass and phycocyanin of *Spirulina fusiformis* V. at 0.15 mg · g\(^{-1}\) and 0.30 mg · g\(^{-1}\) proved to decrease the blood glucose level.

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**Keywords:** Antihyperglycemic; microalgae; phycocyanin; *Spirulina fusiformis* V.

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1. Introduction

*Spirulina* is one of the microalgae containing nutrients used as functional food and therapeutic (active compound), so that it is good for health. It also has potentiality as hypcholesterol and immunostimulant. *Spirulina* has therapeutic properties which include prevention and inhibition of cancer, ability to decrease blood cholesterol levels, to stimulate the immunological system, and to reduce nephrotoxicity of pharmaceutical and toxic metal. *Spirulina* is used as food supplement and it contains biopigment phycocyanin which has a potential as source of nutraceutical and pharmaceutical substances.

Blue green alga *Spirulina platensis* Voronikhin (*S. platensis*) with a dose of 150 mg · kg⁻¹ body weight can lower blood glucose levels up to 33 % of the control condition, thus, it can be used for diabetes mellitus (DM) therapy. DM is a metabolic disorder characterized by chronic hyperglycemia with disturbance of carbohydrate, protein or fat metabolism resulting from defects in insulin secretion, insulin action or both. Although several synthetic hypoglycemics are developed but a truly safe and effective treatment paradigm has not been developed yet.

Several studies have been carried out to find α-glucosidase inhibitors from natural products, including plant materials, as alternative hypoglycemic agents for diabetes. The methanol extract of *Euonymus alatus* inhibited α-glucosidase activity by 69.4 % up to at concentrations of 0.50 mg · mL⁻¹ in vitro.

The prevalence DM in Indonesia is ranked number four or around 8.6 % from the total population. Patients with type–2 DM require oral hypoglycemic drugs (OHO) since the diet does not control their blood glucose levels. Using OHO cause side–effect abnormal hypoglycemic. Traditional medicine have to be proved scientifically. *Spirulina* is a natural products that has potential to overcome hyperglycemic, but still needs more research. One of the natural pigment in *Spirulina* is phycocyanin that has antioxidant activity. Research on antihyperglycemic activity of phycocyanin is scarce, thus, further research is needed to determine the benefit of phycocyanin. The presence of phycocyanin in *Spirulina* depends on its growth and harvesting time. There are only few strains of cyanobacter that have ability to produce high amount of phycocyanin. Phycocyanin content in *Spirulina* can be improved by manipulating culture condition. The purposes of the study are to determine the best harvesting time of *S. fusiformis* with the highest phycocyanin level and to test antihyperglycemic activity of biomass and phycocyanin of *S. fusiformis* by in-vivo to evaluate its possible use as an anti-diabetic agent. This study was expected to serve as baseline information for development of *Spirulina fusiformis* and phycocyanin to become nutraceuticals and pharmaceuticals.

2. Materials and methods

Materials used in the study such as *Spirulina fusiformis* V., was obtained from Indonesia Institute of Science (LIPI) Cibinong – West Java, Zarrouk media, male rat aged 6 wk to 8 wk, standar rations feed, sucrose, glucobay tablet, mineral water and other chemical for analysis. The equipment that was used in this study include glass jars, aquarium, lux meter, aerator, oven, spectrophotometer, freezer, glucose strip test, centrifuse and others glass ware.

The study involved culturing of *S. fusiformis*, harvesting cell, extraction of phycocyanin, antihyperglicemic activity test in vivo.

2.1 Cultivation of *S. fusiformis* and extraction of phycocyanin

Cultivation of *S. fusiformis* was conducted in Zarrouk medium at 28 °C to 30 °C, pH 9 to pH 10, providing the lighting 5 500 lux (1 lux = 1 lm · m⁻²) using a tube lamp Phillip 40 W with non–stop aeration. Sampling of the culture was conducted every day for determining of the growth curve based on the optical density (OD), which was measured at 480 nm using spectrophotometer. The scaling up to 100 L was conducted in aquarium with the same condition as in semi-batch culture. A 10 % to 20 % of *S. fusiformis* inoculant was added to media culture.

The culture was harvested on the initial and at the end of the logarithmic phase, and at the initial and the end of the stationary phase for obtaining the biomass. Separation of biomass was done using nylon filter with 20 μm size. The nylon filter was put onto the cover of glass, then the culture was poured. Biomass left in the nylon filter was rinsed using freshwater and dried by using a fan in a room temperature for 5 h. The biomass obtained was weighted for the next analysis.
Extraction method of phycocyanin was conducted by using freeze thawing method. Biomass of *S. fusiformis* was extracted with distilled water. The biomass was suspended to the distilled water, then stirred using magnetic stirrer and freezeed using a *National* freezer for 48 h. The sample was thawed and centrifuged in a refrigerated centrifuge (4 °C) to obtain crude phycocyanin as supernatant. Then phycocyanin was dried by freeze dryer (−77 °C). The phycocyanin will be used for next analysis.

