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Functional evaluation of spirulina extract: antioxidant properties and immune regulation in aged mice models

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ABSTRACT

Background: *Spirulina platensis* is a cyanobacterium rich in protein, commonly regarded as a functional food due to its exceptional nutritional profile. Among its components, phycocyanin (a water-soluble pigment-protein complex) has gained attention for its strong antioxidant, anti-inflammatory, immunoregulatory, and liver-protective properties. Both oxidative stress and chronic inflammation are key drivers of the aging process and play major roles in the development of disease, namely metabolic syndromes and neurodegenerative disorders. Although spirulina has been widely studied for its health-promoting effects, its precise role in regulating systemic redox balance and modulating age-related immune responses remains insufficiently explored. This study aims to evaluate the antioxidant and anti-inflammatory activities of spirulina extract through in vitro assays and a murine aging model.

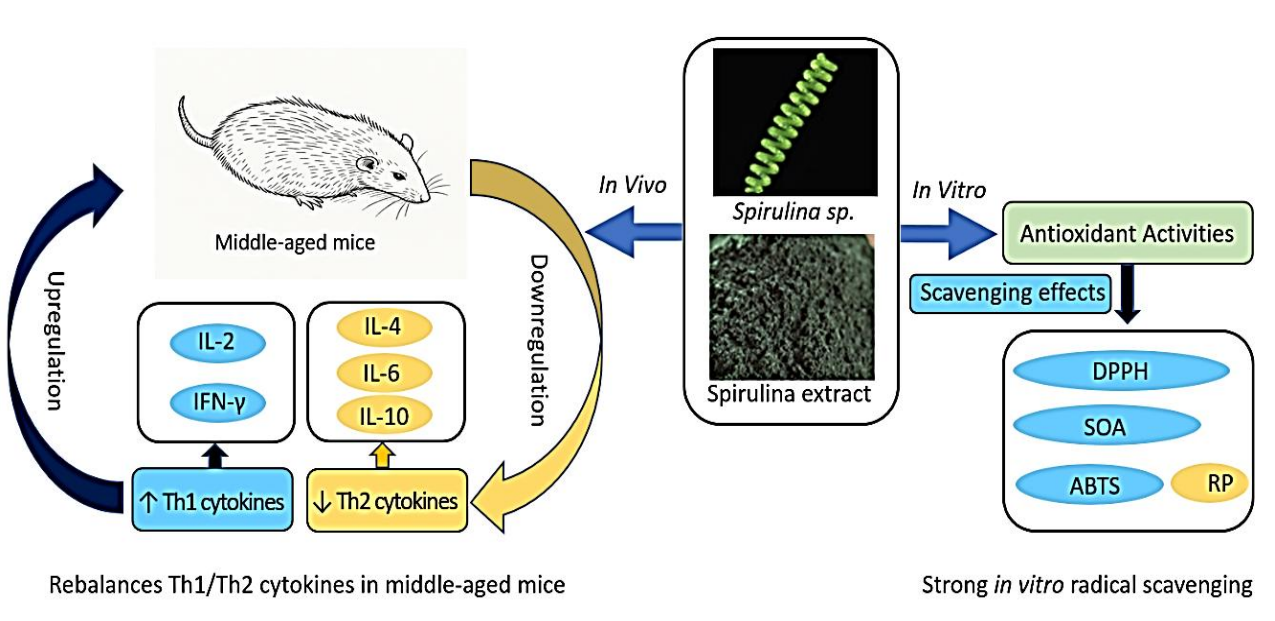
Methods: Spirulina extract was prepared via enzymatic cell wall degradation followed by fermentation. Antioxidant

activity was measured *in vitro* through four key assays: reducing power, total antioxidant capacity, DPPH radical scavenging, and superoxide anion scavenging. Additionally, young and middle-aged mice were orally administered spirulina extract at a dose of 7 mg per day for 28 days. Blood samples were collected to assess levels of GSSG and cytokines associated with Th1/Th2 immune regulation.

Results: *In vitro* assays showed strong antioxidant activities, particularly DPPH radical scavenging (88.58 ± 3.68%). Spirulina extract significantly increased the GSH/GSSG ratio in middle-aged mice, indicating improved antioxidant status, while no changes were seen in young mice. Additionally, spirulina upregulated Th1 cytokines and downregulated Th2 cytokines in middle-aged mice, with significant reductions in IL-6 and -10. Spirulina extract demonstrated potent antioxidant and immunomodulatory effects, alleviating oxidative stress and correcting Th1/Th2 imbalance during aging. These results support its potential as a supplement for promoting healthy aging in the mouse model.

