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Review: Bioactivity of Spirulina

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ABSTRACT
Spirulina, a type of microalgae, has great potential due to its high protein content and functional properties. This spiral-shaped, blue-green organism contains various compounds, including phycocyanin, chlorophyll, carotenoids, and flavonoids, which exhibit significant bioactive potential. Numerous studies have demonstrated Spirulina's antioxidant, antidiabetic, anti-cancer, anti-inflammatory, antihypertensive, and antibacterial activities.
Keyword: Spirulina, microalga, bioactivity

I. INTRODUCTION

Spirulina is a type of one-celled blue-green algae that has no cell wall and is shaped like a circle (spiral) (Backer, 1994). The name Spirulina is derived from the Latin “helix” or “spiral,” which indicates the organism's physical shape (Figure 1). Spirulina was first discovered in Mexico and Central Africa, where it is known as the most nutrient-rich ancient plant. Spirulina thrives in warm climates and is currently being developed as the 'food of the future' due to its remarkable ability to synthesize high-quality concentrated food more efficiently than other algae (Christwardana, 2013). It is recommended by the FAO and WHO as the best health food of the 21st century.

Figure 1. *Spirulina platensis*
Source: https://algae-lab.com

Spirulina belongs to the blue-green algae group because it contains a lot of chlorophyll (green) and phycocyanin (blue) pigments in its cell structure. The algae can thrive in very hot waters with temperatures of 32-45^{0}C (about 85-112^{0}C), and can even survive temperatures of 60^{0}C (140^{0}C). The nutritional content in spirulina includes protein (leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine), amino acids (alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, proline, serine, tyrosine), minerals (potassium, calcium, zinc, magnesium, manganese, selenium, iron, phosphorus), vitamins (B1, B2, B6, B12, biotin, E, folic acid, inositol, niacin, pantothenic acid), carotenoids (α-carotene, β-carotene, xanthophyll, cryptoxanthin, echinenone, zeaxanthin, lutein), enzymes, fat, sugar, salt, calories and chlorophyll (Konickova, 2014). The pigment that gives spirulina its blue color is phycocyaninwhich is found in large amounts, about 7%. The pigment is related to the pigment bilirubin which is essential for liver health and amino acid digestion. Another pigment found in spirulina is porphyrin, a red-colored compound that forms the active nucleus of hemoglobin.

Spirulina is used as a protein source with protein levels of up to 70% (Phang, et al., 2000). In addition, Spirulina contains vitamins, especially vitamin B12 and pro-vitamin A (β-carotene), as well as minerals, especially iron. Spirulina is also rich in tocopherols, phenolic acid, and γ-linolenic acid, all of which have various benefits for human life.

Spirulina Chemical Content

Spirulina contains 65-70% protein, which is higher than other foods such as eggs (45%), tempeh (45%), soybeans (40%), dry milk (35%) and beef (17%). In addition, spirulina contains 8 types of essential amino acids and 10 types of non-essential amino acids (Li and Qi, 1997). Spirulina contains high concentrations of nutrients, namely

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mineral chelates, pigments, rhamnose sugar, enzymes and other essential elements. The content of Spirulina is shown in Table 1.

Table 1. The content of Spirulina

Parameter	Content (%)
Proteins	65-70
Lipid	4-6
Carbohidrate	17-25
Linoleate acid (gamma)	0,8
Chlorophyll	0,8
Phycocyanin	6,7-11,7
Caroten	0,43
Zeaxanthin	0,1
Water	3-6

Source:Christwardana dkk (2013)

The main pigment in spirulina is beta-carotene which, when absorbed by the body, is converted into vitamin A. Two grams of spirulina provides more than 100% of the RDI of vitamin A, plus calcium, iron, phosphorus, potassium, zinc and many other minerals. Spirulina is also rich in vitamins including the entire group of B-complex vitamins. Previously thought to be found only in animals, spirulina contains twice the amount of vitamin B_{12} found in liver. Today, spirulina is also a natural source of B vitamins that can replace the need for synthetic B vitamins.