2.2 Antihyperglycemic activity test by in vivo

An antihyperglycemic activity test was conducted by oral glucose tolerance test (OGTT) to the animal. Males of *Sparague–Dawley* rats weighing between 180 g to 200 g were purchased at the Veterinary Institute, Bogor. The rats were housed individually in plastic cages and placed in a room which the temperature of 26 °C to 28 °C. The animals were given feed *ad libitum* with a standard rations during the test. The cages were cleaned every 3 d. In this study, 30 animals test were divided to six groups including rats which were given acarbose 0.001 mg · g⁻¹ body-weight (P), rats that were given dried biomass of *S. fusiformis* 0.15 mg · g⁻¹ (B1), rats that were given dried biomass of *S. fusiformis* 0.30 mg · g⁻¹ (B2), rats that were given phycocyanin 0.15 mg · g⁻¹ (F1), rats that were given phycocyanin 0.30 mg · g⁻¹ (F2), and rats that were not given dried biomass and phycocyanin of *S. fusiformis* (N). Each sample was dissolved in 2 mL mineral water.

Before test, rats fasted for 18 h then they were taken the blood to measure the fasting blood glucose level. Acarbose, dried biomass and phycocyanin of *S. fusiformis* were administered orally to the rats, except for the N group. After 5 h, the rats were administered 1 mL sucrose 80 % (w/ v), and blood glucose level was measured after 0 h, 0.5 h, 1 h, and 2 h, by using a glucose meter (*One Touch Ultra*).

3. Result and discussion

3.1 Growth of *S. fusiformis*

*S. fusiformis* which was cultivated in Zarrouk media had five growth phases including lag phase reached day 0 to day 7 culture, logarithmic phase reached on day 8 to day 31 of culture, declined phase reached on day 32 to day 34 of culture, stationary phase reached on day 35 to day 75 of culture, and death phase starting from day 76 of culture (Figure 1). On the lag phase, microalgae cell still adapted to the environment. However, on the logarithmic phase, it grew fastly and produced biochemical compounds. Decrease in the cell density was due to the death cell and lysis. The lysis occurred due to the difference in the osmotic pressure between the cell and environment.

![Fig. 1. Growth curve of *S. fusiformis* in Zarrouk medium](image-url)
3.2 Biomass and phycocyanin of *S. fusiformis*

The culture age affected the amount of the cell biomass. Harvesting the culture was conducted on day 8 (initial of the logarithmic phase), day 15 (middle of the logarithmic phase), day 31 (end of the logarithmic phase), day 35 (initial of the stationary phase), and day 75 (end of the stationary phase). The dried biomass cell of the *S. fusiformis* that were harvested on day 8, day 15, day 31, day 35, and day 75 were 81.4 g, 82.1 g, 110.6 g, 117 g, and 123.5 g, respectively. The water content of them were 8.14 %, 8.21 %, 8.46 %, 8.48 %, and 8.77 %, respectively. *S. fusiformis* has green biomass (due to chlorophyll). The biomass *S. fusiformis* that was harvested on stationary phase was used for further analysis. The highest amount of harvestes biomass was obtained during the stationary phase.

Biomass was dried by using a fan at 27 °C to 28 °C. Mohammad et al. reported that drying at room temperature can maintain the quality of phycocyanin.

3.3 Antihyperglycemic activity of cell biomass and phycocyanin

Inhibition of α-glucosidase is one of the approaches for controlling the blood glucose level. α-Glucosidase is a key enzyme in carbohydrate digestion in the small intestine. Therefore, attention has been given to natural substances that have potential as inhibitors against α-glucosidase and have a few side effects.

Average blood glucose level of rat at 0.5 h after administration of sucrose and biomass of *S. fusiformis* increased, as well as administration of sucrose and phycocyanin from *S. fusiformis* also increased, but after 2 h it decreased. The increase of the blood glucose level at the N group was longer than in the K, B1 and B2 groups. It showed that acarbose and biomass *S. fusiformis* have ability to inhibit the increase of blood glucose level in rats. The decreasing mechanism of blood glucose levels in rats is still unknown. Pandey et al. reported that diabetic rats treated with *S. maxima* showed increase in body weight which may be explained by an increased insulin secretion or increased food consumption. Oral administration of *S. maxima* could reverse the diabetic effect.

