Investigation of antioxidants from wild *Spirulina platensis* in Iran

Asghari A.[1]; Choopani A.[1, 2*]; Ghaedamini N.[2]; Fazilati M.[1]; Latifi A.M.[2]; Salavati H.[1]

Received: October 2024 Accepted: January 2025

Abstract

Spirulina micro-algae is a valuable green-blue algae, which due to its nutritional value, its medicinal properties, high protein, vitamins, minerals, and natural pigments It is widely used in various food, health and beauty industries, human food supplements, livestock, poultry, And aquatic animals. Free radicals cause many diseases in humans. Antioxidants eliminate free radicals that are active and destructive. Experts predict that our communities will face a cancer tsunami in the future, with new and modern uses of these algae having anticancer compounds to cope with cancerous diseases. In this study, the total antioxidant activity of the methanolic extract of *Spirulina* in three concentrations (10, 20, 30 ppm) was calculated using the DPPH method and was compared with ascorbic acid. Also, the assessment of Chlorophyll a, carotenoid, and Phycobiliproteins measurements of proteins measured in a 5-to-20-day growth period under laboratory conditions. Results: Based on the results of antioxidant activity measurements, the total alcoholic extract of algae at 10, 20, 30 ppm concentration was 23.18%, 40%,71.42%, chlorophyll a, carotenoids, allophycocyanin, phycocyanin, and phycoerythrin was calculated respectively 6.8, 3. 2, 41.08, 28, 14.8 µg/mgdw. In DPPH, the amount of ic50 of *spirulina* platensis calculated to be 23.69%. Conclusion: In this study, the antioxidant activity of the *spirulina platensis* strain of Iran has been investigated. According to studies, this strain was introduced as a rich and new source of natural antioxidants for further research and industrialization.

Keywords: *Spirulina* algae, Antioxidant activity, Carotenoids, Chlorophyll a, Phycobilyproteins

1-Department of Biochemistry, Faculty of Biologic Science, Payame noor University, Tehran, Iran
2-Applied Biotechnology Research Center, Bqiyatallah University of Medical Sciences, Tehran, Iran
*Corresponding author's Emial: choopani.ali3266@gmail.com

Introduction

Spirulina has antioxidant properties that can cope with free radicals and strengthen cells that cure cancer (Siramdas *et al*., 2024). These algae are rich in nutrients useful in the treatment of cancer and immune system diseases (such as beta-carotene and chlorophyll) (Amin *et al.*, 2024; Çelekli *et al*., 2024). Several studies have shown that *Spirulina* is a potent antioxidant and its health-promoting properties are associated with antioxidant pigments, carotenoids, chlorophyll, and its unique phycocyanin blue pigment (Karkos *et al*., 2010). The biochemical analysis of *Spirulina* strains shows that this algae has a significant potential for human nutrition (Giri *et al*., 2023). *Spirulina* has high levels of high-quality protein, including essential amino acids with high digestibility, pigments such as carotenoids and phycocyanin, vitamins and minerals such as calcium and iron (Gupta *et al.*, 2011). Among these pigments, xanthophylls, zeaxanthin are scarce in nature, and 3 grams of *spirulina* can provide enough amount of it (Çelekli *et al*., 2023). Its health benefits include anti-cancer, antibacterial and anti-bacterial properties in allergies, gastric ulcer, anemia, heavy metals poisoning, and radioactive toxicity. *Spirulina*'s antioxidant properties are mainly attributed to phycocyanin, beta-carotene, and phenolic compounds (Gupta *et al*., 2011). *Spirulina* reduces the number of cancerous tumors, cures some cancers, and stimulates the immune system. Some studies have shown that this micro-alga has been able to effectively treat skin cancers and eliminate tumors. These algae deal with skin, thyroid, pancreas, and lung cancers (Siramdas *et al*., 2024). Reducing blood lipids, fighting fatigue, increasing the number of immunoglobulins, increasing white blood cell count after chemotherapy and radiotherapy, improving the function of the immune system, and reducing the growth of cancer cells in people with leukemia are among the therapeutic properties of *spirulina* (Alagawany *et al*., 2021). Oxidative damage can damage our DNA and our cells. *Spirulina* is an excellent source of antioxidants that protects oxidative damage. The main active ingredient is called phycocyanin. This antioxidant agent gives *Spirulina* a unique blue-green color (Çelekli *et al*., 2024; Lafarga *et al*., 2020). C-Phycocyanin is a water-soluble, highly fluorescent compound with antioxidant properties. Phycocyanin and other Phycobilyproteins have many properties in the food industry, cosmetics, sanitation, biotechnology, diagnosis, and treatment. Currently, 11 companies in the world are producing and selling Phycobilyproteins and their derivatives (Jha *et al*., 2024). Phycocyanin can fight free radicals, prevent the production of inflammatory molecules, and provide effective antioxidants and anti-inflammatory effects, and has anti-cancer effects (Pentón-Rol *et al*., 2024). It plays a very important role in the prevention of skin-mucosal cancers and chronic myeloid leukemia in humans (Mikhailova *et al*., 2023). By inhibiting