The protein in spirulina contains high biological value because it contains essential amino acids. In addition to protein, spirulina contains minerals (iron, potassium, calcium, sodium, phosphorus, manganese, copper, zinc, iodine, chromium, and magnesium), vitamins (A, B1, B2, B5, B6, B12, D, E, and K), chlorophyll, fiber and phycocyanin. Other contents are unsaturated fatty acids (gamma-linolenic acid, linoleic acid, docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, stearidonic acid), beta-carotene, thiamine, nicotinamide, pyridoxine, riboflavin, folic acid, phenolics, phycobiliproteins, catechin hydrate, epicatechin, pyrocatechol, C-phycocyanine and superoxide dismutase (SOD) enzymes.

Various studies around the world have reported that Spirulina can regulate diabetic processes, such as hypercholesterolemia activity, and has antioxidant effects and radical-catching properties that provide significant multiorgan protection and improve the effects of many drugs and toxic attacks.

Antioxidant

Nasirian et al., (2018) found that Wistar rats supplemented with Spirulina platensis extract at doses of 20 and 30 mg/kgBB showed decreased levels of malondialdehyde, glucose, lipid parameters, AST, ALT, TNF-α, and IL-6 as well as an increase in several minerals and antioxidant enzymes. These supplements may also provide minerals for the formation of antioxidant enzymes, which in turn may reduce lipid profiles, glucose concentrations, and anti-inflammatory responses.

Zaid et al., (2015) tested the antioxidant activity of Spirulina platensis water extract. Testing with the DPPH method showed that at 1.5 g/100 ml of Spirulina platensis water extract produced the highest antioxidant percentage of 81.1%.

Tests using DPPH for the antioxidant activity of Spirulina platensis methanol extract were conducted (Gheda et al., 2021). It was found that at a concentration of 1.5 g/100 ml the extract showed the highest antioxidant activity (59.44%) and the highest inhibitory effect against the enzymes diabetes-amylase (96.46%) and glucosidase (97.42%).

ABTS and DPPH methods were used to test the antioxidant activity of Spirulina platensis extracts at 40 grams/200 ml (methanol, hexane and acetone), showing that methanol extracts had the highest ABTS and DPPH radical inhibition of 93% and 90%, respectively, followed by acetone extracts of 88% and 82% and hexane extracts of 60% and 55%, respectively, compared to standard compounds of 96% and 94%, respectively (Abdel-Moneim et al., 2022).

DPPH technique used for antioxidant activity on methanol extract of Spirulina platensis of 60 µg/ml, the results are expressed in percentage (%) and IC_{50} is the amount of extract needed to inhibit 50% of DPPH radicals. The lowest IC_{50} identifies the strongest level of antioxidant activity, obtained the highest DPPH radical inhibitory activity with methanol extract of Spirulina platensis IC_{50} of 28 mg/ml. DPPH antioxidant activity is relatively the same when compared to ascorbic acid as the reference antioxidant, with IC_{50} = 22 mg/ml. The methanol extract of Spirulina platensis had a DPPH radical inhibitory activity of 79.7%. It was found that increasing the concentration of the extract increased the inhibition of DPPH radicals (Gheda et al., 2023).

Anti-obesity

According to Zhao et al., (2019)Spirulina platensis protein hydrolysate at a dose of 2 g/kg

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tested on rats fed a high-fat diet showed a good anti-obesity effect of reducing 39.8% ± 9.7% body weight, lowering 23.8% ± 1.6% serum glucose, and lowering 20.8% ± 1.4% total cholesterol. So the anti-obesity effect of Spirulina platensis protein hydrolysate may have a similar mechanism of action with the orlistat drug in lowering high-density lipoprotein cholesterol.

Several clinical and preclinical trials have been conducted to test the benefits of spirulina on weight loss. Yousefi et al., (2018) studied 52 obese participants with body mass index (BMI) ≥ 25-40 kg/m^2 who were randomly assigned 2 grams of spirulina per day with a calorie-restricted diet as well as a placebo consisting of a calorie-restricted diet for 12 weeks. Participants in the spirulina group had significantly lower body weight of -3.22 + 1.97 kg, waist circumference -3.37 ± 2.65 kg, body fat -2.28 = 1.74 kg and BMI - 1.23 ± 0.79 kg/m^2. In addition, triglycerides were reduced by -18 mg/dL and high-sensitivity reactive protein levels were lower at -1.66 ± 1.9 ng/ml.