Conclusion: Combining cell-based experiments with an aging mouse model, this research reveals new perspectives on spirulina's impact on maintaining redox balance and modulating immune function. The results indicate that spirulina extract could be a beneficial supplement for addressing oxidative stress and immune aging during midlife. Further clinical studies are warranted to explore its potential as a functional food or nutraceutical aimed at reducing age-associated immune dysfunction and inflammation.

Keywords: spirulina extract, antioxidant activity, cytokine, immune regulation.



Graphic abstract: Antioxidant properties and immune regulation of spirulina extract in aged mice

INTRODUCTION

Spirulina platensis is a highly nutritious cyanobacterium that has been widely utilized in the global health food and dietary supplement industries. It contains over 60% high-quality protein and is rich in a variety of active compounds, including β-carotene, lutein, and phycocyanin [1]. Among these, phycocyanin is a water-soluble phycobiliprotein predominantly located within the phycobilisomes, exhibiting natural fluorescence and high aqueous solubility. Phycocyanin is primarily composed of C-phycocyanin (CPC) and allophycocyanin (APC), both of which consist of chromophore-bearing α- and β-polypeptide chains, with CPC accounting for approximately 20% of the total phycobiliprotein content. Phycocyanin has also expanded into the cosmetics industry, further highlighting its commercial and functional value [2-3].

The value of phycocyanin extends beyond its use as a natural colorant. Phycocyanin demonstrates diverse pharmacological properties, notably its antioxidant, anti-inflammatory, and immune-regulating capabilities. Previous studies have demonstrated that phycocyanin effectively scavenges free radicals, inhibits HIV-1 replication within T cells, and possesses the potential to regulate cytokine production and mitigate inflammatory responses [3]. Phycocyanin can protect against inflammation and regulate macrophages in the mucosal immune responses in the Caco-2 cell models [4]. Proteins extracted from spirulina can attenuate inflammation triggered by lipopolysaccharide (LPS) by downregulating pro-inflammatory cytokines, like interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), a process linked to modulation of histone deacetylase (HDAC) activity [3]. Moreover, the extracts from *Spirulina platensis* have been reported to activate the Nrf2/HO-1 signaling pathway, thereby alleviating radiation-induced oxidative stress and DNA damage, suggesting a potential hepatoprotective effect [5, 6]. Recent animal studies have further demonstrated that spirulina extracts can ameliorate age-related vascular dysfunction through their antioxidant properties [7].

Spirulina species are rich in various bioactive functional components, such as phycocyanin, polysaccharides, and small peptides, which possess potent antioxidant, anti-inflammatory, and immunomodulatory properties [5-8]. However, these functional components are typically encapsulated within the rigid structure of algal cell walls, whose dense architecture and high mechanical strength make the efficient release and extraction of intracellular substances challenging [9]. This limitation has hindered the broader industrial application of algal bioactives [10-12]. Therefore, the development of mild cell-wall disruption and efficient extraction technologies is critical for improving the recovery yield and preserving the bioactivity of these valuable compounds [13].

In this study, the antioxidant capacity of spirulina extract was systematically evaluated. Furthermore, 3-month-old and 12-month-old mice were orally administered spirulina extract for 28 consecutive days, after which serum samples were collected to assess the concentrations of reduced and oxidized glutathione, as well as T helper cell-associated cytokines. This study aimed to investigate whether spirulina extract could alleviate oxidative stress and attenuate the pro-inflammatory responses associated with aging in middle-aged mice. Overall, the objective of this research was to validate the antioxidant and anti-inflammatory potential of spirulina extract both *in vitro* and *in vivo*, and to explore its regulatory effects on immune imbalance during aging, thereby demonstrating its functional benefits in living organisms.

Materials and Methods

***In vitro* antioxidant and anti-inflammatory activity of spirulina extract:** The spirulina extract used in this study

was kindly donated by Food Wealth Biotech Co (Taiwan). The antioxidant activities of the spirulina extract were evaluated using two different assays. The reducing power of the extract was determined based on the formation of Prussian blue, following previously described protocols [14,15]. The amount of Prussian blue generated positively correlates with the sample's reducing capacity, indicating stronger reducing power with higher absorbance. The total antioxidant capacity was assessed using the ABTS radical cation decolorization assay, according to established methods [16]. In addition, two assays were conducted to evaluate the free radical and reactive oxygen species (ROS) scavenging abilities. The DPPH radical scavenging capacity was measured based on the method described in previous literature [14,17]. The scavenging effect on superoxide anion radicals was evaluated following the method reported by Robak et al., assessing the ability of the extract to neutralize superoxide radicals [15].

