

# Evaluation of *Kappaphycus striatus* Lectin on SARS-CoV-2 Spike Protein-Specific IgG Responses

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**Abstract.** The COVID-19 pandemic highlighted the urgent need for novel therapeutics that would balance vaccines and antivirals. Lectins, which are carbohydrate-binding proteins, can interfere with viral glycoproteins and modulate host immune responses. Seaweed lectins are of particular interest due to their specificity for complex glycans present on viral spikes, including SARS-CoV-2. *Kappaphycus striatus*, widely cultivated in Southeast Asia, is known for its carrageenan but also contains lectins with promising bioactivities. Little is known about the *in vivo* immunological role of *K. striatus* lectins. This study evaluates whether lectin from *K. striatus* affects IgG responses to the SARS-CoV-2 spike protein in mice.

## 1 Introduction

An urgent need for novel therapeutic and preventative strategies beyond conventional vaccines and antiviral drugs has increased due to the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that caused COVID-19 pandemic in 2020. Although vaccines have played a central role in reducing morbidity and mortality, some have said it might have been too late as emerging variants continued to pose challenges. This situation highlights the importance of identifying therapeutic treatments that are capable of modulating host immune responses and interfering with the viral entry mechanism.

*Kappaphycus* seaweed is widely cultivated in Southeast Asia for its carrageenan content, which is used in the food industry as a thickening, gelling, and emulsifying agent [1, 2]. Seaweed is also known to contain lectins, and these lectins have a proteinaceous content comparable to terrestrial plants. In general, they have low molecular masses, no affinity for monosaccharides, and a high specificity for complex oligosaccharides and glycoproteins [3]. Seaweed lectins have also been reported to have anticancer, antiviral, and antibacterial properties [4, 5, 6]. Lectins isolated from *Kappaphycus* species, such as *Kappaphycus alvarezii*, are reported to bind with N-acetyl-D-galactosamine (GalNAc) residues with high specificity and exhibit activity against viral glycoproteins, including the SARS-CoV-2 spike protein [7]. However, little is known about the immunological effects of *Kappaphycus* seaweed lectins *in vivo*, particularly in relation to their ability to modulate IgG responses following exposure to viral antigens.

In this study, the effects of *Kappaphycus striatus* lectin on IgG levels specific to SARS-CoV-2 spike protein were evaluated in BALB/c mice. By combining lectin administration with a spike protein challenge, we investigated whether the lectin could enhance antibody production and offer clues about its potential as an immunomodulatory agent against SARS-CoV-2. Findings from this preliminary *in vivo* study contribute to the growing body of evidence supporting the antiviral and immune-modulating properties of algal lectins and provide a foundation for their further development as sustainable, seaweed-derived therapeutic candidates.

## 2 Materials and Methods

### 2.1 *Kappaphycus* seaweed sampling

*Kappaphycus striatus* seaweeds were collected by local farmers from seaweed farms located 3.6 km from Omadal Island (coordinates: 4°23'58.8"N, 118°43'39.5"E), Semporna, Sabah. The seaweeds were then maintained in the Plant *In Vitro* Laboratory at the Biotechnology Research Institute, Universiti Malaysia Sabah.

### 2.2 Animal ethics

All experiments involving mice performed in this study were approved by the Animal Ethics Research Committee of Universiti Malaysia Sabah (file number AEC001/2022).

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### 2.3 Lectin extraction and purification

Crude lectin was extracted according to the procedure described by Field et al. [8]. The modification involved removing the secondary incubation with  $\beta$ -mercaptoethanol ( $\beta$ -ME) to reduce the toxicity of the end products, and phosphate-buffered saline (PBS) was used to incubate the seaweed instead of ultrapure water. The extracted crude lectin was lyophilized and stored in  $-20^{\circ}\text{C}$  freezer until use.

Lyophilized crude protein was purified by bio-specific affinity chromatography on a Sepharose 6B-GalNAc column following the procedure by Maliki et al. [7]. A total volume of 5 mL (1 mg/mL) of crude protein was applied to the column. Collected fractions were pooled together and lyophilized before being subjected to a hemagglutination inhibition assay and characterization.

