JPPIPA 11(4) (2025)

Jurnal Penelitian Pendidikan IPA

http://jppipa.unram.ac.id/index.php/jppipa/index

Amino Acid Content Profile and Antioxidant Activity Test of Spirulina Platensis Bioactive Protein Extract Using DPPH Method

Tahirah Hasan[1*], Nur Ida[2], Yasnidar Yasir[1]

[1] Program Studi Kimia, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Islam Makassar, Makassar, Indonesia
[2] Program Studi Farmasi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Islam Makassar, Makassar, Indonesia.

Received: December 06, 2024
Revised: February 18, 2025
Accepted: April 25, 2025
Published: April 30, 2025

Corresponding Author:
Tahirah Hasan
tahirah.dty@uim-makassar.ac.id

DOI: 10.29303/jppipa.v11i4.10916

Abstract: The study of Amino Acid Content Profile and Antioxidant Activity Test of Spirulina platensis Bioactive Protein Extract with DPPH Method has been conducted. This study aims to determine the protein content and IC_{50} value of Spirulina platensis bioactive protein extract with DPPH method. The research method includes extraction of Spirulina platensis powder using buffer A solvent. Determination of protein content with Lowry method using Bovine Serum Albumin (BSA) as standard solution and antioxidant activity test with DPPH method. The results of the analysis of protein content of Spirulina platensis extract obtained a level of 39.51%. The antioxidant activity test against DPPH free radicals showed that Spirulina platensis protein extract has antioxidant activity with IC50 value of 599.17 ± 3.75 ppm.

Keywords: Antioxidant; DPPH; Protein; Spirulina platensis.

Introduction

The development of the era causes most people to spend time working so that some people live an unhealthy lifestyle, coupled with human activities such as smoking, cooking, use of pesticides, burning fuel in engines and motor vehicles, and continuous exposure to ultraviolet rays, causing free radicals to spread everywhere (Pandya, 2023). Biochemists say that free radicals are a form of reactive oxygen compounds which are generally compounds that have unpaired electrons (Kaur et al., 2025; Sadiq, 2023).

Free radicals are also produced normally by the body as a result of biochemical processes, excessive free radicals can cause degenerative diseases, such as cardiovascular, cancer, atherosclerosis, and osteoporosis, therefore a compound is needed that can inhibit free radicals by consuming foods containing antioxidant compounds (Engwa et al., 2022; Winarsi, 2007). Nowadays, many kinds of natural ingredients have been found such as plants, spices, fruits that can be used as medicinal ingredients to cure a disease (Chaachouay & Zidane, 2024; Mayekar et al., 2021; Sun & Shahrajabian, 2023). As in the words of the Prophet Shallallaju Alaihi Wasallam that, "For every disease there is a cure. If a medicine is suitable for a disease, then the person is cured with the permission of Allah Ta'ala" (Cholissodin et al., 2021). Based on the information from the hadith above, it can be concluded that there is no disease that has no cure. When a disease appears, Allah creates an antidote along with the creation of the disease. So with the permission of Allah SWT all kinds of diseases can be cured".

Antioxidants are compounds that can neutralize free radicals (Martemucci et al., 2022; Unsal et al., 2021). The human body also naturally has an antioxidant system to counteract the reactivity of free radicals continuously, but if the number of free radicals in the body is excessive, additional antioxidants are needed from outside the body (Aribi & Hameed, 2025; Halliwell,

How to Cite:
Hasan, T., Ida, N., & Yasir, Y. (2025). Amino Acid Content Profile and Antioxidant Activity Test of Spirulina Platensis Bioactive Protein Extract Using DPPH Method. *Jurnal Penelitian Pendidikan IPA*, *11*(4), 195–201. https://doi.org/10.29303/jppipa.v11i4.10916

2024). There are three types of antioxidants, namely endogenous antioxidants, for example superoxidase dismutase, glutathione peroxidase and catalase, natural antioxidants such as tocopherol, vitamin C, beta-carotene, flavonoids and phenolic compounds, artificial antioxidants (synthetic) such as Butylated hroxyanisole (BHA), BHT, TBHQ PG and NDGA (Yunanto et al., 2009). Currently, antioxidants that are widely used in food ingredients are generally synthetic antioxidants, the use of synthetic antioxidants as food ingredients is not recommended by the Ministry of Health because it is suspected of causing cancer (carcinogenic) (Barus, 2009) so that other safe antioxidant alternatives are needed for use, one potential source is antioxidants derived from plants.