Isolation and purification of peptides for anti-obesity in Spirulina platensis protein. Five enzymes (trypsin alcalase, pepsin, papain and protamex) were used for hydrolysis of the extracted proteins. The inhibitory effect on pancreatic lipase and 3T3-L1 preadipocytes showed that the 3-5kDa fraction from pepsin digestion showed good inhibition on lipase by 72% and the chromatographed fraction showed strong inhibition on 3T3-L1 preadipocytes by 72.7-88.1% but low inhibitory or stimulatory activity on normal liver L-O2 cells by -26.02 to 12.16%. Results from mass spectrophotometry showed four novel peptides NALKCCHSCPA, LNNPSVCDCDC-MMKAAR, NPVWKRK and CANPHELPNK were identified to exhibit inhibitory effects on the proliferation of 3T3-L1 preadipocytes by 32.29-60.08%. In addition, NPVWKRK and CANPHELPNK also significantly reduced triglyceride accumulation at 600 µg/ml ($p < 0.05$), by 23.7% and 19.5%, respectively. This indicates the potential of Spirulina platensis hydrolysate and peptide derivatives as anti-obesity ingredients. (Fan et al., 2018).

Anti-cancer

Cyanobacteria classified in the genus Arthrospirahave been described as potential agents with protective and beneficial effects on the human respiratory tract. In addition, there is anti-cancer activity of some Spirulina components against lung carcinoma cells. (Czerwonka et al., 2018) reported aqueous extract of Spirulina platensis exerted cytotoxic and anti-proliferative activity against A549 human lung adenocarcinoma cells. In contrast, there was no cytotoxic activity of Spirulina platensis aqueous extract against normal human skin fibroblasts. In the literature, the cytotoxicity of Spirulina platensisis considered as one of the mechanisms of anti-cancer action. Spirulina platensis aqueous extract 20 g/200 ml has shown cytotoxic activity against HCT116 colon carcinoma and HEPG2 hepatocellular carcinoma with IC_{50} values of 18.8 µg/ml and 22.3 µg/ml respectively, human acute leukemia Kasumi-1 and chronic myelogenous leukemia K-562 with IC_{50} values of 2.13 mg/ml and 12.68 mg/ml respectively and human chronic myelogenous CM with IC_{50} value of 50.29 mg/ml cell line. Anti-cancer activity against A549 lung cancer cells had an IC_{50} value of Spirulina platensis aqueous extract of 2.42 mg/ml.

Anti-cancer activity of Spirulina platensis aqueous extract using colon carcinoma cells (HCT116) and hepatocellular carcinoma cells (HEPG2). Several studies reported that cancer can be prevented by Spirulina platensis extract, due to its antioxidant properties. Chemotherapy is one of the main treatments used to cure cancer. Spirulina platensis aqueous extract was found to inhibit the proliferation of human colon (HCT116) and liver (HEPG2) cancers. The inhibition concentration IC_{50} is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function and represents the concentration of a substance or drug required for 50% inhibition in vitro. Results showed that the 50% inhibition IC_{50} of HCT116 and HEPG2 cell lines at 18.8 3 µg ml $^{-1}$ and 22.3 µg ml $^{-1}$(Zaid et al., 2015).

Spirulina platensis was isolated and identified microscopically and genetically through detection of phycocyanin gene (cpcBA) and isolated through the detection of microcystin-producing gene (mcyE) gene through PCR technique. To assess the cytotoxic effect of Spirulina platensis methanol extract on human cancer cell lines L20B and MCF7. Various concentrations of Spirulina platensis extract (mg/ml) obtained with 70% methanol solvent were used to treat the cell lines after 24 h and 48 h, the exposure time of MTT assay was achieved for the study of cytotoxic effect. Chemical analysis and GC mass analysis for the crude extract were performed to identify the most active chemical compounds, the methanol extract of Spirulina platensis showed cytotoxicity against the two

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cancer cell lines tested, the highest growth inhibition percentage was 32.5%, treatment using 25 mg/ml, 12.5 mg/ml was 71.5% against L20B and MCF7 respectively. This percentage increased after 48 hours of application to 35.5% against L20B and 78% against MCF7. Phytochemical analysis showed that the active chemical compounds of the extract contained alkaloids, phenols, terpenes, flavone steroids, resins, saponins, amino acids proteins and tannins. GC mass analysis results for the extract proved the presence of many biologically active compounds including 11 anti-cancer compounds. Thus, the methanol extract of Spirulina platensis showed considerable bioactive content as an anti-cancer, namely antiproliferative properties against breast cancer adenocarcinoma cell line (MCF7) and intestinal carcinoma cell line (L20B). (Fayyad et al., 2019).