Antioxidant and anti-inflammatory activity of spirulina extract in mice: All animal experiments were conducted in accordance with the guidelines of the Institutional Animal Care Committee in IMB, Academia Sinica, Taiwan. The committee recognizes that the proposed animal experiment follows the guidelines as shown in the Guide for Laboratory Animal Facilities and Care as promulgated by the Council of Agriculture, Executive Yuan, ROC. Animal usage adheres to the 3Rs principles to minimize animal use and suffering. To evaluate the *in vivo* antioxidant activity of the spirulina extract, young adult and middle-aged mice (n = 5 per group) were orally administered spirulina extract (7 mg per mouse) daily for 28 consecutive days. After the treatment period, serum samples were collected and analyzed for oxidized (GSSG) and reduced glutathione (GSH) levels (Abcam, No. 138881) [18].

To assess the anti-inflammatory activity, cytokine levels in the serum were analyzed. After 28 days of oral administration of spirulina extract (7 mg per mouse), mice were sacrificed, and serum samples were collected. Cytokine concentrations related to Th1 and Th2 responses were measured using enzyme-linked immunosorbent assay by the inflammation core facility of IMB.

Statistical analysis: Statistical comparisons between groups were performed using t-test. A p-value < 0.05 was considered statistically significant ($*p < 0.05$), and $p < 0.01$ was considered highly significant ($**p < 0.01$).

Results and Discussion

***In vitro* Antioxidant Activity of Spirulina Extract:** The major components of the spirulina extract are summarized in Table 1. The results of the *in vitro* antioxidant activity assays are presented in Table 2 and Figure 1. When treated with 1000 µg/mL of spirulina extract, the reducing power, total antioxidant capacity, DPPH radical scavenging activity, and superoxide anion radical scavenging activity were 35.35 ± 2.92, 60.85 ± 3.94, 88.58 ± 3.68, and 73.76 ± 3.15 %, respectively. Among these, the DPPH radical scavenging activity was the most prominent, followed by the scavenging activity against superoxide anions, whereas the reducing power exhibited the weakest performance. Furthermore, the IC_{50} values for the reducing power, total antioxidant capacity, DPPH radical scavenging activity, and superoxide anion scavenging activity were 1514.42 ± 85.71, 846.69 ± 49.81, 584.46 ± 32.15, and 697.87 ± 41.36 µg/mL. These results indicate that the spirulina extract possesses notable antioxidant activities, with particularly strong scavenging effects on DPPH radicals and superoxide anions.

Table 1. Composition of spirulina extract.

Composition	Content (w/w)
phycobiliprotein	2.3%
polysaccharide	32%
sulfated Polysaccharides	12.5%
oligopeptide	2.1%
superoxide dismutase	12500 unit/100g

Table 2. Summary of antioxidative effects of spirulina extract. *

Antioxidative effects	Antioxidant activity (%)
Reducing power	35.35 ± 2.92
Scavenging effect on ABTS radicals	60.85 ± 3.94
Scavenging effect on DPPH radicals	88.58 ± 3.68
Scavenging effect on superoxide anion radicals	73.76 ± 3.15

*The reducing power, scavenging effects on ABTS radicals, DPPH radicals, and superoxide anion radicals were investigated by adding 1000 g/mL extract from *Spirulina platensis*.

