Bioactivity of Acetone Extract from Spirulina Platensis

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Bioactivity of Acetone Extract from *Spirulina Platensis*

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Abstract. *Spirulina platensis* is one of natural product from alkaline water which potential as nutraceutic food. The study was performed to investigate the level of antioxidative and anticancer activity of acetone extracts from *Spirulina platensis*. The microalga was extracted with acetone in an ultrasonic bath. Antioxidant activity was determined by using antioxidant model, 1,1-diphenyl-2picryl hidrazyl (DPPH) radical scavenging activity. The anticancer activities were evaluated against human breast cancer (T47D) and murine leukemia (P388) cell lines. Moreover, the 3(4,5-dimethylthiazol-2-yl) -2,5 -diphenyl tetrazolium bromide (MTT) assay was employed to evaluate cell viability. The results provide information that the extract has low antioxidant activity (IC_{50} 3345 µg/mL). The crude acetone extract showed moderate anticancer activity both in T47D and P388 cell lines, IC_{50} 379.5 and 64.3 µg/mL, respectively.

1. Introduction

Today, popular lifestyle personalities endorse nutraceutical as food that may provide a health benefit beyond basic nutrition. *Spirulina platensis* is a type of blue-green algae that grows naturally in warm water alkaline lakes. The nutritional value of Spirulina is well recognized with its high content of protein (50–70% by dry weight), vitamins, minerals, essential fatty acids, photosynthetic pigments, polysaccharides, β-carotene, astaxanthin, phycocyanobilin, phycoerythrobilin, and other nutrients [1,2]. Those compounds are of potential use in the food, pharmaceutical, and cosmetic industries, due to their numerous biological activities (antioxidant, anticancer, antihypertension, immunomodulatory, and prevention of cardiovascular diseases) [3,4].

Specialty chemicals have higher revenues than bulk compounds. Several companies are focusing in the production and commercialization of compounds from algae. Among functional constituents identified in *Spirulina platensis*, natural pigments have received special attention. The pigments exhibit several biological activities. Acetone has been used to extract pigments, especially chlorophyll *a*, from *Spirulina platensis*. The classical organic solvent extraction techniques, including maceration, percolation, and soxhlet increases the risk of thermal denaturation or transformation of pigments. Ultrasonication gave excellent extraction efficiencies to extract natural pigments [5,6]. In the present study, we used ultrasonication method for extraction of *Spirulina platensis* with acetone in cold condition. The aim of the study was extraction of natural pigments from *Spirulina platensis* and to evaluate the antioxidant activity of *Spirulina platensis* acetone extract and studying their cytotoxicity against cell lines (human breast cancer T47D and murine leukemia P388).

2. Methods

2.1 Materials

Spirulina platensis (Changsha Natureway Co. Ltd., China), murine leukemia P-388 cell lines (Institut Teknologi Bandung), human breast cancer T47D cells lines (Universitas Gadjah Mada), acetone (Merck), RPMI 1640 medium, butylated hydroxytoluene (BHT), phosphate buffer saline (PBS), dimetil sulfoksida (DMSO), 3-(4,5-dimetiltiazo-2-yl)-2,5-difeniltetrazolium bromida (MTT), SDS (stop solution), 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich.

2.2 Extraction of Spirulina platensis

Spirulina platensis powder 10 g was extracted by ultrasonic bath with 100 mL acetone for 30 minutes, frequency of 60 kHz, intensity of 120 W [7]. The supernatant was separated by filtration using Whatman filter paper. Then, the acetone fraction was evaporated using rotary evaporator. All treatments were performed in a condition protected from light.

