International Journal of Agricultural Technology 2025 Vol. 21(6):2229-2242
DOI: https://doi.org/10.63369/ijat.2025.21.6.2229-2242
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Evaluation of plant-based protein products: Tempeh derived from the local edible freshwater alga, *Chara corallina* Willdenow on immune function, toxicity and antioxidant activity

Chankaew, W.[1], Sumana, B.[2], Srimoon, R.[2], Kongthong, S.[3], Ngamphongsai, C.[4] and Boonprab, K.[5*]

[1]Faculty of Agriculture, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat, 80110, Thailand; [2]Faculty of Agro-Industrial Technology, Rajamangala University of Technology Tawan-OK,22210, Thailand; [3]Faculty of Veterinary Science, Rajamangala University of Technology Srivijaya, 80240 Thailand; [4]National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Centre, 113 Thailand Science Park, Paholyothin Road, Klong Luang, Pathumthani, 12120, Thailand; [5]Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand.

Chankaew, W., Sumana, B., Srimoon, R., Kongthong, S., Ngamphongsai, C. and Boonprab, K. (2025). Evaluation of plant-based protein products: Tempeh derived from the local edible freshwater alga, *Chara corallina* Willdenow on immune function, toxicity and antioxidant activity. International Journal of Agricultural Technology 21(6):2229-2242.

Abstract The findings demonstrated that a plant-based protein powder, tempeh derived from the freshwater alga *Chara corallina* (ATF-C), at 3.90–500 µg/mL showed no cytotoxicity, maintaining cell viability above 90%. It significantly promoted cell proliferation and upregulated mRNA expression of key antioxidant enzymes—superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT)—within 62.5–500 µg/mL ($p<0.05$). Moreover, ATF-C enhanced nitrite production and phagocytic activity in a dose-dependent manner. Gene expression profiling revealed marked induction of immune-related markers, including Lysozyme M, tumor necrosis factor-alpha (TNF-α), and interleukin-8 (IL-8), across the same concentration range ($p<0.05$). Collectively, these findings demonstrated that ATF-C is not only safe but also exhibits promising antioxidant and immunomodulatory properties. Its dual role as an immune enhancer and antioxidant is provided a strong foundation for future applications in human health, particularly in immunity regulation and oxidative stress management.

Keywords: Edible algae, Plant-based protein, Cytotoxicity, Immune-potentiator, Antioxidant

Introduction

The trend of consuming food made from plant-based ingredients is continuously increasing, particularly in the area of protein nutrients. Plant-based protein is classified as an alternative protein, and consumers are turning their attention to high-protein plants as a substitute for meat, which helps reduce the

[*]**Corresponding Author:** Boonprab, K; **Email:** ffisksb@ku.ac.th

environmental impact of livestock farming. Traditional livestock farming has high carbon dioxide emissions and long-term effects on consumer health due to the use of chemicals and antibiotics in raising animals (Özbek and Bilek, 2018). Currently, the food industry is researching and developing new sources of raw materials and appropriate processing techniques to create products that meet consumer demands in terms of taste, texture, and nutritional value. Plant-based protein products resemble meat but differ in composition (Rubio *et al*., 2020). Plant-based proteins are particularly beneficial because they are high in fiber, which helps prevent obesity, colon cancer, and diabetes, while also reducing the risk of heart disease (Rubio *et al*., 2020). Numerous studies have reported that plant-based protein products contain significantly more dietary fiber than meat (Thongkorn, 2020). As a result, the global market for these products is expected to grow. Recent research has focused on identifying plant species that are both nutritious and safe for consumption. Plant-based protein products are derived from five main groups: grains, nuts, seeds, dried fruit, non-grain grasses, and vegetables (Curtain and Grafenauer, 2019). The most popular plant protein sources in the industry are high-protein plants such as legumes, as well as smaller plants and seaweed (Naksit, 2020). Algae-based protein products are growing rapidly, with most derived from microalgae, particularly *Spirulina* and *Chlorella* (Youla *et al*., 2023).

