

## ANALYTICAL STUDY ON ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES OF MICROALGAE SPIRULINA

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### ABSTRACT

*Spirulina maxima is a food additive that uses microalgae's high protein and nutritional content. Spirulina includes antioxidants such  $\beta$ -carotene, tocopherols, and phenolic acids. This research assessed the antioxidant capabilities and efficacy of spirulina extract. The antioxidant activity of a methanolic spirulina extract was tested in vitro and in vivo before and after the experiment. For in vitro antioxidant activity testing, brain homogenate was treated at 37 degrees Celsius with and without the extract. The extract's IC50 is 0.18 mg/ml when made this way. This concentration reduces oxidation by 50%. The rats received 5 mg daily for two and seven weeks to assess their liver and plasma antioxidant capability. Plasma antioxidant capacity was measured by incubating brain homogenate at 37°C for an hour. Thiobarbituric acid reactive substances (TBARS) were measured in the control and experimental groups' liver oxidised compounds after two hours at 37 degrees Celsius. The treated group had 71% plasma antioxidant capacity after treatment, whereas the control group had 54%. Liver spontaneous peroxidation investigations across categories yielded similar results. Determining phenolic acids,  $\alpha$ -tocopherol, and  $\beta$ -carotene levels in spirulina extracts is crucial. Spirulina's antioxidant protection was identified in vitro and in vivo.*

**Keywords:** - Antioxidant activity, Microalgae Spirulina, Tocopherol

### INTRODUCTION

Spirulina, also known as Arthrospira in the scientific community, is a kind of symbiotic microalgae that is able to float freely and contains filaments that are shaped like spirals as well. On occasion, you could also hear it referred to as blue-green algae. It has the ability to produce photosynthetic enzymes. It was due to the pigment richness and photosynthetic capabilities of spirulina that it was first acknowledged as a member of the plant kingdom. Within the phylum of cyanobacteria, it is classed as belonging to either the Spirulinaceae or the Pseudonabaenaceae family. Later on, it was classified as belonging to the kingdom of bacteria. Spirulina is able to flourish in the highly salinized and acidic water that is found in the tropics and subtropics. All three species of edible spirulina—Spirulina platensis, Spirulina maxima, and Spirulina fusiformis—are now being researched because of the high nutritional content they have and the potential medical applications they may possibly have.

A number of studies have been conducted to investigate the nutritional value of spirulina as a food source. It is believed that the Mayans, Toltecs, and Kanembu civilization of Mexico used it around 400 years ago. The Chadians have been consuming spirulina cakes for more than a century; the algae that was used to make the cakes was

collected and dried in Lake Texcoco, which is located in Central Africa. Spirulina, which is harvested from Lake Kossorom (Chad), is used to make dishes like as cakes and broths, which are then sold at the neighbourhood market. In the past, nutraceuticals and functional foods have been the focus of a significant amount of research and development. This has been done in the goal of preventing or managing a wide range of illnesses and providing alternative sources of energy. As a result of the amount of essential elements that it contains, such as protein (60–70% by dry weight), minerals, vitamins (B12 and E), carotenoids, antioxidants, and phycocyanin, spirulina is considered to be a superfood. Since the middle of the 1970s, the Intergovernmental Institution for Micro-algae Spirulina Against Malnutrition (IIMSAM) has been promoting for the use of spirulina as a source of nutrient-rich food for pregnant women and their newborn children. Consuming spirulina, which is a nutraceutical food, has been demonstrated to relieve the symptoms of a number of different ailments. According to a number of publications, spirulina has the potential to assist in the management of hypercholesterolemia, allergies, cancer, toxicities resulting from environmental contaminants and drugs, cardiovascular disease, diabetes, and a number of inflammatory illnesses. In addition, the European Space Agency (ESA) and the National Aeronautics and Space Administration (NASA) have both said that spirulina is the best and most sustainable space food for long-term space missions due to the high concentration of macro and micronutrients that it contains.