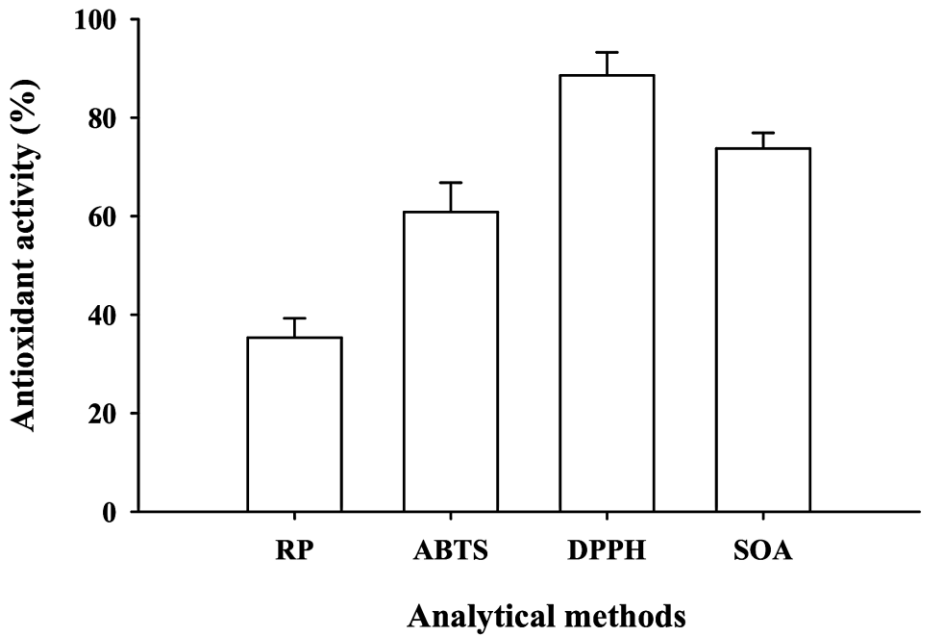


Figure 1. The antioxidative effects of spirulina extract. The reducing power (RP), scavenging effects on ABTS radicals, DPPH radicals, and superoxide anion radicals (SOA) were investigated.

***In Vivo* Antioxidant Activity of Spirulina Extract:** Under oxidative stress, the concentration of GSSG typically increases, whereas the level of GSH decreases. In this study, young adult and middle-aged mice (body weight approximately 30–35 g) were orally administered spirulina extract daily for 28 days. Serum samples were then collected to measure GSH and GSSG concentrations. As shown in Table 3, the baseline GSH concentration was lower in middle-aged mice compared to young mice. After spirulina extract administration, GSH levels in young

mice remained unchanged, whereas a significant increase in GSH concentration was observed in middle-aged mice (Figure 2). As shown in Figure 3, middle-aged mice exhibited notably higher GSSG levels compared to their younger counterparts. While spirulina extract had no measurable effect on GSSG levels in young mice, treatment in middle-aged mice led to a marked reduction in GSSG concentration. In addition, Figure 4 illustrates that the GSH/GSSG ratio, which was initially lower in middle-aged mice than in young ones, significantly increased following spirulina administration in the older group. In contrast, no significant change was detected in young mice. Collectively, these results indicate that spirulina extract enhances intracellular redox homeostasis and mitigates oxidative stress in aging mice.

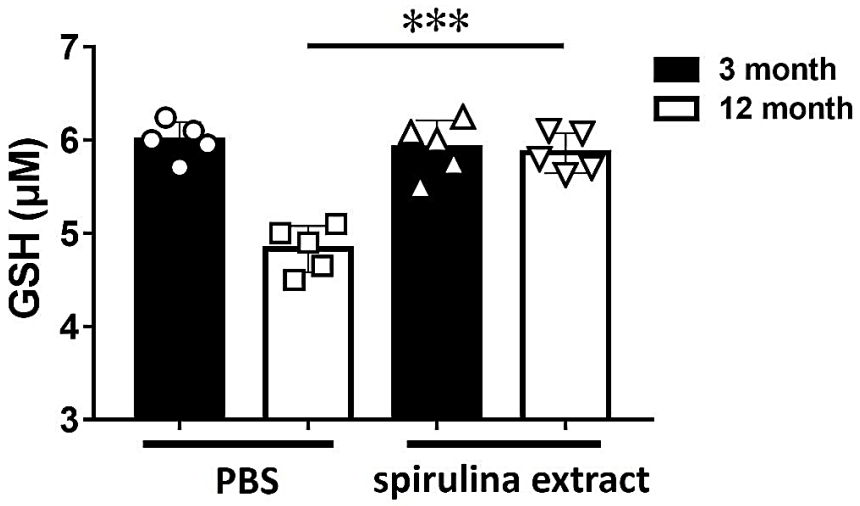


Figure 2. Effects of spirulina extract on the serum concentration of GSH in young and middle-aged mice.