Chara corallina, a native freshwater alga from southern Thailand, especially in Krabi province, is notable for its high protein content (16.8-19.8% dry weight) and also high valuation of phytochemical (Chankaew *et al*., 2020; 2024) making it a promising candidate for plant protein product development (Rapeephon, 2020). In this research, a plant-based protein product was developed: tempeh powder (ATF-C). This is the first plant-based product made from *C. corallina*, offering significant potential for the production of algae-based protein products. This study is investigated the product's efficiency, including its toxicity in cultured cells, antioxidant properties, and its effects on the immune system and assessed cytotoxicity analysis via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, antioxidant activity evaluation by measuring H2O2 levels, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) levels, and conduct qRT-PCR in human dermal fibroblast cells, and immune response assessment in macrophage cells by measuring phagocytic activity and gene expression of Lysozyme M, TNF-alpha, and IL-8 using qRT-PCR.

Materials and methods

A plant-based protein powder product and tempeh (ATF-C) from Chara corallina preparation

The soybeans, weighing 1,000 g, were washed several times with tap water, and any damaged or imperfect beans were discarded. The soybeans were then soaked until they swelled. For the first boil, the soybeans were cooked for 20–30 minutes, the water was changed, and then they were soaked in water for 24 hours. The pH was measured using test paper, ensuring the acidity was around 5 or lower. For the second boil, they were cooked for 5–10 minutes, drained, and left to dry. Once dried, the soybeans were mixed with 2 g of Rhizopus oligosporus spore powder per 1 kg of soybeans. After mixing, the inoculated soybeans were placed into a prepared container. The inoculated soybeans (100 g) were combined with 50 g of C. corallina wet weight, which had been autoclaved, and mixed thoroughly. The mixture was then packed into zip-lock bags (16 cm wide x 22 cm long) with holes punched in them. The bags were sealed tightly, placed on bamboo trays, and covered with muslin cloth. The soybeans were incubated at room temperature for 36–48 hours. Once the tempeh was ready, the samples were freeze-dried and ground into protein powder before undergoing further testing.

Cell culture

Human skin cells, including HDF and L6 cells, as well as murine macrophage RAW 264.7 cells used in this study, were cultured in different media. HDF cells were cultured in DMEM-low glucose medium containing 10% fetal bovine serum (FBS) and 1% (v/v) antibiotics (penicillin/streptomycin). A mixture of keratinocyte-SFM medium containing 5 ng/ml human recombinant EGF and MEM-high glucose, supplemented with 10% FBS and 1% (v/v) antibiotics in a 2:3 ratio, was used as the L6 culture medium. RAW 264.7 cells were maintained in RPMI-1640 medium supplemented with 10% FBS and 1% (v/v) antibiotics. All cell types were incubated under a humidified 5% CO_2 atmosphere at 37°C. The culture medium was typically changed every three days until the cells reached 80% confluency. Confluent cells were routinely sub-cultured by trypsinization using trypsin-EDTA.

Cell viability assay

Cell survival was assessed using thiazolyl blue tetrazolium bromide (MTT) reagent. Viable cells metabolize yellow tetrazolium salts into purple formazan crystals via the mitochondrial reductase enzyme. HDF (8×10^3 cells) and L6 (1.8×10^4 cells) were plated into 96-well plates and grown in their complete medium at 37°C under a humidified 5% CO_2 atmosphere for 24 hours. The cells were then treated with various concentrations of ATF-C (3.90–500 µg/mL) in 100 µL of complete medium, while untreated cells served as controls. After 24 hours of incubation, the cells were washed once with PBS and replaced with 100 µL of fresh medium. Subsequently, 10 µL of 5 mg/mL MTT reagent was added to each well and incubated at 37°C in a CO_2 incubator for 2 hours. After incubation, the culture medium and MTT solution were discarded, and 100 µL of DMSO was added to dissolve the formazan crystals. The optical density of the formazan solution was measured at 570 nm using a microplate reader. The experiment was performed in triplicate, and cell viability was calculated and expressed as a percentage of viable cells (Mahshid *et al*., 2021).