In accordance with the dietary supplements information expert committee (DSI-EC) and the food and drug administration (FDA), spirulina is regarded as being safe for ingestion by human beings. As a result of its antioxidant properties, immune system regulating properties, and cholesterol-lowering capabilities, spirulina is being more recognised as a functional food of increasing popularity. Species of superoxide, hydroxyl, and non-free radicals, such as hydrogen peroxide, have the potential to cause harm to cells and metabolism, accelerate the ageing process, and potentially cause cancer. A variety of antioxidant molecules, on the other hand, may be found in edible green plants, and these compounds have the ability to reduce or even prevent the oxidative damage that occurs.

## **Spirulina**

Spirulina formerly included *A. maxima* and *A. platensis*. Spirulina is the dried biomass of *A. platensis*, a photosynthetic bacterium of the Cyanobacteria and Prochlorophyta families. Spirulina and *Arthrospira* differ scientifically. Tropical and subtropical alkaline brackish and saline waters have yielded *Arthrospira* species. *A. platensis* is the most widespread *Arthrospira* species, occurring mostly in Africa and Asia. *A. maxima* may be in California and Mexico. Historical factors keep spirulina in use. *Arthrospira* species are filamentous, free-floating cyanobacteria with cylindrical, multicellular trichomes in an open left-handed helix. Their native habitat is high-pH tropical and subtropical lakes with high carbonate and bicarbonate concentrations. *A. platensis* is found in Africa, Asia, and South America, whereas *A. maxima* is in Central America. Most spirulina is grown in paddle-wheel-agitated open-channel raceway ponds.

## **OBJECTIVES**

1. The antioxidant and antibacterial properties of spirulina, which is a kind of microalgae, have been the subject of substantial research.
2. Spirulina platensis has both phytonutrients and antioxidants among its characteristics.

## METHOD AND MATERIALS

### Procurement of raw materials

Puducherry, India's culture collection was the source of the *S. platensis*, which was obtained from Aurospirulin. A phytoplankton net, specifically a No. 10 bolting silk cloth with a mesh size of 48  $\mu\text{m}$ , was used to capture the samples. These samples were then recovered by washing them with filtered seawater in order to eliminate any debris that may have been present. Following that, they were transferred to the laboratory in an aseptic setting, which was done in accordance with the approach that Kumar et al. specified. After that, the phytoplankton soup was prepared, and then the cultures were sown in Zarrouk medium to ensure that they remained in contact with one another. Finally, the crops were rinsed with filtered seawater via a net cloth.

### Restrictions on the species

In order to effectively isolate the microalgae *S. platensis*, the serial dilution procedure that was described by Kavisiri et al. was taken into consideration. The samples were then transferred to the containers that were designated for them. In order to determine whether or not any algae had developed in the flasks, the optical microscope was utilized every two days. The flasks that had demonstrated growth were used to make a serial dilution of Zarrouk's medium, if any had been successful in doing so. In addition to performing routine inspections under microscopic identification, subcultures were kept in test tubes, and the purity of the culture was certified by the use of a frequent plating procedure.

### Cultivation of *S. platensis* in a significant quantity

In order to conduct the research, a conical flask with a capacity of 250 millilitres was filled with growing *S. platensis* and 100 millilitres of Zarrouk's medium. At a temperature of thirty degrees Celsius, with a pH ranging from eighteen to eleven, and with a light-to-dark cycle of around twelve to twelve, the culture was allowed to grow. Upon the completion of the treatment, the algal culture had attained a density of  $2 \times 10^5$  cells per millilitre, therefore ensuring a cell density of 100 cells per millilitre or higher.

### The production of spirulina extract with

The collection of the supernatants was accomplished by stirring a mixture consisting of forty grammes of spirulina powder blended with two hundred millilitres of ethanol and water for a period of two hours. The solvents were stored in the rotary evaporator, while the residues were stored at a temperature of 4 degrees Celsius for further research. For the purpose of this experiment, analytical-grade substances were implemented.

Utilising enzymes and several other ways, antioxidant activity is achieved.

Superoxide dismutase (SOD) activity is a molecule.