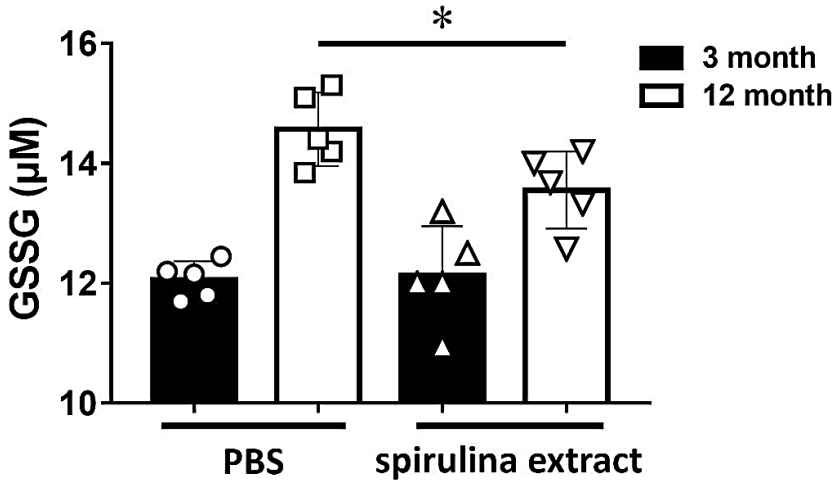


Figure 3. Effects of spirulina extract on the serum concentration of GSSG in young and middle-aged mice.

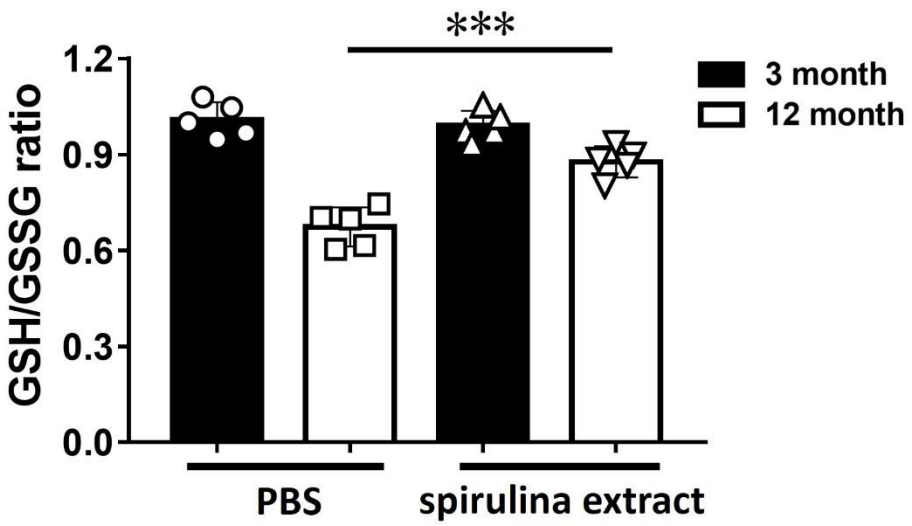


Figure 4. Effects of spirulina extract on the GSH/GSSG ratio in the serum of young and middle-aged mice.

Table 3. Effects of spirulina extract on the serum levels of GSH, GSSG, and the GSH/GSSG ratio in young and middle-aged mice.

	GSM (μM)			GSSG (μM)			GSH/GSSG ratio	
	Control	Experimental		Control	Experimental		Control	Experimental
young adult	6.00 ± 0.20	5.92 ± 0.29		12.06 ± 0.31	12.13 ± 0.82		1.01 ± 0.05	0.99 ± 0.05
middle-aged	4.83 ± 0.25	5.86 ± 0.21***		14.57 ± 0.61	13.55 ± 0.64*		0.67 ± 0.06	0.88 ± 0.05***

n = 5

Anti-Inflammatory Effects of Spirulina Extract in Mice: As organisms age, the immune system experiences functional and structural alterations collectively termed immunosenescence. This gradual decline in immune competency contributes to greater vulnerability to infections, diminished efficacy of vaccinations, and an elevated incidence of chronic inflammatory conditions in older individuals. To evaluate the potential in vivo anti-inflammatory effects of spirulina extract, mice received daily oral doses for a duration of 28 days. After the treatment period, blood was collected, and serum levels of key Th1- and Th2-associated cytokines were measured.