Gene expression of antioxidant enzymes

The expression of antioxidant enzymes (SOD, CAT, and GPx) was evaluated in HDF cells treated with ATF-C. Cells (4×10^5) were seeded in 60 mm culture dishes, maintained in complete medium for 24 h, and serum-starved overnight. They were then exposed to ATF-C (62.5–500 µg/mL) in medium supplemented with 2% FBS for 6 h, while control cells received the same medium without ATF-C. Total RNA was extracted using an RNA isolation kit (FAVORGEN Biotech, Taiwan) and quantified spectrophotometrically, ensuring $OD_{260/280}$ ratios between 1.6 and 2.2. cDNA was synthesized from 500 ng RNA using Oligo$(dT)_{18}$ primers and a reverse transcription system (Solis BioDyne, Estonia).

Quantitative real-time PCR was conducted with HOT FIREPol® EvaGreen® qPCR Mix Plus (Solis BioDyne, Estonia) to determine mRNA levels of the target enzymes. Each reaction mixture (25 µL total volume) contained 2 µL cDNA (250 ng), 0.5 µL of each primer (10 ng/µL), and 5 µL master mix. The cycling protocol included initial activation at 95 °C for 15 min, followed by 40 cycles of denaturation (95 °C, 15 s), annealing (54 °C, 20 s), and extension (72 °C, 30 s). GAPDH served as the housekeeping gene, and untreated controls were used as calibrators. Gene expression was quantified by the comparative CT method, with melting curve analysis confirming product specificity. All assays were performed in triplicate (Khongthong *et al*., 2021; Monosroi *et al*., 2004).

Nitric oxide production for inhibition assay

The inhibitory effect of ATF-C on NO release was examined using a model of murine macrophage-like RAW 264.7 cells, following a slightly modified process from a previous report. The cells (1×10^5 cells/well) were plated into a 96-well plate with complete culture medium and allowed to adhere to the plate for 1 hour at 37°C under a humidified 5% CO_2 atmosphere. The medium was then replaced with 200 µL of 1 µg/mL LPS in fresh medium supplemented with 6.25–500 µg/mL ATF-C, and the cells were cultured for an additional 24 hours. Indomethacin was used as a standard.NO release from LPS-induced RAW 264.7 cells was evaluated by detecting the accumulation of nitrite in the culture medium using Griess reagent (Kim *et al*., 2011). The optical density of the reaction was measured at 570 nm using a microplate reader. The experiment was performed in triplicate.

Gene expression of cytokine

To investigate the molecular mechanisms underlying the immunomodulatory effects of ATF-C, the mRNA expression levels of lysozyme M, TNF, and IL-8 were analyzed. RAW264.7 macrophages (1.5×10^6 cells/well) were seeded in 6-well plates, incubated for 1 h, and subsequently treated with ATF-C (62.5–500 µg/mL) in the presence of 1 µg/mL LPS for 20 h at 37 °C under 5% CO_2. Total RNA was extracted and quantified spectrophotometrically, ensuring $OD_{260/280}$ ratios of 1.6–2.2. cDNA synthesis was performed using 500 ng RNA, Oligo$(dT)_{18}$ primers, and a reverse transcription kit (Solis BioDyne, Estonia). Quantitative real-time PCR was carried out with HOT FIREPol® EvaGreen® qPCR Mix Plus (Solis BioDyne, Estonia) under the following cycling conditions: initial activation at 95 °C for 15 min, followed by 40 cycles of denaturation (95 °C, 15 s), annealing (54 °C, 20 s), and extension (72 °C, 30 s). Each reaction contained 2 µL cDNA (250 ng), 0.5 µL of each primer (10 ng/µL), and 5 µL master mix in a total volume of 25 µL. GAPDH was used as the housekeeping gene, and untreated controls served as calibrators. Relative gene expression was quantified by the comparative CT method, and product specificity was verified by melting curve analysis. All assays were conducted in triplicate**.**

Statistical analysis

Statistical analysis was performed in triplicate and the data analyzed with SPSS with one way ANOVA for significant differences ($p<0.05$) using Duncan's Multiple Range test.

Results

Evaluating the toxicity of protein powder products in cell culture

Evaluation of the cell cytotoxicity of protein powder products from *C. corallina*; ATF-C using MTT assay was conducted in macrophage cells culture (RAW 264.7 cell), fibroblast cell culture (Human dermal fibroblast, HDF cell) and rat skeletal muscle cell (L6 cell). The results showed that ATF-C at 3.90-500 μg/mL had no toxicity on RAW 264.7 cell with the survival rate were more 90% (Figure 1). The ATF-C at also had no toxicity on HDF cell with the survival rate were more 90%. The ATF-C affected on cell proliferation at 99.93±0.50, 109.33±1.01, 111.67±2.32, 113.27±2.58, 118.30±3.00, 121.97±2.92, 127.93±3.25 and 132.56±5.86%, respectively (Figure 2).