One of the commodities that has high potential in the development of herbal medicine is microalgae (Selvam et al., 2024). Microalgae are rich in sources of carbohydrates, proteins, enzymes and fiber (Demarco et al., 2022; Patel et al., 2021). Spirulina platensis is one type of microalgae. Spirulina platensis contains compounds such as carotenoid proteins, chlorophyll and sources of micronutrients (Bortolini et al., 2022; Kumar et al., 2022).

The content of Spirulina platensis compounds consists of protein compounds. Protein compounds consist of several types of amino acids. Glutathione (GSH) is a tripeptide consisting of the amino acids glutamate, cysteine and glycine. Glutathione as an intracellular antioxidant (antioxidant from the body's own cells), is also called a master antioxidant because GSH regulates the work of other antioxidants. The effectiveness of GSH in protecting the body from free radicals is much better than other antioxidants such as vitamins C and E (Blaner et al., 2021).

Several studies on the activity of algae as antioxidants, including protein extracts from Rhodophyta and Chlorophyta from Pari Island waters have the best antioxidant activity in Chlorophyta samples with an IC50 value of 1.6114 mg/ml (Kustiyah et al., 2019), the antioxidant activity of Oscillatoria pigments is stable at pH 7 and a temperature of 280C (Karseno et al., 2013), the antioxidant activity of Naviculla sp is very strong with an IC50 of 41.304 ppm, Oscillatoria sp is very strong with an IC50 of 23.401 ppm, Carteria sp is strong with an IC50 of 51.433 ppm (Kristian et al., 2015), The antioxidant activity of Spirulina fusiformis has an IC value of 1937.41 ppm (Ukhty, 2018). Based on the description above, a problem can be formulated, namely, what is the protein content of Spirulina platensis extract and whether Spirulina platensis protein extract has the potential as an antioxidant.

Method

The purpose of this study was to determine the protein content and IC50 value of spirulina (Spirulina platensis) protein extract using the DPPH method. Spirulina platensis samples were taken at the Center for Research and Development of Marine and Fisheries Product Processing and Biotechnology, Jember, East Java, Indonesia.

Preparation of Bovine Serum Albumin (BSA) Stock Solution

The preparation of Bovine Serum Albumin (BSA) Stock Solution with a concentration of 10 mg/mL was made by weighing 0.1 grams of bovine serum albumin (BSA) then dissolving it with 10 mL of distilled water, then diluting it to a concentration of 1 mg/mL by pipetting 1 mL of the solution and then adding enough water to reach a volume of 10 mL.

Measurement of BSA Maximum Wavelength

A stock solution of 0.32 mg/mL of BSA was taken and added enough water to reach a volume of 2 mL. Furthermore, the absorbance is measured with a UV-VIS spectrophotometer at a wavelength of 600-800 nm to obtain the maximum wavelength. The maximum wavelength is the wavelength at which maximum absorption occurs.

Wavelength Measurement of BSA Concentration Series

A series of bovine serum album (BSA) solution concentrations were made from the stock solution of 1 mg/mL with concentrations of 0.01 mg/mL, 0.02 mg/mL, 0.04 mg/mL, 0.08 mg/mL, and 0.16 mg/mL by pipetting the stock solution of 0.02 mg/mL, 0.04 mg/mL, 0.08 mg/mL, 0.16 mg/mL, and 0.32 mg/mL, then added with distilled water to a volume of 2 mL. Furthermore, the absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 660 nm. .

Extraction of Bioactive Spirulina platensis Proteins

A sample of 100 g of Spirulina platensis powder was added with 400 ml of cold buffer A solvent, then blended. The Spirulina platensis extract was then filtered with a Buchner funnel. The filtrate obtained was frozen and thawed 3 times and then centrifuged at 3500 rpm at a temperature of 40C for 30 minutes, then the volume of the supernatant obtained was measured and stored in a refrigerator.