2.3 Antioxidant activity of Spirulina platensis acetone extract determined by DPPH radical scavenging assay

The antioxidant activity of *Spirulina platensis* acetone extract on DPPH radical was estimated using the method of Pathiranan and Shahidi [8]. A solution of 1500 ppm DPPH in methanol was prepared and 100 µL of this solution was mixed with 200, 400, 800, 1600, and 3200 µL containing 2000 ppm of the extract. The reaction mixture left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 516 nm. BHT was used as reference, with 2,5; 5; 10, 20, and 40 µL containing 1000 ppm BHT in methanol. The ability to scavenge DPPH radical was calculated by the following equation:

$$\%\ inhibition = \frac{(A_0 - A_1)}{A_0} x\ 100 \qquad (1)$$

A_0 is the absorbance of DPPH radical + methanol;
A_1 is the absorbance of DPPH radical + sample extract/reference.

2.4 Spirulina platensis acetone extract on T47D and P388 cell lines by MTT assay

T47D breast cancer cells and P388 leukemia cancer cells that have been subculture were used in the activity test. The cells were seeded into 96-well plates at densities $3x10^3$ cells/well and incubated for 24 hrs. Afterwards, cells were washed with PBS and samples (dissolved in DMSO) were added to the microplate. The mixture shaken with microplate mixer and stored in CO_2 incubator for 48 hours. After being incubated for 48 hours, the medium was discarded and replaced with MTT reagent and incubated for 4 hrs. The reaction was stopped with SDS solution and was incubated overnight in a light protected chamber to dissolve the formazan salt. The absorbance was measured with an ELISA reader at 595 nm. Cell viability was expressed as the percentage of viable treated cells relative to untreated control cells.

3. Results and discussion

3.1 Extraction of Spirulina platensis

Spirulina platensis was extracted by ultrasonic wave. Acetone was used as extractant. The method was selected because of necessary to extract biologically active compound in microalgae in low temperature extraction with less damage of the compounds. Under control condition, acetone easily penetrate into the sample at low temperatures without completely damaging the whole structure [7,9]. The method results higher efficiency than freezing and thawing methods for photosynthetic pigments extraction [10]. All steps require protection from light to minimize biologically active compound damage. *Spirulina platensis* is known contain a chlorophyll pigment and beta carotene that is unstable to light [11]. Extraction yield (mass of extract/mass of dry matter) was 6.48%.

3.2 Antioxidant activity of Spirulina platensis

DPPH assay was used for determination of antioxidant activity of *Spirulina platensis*. The compound, DPPH is stable at room temperature, is reduced in the presence of an antioxidant substance. The method provide an easy and rapid way to evaluate antioxidant by spectrophotometry [12]. DPPH assay antioxidant capacity of the extract was expressed in terms of IC_{50} value. The changes in DPPH colour (from deep violet to light yellow) was read at 516 nm with operating time about 30 menit. The result is presented in Figure 1.

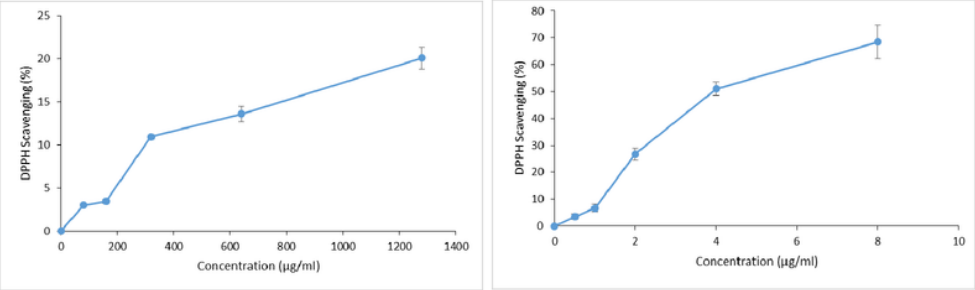

Figure 1. DPPH scavenging activities of the acetone extract of the *Spirulina platensis* (left) compared with BHT (right).