mitotic cell activity, this protein can stop the expression of CD59 and thus cause apoptosis, which itself justifies the anti-cancer activity of this pigment (Salgado *et al*., 2024). Chlorophyll is an essential ingredient in many everyday products, which are not only used as an additive in pharmaceutical and cosmetic products but also as natural food colors. In addition, it has antioxidant, anti-mutagenic and anti-cancer properties (Lafarga *et al*., 2020; Ferruzzi *et al*., 2007; Asghari *et al*., 2016). Carotenoids are a group of pigments that play an antioxidant role, and animals and humans are not able to synthesize them, and they must be fed through diet, and then they can be transformed into carotenoids in another form (Landrum *et al*., 2009) *Spirulina* pigments are used in the pharmaceutical and food industries. Blue pigment, Phycocyanin is used in Japan for food color. Now artificial colors are replaced by natural colors, and tiny algae are the largest source of chlorophyll, and chlorophyll *spirulina* is very suitable for this usage (Kurima *et al.*, 2024). Among the many nutrient compounds derived from microalgae, pigments have been increasingly commercialized due to their high utilization and easy extraction. Many pigments are now commercially produced from non-fossil sources, due to the toxic effects of synthetic pigments; there are various reasons for the use of natural pigments in pharmaceutical and nutritional uses. Therefore, there is a significant increase in the tendency to replace the cyanobacteria pigments, especially *Spirulina* (Anusree *et al*., 2023). With the use of low-cost, ultra-low-energy micronutrients, *Spirulina platinum* can produce a desirable nutritional product in the food industry. Due to the increasing demand for non-food products, the adoption of more micro-algae *Spirulina platensis* in the world, low production costs the high nutritional value of this micro-algae, research, and development in this field is necessary to increase its application in the Iranian food industry. This study aimed to study the amount of useful pigments of indigenous *spirulina*, especially carotenoids, chlorophyll, and phycocyanin, as antioxidants, due to the great properties and applicability of *spirulina* and the presence of natural pigments.

Materials and methods

Samples and chemicals: To conduct experiments, *Spirulina* algae was prepared from an Iranian environment in the north of the country and it was identified in Shahid Beheshti University. And other laboratory works, cultivation, powder preparation, pigment measurements, and examination Total antioxidant properties have been performed at the Applied Biotechnology Research Center of medical sciences university Bqiyatallah, of Iran. Materials required prepared from Sigma-Aldrich. To determine the antioxidant properties of *spirulina*, a methanol extract was first prepared. 1g of *spirulina* powder is poured inside the falcon, 40 cc methanol is added to it and mixed with high intensity after 24 hours stirring, the extract Using Watman Flat Filter, the flat

plate 1 is placed under a hood for 12 hours to evaporate methanol (this was done in four steps in a Bio Shaker BR-42 FL incubator at 24°C and 190 rpm) and the direction Completely solvent removal is performed in the incubator at 60°C for 24 hours Set. Concentrations of 10, 20, 30, ppm were prepared from the extracted extract to measure its inhibitory activity. (Also, the aqueous extract of algae was prepared to compare the activity to the dumping of free radicals DPPH).The concentrations of 10, 20, and 30 ppm of the extract mixed with1.5mL of methanol solution (0.002% DPPH) and stomped for 30 minutes in darkness and room temperature. After this stage, its absorption was read at 517 nm. Finally, using the following formula, activity is measured by dumping DPPH free radicals. The antiradical activity was defined as the relative standard concentration required to reduce the initial concentration of DPPH to 50% (IC50 (Matys *et al.*, 2023; Maikai *et al*., 2010):

$$\text{Inhibition\%} = (A517_{control} - A517_{sample})/A517_{control} \times 100$$