Beauchamp and Fridovich were the ones who determined the SOD activity. Following a 15-minute centrifugation at 1,600 revolutions per minute, the spirulina extract was homogenized in a phosphate buffer with a pH of 7.8 and

a concentration of 50 mmol/L using a Polytron homogenizer. Following the addition of 20 ml of a pyrogallol solution with a concentration of 10 mmol/L to various amounts of the extract supernatants, we utilised a spectrophotometer to determine the rate of autoxidation at 420 nm. A measure of the enzyme's ability to prevent pyrogallol from being autoxidated by fifty percent is the superoxide dismutase (SOD) activity, which is expressed as SOD per milligramme of protein.

The catalase (CAT) enzyme works.

After this, Kar and Mishra conducted an experiment to determine the catalase activity, adding a few modifications. An isotonic buffer with a pH of 7.4 was utilised in order to attain homogenization of the spirulina extracts. The homogenate was spun in a centrifuge for ten minutes at a speed of one thousand revolutions per minute. In a test mixture consisting of 980  $\mu$ l, 20  $\mu$ l of extracts that had been diluted 100 times were mixed with the supernatant. This mixture was then added to 900  $\mu$ l of 10mmol/L hydrogen peroxide, 50  $\mu$ l of Tris HCl buffer with a pH of 8.0, and 30  $\mu$ l of water that had been well cleaned. Spectrophotometric analysis was performed at 240 nm to evaluate the rate of H<sub>2</sub>O<sub>2</sub> breakdown. Within the context of quantifying the activity of CAT, units of k/mg protein are utilised, where k represents the first-order rate constant.

GPx, which stands for glutathione peroxidase, is an enzyme.

Mills' method underwent certain modifications in order to make it possible for it to examine the behaviour of GPx. As a result of the degradation of H<sub>2</sub>O<sub>2</sub> by GPx in the presence of GSH, the amount of GSH is reduced. In the following step, 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) is utilised in order to ascertain the residual GSH. At a temperature of 37 degrees Celsius, an incubation mixture was utilised that included elements such as 80 mM sodium phosphate buffer with a pH of 7.0, 80 mM EDTA, 1 mM NaN<sub>3</sub>, 0.4 mM GSH, 0.25 mM H<sub>2</sub>O<sub>2</sub>, and the extracts. After the extraction process had been completed for three minutes, a precipitation solution that included metaphosphoric acid was added to this solution. In order to determine the amount of GSH present in the protein-free filtrate, a solution consisting of 0.4M Na<sub>2</sub>HPO<sub>4</sub> and 1mM DTNB in 1% trisodium citrate was used. An absorbance measurement was taken of the solution at a wavelength of 412 nm. As a result of the fact that H<sub>2</sub>O<sub>2</sub> oxidises GSH in a non-enzymatic manner during the incubation process, a blank was performed in parallel with the samples. For the purpose of measuring enzyme activity, GPx per milligramme of GSH consumed per minute was the unit of measurement.

Glutathione (GSH) activity that is more low than normal

The GSH content was possible to be determined by Silber et al. by the reaction of algal buffer extracts with 5,5'-dithiobis-2-nitrobenzoic (DTNB). Homogenization of the algal extract was accomplished with the use of Tris HCl buffer (50 mmol/L, pH 7.4). The homogenate was spun in a centrifuge for twenty minutes at a speed of 10,000 revolutions per minute, and then again for sixty minutes at a speed of 100,000 revolutions per minute. When 0.5 ml of material supernatant was combined with 7.9 ml of methanol, 1.5 ml of 0.2 mol/L Tris HCl buffer (20 mmol/L EDTA, pH 8.2), and 0.1 ml of 0.01 mol/L 5,5'-dithiobis-(2-nitrobenzoic acid were added to the mixture. Over the course of thirty minutes, the ingredients were cooked in an oven that had been prepared to 37 degrees Celsius and stirred on occasion. After incubation, the mixture was centrifuged at a speed of 3,000 revolutions per minute for

fifteen minutes, and the absorbance of the supernatant that was produced was measured at a wavelength of 412 nanometers. For the purpose of calculating the GSH concentrations, a regular curve was created using various amounts of GSH that were already known.