Anti-inflammatory

Jiang et al (2023) have examined that proteins in Spirulina platensis containing selenium have anti-inflammatory activity. Selenium suppresses inflammatory cytokines, where there is a 74% decrease in interleukin 6 (IL-6), 42.28% tumour necrosis factor-α (TNF-α), 69.07% malondialdehyde (MDA) content, 40.45% interleukin- 1β (IL-1β) content relative to the LPS group.

Abu Taweel et al. (2019) reported the anti-inflammatory effect of S. platensis extract powder by using carrageenan-induced edema rats, and cotton pellet-induced granuloma formation. The results showed that S. platensis extract significantly attenuated carrageenan-induced hind paw edema, and cotton pellet-induced granuloma. Preliminary molecular mechanistic studies established that S. platensis extract decreased the production of TNF-, IL-1, IL-6, PGE2 and NO, and suppressed the activities of COX-2 and iNOS.

S. platensis extract at doses of 20 and 30 mg/kgBB can reduce malondialdehyde, glucose, lipid parameters, AST, ALT, TNF-α and IL-6 levels in Wistar rats. S. platensis extract is given because it can increase some minerals and antioxidant enzymes. The supplement can provide minerals for the synthesis of antioxidant enzymes which can further reduce lipid profile, glucose concentration and anti-inflammatory response (Nasirian et al. 2018).

Antihypertension

The antihypertensive effect of S. maxima was reported by Sulibursk et al. (2016) in a study involving 50 patients with hypertension, each of whom was given 2 g of spirulina daily for 3 months. Plasma concentrations of calcium, magnesium, iron, and zinc were assessed at the beginning of the study and after the treatment period. The results indicated that supplementation with S. maxima for 3 months led to a significant reduction in plasma iron levels in obese patients with hypertension, with average levels decreasing from approximately 16.58 to 13.75. Specifically, it was demonstrated that phycocyanin isolated from S. maxima can bind iron ions from FeSO4 and FeCl3. Furthermore, S. maxima is known to accumulate heavy metals and act as a bioadsorbent for these metals. Consequently, spirulina may reduce iron absorption from food. Additionally, the decreased iron bioavailability may partly result from the interaction between heavy metals in spirulina and iron, as certain metals, such as cadmium, are known to inhibit iron absorption.

Antibacterial

Abdel-Moneim et al (2022) methanol, acetone, and hexane extracts of spirulina have antibacterial activity against Gram-positive bacteria (B. cereus, S. aureus, and L. monocytogenes). The activity against Gram-positive bacteria was greater than Gram-negative. Nayyef & Thalij (2020) reported that the antibacterial effect of Spirulina sp. water extract was evaluated against bacterial isolates using the disc diffusion method, the results were read to determine the diameter of the inhibition zone of each sample using the inhibition concentration formula. Spirulina platensis aqueous extract had inhibition zones ranging from 11-26 mm at a dose of 200 mg/mL with inhibitory effects on Streptococcus sp., E. coli, Pseudomonas spp., P. aeruginosa, K. pneumonia, and G. adicans. In the research of Usharani et al. (2015), the antibacterial effect of Spirulina water extract was evaluated against bacterial isolates using the disc diffusion method. As a result, Spirulina platensis water extract had inhibition zones ranging from 11-26 mm at a dose of 200 mg/mL with inhibitory effects on Streptococcus sp., Staphylococcus aureus, and Bacillus cereus bacteria.

II. CONCLUSION

Spirulina is a type of microalgae. These organisms are rich in very useful compounds. Compounds in Spirulina have bioactivities such as

antioxidant, antiobesity, anticancer, antiinflammatory, antihypertensive and antibacterial.

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