As illustrated in Figure 5 and Table 4, untreated (control) middle-aged mice showed a reduction in serum IL-2 levels, decreasing from an average of 16 pg/mL in young mice to 10 pg/mL, while IFN-γ dropped from 1.55 pg/mL to 0.6 pg/mL. However, in spirulina-supplemented middle-aged mice, IL-2 levels rose from 10 pg/mL to 12.5 pg/mL, and IFN-γ increased from 0.6 pg/mL to 0.8 pg/mL. Notably, spirulina administration had no marked effect on IL-2 or IFN-γ concentrations in the younger cohort.

Additionally, as illustrated in Figure 6 and Table 5, the control group showed that IL-4 concentration increased from an average of 0.35 pg/mL in young mice

to 0.55 pg/mL in middle-aged mice; IL-6 concentration rose from 10 pg/mL to 20 pg/mL; and IL-10 concentration increased from 4 pg/mL to 10 pg/mL. However, in the spirulina-treated group, middle-aged mice exhibited a reduction in IL-4 concentration from 0.55 pg/mL to 0.5 pg/mL, IL-6 concentration from 20 pg/mL to 15 pg/mL, and IL-10 concentration from 10 pg/mL to 7 pg/mL. Similar to the Th1 cytokines, no significant changes in IL-4, -6, or -10 levels were observed in young mice following spirulina supplementation. These findings suggest that spirulina extract supplementation can partially reverse the age-associated imbalance in Th1/Th2 cytokine profiles, enhancing Th1 responses while attenuating Th2-mediated pro-inflammatory cytokine production in middle-aged mice.

Table 4. Effects of spirulina extract on the serum concentrations of IL-2 and IFN-γ in young and middle-aged mice (3-month-old and 12-month-old, respectively).

	IFN-γ(pg/ml)			IL-2 (pg/ml)	
	Control	**Experimental**		**Control**	**Experimental**
young adult	1.55 ± 0.30	1.69 ± 0.19		15.94 ± 1.54	16.00 ± 1.32
middle-aged	0.57 ± 0.18	0.87 ± 0.20*		10.20 ± 1.65	12.70 ± 0.80*

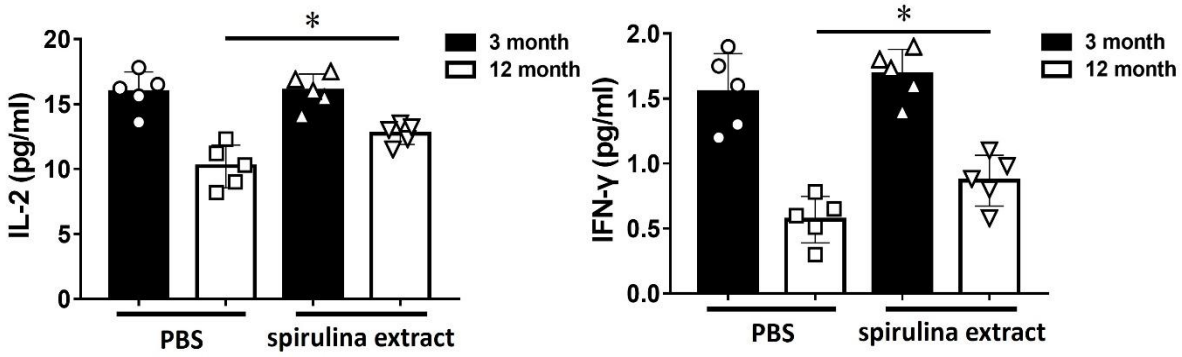


Figure 5. Effects of spirulina extract on the serum concentrations of IL-2 and IFN-γ in young and middle-aged mice (3-month-old and 12-month-old, respectively).