The ATF-C at 3.90-500 μg/mL had no toxicity on rat skeletal muscle cell (L6 cell) with the survival rate were more than 90% (Figure 3). Moreover, the ATF-C affected on cell proliferation at 103.96±1.19, 111.00±2.07, 114.67±1.17, 116.62±2.32, 128.37±1.95, 131.71±3.28, 133.01±5.09 and 132.56±5.86%, respectively.

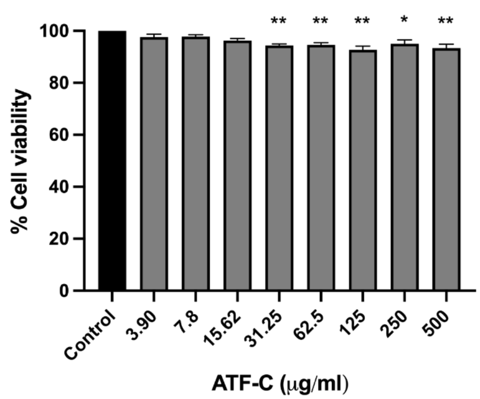

Figure 1. Percentage of cell viability of ATF-C in RAW264 cell macrophage (% survival at n=3, *p<0.05, **p<0.01)

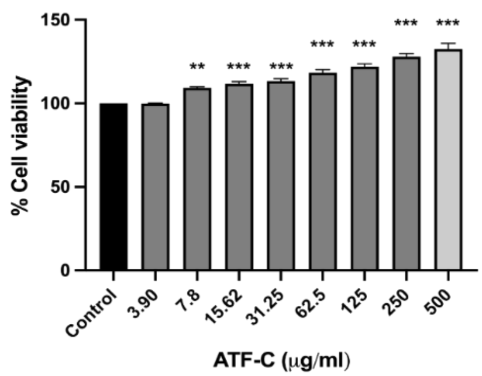

Figure 2. Percentage of cell viability of ATF-C in HDF cell (% survival at n=3, *p<0.05, **p<0.01, ***p<0.001)

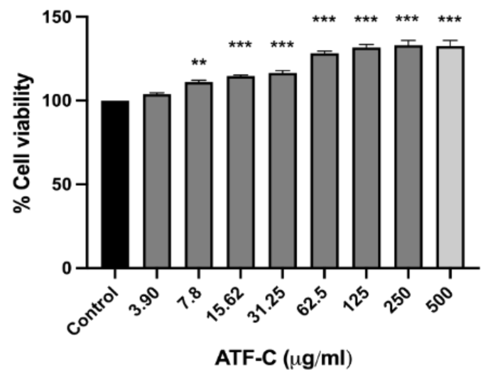

Figure 3. Percentage of cell viability of ATF-C in L6 cell (% survival at n=3, *p<0.05, **p<0.01, ***p<0.001)

Antioxidant activity of protein powder from Chara corallina

The qRT-PCR in HDF cell was used to evaluate antioxidant activity of protein powder from *C. corallina*. The results showed that the ATF-C at 62.5-500 µg/mL stimulated the gene expression at the mRNA of SOD, GPx and CAT significantly ($p<0.05$), compared with the control group (Figure 4). The relative gene expression values were SOD (1.00±0.02, 1.10±0.02, 1.15±0.04, 1.17±0.02 and 1.23±0.04, respectively) GPx (1.00±0.03, 1.04±0.02, 1.09±0.03, 1.13±0.05 and 1.16±0.08, respectively) and CAT (1.00±0.04, 1.03±0.04, 1.07±0.06, 1.07±0.04, 1.13±0.09 and 1.14±0.06, respectively).