Determination of Protein Content

Determination of protein levels is determined based on the Lowry method using bovine serum albumin (BSA) as a standard. Pipette 0.1 mL of the sample then add enough distilled water to 10 mL then pipette 0.2 mL of the 10 mL sample added with distilled

water to a volume of 2 mL after that put into a beaker, then add 2.75 mL of Lowry B reagent, homogenize then leave for 15 minutes at room temperature, then add 0.25 mL of Lowry A reagent, homogenize and leave for 30 minutes at room temperature. The absorbance is measured at a wavelength of 660 nm (Alamgeer et al., 2017). Determination of protein levels is done by substituting the absorbance of the solution into the regression equation of the calibration curve of the protein standard solution. Linear regression equation: Y = bx + a

Preparation of DPPH Solution

DPPH solution 0.4 mM is made by weighing 15.8 mg of DPPH crystals and dissolving it with a little methanol p.a in a beaker then put into a 100 ml volumetric flask, then the volume is filled with pro-analysis methanol to the limit mark (Azizah, 2023).

DPPH Wavelength Measurement

The blank solution was made by pipetting 1 ml of 0.4 mM DPPH solution then making the volume up to 5 ml with pro-analysis methanol, measuring the absorbance with a UV-VIS spectrophotometer at a wavelength of 500-600 nm, so that the maximum wavelength was obtained (Azizah, 2023). The maximum wavelength is the wavelength at which maximum absorption occurs.

Making Spirulina platensis Mother Solution. The 5000 ppm Spirulina platensis mother solution was made by diluting a 197,570 ppm protein extract sample of 0.51 mL then making the volume up to 20 mL with p.a methanol. Measurement of Antioxidant Activity of Spirulina platensis Extract using DPPH Method

The stock solution was made into a series of concentrations of 200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm by pipetting the stock solution of 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1.0 mL, respectively, into a 5 mL volumetric flask wrapped in aluminum foil. Then 1 ml of 0.4 mM DPPH solution was added and the volume was made up with pro-analysis methanol to the limit mark, then closed and left for 30 minutes at room temperature and in a room protected from sunlight. Furthermore, the absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength of 515 nm (Azizah, 2023).

Making Ascorbic Acid Stock Solution

Ascorbic acid solution is made 1000 ppm as stock by weighing 0.01 grams of ascorbic acid dissolved in pro-analysis methanol while homogenizing, then the volume is made up with pro-analysis methanol to 10 ml. A 50 ppm dilution is made by pipetting 1 mL of ascorbic acid from the stock solution then making up the volume with pro-analysis methanol to 20 ml. Re-dilution is carried out with a concentration of 5 ppm made by pipetting 1 mL of the solution then making up the volume with pro-analysis methanol to 10 mL. Measurement of Antioxidant Activity of Ascorbic Acid Reference Solution.

Testing the antioxidant activity of ascorbic acid solution was carried out by making a series of concentrations of 0.25 ppm, 0.5 ppm, 1 ppm, 2 ppm and 4 ppm by pipetting 0.25 mL, 0.5 mL, 1 mL, 2 mL, and 4 mL standard solutions into a 5 mL volumetric flask wrapped in aluminum voil. Then 1 ml of 0.4 mM DPPH solution was added and the volume was made up with pro-analysis methanol to the boundary mark, closed and left for 30 minutes at room temperature and in a room protected from sunlight. Furthermore, the absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 515 nm (Azizah, 2023).

Result and Discussion

Result

The results of the analysis of protein content and antioxidant activity test of Spirulina platensis using the DPPH method obtained the following research results:

Table 1. Data on the Results of BSA Solution Measurements

BSA (mg/mL)	V BSA (mg/ml)	V Total (mL)	Absorbance (λ= 660)
0.01	0.02	2	0.071
0.02	0.04	2	0.128
0.04	0.08	2	0.212
0.08	0.16	2	0.363
0.16	0.32	2	0.608

The results of the analysis of the protein content of Spirulina platensis extract using the Lowry Method can be seen in the following table:

Table 2. Results of the Analysis of the Protein Content of Spirulina platensis Extract

Sampel Code	Absorbance	Dilution Factor	Measurable Protein (mg/ml)
Simplo	0.750	1000	196.53
Duplo		1000	197.95
	0.755		
Triplo	0.756	1000	198.23

Table 3. Data from the Results of Analysis of Protein Content of Spirulina platensis Extract

Number of Samples (gram)	Supernatant volume (mL)	Protein Content (mg/mL)	Protein Content (ppm)	Protein Content (%)
100	200	197.57	197.570 ± 0.92	39.51