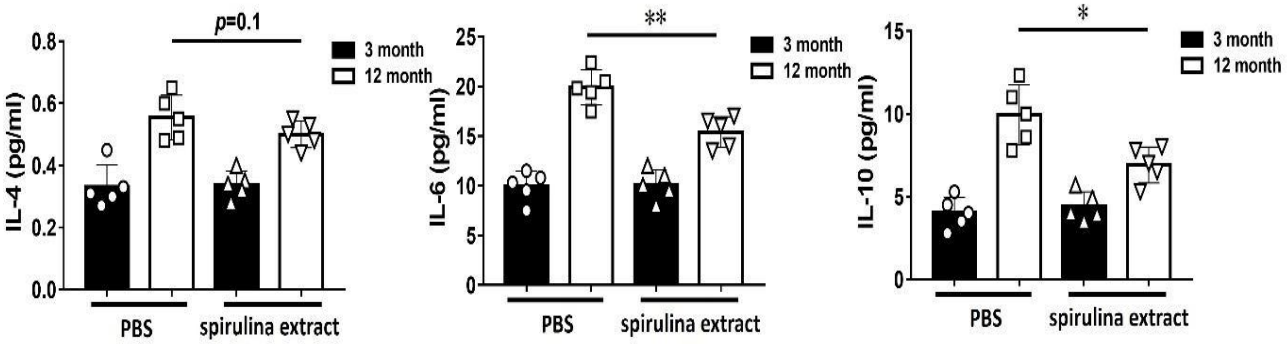


Figure 6. Effects of spirulina extract on the serum concentrations of IL-4, IL-6, and IL-10 in young and middle-aged mice

(3-month-old and 12-month-old, respectively).

Table 5. Effects of spirulina extract on the serum concentrations of IL-4, IL-6, and IL-10 in young and middle-aged mice (3-month-old and 12-month-old, respectively).

	IL-4 (pg/ml)			IL-6 (pg/ml)			IL-10 (pg/ml)	
	Control	Experimental		Control	Experimental		Control	Experimental
young adult	0.33 ± 0.07	0.34 ± 0.04		9.92 ± 1.54	10.10 ± 1.52		4.02 ± 0.95	4.40 ± 0.89
middle-aged	0.55 ± 0.07	0.50 ± 0.04		19.92 ± 1.78	15.40 ± 1.56**		9.94 ± 1.81	6.92 ± 1.09*

DISCUSSION

This study assessed the antioxidant and anti-inflammatory potential of spirulina extract through both *in vitro* and *in vivo* experiments. The *in vitro* analysis demonstrated that the extract exhibited significant reducing power, high total antioxidant capacity, and strong scavenging activities against both DPPH and superoxide anion radicals. Notably, the ability to neutralize DPPH radicals was the most prominent, followed closely by activity against superoxide radicals. In the *in vivo* model, baseline measurements showed that the GSH/GSSG ratio in young mice was around 1.0, while middle-aged mice exhibited a reduced ratio of approximately 0.7, reflecting elevated oxidative stress with age. After 28 days of spirulina supplementation, the GSH/GSSG ratio remained stable in the young group but significantly improved to about 0.85 in the middle-aged group, suggesting a reduction in oxidative stress.

Glutathione (GSH) is a vital molecule involved in cellular defense, playing a central role in signal transduction regulation and the neutralization of reactive oxygen species (ROS), free radicals, and toxic metals [19]. During oxidative stress, oxidized glutathione (GSSG) levels rise, while reduced GSH levels decline. As such, the GSH/GSSG ratio serves as a dependable indicator of oxidative damage and cellular toxicity [20]. An imbalance caused by excessive ROS generation and insufficient antioxidant response is closely linked to the aging process and a range of chronic conditions, including diabetes, cardiovascular disease, autoimmune disorders, neurodegenerative diseases [21].

However, during the aging process, an imbalance between Th1 and Th2 immune responses tends to occur. The secretion of IL-2 and IFN-γ by Th1 cells declines with age, leading to a weakened cellular immune response, thereby reducing the body's ability to resist viral and bacterial infections and to suppress tumorigenesis. Conversely, the secretion of IL-4, IL-6, and IL-10 by Th2 cells increases, promoting humoral immune responses associated with allergic reactions and chronic inflammation. With advancing age, the immune balance gradually shifts from a Th1-dominant cellular immunity toward a Th2-dominant humoral immunity, which favors a pro-inflammatory environment [22].

Th1 and Th2 cells are two subsets of T helper cells responsible for orchestrating immune responses. Th1 cells primarily secrete IFN-γ, TNF-α, and IL-2, which activate macrophages to eliminate intracellular pathogens and promote the differentiation of cytotoxic T cells to clear infected cells, thus mediating cellular immunity. In contrast, Th2 cells secrete IL-4, -5, -6, and -10, which activate B cells to produce antibodies and recruit eosinophils and mast cells to defend against parasites and contribute to allergic reactions, thus mediating humoral immunity (23). There is a reciprocal regulatory mechanism between Th1 and Th2 responses; for instance, IFN-γ inhibits Th2 differentiation, whereas IL-10 suppresses Th1 responses. Maintaining the balance

between Th1 and Th2 immunity is critical for immune homeostasis. Disruption of this balance may lead to immune-related disorders, such as autoimmune diseases (excessive Th1 activity) or allergic diseases (excessive Th2 activity).