The approach developed by Roe and Kuether was utilised in order to acquire an estimation of the amount of vitamin C ascorbic acid. After combining 0.5 grammes of spirulina extract with 10% of 1.5 millilitres of ice-cold TCA, the mixture was centrifuged for ten minutes at a speed of 1800 revolutions per minute. Next, 0.1 millilitres of thiourea-copper sulphate reagent (DTC) was added to 0.5 millilitres of the supernatant after the mixture had been thoroughly mixed. After three hours of incubation at 37 degrees Celsius, 0.75 millilitres of ice-cold 65% hydrogen sulphide was introduced to the tubes, and another half an hour was allowed for the tubes to cool down. Using the same procedure as a blank that contains 0.5 ml of 10% TCA, a series of solutions that include 10-50 ml of ascorbic acid are prepared to a concentration of 0.5 ml. These solutions are then used to replicate the blank. The wavelength of 520 nm was utilised in order to determine the hue that was produced. In terms of  $\mu\text{mol}/\text{mg}$  protein, the amount of ascorbic acid that was present in the materials was assessed.

The Albert et al. approach was utilised in order to ascertain the amounts of vitamin E. The estimation of vitamin E took place. I mixed the extracts, 1.5 ml of ethanol, and 2.0 ml of petroleum ether together, and then I centrifuged them for five minutes at a speed of one thousand revolutions per minute. At a temperature of 80 degrees Celsius, the liquid that was above was evaporated to dryness. Following the addition of 0.2 millilitres of butanol to the ultraviolet visible spectrophotometer (UV-Visible spectrophotometer UV 2450 B, obtained from LabTech), the vivid red hue was observed at 520 nanometers. We used  $\beta$ -tocopherol as a control in our experiment. In order to determine the amount of  $\beta$ -tocopherol present in the extract, the concentration was measured in grammes of protein per millilitre.

### Quantitative Phytochemical Analysis

#### The determination of the total phenolic content

A determination was made about the total soluble phenolics of the spirulina extracts by using the approach that was defined by Swain and Hill. It was placed in a dark container at 0 degrees Celsius for twenty-four hours after being submerged in ten millilitres of ethanol that was eighty percent. After going through three rounds of re-extraction, the extracted substance was brought down to a volume of fifty millilitres using eighty percent ethanol. After the Folin-Denis reagent and the extract had been well combined and the test tube had been violently shaken, one millilitre of the extract was added to the mixture. Following the completion of the chilling process, the solution was left to accumulate at room temperature for a full night before being filtered. After heating the mixture for three minutes, one millilitre of saturated sodium bicarbonate, which is equivalent to thirty-three grammes of anhydrous salt, was dissolved in one hundred millilitres of deionized water. A spectrophotometer (UBVisible spectrophotometer UV 2450 B, LabTech) was utilised in order to determine the absorbance at the wavelength of 725 nm. A gallic acid standard curve was employed in order to determine the total amount of phenols that were soluble in the solution. Gallic acid equivalents per gramme of dry sample weight were the method that we utilised in order to determine the total phenols.

### Analysis of the Total Flavonoids Captured

For the purpose of determining the total flavonoid content, Quettier-deleu and colleagues utilised a colorimetric estimation method. This mixture contains 0.1 millilitres of 10% aluminium nitrate, 4.3 millilitres of 80% ethanol, 1 millilitre of spirulina extract, and 0.1 millilitres of potassium acetate in water at a concentration of 1 concentration. After maintaining the temperature at room temperature for forty minutes, we measured the absorbance at a wavelength of 415 nm. It was necessary to compare the quantity of rutin equivalents (RE mg/g) present in the extract in order to ascertain the total flavonoids from the sample.

### The Evaluation of Tannin

The method that was established by Reynolds and colleagues is utilised extensively for the estimation of tannin. The extract solution was blended after one to two drops of ferric chloride solution were added to one millilitre of distilled water. The mixture was then observed for the presence of a blue or green-black coloration.