The results of measurement I and measurement II of antioxidant activity and IC50 value of Spirulina platensis protein extract using the DPPH method can be seen in the following table:

Table 4. Results of Measurement I of Antioxidant Activity of Spirulina platensis Extract using the DPPH Method

Concentration (ppm)	Absorbance (A)	Antioxidant Activity (%)	Value IC_{50} (ppm)
200	0.347	29.33	596.51
400	0.279	43.18	
600	0.239	51.32	
800	0.199	59.47	
1000	0.159	67.62	
Control	0.491		

Table 5. Results of Measurement II of Antioxidant Activity of Spirulina platensis Extract with the DPPH Method

Concentration (ppm)	Absorbance (A)	Antioxidant Activity (%)	Value IC_{50} (ppm)
200	0.350	28.72	601.82
400	0.284	42.16	
600	0.246	49.90	
800	0.195	60.29	
1000	0.155	68.43	
Control	0.491		

The results of measurement I, measurement II and measurement III of antioxidant activity and IC50 value of the Reference Solution (Ascorbic Acid) can be seen in the following table:

Table 6. Results of Measurement I of Antioxidant Activity of Vitamin C Reference Solution (Ascorbic Acid)

Concentration (ppm)	Absorbance (A)	Antioxidant Activity (%)	Value IC_{50} (ppm)
0.25	0.304	27.10	2.61
0.50	0.289	30.70	
1.00	0.27	35.25	
2.00	0.239	42.69	
4.00	0.15	64.03	
Control	0.417		

Table 7. Results of Measurement II of Antioxidant Activity of Vitamin C (Ascorbic Acid) Comparison Solution using the DPPH Method

Concentration (ppm)	Absorbance (A)	Antioxidant Activity (%)	Value IC_{50} (ppm)
0.25	0.301	27.82	2.48
0.5	0.287	31.18	
1.0	0.270	35.25	
2.0	0.239	42.69	
4.0	0.140	66.43	
Control	0.417		

Table 8. Results of Measurement III of Antioxidant Activity of Vitamin C (Ascorbic Acid) Comparison Solution using the DPPH Method

Concentration (ppm)	Absorbance (A)	Antioxidant Activity (%)	Value IC_{50} (ppm)
0.25	0.300	28.06	272
0.50	0.280	32.85	
1.00	0.272	34.77	
2.00	0.240	42.45	
4.00	0.160	61.63	
Control	0.417		

Discussion

The sample used in this study was Spirulina platensis obtained from the Center for Research and Development of Marine and Fisheries Product Processing and Biotechnology, Jember, East Java, Indonesia. Spirulina platensis samples were extracted using cold buffer a solution. The composition of buffer a consists of triton X-100 which functions to assist the cell lysis process and β-mercaptoethanol functions to prevent the oxidation process in the sulfhydryl group of the protein (Pulikkottil Rajan, 2024). Determination of the protein content of Spirulina platensis extract was carried out using the Lowry method with bovine serum album (BSA) as a standard. BSA is used as a standard because BSA also contains amino acids such as glutamate, cysteine and glycine, these three amino acids are precursors for the formation of glutathione which is responsible for antioxidants. Spirulina platensis Protein Extract in protein testing is added with Lowry B reagent. The materials used in Lowry reagent consist of $CuSO_4$ which functions to reduce phosphotungstate-phosphomyl phosphate, Na-K-tartrate functions to prevent the precipitation of cuprous oxide in Lowry B reagent, while Na_2CO_3 is used as a salt that coordinates the reaction in a basic atmosphere with NaOH (Lucarini & Kilikian, 1999). The results of the analysis of the protein content of Spirulina platensis extract obtained a protein content of 39.514%.

This study used the DPPH method to test the antioxidant activity of Spirulina platensis protein

extract. The DPPH method was chosen because it has several advantages, including easy, simple, fast, sensitive, suitable for polar compounds and the process only requires a small sample. The working principle of the DPPH method is through the mechanism of taking hydrogen atoms from antioxidant compounds by free radicals. DPPH compounds react with antioxidant compounds by taking hydrogen atoms from antioxidant compounds to obtain electron pairs (Molyneux, 2004). The parameter used to determine antioxidant activity is the IC50 value which is defined as the concentration of antioxidant compounds that causes a loss of 50% of the activity of oxidant compounds.