Chlorophyll a

1 mL of algae suspension, which was uniform, is removed for 10 minutes at a speed of 12000 RPM and its upper surface. Then 1 mL of pure methanol is added to the remaining deposition and with high vortex strength. The specimens were placed in the dark for 24 hours at 4°C. After this time, the samples were centrifuged again for 10 minutes at a speed of 12,000 rpm, and the absorbance spectrum at the wavelength of 665 nm was recorded against methanol control. Chlorophyll concentration was calculated using the following formula in terms of a microgram of chlorophyll per mg dry weight (Lefebvre *et al*., 2020):

$$\text{Chlorophyll a} = 13.14 \times A665nm/DW$$

Amount of carotenoids

1 mL of homogeneous alga suspension placed for 10 minutes at a speed of 12000 rpm and centrifuged to the remaining pellet of 1 mL of acetone 80% and high-intensity vortex and samples at 4°C for 24 hours. In the next step, the absorption spectra of the overlying unit were recorded at 10,000 rpm at 12000 rpm at 461 nm λ and 665 nm λ=vs. 80% acetone. The concentration of carotenoids in terms of micrograms per mL was calculated based on the following formula (Kato *et al*., 2021):

$$Car = ([OD461nm - (0.046 \times OD665nm)] \times 4) / DW$$

The amount of Phycobilyproteins

1mL of algae suspension was completely homogenized, centrifuged for 10 minutes, and the supernatant was removed. On the remaining pellet, 60 to 150 µL of pure glycerol is added green-blue and the vortex is high-intensity. The mixture was placed at least 24 hours in

darkness at 4°C. Then, to the mixture, add some distilled water to give a concentration of 10% glycerol. This action causes osmotic shock, fibrinous cells, and Phycobilyproteins are released. The sodium acetate solution is added to a concentration of 200 mM. The resulting mixture was recorded for centrifugation and absorbance for 10 minutes at 562, 615, 652, 750 nm. The concentration of Phycobilyproteins was calculated using the following formulas in micrograms per milliliter (in this study, each experiment was repeated three times.) (Xu *et al*., 2021):

$$APC = ([1000 \times (A_{652} - A_{750}) - 208 \times (A_{615} - A_{750})] / 5.09) / DW$$
$$PC = ([1000 \times (A_{615} - A_{750}) - 474 \times (A_{652} - A_{750})] / 5.34) / DW$$
$$PE = ([1000 \times (A_{562} - A_{750}) - 2.41 \times (PC) - 0.949 \times (AP)] / 5.09) / DW$$

Results

Spirulina antioxidant properties

Detection of DPPH free radicals is one of the methods for determining the antioxidant properties. In this method, the purple color of DPPH radicals at a wavelength of 517 nm reduced by antioxidants and converted to pale yellow. The degree of coloration of this compound represents the power to free radical scavenging by the relevant antioxidant. In Figure 1, the DPPH radical scavenging rate at concentrations of 10, 20, and 30 ppm *Spirulina* algae extract and DPPH as a control sample are presented. The results show that the inhibitory concentration of algae extract has increased with increasing concentrations.

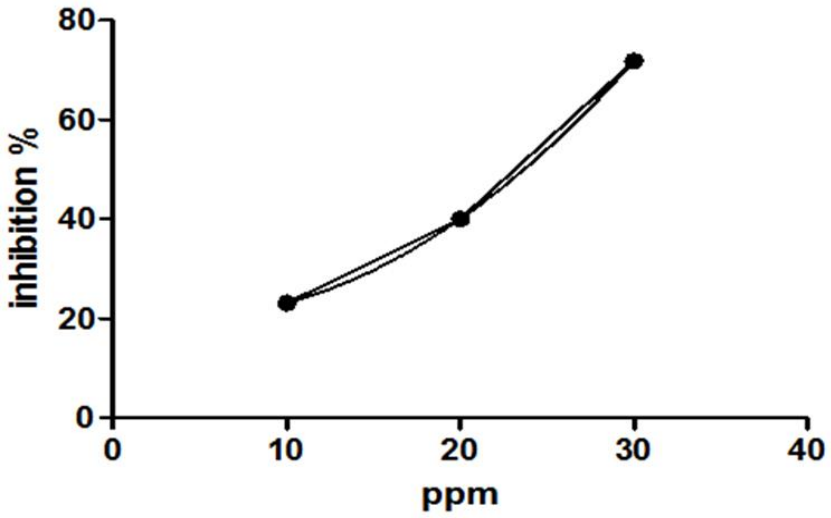

Figure 1: Activity to free radical scavenging DPPH at concentrations (10, 20, 30 ppm) of microalgae extract.

The IC50 or the required concentration of the extract was calculated to contain

50% DPPH radicals, which is 23.69%, the lower the IC50 value, the greater the antioxidant capacity.