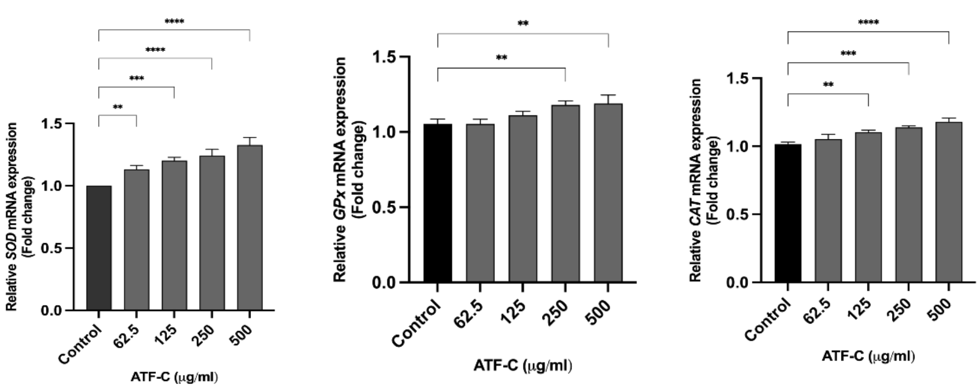

Figure 4. Effects of ATF-C at 62.5-500 µg/mL on the gene expression at the mRNA of SOD, GPx and CAT enzymes using qRT-PCR technique compared with the control group (*p<0.05, **p<0.01, **p<0.001)

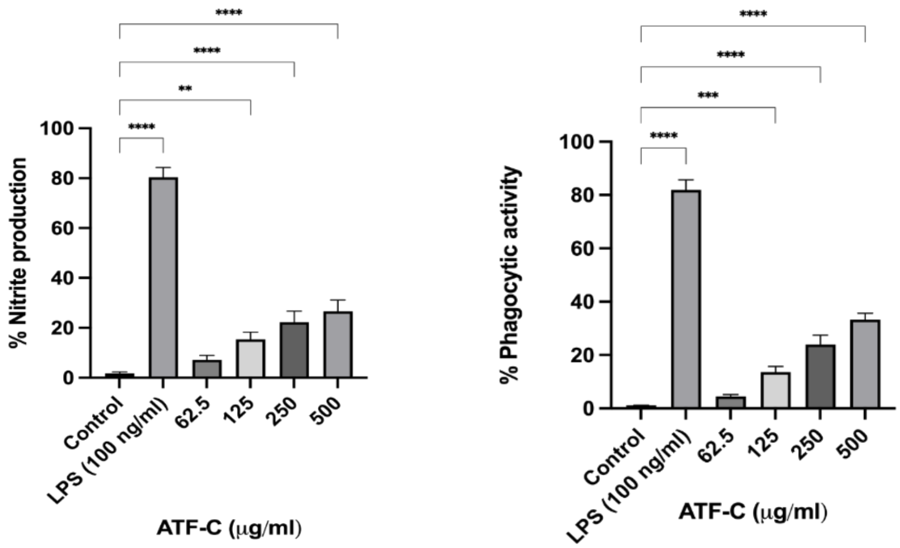

Figure 5. Effect of ATF-C at 62.5-500 μg/mL on the nitrite production using Griess assay, and phagocytic activity using NBT assay compared with the control group (*p<0.05, **p<0.01, ***p<0.001)

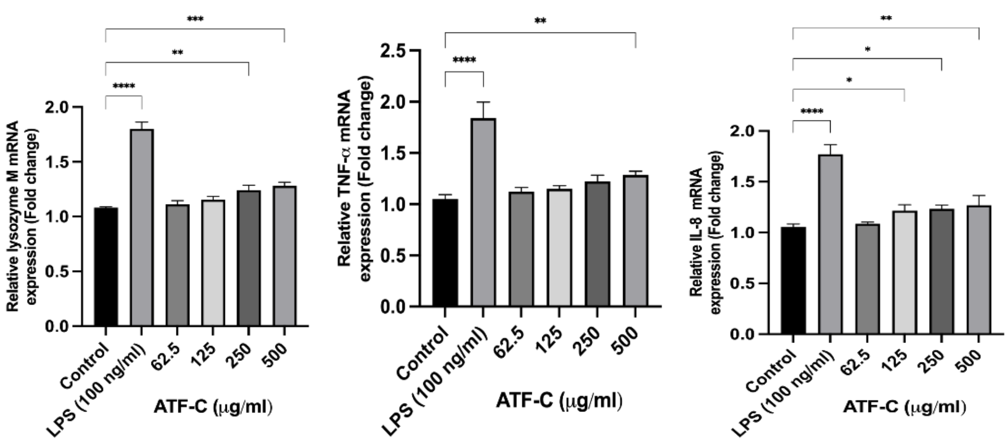

Figure 6. Effect of ATF-C at 62.5-500 μg/mL on the gene expression of the lysozyme M, TNF-alpha and IL-8 using qRT-PCR technique compared with the control group (*p<0.05, **p<0.01, ***p<0.001)