DPPH free radical scavenging reaction mechanism:

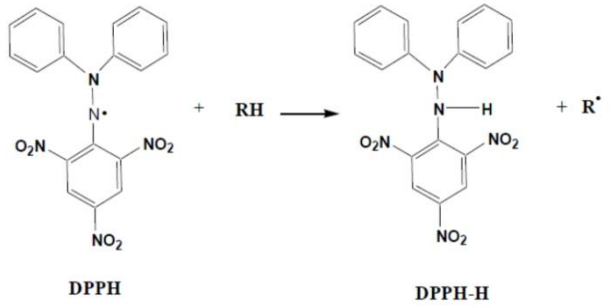

Figure 1. DPPH reduction of free radical scavenging compounds (Prakash et al., 2001).

DPPH solution is purple. When DPPH solution is mixed with a compound that can donate electrons, it makes DPPH become reduced. This reduced form makes DPPH lose its purple color. This is the basis for measurement based on the DPPH method because the intensity of the purple color is directly proportional to the concentration of DPPH. The decrease in color intensity that occurs is caused by the capture of one electron by the DPPH radical compound from the antioxidant substance. Antioxidants are compounds that can neutralize free radicals. The human body also naturally has an antioxidant system to counteract free radical reactivity (Chaudhary et al., 2023). Protein is one of the compounds that has biological activity, including as an antioxidant. The antioxidant activity of Spirulina platensis protein is possible because of the presence of hydrogen and sulfhydryl functional groups in proteins that can donate protons to free radicals, in addition, the composition of amino acids in Spirulina platensis extract can also affect antioxidant activity. Amino acids such as glycine, cysteine and glutamate are precursors for the formation of glutathione compounds that function as antioxidants in the body (Bon et al., 2023).

The results of the analysis of the amino acid composition of Spirulina platensis bioactive protein extract using the HPLC (High Performance Liquid Chromatography) method can be seen in Table 9.

Table 9. Amino Acid Composition of Bioactive Proteins of Spirulina platensis by HPLC Method

Asam amino	Kadar Asam Amino (%)
L-Histidine	0.14
L-Serine	0.44
L- Glutamin	tda
L-Asparagin	tda
L-Arginine	0.21
L-Glycine	0.47
L-Aspartic Acid	0.66
L-Glutamic acid	0.95
L-Threonine	0.48
L-Alanine	0.49
L-Proline	0.28
L- Triptopan	Tda
L-Cystine	0.08
L-Lysine	0.28
L-Tyrosine	0.35
L-Methionine	0.14
L-Valine	0.52
L-Isoleucine	0.45
L-Leucine	0.99
L-Phenylalanine	0.47

Description tda = not analyzed

The results of the antioxidant activity test using the DPPH method showed that the Spirulina platensis protein extract had an activity with an IC50 value of 599.17 ppm. The results obtained showed that the Spirulina platensis protein extract has the potential as an antioxidant but is still low compared to the antioxidant activity of the comparison. This is likely because the protein extract used is still in the form of a crude extract. The lysis factor with the freeze-thaw process can also cause the compound content in the cells not to be extracted optimally, thus affecting antioxidant activity.

Conclusion

Based on the data and discussion of the research results, it can be concluded that the protein content of Spirulina platensis extract is 39.51% and the antioxidant activity with the DPPH method obtained an IC50 value of 599.17 ppm. This shows that Spirulina platensis protein extract has the potential as an antioxidant.

Acknowledgments

The author team would like to thank all parties who have been involved in this research, both directly and indirectly.

Author Contributions

This article was written by three authors, namely T. H., N. I., and Y. Y. All authors worked together in carrying out each stage of the article writing.

Funding

This research received no external funding.

Conflicts of Interest
The authors declare no conflict of interest.