The results show that the amount of inhibitory concentration of *spirulina* alga extract increased with increasing concentrations, while this level did not differ significantly in ascorbic acid. There is a significant difference between the inhibition concentration of methanol extract of algae at concentrations of 10, 20, 30 ppm, while in concentrations of 10, 20, 30 ppm ascorbic acid did not show significant differences in free radical scavenging. Also, the results of the graph show that the alcoholic extract (Alcohol 70%) of alcohol is less than that of alcoholic extract (Table 1; Fig. 2).

Table 1: Antiradical activity percentage of algae extract compared to ascorbic acid.

Concentration ppm	Alcoholic Extract	Hydro-alcoholic extract	Ascorbic acid
10	23.18	12	78

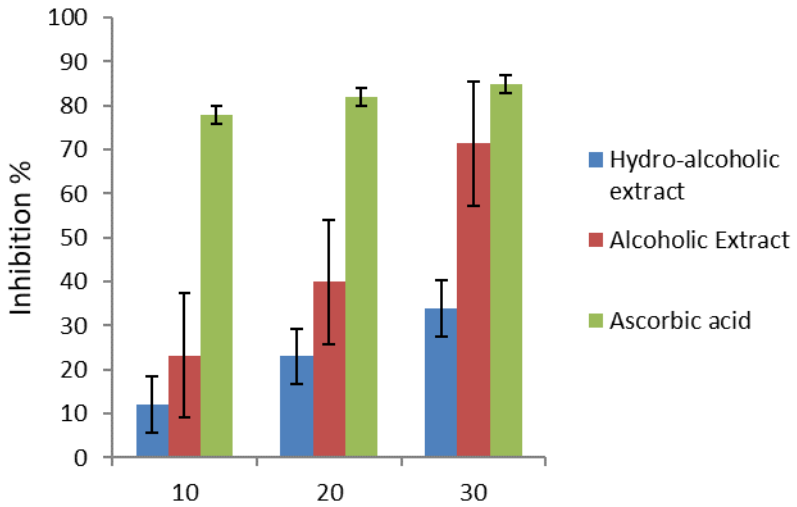

Figure 2: anti-radical activity with comparing by ascorbic acid.

Chlorophyll a

To measure the amount of chlorophyll-a in *spirulina*, after reading at 665 nm, the numbers obtained in the formula are given and the chlorophyll content from the fifth day of growth is reached by the twentieth day. Moreover, its rate has risen from the fifth to the twentieth day. As shown in Figure 3. On the fifth day, its value is 2.2µg/mg DW, and on the twentieth day, its value is 6.8µg/mg DW.

Carotenoids

To measure the amount of carotenoids in *Spirulina platensis*, after reading the absorbance value at wavelengths of nm 461 and 665 nm, the numbers obtained are in the formulas and the carotenoids are calculated from day 5 to the twentieth day. As shown in Figure 4, its rate is increasing over time, with the rate on the fifth day Being 0.6µg/mg DW and on the twentieth day, 2.3µg/mg DW is obtained.

The amount of phycobilyproteins

To measure the amount of three Phycobilyproteins in *Spirulina platensis*, after reading at 562, 615, 652, 750 nm, and the numbers were obtained in the corresponding formulas and the sum of the finest and the phycoerythrin and phycocyanin and the allophycocyanin were calculated. The numbers obtained, as It has been reported in Figure 5, shows that, with time, the amount of Phycobilyproteins has increased, with the amount of allophycocyanin on the 5 days, 6.01 and the 20 days, 28.08µg/mg DW, Phycocyanin on the 5 days, 9.77 and on the 20 days, 41µg/mg DW and phycoerythrin on the 5 days, 0.73 and on the 20 days, 14.8µg/mg DW is reached.

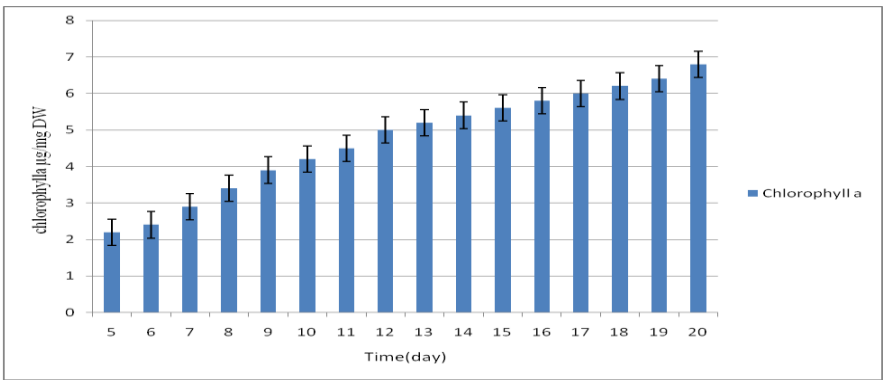

Figure 3: Chlorophyll a changes from 5th to 20th day of growth.