### Calculation of Potential Carbohydrates

It was determined that the phenol-sulfuric acid method, which was developed by Masuko and colleagues, was useful for the analysis of carbohydrates. The sample was first dried with air, and then it was hydrolyzed in boiling water with 1N hydrochloric acid for a period of six hours. After the solution had been filtered and neutralised, one hundred millilitres of distilled water was added to it. For the purpose of determining the total amount of reducing sugars, a UV-VIS spectrophotometer was used to heat one millilitre of the sample with an alkaline potassium ferricyanide reagent at 420 nanometers (nm).

### Calculation of Protein Content

The Bradford assay was used to determine the amount of protein present in spirulina extract. The Coomassie brilliant blue dye (G 250) binding method was utilised, and bovine serum albumin was used as the reference curve. The measurement was performed at 595 nm.

### Analysis of data using statistical methods

For each sample, the findings were calculated using the IBM SPSS 25.0 version software. The results were provided as the mean plus or minus the standard error. Student t-tests were used to assess whether or not the difference between the ethanolic and aqueous extracts was statistically significant. This was done when the p-value was less than or equal to 0.05.

## RESULTS AND DISCUSSION

Antioxidants protect cells from free radicals by donating hydrogen and electrons, breaking down peroxide and singlet oxygen, inhibiting enzymes, promoting synergy, and chelating metals. Enzymatic and non-enzymatic antioxidants neutralise reactive oxygen species (ROS) within and outside cells.

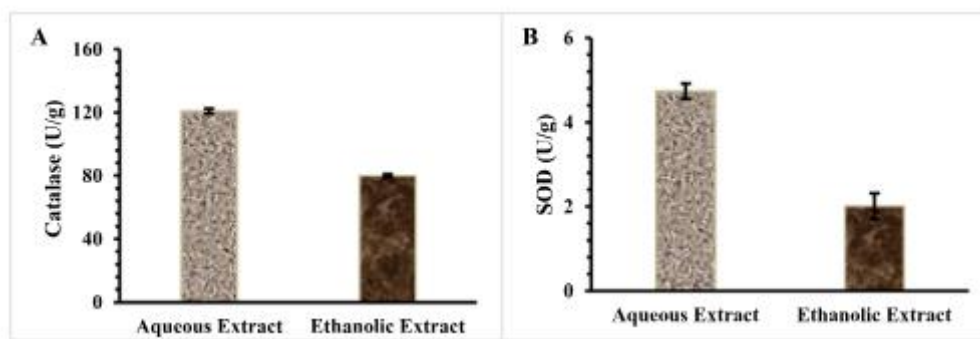


Possible antioxidant capabilities of *S. platensis* Antioxidants reduce free radical damage by giving them an electron. Antioxidants delay or prevent cell damage by scavenging free radicals. Spirulina's antioxidant properties have garnered attention, and various studies have shown that it may greatly lower oxidative stress. In addition to phycocyanin and beta-carotene, spirulina contains other minerals and vitamins that boost its antioxidant and protective qualities. ROS cause metabolic problems, tissue damage, and cell death. These problems result from DNA, RNA, protein, and lipid damage. Oxidative stress and ROS can affect cancer, hypertension, diabetes, atherosclerosis, and ischemic illness. MDA, LPOs, and 4-hydroxynonenal are major oxidative stress indicators.

Enzymatic and non-enzymatic spirulina extracts from ethanolic and aqueous plants were tested for antioxidant capabilities. Figure 1-2 and Table 1-2 show the antioxidant, catalase, glutathione peroxidase, reduced glutathione, vitamin C, and vitamin E activity for *S. platensis* aqueous and ethanolic extracts.

Three of the most essential antioxidant enzymes in algal cells are SOD, CAT, and GPx. Many believe that superoxide dismutase (SOD) is the earliest natural defence mechanism for stressed cells. It does this by converting damaging superoxide anion ( $O_2^-$ ) into oxygen and hydrogen peroxide. Antioxidant enzymes neutralise hydrogen peroxide and inhibit peroxynitrite formation in cells.