Figure 1 shows the dose-response curve of DPPH radical scavenging activity of the extract. The extract exhibited antioxidant activity that increased with increasing amount of extract concentration, which was compared with standard BHT at different concentrations as extract. The activity is thought to be due to its hydrogen donating ability. The result showed that the extract has the proton-donating ability but low ability. It was observed that the DPPH radical scavenging ability of the extract (IC_{50} 3345 µg/mL) was less than those of BHT (IC_{50} 5.3 µg/mL).

Spirulina platensis acetone extract on T47D and P388 cell lines

In vitro anticancer activity of acetone extract of *Spirulina platensis* was evaluated by the MTT assay using T47D (human breast cancer) and P388 (murine leukemia) cell lines. MTT is considered one of the most economic, reliable and convenient methods. This is based on their ease of use, accuracy, rapid indication of toxicity, sensitivity, and specificity. The assay is in vitro whole cell toxicity assay that employ colorimetric methods for determining viability cell based on mitochondrial dehydrogenase activity measurement. The reagent is bio reduced by dehydrogenase inside living cells to form a coloured formazan dye [13].

Acetone extract of *Spirulina platensis* was dissolved in DMSO. DMSO is an amphipathic molecule, making it soluble in both aqueous and organic media. It is commonly used as a very efficient solvent for water-insoluble compounds in biological studies. DMSO also had been used for treatment of leukemia for several years as it induces cellular differentiation, causing leukemia cells to lose their proliferative properties [14]. On in vitro cultured CL1–5 cells (lung adenocarcinoma cells), low and moderate DMSO treatment did not cause cytotoxicity up to a 2% (v/v) concentration [15].

Cytotoxic effect of *S. platensis* acetone extract on T47D cell lines viability was shown in Figure 2. As the concentration of the extract increased, the percentage of live cells decreased. *Spirulina platensis* acetone extract was found to inhibit proliferation of human breast cancer T47D. Results showed that acetone extract of *S. platensis exhibited* 50% inhibition (IC_{50}) of T47D cell lines at 379.5 µg/mL. The IC_{50} of extract was higher than doxorubicin (4.6 µg/mL) as positive control. It shows the acetone extract of *S. platensis* has lower activity than doxorubicin.

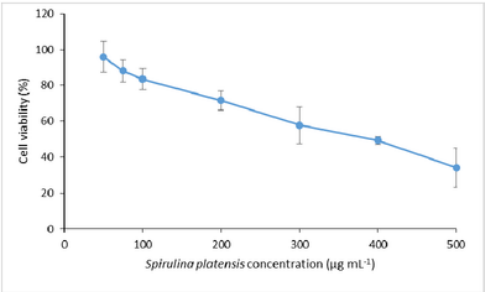

Figure 2. Inhibitory activity of *Spirulina platensis* against T47D cell lines.

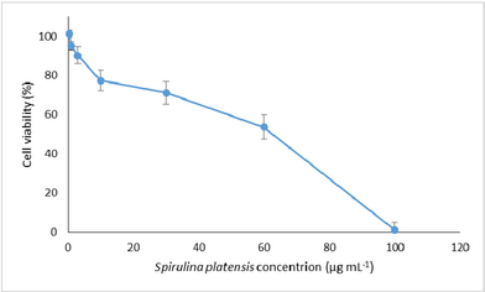

Figure 3. Inhibitory activity of *Spirulina platensis* against P388 cell lines.

The acetone extract of *Spirulina platensis* has anticancer activity against murine leukemia cell P-388 with IC_{50} 64.27 µg/mL. The IC_{50} value is higher than IC_{50} of artonin E as a positive control (0.66 µg/mL). Therefore, the cytotoxic activity of acetone extract of *Spirulina platensis* has lower anticancer activity than artonin E. The crude acetone extract showed moderate anticancer activity both in T47D and P388 cell lines.

4. Conclusion

Our results suggest that the acetone extract of *Spirulina platensis* by ultrasonication exhibited low antioxidant and moderate anticancer activity. *Spirulina platensis* therefore be a good source as nutraceutical.

Acknowledgement

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