References

Alamgeer, Uttra, A. M., & Hasan, U. H. (2017). Anti-arthritic activity of aqueous-methanolic extract and various fractions of Berberis orthobotrys Bien ex Aitch. *BMC Complementary and Alternative Medicine*, *17*, 1–16. https://doi.org/10.1186/s12906-017-1879-9

Aribi, M. A. B., & Hameed, S. A. (2025). Antioxidants, free radicals, functional foods and their impact on human health (A Review). *Alnakhla Journal of Science (AJOS)*, *14*(1), 7–18. Retrieved from https://alnakhlajos.com/index.php/AJOS/article/view/66

Azizah, S. (2023). Uji Aktivitas Antioksidan Ekstrak Etanol Daun Jahe Merah (Zingiber Officinale Var. Rubrum) Dengan Metode ABTS. *Jurnal Kesehatan Borneo Cendekia*, *7*(1,), 132–141. Retrieved from http://journal.stikesborneocendekiamedika.ac.id/index.php/jbc/article/view/379

Barus, P. (2009). *Pemanfaatan bahan pengawet dan antioksidan alami pada industri bahan makanan*. Medan: Universitas Sumatra Utara.

Blaner, W. S., Shmarakov, I. O., & Traber, M. G. (2021). Vitamin A and vitamin E: will the real antioxidant please stand up? *Annual Review of Nutrition*, *41*(1), 105–131. https://doi.org/10.1146/annurev-nutr-082018-124228

Bon, L. I., Maksimovich, N. Y., & Burak, I. N. (2023). Amino Acids that Play an Important Role in the Functioning of the Nervous System Review. *Clinical Trails and Clinical Research*, *2*(3). https://doi.org/10.31579/2834-5126/026

Bortolini, D. G., Maciel, G. M., Fernandes, I. de A. A., Pedro, A. C., Rubio, F. T. V., Branco, I. G., & Haminiuk, C. W. I. (2022). Functional properties of bioactive compounds from Spirulina spp.: Current status and future trends. *Food Chemistry: Molecular Sciences*, *5*, 100134. https://doi.org/10.1016/j.fochms.2022.100134

Chaachouay, N., & Zidane, L. (2024). Plant-derived natural products: a source for drug discovery and development. *Drugs and Drug Candidates*, *3*(1), 184–207. https://doi.org/10.3390/ddc3010011

Chaudhary, P., Janmeda, P., Docea, A. O., Yeskaliyeva, B., Abdull Razis, A. F., Modu, B., Calina, D., & Sharifi-Rad, J. (2023). Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. *Frontiers in Chemistry*, *11*, 1158198. https://doi.org/10.3389/fchem.2023.1158198

Cholissodin, I., Soebroto, A. A., Muallif, M., Nagara, A. Y., Nova, R., & Ebtavanny, T. G. (2021). Design Framework as a Prototype of Islamic Medicine Engine to any Disease Especially for Covid-19 Based Al-Qur'an and Hadith Using Meta-Deep AI and Particle Swarm Optimization. *International Conference on Engineering, Technology and Social Science (ICONETOS 2020)*, 184–189. https://doi.org/10.2991/assehr.k.210421.026

Demarco, M., de Moraes, J. O., Matos, Â. P., Derner, R. B., de Farias Neves, F., & Tribuzi, G. (2022). Digestibility, bioaccessibility and bioactivity of compounds from algae. *Trends in Food Science \& Technology*, *121*, 114–128. https://doi.org/10.1016/j.tifs.2022.02.004

Engwa, G. A., Nweke, F. N., & Nkeh-Chungag, B. N. (2022). Free radicals, oxidative stress-related diseases and antioxidant supplementation. *Alternative Therapies in Health & Medicine*, *28*(1). Retrieved from https://shorturl.asia/tJjEr

Halliwell, B. (2024). Understanding mechanisms of antioxidant action in health and disease. *Nature Reviews Molecular Cell Biology*, *25*(1), 13–33. Retrieved from https://www.nature.com/articles/s41580-023-00645-4

Karseno, K., Handayani, I., & Setyawati, R. (2013). Aktivitas dan Stabilitas Antioksidan Ekstrak Pigmen Alga Oscillatoria sp. *Agritech*, *33*(4), 371–376. https://doi.org/10.22146/agritech.9531

Kaur, G., Rani, L., Sood, P., Sharma, V. K., Mittal, N., Devi, S., & Singh, T. G. (2025). Introduction to Free Radicals. In *Role of Free Radicals in Pathology* (pp. 1–27). Apple Academic Press. https://doi.org/10.1201/9781003560579-1

Kristian, R., Raharjo, S., & Sulastrianah, S. (2015). Uji aktivitas antioksidan mikroalga air tawar. *Medula: Jurnal Ilmiah Fakultas Kedokteran Universitas Halu Oleo*, *3*(1), 152092. https://doi.org/10.33772/medula.v3i1.2537