Overexposure to light and carbon deprivation are two stress factors that enhance ROS generation. Both plants and animals use CAT for proximal  $H_2O_2$  scavenging. An organism may produce antioxidant enzymes to defend against a hazardous environment. the water extract had greater CAT activity than the equivalent ethanolic extract. Low doses of catalase do not eliminate hydrogen peroxide from cells, while large levels do. A range of defence mechanisms activated by illnesses and stress depend on CAT activity. Reducing overall hydrogen peroxide levels indicates that improved CAT activity has avoided ROS buildup. the two extracts had significant differences in SOD activity. Due to its increased acetic acid content, the aqueous extract exhibited 4.735 U/mg SOD activity, while the ethanolic extract had 2.009 U/mg. However, there are still



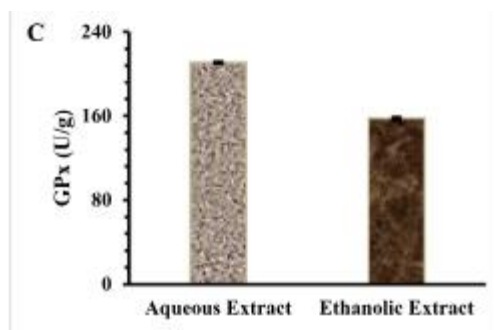


Figure 1. Graphical representation of Enzymatic antioxidant (A. Catalase, B. Superoxide dismutase, and C. Glutathione peroxidase) activity of aqueous and ethanolic extract of *S. platensis*.

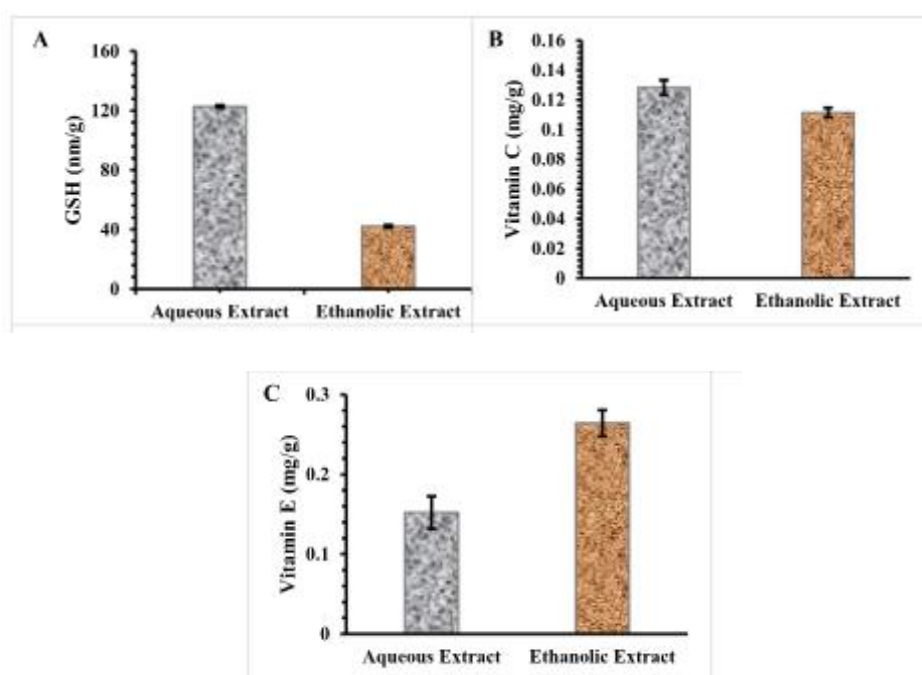


Figure 2. Graphical representation of Non-Enzymatic antioxidant (A. Reduced glutathione, B. Vitamin C, and C. Vitamin E, and) activity of aqueous and ethanolic extract of *S. platensis*.