Table 1. Average blood glucose levels of rats given biomass of *S. fusiformis*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 h*</th>
<th>0.5 h*</th>
<th>1 h*</th>
<th>2 h*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (sucrose)</td>
<td>72.8 ± 0.837</td>
<td>116.4 ± 2.702</td>
<td>133.8 ± 0.837</td>
<td>107.8 ± 1.643</td>
</tr>
<tr>
<td>K (acarbose 0.001 mg · g⁻¹ + sucrose)</td>
<td>72.6 ± 0.849</td>
<td>84.2 ± 1.095</td>
<td>94.4 ± 0.548</td>
<td>88.4 ± 0.548</td>
</tr>
<tr>
<td>B1 (biomass 0.15 mg · g⁻¹ + sucrose)</td>
<td>73.4 ± 0.548</td>
<td>97.6 ± 0.548</td>
<td>112.8 ± 1.304</td>
<td>80.0 ± 0.707</td>
</tr>
<tr>
<td>B2 (biomass 0.30 mg · g⁻¹ + sucrose)</td>
<td>73.4 ± 0.548</td>
<td>91.8 ± 0.447</td>
<td>99.8 ± 0.447</td>
<td>86.0 ± 1.000</td>
</tr>
</tbody>
</table>

Note: *value ± s.e.*

Table 2. Average blood glucose levels of rats given phycocyanin of *S. fusiformis*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 h*</th>
<th>0.5 h*</th>
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<td>88.4 ± 0.548</td>
</tr>
<tr>
<td>F1 (phycocyanin 0.15 mg · g⁻¹ + sucrose)</td>
<td>73.4 ± 0.548</td>
<td>98.0 ± 0.548</td>
<td>112.8 ± 1.304</td>
<td>86.4 ± 0.707</td>
</tr>
<tr>
<td>F2 (phycocyanin 0.30 mg · g⁻¹ + sucrose)</td>
<td>73.2 ± 0.588</td>
<td>96.0 ± 0.447</td>
<td>107.8 ± 0.447</td>
<td>84.8 ± 1.000</td>
</tr>
</tbody>
</table>

Note: *value ± s.e.*

Phycocyanin of *S. fusiformis* has ability to inhibit the increase of the blood glucose level, but its ability was lower when compared to acarbose. This requires more study. The effect of phycocyanin at a dose of 0.30 mg · g⁻¹ body weight was higher than the dose of 0.15 mg · g⁻¹ body weight. According to Gershwin and Belay, biopigments have potential as an antihyperglycemic agents, such as anthocyanin and phycocyanin.

The effect of *S. maxima* at a dose of 15 mg · kg⁻¹ of body weight was significantly higher than the dose of 5 mg · kg⁻¹ and 10 mg · kg⁻¹ body weight. *S. maxima* has hypoglycemic effect which helps to control blood glucose levels and also increased body weight. Active compound in *Spirulina* biomass which is used to antihyperglycemic include chromium mineral (Cr), fatty acid gamma linoleat, and flavonoid compound. Based on qualitative test it was found that ethanol extract of *S. fusiformis* containt flavonoids, which have the antihyperglycemic activity.
The administration of sucrose to oral glucose tolerance test raises blood glucose in 60 min\textsuperscript{15}. In this study, blood glucose level in rats increased in 0.5 h after administration of sucrose and the highest level occurred after 1 h. Average blood glucose level increased from 72.8 mg · dL\textsuperscript{–1} to 133.8 mg · dL\textsuperscript{–1}. Mridha et al reported that digestion of sucrose (carbohydrate) occurs in the small intestine giving glucose and fructose\textsuperscript{4}. The biomass and phycocyanin of the \textit{S. fusiformis} at dose of 0.15 mg · g\textsuperscript{–1} body weight and 0.30 mg g\textsuperscript{–1} body weight have effect to control on blood glucose level\textsuperscript{16}.

4. Conclusion

\textit{S. fusiformis} which was cultivated in Zarrouk media had five growth phases including lag phase reached on day 0 to day 7 culture, logarithmic phase reached on day 8 to day 31, declined phase reached on day 32 to day 34, stationary phase reached on day 35 to day 75, and death phase starting from day 76. The culture age affects the amount of the biomass. The biomass and phycocyanin of the \textit{S. fusiformis} have ability to inhibit increasing blood glucose level in rats. The effects of biomass and phycocyanin at a dose of 0.30 mg · g\textsuperscript{–1} body weight was higher level than the doses of 0.15 mg · g\textsuperscript{–1} body weight.

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