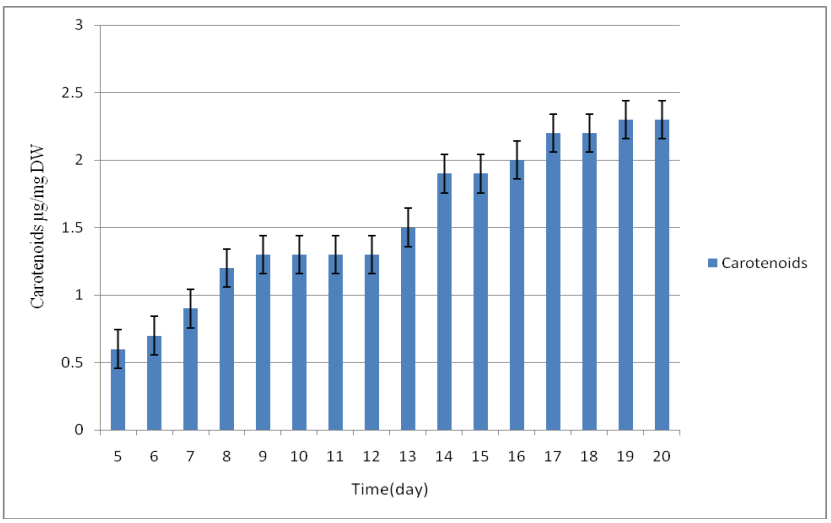

Figure 4: Assessing the variation of carotenoids from 5th to 20th day of growth.

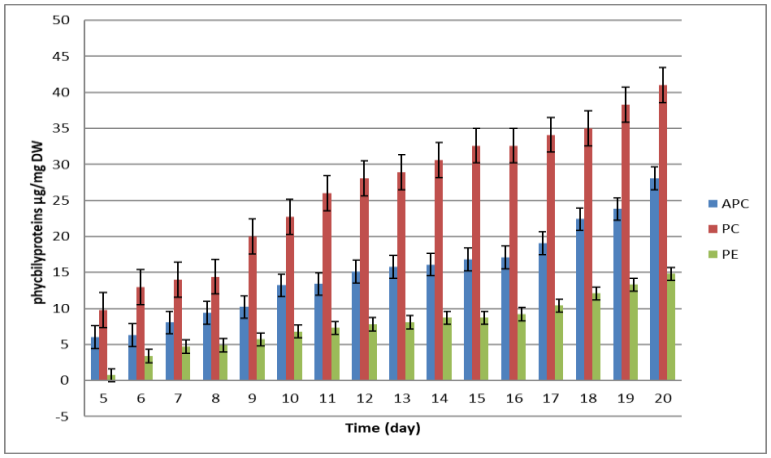

Figure 5: Changes in Phycobilyproteins from the fifth to the twentieth day of growth.

Discussion

In a biological system, antioxidants are a substance that, when present at a low concentration in the oxidation material, delayed or significantly inhibited its oxidation. Over many centuries, antioxidant activity has been used to enhance the quality of food through delayed lipid oxidation (Pokorny *et al*., 2001). The body acts on the oxidation processes and, while metabolizing the body, the active oxygen species are produced. In addition to active oxygen species, active nitrogen species are also produced under oxidative stress conditions. An effective way to remove free radicals is to use antioxidant compounds that act as a free radical binder, so special attention has been paid to the use of antioxidants to protect cells from the biological damage of free radicals (Nickavar *et al*., 2010).In this study, DPPH (diphenyl-1-picyrilhydrazil) has been used to evaluate antioxidant capacity and potency. DPPH is a free radical-nitrogen compound that is readily destroyed by a free radical bubble, so it can be used to test antioxidant compounds. This is a simple, fast, and reliable method for antioxidant evaluation. The antioxidant activity of the methanolic extract was calculated at three concentrations (30, 20, 10 ppm) using the DPPH method and compared with ascorbic acid (reported in Table 1, Butterfly 2). In the study, it was observed that the inhibition of methanolic extract of *spirulina* algae was increased with increased concentrations, while this level was not significantly different in ascorbic acid. The results of this study showed that increasing the concentration of *Spirulina* extract, the antioxidant activity, and the DPPH radical inhibition were also increased. The results of the graph also show that the inhibition of alcoholic extract (alcohol 70%) of *spirulina* algae is very low compared with alcoholic extract (Table 1; Fig. 2).