Table 1.1 Enzymatic antioxidant potential of *S. platensis* in aqueous and ethanolic extract

	Aqueous Extract	Ethanolic Extract
Catalase a (U/g)	120.827±0.672	79.907±0.447
SODb (U/g)	4.735±0.179	2.009±0.305*
GPxc (U/g)	211.098±0.563	157.878±0.790*



a =  $\mu$ mole of H<sub>2</sub>O<sub>2</sub> decomposed / min/g

b = amount of enzyme required for 50% inhibition of nitro blue tetrazolium (NBT) reduction.

c =  $\mu$ g of glutathione utilized / min/g.

The results are shown as the mean $\pm$ SE. The significant difference is indicated by \*( $P \leq 0.005$ ).

The relationship between protein content and SOD activity is inverse when comparing the percentage inhibitions of various SOD activities to their volume. SOD activity has been studied by many authors via experiments. Al Zoubi found 2.30 percent inhibition from *S. maxima* extract after seven days of culture. Temperate phytoplankton cultures had different inhibition levels. The increased activity of superoxide dismutase (SOD) inhibited SOD 32% for *Nannochloropsis* at 1°C and 5% for *Synechococcus* at 30°C. *Synechococcus* inhibited SOD 99%. SOD has been found in anoxygenic photosynthetic bacteria for chromium and chlorobium. Before cyanobacteria appeared, anaerobic bacteria relied on superoxide dismutase (SOD) to scavenge low dioxygen levels in the air, according to Kanematsu and Asada. Darbyshire and Henry observed that cyanobacteria (*Anabaena cylindrica*) had lower SOD levels than vegetable cells. Since this enzyme is commonly linked to oxygen, they assumed it was because cyanobacteria had less oxygen. The photosynthetic cyanobacterium *S. platensis* is a good model organism for studying how the environment affects plant and microorganism oxidative stress.

*S. platensis* increased reduced glutathione and maintained cellular antioxidant enzyme activity (total GPx, GPx-Se, and GR). Due to environmental stress, *S. platensis* may produce more antioxidants. Hussein et al. investigated if *S. platensis* cells could be grown in hydrogen peroxide-containing mediums to increase bioactive substances. The present experiment shows that the aqueous extract had a greater GPx than the other extracts (Figure 1(C) and Table 1). NADPH-dependent GPx activity protected cyanobacterium membranes against lipid peroxidation. GPx in spirulina protects against oxidative stress as an antioxidant. In addition, *S. platensis* antioxidant enzymes including catalase, peroxidase (PX), superoxide dismutase (SOD), and ascorbate peroxidase (APx) increased significantly and linearly with hydrogen peroxide levels.

Kurutas found a significant correlation between rising H<sub>2</sub>O<sub>2</sub> levels and increased levels of hydrophilic antioxidants (glutathione and ascorbic acid) and cellular lipophilic antioxidants (total carotenoids and  $\alpha$ -tocopherol). Glutathione peroxidase (GPx) and ascorbate peroxidase scavenge hydrogen peroxide. GPx in mammals reduces alkyl and lipid hydroperoxides with selenocysteine. Mammalian GPx retains this residue. Glutathione peroxidases oxidise glutathione to reduce peroxides. Glutathione reductase reduces glutathione's oxidised state by donating electrons. Many GPx gene homologs are found in higher plants. *S. platensis* is a popular nutritional supplement for humans, poultry, and fish with dietary requirements due to its high protein, fatty acid, mineral, and vitamin content. Its cysteine residue makes it less efficient than mammalian GPx, although having the same structure. Following what was mentioned in

**Table 1.2 Non-enzymatic antioxidant potential of *S. platensis* in aqueous and ethanolic extract.**

	<b>Aqueous Extract</b>	<b>Ethanollic Extract</b>
Vitamin C (mg/g)	0.128±0.005	0.111±0.003
Vitamin E (mg/g)	0.152±0.010	0.264±0.163*
Reduced GSH (nm/g)	122.758±0.793	42.081±0.913

The results are shown as the mean±SE. The significant difference is indicated by \*( $P \leq 0.005$ ).