Moreover, age-related immune dysfunction is characterized by a decline in Th1 activity and a relative enhancement of Th2 responses, further compromising immune system functionality and increasing susceptibility to various diseases [8].

Plants serve as important dietary sources of both proteins and polysaccharides, that contribute significantly to human health and daily nutritional needs [24]. Plant extracts, which may be naturally derived or modified through fortification or biotechnology, offer additional health benefits, extending their role beyond basic nourishment [25]. Herbal extracts and marine algae extracts are both natural antioxidants that show promising immunomodulatory potential, but they differ in origin, composition, mechanisms of action, and application value [26]. The plant-derived compounds such as curcumin, epigallocatechin gallate, and rosmarinic acid possess strong free radical scavenging activity and are known to suppress pro-inflammatory factors (e.g., NF-κB and TNF-α), thereby reducing chronic inflammation and immune dysregulation. Their long history of dietary use also supports their safety. However, plant-derived compounds often suffer from low bioavailability and poor stability, which limits their clinical applications [27].

In contrast, spirulina extracts containing phycocyanin and sulfated polysaccharides, not only exhibit antioxidant and anti-inflammatory effects but also modulate immune cell activities, including those of macrophages and T cells, enhancing host immune defense. Their unique molecular structures, especially the presence of sulfated polysaccharides, show great promise in immune activation and antiviral applications. Additionally, algae are fast-growing and can be sustainably cultivated on a large scale, offering strong potential for industrial development.

CONCLUSION

This study presents novel evidence that spirulina extract can effectively restore immune balance and reduce inflammation associated with aging, highlighting its unique dual role as an antioxidant and immunomodulator. Using a middle-aged mouse model, we demonstrated that spirulina supplementation significantly upregulated Th1 cytokines, reversing the age-related decline in cellular immunity. Concurrently, it downregulated Th2 cytokines, which are typically elevated in aging and contribute to chronic inflammation. The observed reduction in IL-10 and IL-6 levels was statistically significant, while IL-4 showed a non-significant downward trend. These immune-modulating effects were supported by strong *in vitro* antioxidant activity, particularly DPPH radical scavenging, and improved in vivo redox status, as indicated by an increased GSH/GSSG ratio. The ability of spirulina to rebalance Th1/Th2 responses and enhance antioxidant defenses introduces a novel therapeutic angle for managing immunosenescence. Importantly, this is among the first animal studies to demonstrate that dietary spirulina can reverse specific biomarkers of immune aging in a controlled experimental model. These findings support the development of spirulina-based functional foods or nutraceuticals aimed at preventing or mitigating age-related immune decline and inflammation, offering a promising natural strategy for promoting healthy aging.

Abbreviation: *S. platensis*: *Spirulina platensis,* TNF-α: Tumor Necrosis Factor-alpha, DPPH: 2, 2-Diphenyl-1-picrylhydrazyl, ABTS: 2, 2-Azino-bis-(3-

ethylbenzothiazoline-6-sulfonic acid), SOA: Superoxide Anion Radicals, ROS: Reactive Oxygen Species, GSH: Glutathione, GSSG: Glutathione disulfide, Th1: T helper type 1, IL-2: Interleukin-2, IFN-γ: Interferon gamma, Th2: T helper type 2, IL-4: Interleukin-4, IL-6: Interleukin-6, IL-10: Interleukin-10, CPC: C-phycocyanin, APC: Allophycocyanin, HIV-1: Human immunodeficiency virus type 1, LPS: Lipopolysaccharide, TNF-α: Tumor Necrosis Factor-α, HDAC: Histone Deacetylase, Nrf2: Nuclear Factor Erythroid 2-Related Factor, HO-1: Heme Oxygenase 1, DNA: Deoxyribonucleic Acid, ELISA: Enzyme-Linked Immunosorbent Assay, PBS: Phosphate Buffered Saline, RP: Reducing Power

Authors' contributions: YCH designed and conducted the *in vitro* experiments and prepared the spirulina extract. JPW was responsible for the design and execution of the animal studies. YCH and CHL contributed to drafting and revising the manuscript. Statistical analysis was designed and performed by YCH and JPW. All authors reviewed and approved the final version of the manuscript.

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Competing interests: The authors declare that they have no competing interests.

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