In the DPPH test, the amount of IC50 of *Spirulina platensis* algae was calculated to be 23.69% (Fig. 1). The lower the IC50 value, the greater the antioxidant capacity. In research, Shalaby and Shanab in 2013 found the antioxidant activity of *spirulina* (methanol, methanol 50% and aqueous)

extracts in the evaluation of the DPPH method by the presence of phenols due to High levels of biologically active phytochemicals (sterols, flavonoids, reducing sugars, tannins, and anthraquinones) (Shalaby *et al*., 2013). Jaime et al. Have isolated and evaluated the antioxidants produced by *spirulina* micronutrient by a combination of pressure-extraction, TLC and DAD-HPLC methods, They introduced carotenoids, phenolic compounds, and decomposed products of chlorophyll as the most important antioxidant compounds of *spirulina* extracts (Shalaby *et al.,* 2005). In the study of total antioxidant capacity, *spirulina* extract was 17.98 mg ascorbic acid per gram of *spirulina* (Apak *et al*., 2004). In the research, *spirulina* alcoholic extract (65%), inhibition against the chemical effects of antioxidants such as alpha-tocopherol (35%), hydroxyanisole butyl (45%), and beta- carotene (48%) and its aqueous extract (76%) compared to acid glycine (54%) and chlorogenic acid (56%), Has a higher antioxidation (Mostolizadeh *et al*., 2020). According to a review carried out in this study and review articles, *spirulina platensis* algae can consider as a supplement of food with antioxidant potency.

In the studies, the carotenoid content of *spirulina platensis* powder was 5.9 to 6 g/kg and its chlorophyll content was 6.6 to 9.2g/kg (Jiménez *et al*., 2003). And also obtained chlorophyll content in the *Spirulina platensis* 1.37% (Kumar *et al*., 2011). the chlorophyll content was determined by changing the light intensity, temperature, and potassium nitrate, which had the best result at 30°C and 24 μm photon light intensity (Danesi *et al*., 2011). The total carotenoid content of *Spirulina platensis* is 3.51mg of dry weight (Choudhury and Biswas, 2022). The amount of chlorophyll was 6 mg/l, which was reduced by adding lead to treatments (Arunakumara *et al*., 2007. In the study, the production of phycocyanin by glucose, ethanol, and acetic acid was 29.33, 27.05, 25.05 (Colla *et al*., 2007; Uba *et al*., 2024). In this study, the amount of chlorophyll a and *spirulina* were 6.8μg/mg and the carotenoid content was 2.3μg/mg and the phycocyanin content was 41μg/mg and the allophycocyanin content was 28.08 μg/mg And the phycoerythrin content of 14.8 μg/mg dry weight was obtained. In general, the result of the evaluation of this study with other work by the researchers showed that the amount of *spirulina* pigments produced in Iran was higher than other studies, probably due to the conditions for the development of *spirulina*, indicating that the culture conditions were different Because the ability of algae cells to make valuable compounds depends on different environmental parameters such as salinity, light, temperature and type of strain. It is also possible to produce a specific pigment in *spirulina* with the changing environmental conditions and physical and chemical stresses.

Conclusion

Given the high cost of cancer treatment and the emergence of low-income groups, public education and nutritional behavior can prevent the occurrence of

many new cancers. Based on the results, *Spirulina* algae Platensis can be considered as a rich and new source of natural antioxidants as a food additive in the food industry. In general, the presence of compounds such as carotenoids, chlorophyll and Phycobilyproteins, and total antioxidant capacity and free radical inhibitory ability showed that *Spirulina* Iranian strain has antioxidant properties and, due to its high antioxidant load, could be a market food ingredient that will be effective in controlling the cancer tsunami.

Recommendation

With the increasing incidence of cancer in advanced societies and developing countries, it is recommended that, given the anti-cancer potential, these microalgae in troubled areas should be included in the family basket of foods.

References

Ahmad, M., Kulshreshtha, J. and Singh, G., 2011. Growth and pigment profile of *Spirulina platensis* isolated from Rajasthan, India. *Research Journal of Agricultural Sciences*, 2(1), pp. 83-86. https://doi.org/10.1007/978-3-031-45523-0_3

Alagawany, M., Ayman, E., Taha, A., Noreldin, A., El-Tarabily, K.A. and Abd El-Hack, M.E., 2021. Nutritional applications of species of *Spirulina* and *Chlorella* in farmed fish: A review. *Aquaculture*, 542P. 736841. ISSN 0044-8486. https://doi.org/10.1016/j.aquaculture.2021.736841