### 2.4 Lectin characterization

Hemagglutination inhibition (HI) assay was done according to the procedures reported by Sun et al. [9]. The HI assay was done in a 96-well microtiter U-plate using a 2% (v/v) mouse erythrocyte suspension treated with papain. Papain-treated mice's erythrocytes with no treatment were used as a negative control. Lectin activity was determined by mat formation in the wells, while button formation at the bottom of the cavity indicates the absence of lectin activity.

The effects of temperature, pH, and divalent cation dependence were tested according to the procedures described by Dinh et al. [10] and Souza et al. [11], with minor modifications. Papain-treated mice's erythrocytes with no treatment were used as a negative control in these studies. The HI assay was done as described previously, and the presence of a visible mat was used to indicate agglutination, with the degree of agglutination activity quantified as 100% hemagglutination activity.

For temperature effects on lectin, the purified lectin was incubated at  $30$ – $80^{\circ}\text{C}$  for 10 and 30 minutes, respectively. The effects of pH on lectin activity were determined by dialyzing the purified lectins at different pH levels (3–9) overnight. Next, the effects of divalent cations on hemagglutination activity were investigated using 500  $\mu\text{L}$  of purified lectin dialyzed overnight against 100 mL of 50 mM EDTA in PBS at  $4^{\circ}\text{C}$ . Non-dialyzed fractions were recovered, and 25  $\mu\text{L}$  of the fractions were incubated with 25  $\mu\text{L}$  of 20 mM  $\text{CaCl}_2$  and  $\text{MgCl}_2$  for one hour at room temperature.

Sugar-binding specificity tests were conducted following the methods described by Maliki et al. [7]. Sugars (100 mM) or glycoprotein solutions (100  $\mu\text{g}/\text{mL}$ ) were diluted in 0.15 M NaCl by 2-fold serial dilutions with a final volume of 100  $\mu\text{L}$ . An equal volume of lectin (0.1  $\text{g}/\text{mL}$ ) was incubated with the sugar or glycoprotein for one hour at room temperature. Sugars and glycoproteins used in this test include D-(+)-galactose, D-(-)-fructose, D-mannitol, lactose, sucrose, D-(+)-maltose, mannose, fucose, N-acetyl-D-galactosamine (GalNAc), N-acetyl-D-glucosamine (GlcNAc), and sialic acid (Neu5Ac). Positive lectin

activity was confirmed by the formation of a distinct dot in the well, indicating specific binding between the lectin and the tested sugars or glycoproteins.

### 2.5 *In vivo* effects of *K. striatus* lectin on the immune response to the SARS-CoV-2 spike (S) protein in BALB/c mice

Male mice (6–12 weeks,  $n=3$  per group) were divided into four groups: negative control (PBS), *K. striatus* control (KSC), *K. striatus* and S protein (KST), and S protein only (Sp7). The chosen sample size of three (3) mice per group was appropriate for a preliminary study, providing initial insights into lectin effects while adhering to ethical guidelines and feasibility constraints. Treatments were administered once daily for 7 days, with lectin given by oral gavage and PBS given to controls. Mice were fasted 2 h prior, and 1 h post administration, with food and water otherwise available *ad libitum*.

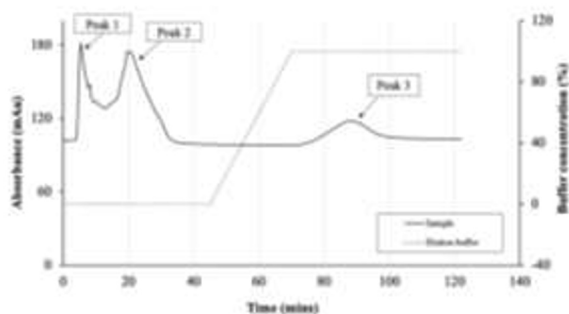
For the S protein challenge, mice in the KST and Sp7 groups received an intramuscular injection of 5  $\mu\text{g}$  recombinant SARS-CoV-2 spike receptor-binding domain (RBD) protein (Sino Biological, China) in 50  $\mu\text{L}$  PBS on day 1, following Wörzner et al. [12] with minor modifications. Mice were monitored for up to 2 hours post-injection for any adverse effects. On day 8, mice were anesthetized with a ketamine (80 mg/kg) and xylazine (10 mg/kg) cocktail before blood collection via cardiac puncture for serum collection. Mice were then euthanized by cervical dislocation, and organs (heart, liver, kidneys, and lungs) were harvested and weighed for relative organ weight analysis.