The cell immunomodulation test

The results of cell immunomodulation showed the ATF-C at 62.5-500 µg/mL increased the nitrite content in concentration-dependent manner. It also stimulated the phagocytic activity significantly ($p<0.05$), compared with the control group (Figure 5). The gene expression of the lysozyme M, TNF-alpha and IL-8 using qRT-PCR technique revealed that ATF-C at 62.5-500 µg/mL stimulated the gene expression at mRNA of Lysozyme M, TNF-alpha and IL-8 significantly ($p<0.05$), compared with the control group (Figure 6).

Discussion

Plant-based protein products (ATF-C) from *C. corallina* are receiving growing interest from scientists due to their immunostimulatory and antioxidant activities (Wongprasert *et al*., 2014; Lins *et al*., 2009; Marinho-Soriano and Bourret, 2005). In this species, proteins have been attributed with stimulatory effects on immune responses in shrimp (Wongprasert *et al*., 2014). In the present study, the immunomodulatory and antioxidant activities of ATF-C from this species were examined in RAW264.7 murine macrophage cells and human dermal fibroblast cells. Additionally, the signaling pathways involved in the macrophage-mediated immune response and the fibroblast-mediated antioxidant response were analyzed.

Nowadays, the efficacy of the biological activity of natural products is of great interest. However, their safety remains a significant concern before conducting experiments. The MTT assay is one of the most popular methods for assessing cell viability in response to tested chemical compounds. Since this study aimed to evaluate the effects of ATF-C on skin cells, their safety was examined in HDF, L6, and RAW264.7 cells using the MTT assay. Non-treated cells were counted as 100% cell viability. The percentages of cell viability for ATF-C are shown in the results. According to ISO 10993-5, a non-cytotoxic agent should demonstrate above 80% cell viability. Compounds that yield cell viability within the ranges of 80-60%, 60-40%, and below 40% are considered weak, moderate, and strong cytotoxic substances, respectively (ISO 10993-5; López-García *et al*., 2014).

Antioxidant enzymes are the primary defense mechanism of cells against oxidative damage. SOD, CAT, and GPx are major cellular antioxidant enzymes that prevent the formation of oxidants and eliminate formed radicals (Velioglu *et al*., 1998). Thus, an increase in the levels of antioxidant enzymes in cells is important for controlling cellular oxidants. Since the plant-based proteins ATF-C demonstrated ROS scavenging effects in HDF cells, their induction of antioxidant enzyme expression was further investigated. The effects of ATF-C on the gene expression of antioxidant enzymes, including SOD, CAT, and GPx, in human skin cells were monitored using real-time PCR. The transcriptional responses were presented in terms of relative mRNA expression. The results in HDF cells showed that both ATF-C could up-regulate the gene expression of SOD, CAT, and GPx. Regarding the levels of antioxidants in the skin, the epidermis layer contained significantly greater levels of antioxidant enzymes than the dermis. This is likely because the epidermis is the outermost layer of skin and is directly exposed to various environmental oxidative stressors (Shindo *et al*., 1994; Ceriello *et al*., 2000).