Amin, M., ul Haq, A., Shahid, A., Boopathy, R. and Syafiuddin, A., 2024. *Spirulina* as a Food of the Future. In: Mehmood, M.A., Verma, P., Shah, M.P. and Betenbaugh, M.J. (eds) *Pharmaceutical and Nutraceutical Potential of Cyanobacteria*. Springer, Cham. pp. 53-83. https://doi.org/10.1007/978-3-031-45523-0_3

Anusree, M.K., Leela, K.M., Sreehari, M., Raj, S., Sreenikethanam, A. and Bajhaiya, A.K., 2023. Chapter 14 - Marine microalgae: an emerging source of pharmaceuticals and bioactive compounds. In: Meena, S.N., Nandre, V., Kodam, K. and Meena, R.S. (eds) *New Horizons in Natural Compound Research*. Academic Press, pp. 251-265. ISBN 9780443152320. https://doi.org/10.1016/B978-0-443-15232-0.00025-4

Apak, R., Güçlü, K., Özyürek, M. and Karademir, S.E., 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry*, 52(26), pp. 7970-7981.

Arunakumara, K., Xuecheng, Z. and Yijing, Z., 2007. Growth and pigment biosynthesis of *Spirulina platensis* as affected by Pb^{2+} concentrations. *Bangladesh Journal of Botany*, 36(2), pp. 177-179.

Asghari, A., Mohammadi, M., Mahmoodi, M. and Khosravi, A., 2016. A review on antioxidant properties of *Spirulina*. *Journal of Applied Biotechnology Reports*, 3(1), pp. 345-351.

Çelekli, A., Özbal, B. and Bozkurt, H., 2024. Challenges in Functional Food Products with the Incorporation of Some Microalgae. *Foods*, 13(5), 725P. https://doi.org/10.3390/foods13050725

Choudhury, A.K. and Biswas, R.K., 2022. Algal Phytochemicals from Different Algal Forms with an Emphasis on Genomic Insights into Their Nutraceutical and Pharmaceutical Applications. In: Swamy, M.K. and Kumar, A. (eds) *Phytochemical Genomics*. Springer, Singapore. pp. 175–215. https://doi.org/10.1007/978-981-19-5779-6_8

Colla, L.M., Reinehr, C.O., Reichert, C. and Costa, J.A.V., 2007. Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. *Bioresource Technology*, 98(7), pp. 1489-1493.

Danesi, E.D.G., Rangel-Yagui, C.O., Carvalho, J.C.M. and Sato, S., 2011. Growth and content of *Spirulina platensis* biomass chlorophyll cultivated at different values of light intensity and temperature using different nitrogen sources. *Brazilian Journal of Microbiology*, 42(1), pp. 362-373.

Ferruzzi, M.G. and Blakeslee, J., 2007. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutrition Research*, 27(1), pp. 1-12.

Giri, S.U., Hadapad, N.G., Akhade, A. and Bhilare, P., 2023. Algae as Superfood. In: Rathoure, A. and Khade, S. (eds) *Biomass and Bioenergy Solutions for Climate Change Mitigation and Sustainability*. IGI Global, pp. 129-147. https://doi.org/10.4018/978-1-6684-5269-1.ch008

Gupta, M., Dwivedi, U.N. and Khandelwal, S., 2011. C-Phycocyanin: an effective protective agent against thymic atrophy by tributyltin. *Toxicology Letters*, 204(1), pp. 2-11.

Jaime, L., Mendiola, J.A., Herrero, M., Soler-Rivas, C., Santoyo, S., Señorans, F.J., Cifuentes, A. and Ibáñez, E., 2005. Separation and characterization of antioxidants from *Spirulina platensis* microalga combining pressurized liquid extraction, TLC, and HPLC-DAD. *Journal of Separation Science*, 28(16), pp. 2111-2119.

Jha, S., Singh, V.K., Singh, A.P., Gupta, A., Rana, P. and Sinha, R.P., 2024. The Radiant World of Cyanobacterial Phycobiliproteins: Examining Their Structure, Functions, and Biomedical Potentials. *Targets*, 2(1), pp. 32-51. https://doi.org/10.3390/targets2010002

Jiménez, C., Cossío, B.R., Labella, D. and Niell, F.X., 2003. The feasibility of industrial production of *Spirulina* (*Arthrospira*) in Southern Spain. *Aquaculture*, 217(1), pp. 179-190.

Karkos, P.D., Leong, S.C., Karkos, C.D., Sivaji, N. and Assimakopoulos, D.A., 2010. *Spirulina* in clinical practice: evidence-based human applications. *Evidence-Based Complementary and Alternative Medicine*, 2011.