**Table 1.3 Phytonutrient contents of *S. platensis* in aqueous and ethanollic extract.**

	<b>Aqueous Extrac Aqueous Extrac</b>	<b>Ethanollic Extract</b>
T. Phenol (mg/g)	9.919±0.449	3.476±0.362
Flavonoid (mg/g)	1.047±0.004	0.585±0.054*
Tannin (mg/g)	0.792±0.006	0.568±0.061*
Carbohydrates (g/g)	0.153±0.008	0.248±0.009
Protein (g/g)	0.707±0.046	0.775±0.047*

The results are shown as the mean±SE. The significant difference is indicated by \*( $P \leq 0.005$ ).

The findings show that *S. platensis* aqueous and ethanollic extracts contained considerable vitamin C and E. Figures (A and B) show this. Aqueous extract had the greatest vitamin C content at 0.128 mg/g, whereas ethanollic extract had the highest vitamin E content at 0.264 mg/g, which was statistically significant. Vitamin levels in this research match those of other *Arthrospira* species. *Spirulina* vitamins and coenzymes regulate bone marrow hematopoietic oxidative activity and haemoglobin synthesis.

GSH has several economic applications owing to its antioxidant capabilities. It excels in detoxification, immunological response, and ROS protection. The aqueous and ethanollic extracts' GSH activity differed greatly in this study, as shown in Table 2. At 42.081 nm/g, the ethanollic extract had the second-highest GSH content after the water-based extract (122.758). Non-enzymatic antioxidants need GSH, and free radical reduction requires ascorbic acid. Research indicates that glutathione may neutralise ROS such as hydroxyl radicals (OH), superoxide radicals ( $O_2 \cdot^-$ ), hydrogen peroxide ( $H_2O_2$ ), and singlet oxygen ( $^1O_2$ ), while protecting protein thiol groups from oxidation. *Spirulina* may oxidise glutathione to oxidised glutathione via reactive oxygen species (ROS) and ascorbate to MDA and DHAR under oxidative stress. Oxidised glutathione, malondialdehyde, and dehydroascorbate are converted into glutathione and ascorbate, which neutralise ROS [88]. Glutathione, the major

low-molecular-weight thiol in prokaryotic and eukaryotic cells, stores reduced sulphur from non-protein sources. In plant cells, phytonutrients are the main water-soluble antioxidants.

## CONCLUSION

In terms of antioxidant and phytonutrient characteristics, *S. platensis* demonstrated good results. In addition to having higher amounts of all other enzyme activities and phytonutrients, the aqueous extract has a significant degree of antioxidant activity. Vitamins have the potential to boost the health of whatever that they are applied to. It was discovered that the ethanolic extract had much higher levels of vitamins C and E. It has been shown that vitamins are responsible for promoting cell proliferation, inflammation, the formation of prostaglandins, the synthesis of cholesterol, and the regulation of blood pressure. Due to the high levels of phytonutrients and antioxidants that they contain, spirulina products may be available that are suitable for consumption on a regular basis. The term "wonder food supplement" has been bestowed to spirulina by a number of well-known dietary supplement organisations. Based on the findings of scientific study and further validation studies, it is anticipated that spirulina will be able to successfully complete the various stages of clinical trials that are required in order to be authorised by the United States Food and Drug Administration (USFDA) as a nutritional and dietary supplement. When it comes to herbal cosmetics, the phycocyanin pigment that is present in spirulina has been used in the production of a broad range of products, including cosmetics for the face, hair, and body. Among the potential applications of beta-carotene and other carotenoids in the future are the prevention of cancer in humans, the enhancement of egg-laying in chicken, the enhancement of meat production, and the addition of a supplement to aquaculture feed.

Because of the significant phytonutrient and antioxidant content of spirulina, it is conceivable to employ spirulina extracts for commercial purposes in the pharmaceutical, food additive, and cosmetic industries. In addition, it could one day be of assistance in the hunt for novel therapeutic components that have the potential to treat a broad variety of disorders.

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