Macrophage-mediated pathogen uptake and activation are pivotal events in the innate immune defense against infectious agents. Once stimulated, macrophages engage in phagocytosis and release a broad spectrum of pro-inflammatory and cytotoxic mediators. In this study, we demonstrate that plant-derived protein from *C. corallina* (ATF-C) enhances both phagocytic activity and nitric oxide (NO) production in RAW 264.7 macrophages in a concentration-dependent manner. Macrophages typically respond to foreign particles or immune activators such as lipopolysaccharide (LPS) by inducing several immune-related genes, including lysozyme, a critical component of the oxygen-independent bactericidal pathway within phagosomes. This enzyme is widely recognized as a marker of macrophage activation. During differentiation, macrophages gradually upregulate lysozyme M gene expression, leading to its pronounced presence in mature cells, and its expression is further elevated upon LPS stimulation (Markart et al., 2004). Earlier studies have also highlighted the essential role of lysozyme M in host defense against bacterial pathogens (Goethe and Phi-van, 1998). Consistent with these findings, our results show that ATF-C significantly increases lysozyme M mRNA expression in a dose-responsive manner, indicating that polysaccharides from *C. corallina* can potentiate macrophage immune functions. Beyond pathogen clearance, phagocytosis triggers signaling pathways that drive the production of molecules linking innate and adaptive immunity, including cytokines, enzymes, reactive oxygen and nitrogen species, and surface receptors. Our data further confirm that ATF-C modulates immune activity by promoting the secretion of cytokines and NO. Notably, interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-α), together with NO, serve not only as central immune mediators but also enhance the tumoricidal potential of activated macrophages (Marinho-Soriano and Bourret, 2005; Craigie and Wen, 1984).

In this result, plant-derived protein was extracted from Chara corallina and its immunomodulatory effects on murine macrophages were systematically evaluated. The results revealed that the protein fraction stimulated macrophage activation, enhanced phagocytic capacity, and upregulated the expression of multiple cytokines and related molecules, many of which are linked to immune regulation and antioxidative defense. These observations identify plant-based proteins from *C. corallina* as bioactive components contributing to both antioxidative and immune-enhancing activities. Collectively, the findings highlight their potential utility as natural immunopotentiators and antioxidant agents in human health. Nevertheless, further investigations are required to elucidate the precise structural characteristics and biological functions of these proteins.

This study is provided the novel insights into the immunomodulatory effects of plant-based protein products, particularly ATF-C derived from *C. corallina*. These findings are not only broaden our understanding of dietary influences on immune responses but also pave the way for developing functional foods aimed at enhancing human health. Given the increasing demand for sustainable and plant-based protein sources, our findings underscore the potential of *C. corallina* as a viable option in food technology. This aligns with global efforts to promote plant-based diets that support environmental sustainability while offering health benefits. Future studies should investigate the long-term effects of ATF-C on various immune pathways and their potential synergistic effects when combined with other dietary components. Additionally, exploring the application of these products in clinical settings could further validate their health benefits. The successful development of these plant-based products opens avenues for commercialization within the health food sector, particularly in addressing consumer demand for nutritious and functional foods. As the global community increasingly shifts towards plant-based diets, our research advocates for the incorporation of innovative food products like ATF-C into mainstream nutrition, urging stakeholders in food science, public health, and policy to consider the broader implications of integrating such solutions into dietary guidelines.

Acknowledgements

The authors are thankful to Thailand Science Research and Innovation; Fundamental Fund (2023) for providing financial support.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

Chankaew, W., Yangtong, M. and Seangkaew, J. (2020). Evaluation of antioxidant activity and phytochemical from Kam Kung (*Chara corallina* Klein ex C. L. Willnenow). Princess of Naradhiwas University Journal, 12:296-314.

Chankaew, W., Phetkul, U. and Srimoon, R., (2024). Phytochemicals, α-glucosidase and α-amylase inhibitory efficiency of brittle wort (*Chara corallina*) extract. Current Applied Science and Technology, 24:1-10.

Ceriello, A., Morocutti, A., Mercuri, F., Quagliaro, L., Moro, M. and Damante, G. *et al.* (2000). Defective intracellular antioxidant enzyme production in type 1 diabetic patients with nephropathy. Diabetes, 49:2170-2177.

Craigie, J. S. and Wen, Z. C. (1984). Effects of temperature and tissue age on gel strength and composition of agar from *Gracilaria tikvahiae* (Rhodophyceae). Canadian Journal of Botany, 62:1665-1670.

Curtain, F. and Grafenauer, S. J. N. (2019). Plant-based meat substitutes in the flexitarian age: an adult of products on supermarket shelves. Nutrients, 11:2603.