Kato, Y. and Hasunuma, T., 2021. Metabolic Engineering for Carotenoid Production Using Eukaryotic Microalgae and Prokaryotic Cyanobacteria. In: Misawa, N. (eds) *Carotenoids: Biosynthetic and Biofunctional Approaches*. Advances in Experimental Medicine and Biology, vol 1261. Springer, Singapore. https://doi.org/10.1007/978-981-15-7360-6_10

Kurima, K., Jimbo, H., Fujihara, T., Saito, M., Ishikawa, T. and Wada, H., 2024. High Myristic Acid in Glycerolipids Enhances the Repair of Photodamaged Photosystem II under Strong Light. *Plant and Cell Physiology*, pcae021. pp. 790–797. https://doi.org/10.1093/pcp/pcae021

Kumar, M., Kulshreshtha, J. and Singh, G., 2011. Growth and pigment profile of *Spirulina platensis* isolated from Rajasthan, India. *Research Journal of Agricultural Sciences*, 2(1), pp. 83-86.

Lafarga, T., Fernández-Sevilla, J.M., González-López, C. and Acién-Fernández, F.G., 2020. *Spirulina* for the food and functional food industries. *Food Research International*, 137. 109356. ISSN 0963-9969. https://doi.org/10.1016/j.foodres.2020.109356

Lefebvre, T., Talbi, A., Atwi-Ghaddar, S., Destandau, E. and Lesellier, E., 2020. Development of an analytical method for chlorophyll pigments separation by reversed-phase supercritical fluid chromatography. *Journal of Chromatography A*, 1612. 460643. ISSN 0021-9673. https://doi.org/10.1016/j.chroma.2019.460643

Maikai, V.A., 2010. In vitro and in vivo evaluation of anti-trypanosomal activity of stem bark of *Ximenia americana*. *International Journal of Biology*, 2(2), pp. 50-56.

Mikhailova, E.O., 2023. Selenium Nanoparticles: Green Synthesis and Biomedical Application. *Molecules*, 28(24), 8125P. https://doi.org/10.3390/molecules28248125

Mostolizadeh, S.S., Moradi, Y., Mortazavi, M.S. and Ghaeni, M., 2020. Effects of incorporation *Spirulina platensis* (Gomont, 1892) powder in wheat flour on chemical, microbial and sensory properties of pasta. *Journal of Food Science and Technology*, 19(1), pp. 410-420.

Nickavar, B., Alinaghi, A. and Kamalinejad, M., 2010. Evaluation of the antioxidant properties of five *Mentha* species. *Iranian Journal of Pharmaceutical Research*, pp. 203-209.

Pentón-Rol, G., Marín-Prida, J., Sarduy-Chávez, R.C. and Hernández-González, I., 2024. Chapter 16 - C-Phycocyanin and Phycocyanobilin for neuroprotection: a deep dive into the biological processes involved. In: de Oliveira, M.R. (ed) *Natural Molecules in Neuroprotection and Neurotoxicity*. Academic Press, pp. 385-401. ISBN 9780443237638. https://doi.org/10.1016/B978-0-443-23763-8.00016-6

Pokorny, J., Yanishlieva, N. and Gordon, M.H., 2001. *Antioxidants in Food: Practical Applications*. CRC Press.

Salgado, M., Silva, M.C.S., Fratelli, C., Braga, A.R.C., Lopes, T.B.G., Ferreira, E., da Silva, I.L.D., Paiva, L.S. and Votto, A.P.S., 2024. Bioactive C-phycocyanin exerts immunomodulatory and antitumor activity in mice with induced melanoma. *Toxicology and Applied Pharmacology*, 484. 116874. ISSN 0041-008X. https://doi.org/10.1016/j.taap.2024.116874

Sahil, S., Bodh, S. and Verma, P., 2024. *Spirulina platensis*: A Comprehensive Review of Its Nutritional Value, Antioxidant Activity and Functional Food Potential. *Journal of Functional Foods*, pp. 1-14.

Shalaby, E.A. and Shanab, S.M., 2013. Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of *Spirulina platensis*. *Journal of Applied Phycology*, 25(3), pp. 803-809.

Uba, K.I.N., Gaid, G.D., Perales, J.M.L. et al., 2024. From Laboratory to Production: Innovating the Small-scale Mass Production of *Spirulina* (*Arthrospira platensis*) with an Alternative Culture Medium and Refined Culture Conditions. *Agricultural Research*. Volume 13, pages 465–476. https://doi.org/10.1007/s40003-024-00709-7

Xu, L., Pan, W., Yang, G. et al., 2021. Impact of light quality on freshwater phytoplankton community in outdoor mesocosms. *Environmental Science and Pollution Research*, 28, pp. 58536-58548. https://doi.org/10.1007/s11356-021-14812-7