Kumar, A., Ramamoorthy, D., Verma, D. K., Kumar, A., Kumar, N., Kanak, K. R., Marwein, B. M., & Mohan, K. (2022). Antioxidant and phytonutrient activities of Spirulina platensis. *Energy Nexus*, *6*, 100070. https://doi.org/10.1016/j.nexus.2022.100070

Kustiyah, E., Saingin, B., Widodo, H., & Piti, V. (2019). Ekstraksi Protein dari Rhodophyta dan Chlorophyta Dari Perairan Pulau Pari Sebagai Alternatif Antioksidant. *Jurnal Jaring SainTek*, *1*(1), 24–31. https://doi.org/10.31599/jaring-saintek.v1i1.186

Lucarini, A. C., & Kilikian, B. V. (1999). Comparative

study of Lowry and Bradford methods: interfering substances. *Biotechnology Techniques*, *13*, 149–154. https://doi.org/10.1023/A:1008995609027

Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P., & D'Alessandro, A. G. (2022). Free Radical Properties, Source and Targets, Antioxidant Consumption and Health. *Oxygen*, *2*(2), 48–78. https://doi.org/10.3390/oxygen2020006

Mayekar, V. M., Ali, A., Alim, H., & Patel, N. (2021). A review: Antimicrobial activity of the medicinal spice plants to cure human disease. *Plant Science Today*, *8*(3), 629–646. https://doi.org/10.14719/pst.2021.8.3.1152

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol*, *26*(2), 211–219. Retrieved from https://shorturl.asia/RTcIm

Pandya, J. B. (2023). *General concept of environmental sciences*. Academic Guru Publishing House.

Patel, A. K., Singhania, R. R., Awasthi, M. K., Varjani, S., Bhatia, S. K., Tsai, M.-L., Hsieh, S.-L., Chen, C.-W., & Dong, C.-D. (2021). Emerging prospects of macro-and microalgae as prebiotic. *Microbial Cell Factories*, *20*(1), 112. https://doi.org/10.1186/s12934-021-01601-7

Prakash, A., Rigelhof, F., & Miller, E. (2001). Antioxidant activity medallion laboratories analitical progres. *Minnesota*, *19*(2), 3. Retrieved from https://www.yumpu.com/en/document/view/6157100/antioxidant-activity-medallion-labs

Pulikkottil Rajan, D. (2024). Extraction, isolation, and characterization techniques of structural proteins. In *Fish Structural Proteins and its Derivatives: Functionality and Applications* (pp. 37–72). Springer. https://doi.org/10.1007/978-981-97-2562-5_3

Sadiq, I. Z. (2023). Free radicals and oxidative stress: Signaling mechanisms, redox basis for human diseases, and cell cycle regulation. *Current Molecular Medicine*, *23*(1), 13–35. https://doi.org/10.2174/1566524022666211222161637

Selvam, J., Mal, J., Singh, S., Yadav, A., Giri, B. S., Pandey, A., & Sinha, R. (2024). Bioprospecting marine microalgae as sustainable bio-factories for value-added compounds. *Algal Research*, *79*, 103444. https://doi.org/10.1016/j.algal.2024.103444

Sun, W., & Shahrajabian, M. H. (2023). Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. *Molecules*, *28*(4). https://doi.org/10.3390/molecules28041845

Ukhty, N. (2018). Komponen Metabolit Sekunder dan Aktivitas Antioksidan Spirulina Fusiformis yang Dikultur Pada Media Campuran (Pupuk R1, Urea dan Katalis). *Jurnal Perikanan Tropis*, *5*(2), 161–168. https://doi.org/10.35308/jpt.v5i2.1035

Unsal, V., Cicek, M., & Sabancilar, I. (2021). Toxicity of carbon tetrachloride, free radicals and role of antioxidants. *Reviews on Environmental Health*, *36*(2), 279–295. https://doi.org/10.1515/reveh-2020-0048

Winarsi, H. (2007). *Antioksidan Alami & Radikal Bebas*. Yogyakarta: Kanisius.

Yunanto, A., Bambang, S., & Eko, S. (2009). *Kapita Selekta Biokimia: Peran Radikal Bebas pada Intoksikasi dan Patobiologi Penyakit*. Banjarmasin: Penerbit Pustaka Benua.