Goethe, R. and Phi-van, L. (1998). Posttranscriptional lipopolysaccharide regulation of the lysozyme gene at processing of the primary transcript in myelomonocytic HD11 cells. The Journal of Immunology, 160:4970-4978.

ISO 10993-5. (2009) Biological evaluation of medical devices Part 5: tests for in vitro cytotoxicity. ISO.2009.

Khongthong, S., Theapparat, Y., Roekngam, N., Tantisuwanno, C., Otto, M. and Piewngam, P. (2021). Characterization and immunomodulatory activity of sulfated galactan from the red seaweed Gracilaria fisheri. International journal of biological macromolecules, 189: 705-714.

Kim, J. K., Cho, M. L. S., Karnjapratum, I. S. and Shin, S. G. (2011). In vitro and in vivo immunomodulatory activity of sulfated polysaccharides from *Enteromorpha prolifera*. International of Journal of Biological Macromoleccules, 49:1051-1058.

Lins, K. O., Bezerra, D. P., Alves, A. P., Alencar, N. M., Lima, M. W. and Torres, L. M. (2009). Antitumor properties of a sulfated polysaccharide from the red seaweed Champia feldmannii (Diaz-Pifferer). Journal of Applied Toxicology, 29:20-26.

López-García, J., Lehock, M., Humpolíek, P. and Sáha, P. (2014). HaCaT keratinocytes response on antimicrobial atelocollagen substrates: extent of cytotoxicity, cell viability and proliferation. Journal of Functional Biomaterial, 5:43-57.

Mahshid, G., Tyron, T., Sonia, S. and Kempson. I. (2021). The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis. International Journal of Molecular Science, 22:1-30.

Marinho-Soriano, E. and Bourret, E. (2005). Polysaccharides from the red seaweed *Gracilaria dura* (Gracilariales, Rhodophyta). Bioresource Technology, 96:379-382.

Markart, P., Faust, N., Graf, T., Na, C. L., Weaver, T. E. and Akinbi, H. T. (2004). Comparison of the microbicidal and muramidase activities of mouse lysozyme M and P. Biochemical Journal, 380:385-392.

Monosroi, A., Saraphachotiwitthaya, J. and Monosroi, J. (2004). Immunomodulatory activity of fractions from hot aqueous extract of wood from *Clausena excavate*. Fitoterapia, 75:302-308.

Naksit, P. (2020). Plant-based Proteins: Nutrition, Structure, Functionality and Applications in Food Industry. Rajabhat Agriculture Journal, 19:61-69.

Özbek, A. G. and Bilek, S. E. (2018). Plant Based Protein Sources and Extraction, Current Investigations in Agriculture and Current Research, 2:169-171.

Rapeephon, S. (2020). Alternative Proteins: Market Research on Consumer Trends and Emerging Landscape. Meat and Muscle Biology, 4:16-11.

Rubio, N. R., Xiang, N. and Kaplan, D. L. (2020). Plant-based and cell-based approaches to meat production. Nature Communications, 11:1-9.

Shindo, Y., Witt, E., Han, D., Epstein, W. and Packer, L. (1994). Enzymic and non-enzymic antioxidants in epidermis and dermis of human skin. Journal of Investigative Dermatology, 102:122-124.

Thongkorn, P. (2020). Research guidelines for Plant-based Protein. Journal of Science and Technology, 38:36-39.

Velioglu, Y. S., Mazza, G., Gao, L. and Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agriculture and Food Chemistry, 46:4113-4117.

Wongprasert, K., Rudtanatip, T. and Praiboon, J. (2014). Immunostimulatory activity of sulfated galactans isolated from the red seaweed *Gracilaria fisheri* and development of resistance against white spot syndrome virus (WSS) in shrimp. Fish Shell fish Immunology, 36:52-60.

Youla, A. A., Nelly, M., Reggie, S, Nurpudji, A. T., William, B. G., Mrinal, S., Felicia K., Aurielle, A. S., Alfredo, W. and Fahrul, N. (2023). Soy and Algae Combination Using Tempe Fermentation Method: A Proposed Opinion for the Development of Functional Food. Revista Nutricion Clínica Diet Hosp, 43:30-35.

(Received: 21 November 2024, Revised: 30 September 2025, Accepted: 1 November 2025)