Serum IgG responses against SARS-CoV-2 spike protein were evaluated using a commercial ELISA kit (Elabscience), with modifications to the secondary antibody. The procedure was performed according to the kit manual, and HRP-conjugated rabbit anti-mouse IgG (1 $\times$ , prepared from 100 $\times$  stock) was used in place of the kit-supplied anti-human antibody. Optical density was measured at 450 nm using a microplate reader.

## 3 Results and Discussion

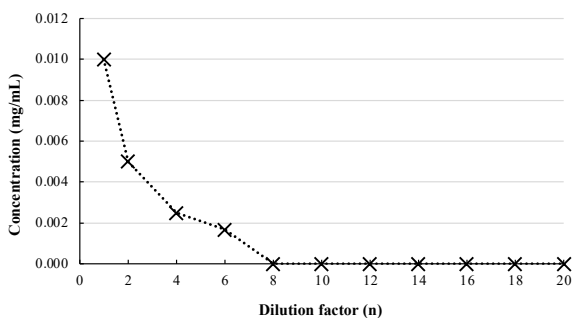
### 3.1 Lectin purification and characterization

The Sepharose 6B-GalNAc column was able to isolate GalNAc-specific proteins from the crude protein samples. In Figure 1, the chromatographic profile depicted that the target lectin from *K. striatus* was eluted as a wide and low peak (Peak 3). However, this finding is consistent with other literature that also uses bio-affinity chromatography [13, 14]. This type of peak shape typically indicates a gradual increase of target protein over several fractions, usually due to moderate affinity between ligand and target protein or a relatively low target protein concentration in the sample [15].



**Fig. 1.** Chromatography profile of *Kappaphycus striatus* crude protein on Sepharose 6B~GalNac column. Unbound protein was washed using 0.9% NaCl and elution buffer (0.85% NaCl with 25mg/mL lactose) was applied as indicated by the arrow to elute purified protein. Fractions were collected every 1 mL and peak 3 appeared approximately from minute 71 to 101.

The hemagglutination activity of purified *K. striatus* lectins was evaluated using papain-treated mouse erythrocytes, as native erythrocytes are resistant to lectin binding. The lectin successfully agglutinated papain-treated erythrocytes, consistent with previous reports of RSL, PA-III, and Con A lectins [16]. The lectin from *K. striatus* exhibited a minimum inhibitory concentration of  $1.67 \times 10^{-3}$  mg/mL (Figure 2).



**Fig. 2.** The HI assay of *Kappaphycus striatus* lectin shows that the minimum inhibitory concentration is  $1.67 \times 10^{-3}$  mg/mL. The x-axis represents the dilution factor of the HI assay (1:n, where n is the dilution factor).

The lectin purified from *K. striatus* is a thermostable lectin, as agglutination activity can be observed throughout the range of 30–80°C. A wide range of pH also did not change the agglutination activity of the lectin. pH and thermostability of small proteins may be attributed to their small peptide size, enabling them to be easily refolded to their original structure when temperature changes [17]. Furthermore, red alga hemagglutinin does not require divalent cations to exert its agglutination activity, and this property is well presented by this lectin, as the absence or presence of divalent cations did not affect its agglutination activity [11]. The thermostability and pH resistance of *K. striatus* lectin suggest their potential for biomedical and pharmaceutical applications, such as antiviral therapeutics or drug delivery systems, where stability under varying physiological conditions is crucial.

From Table 1, sugar-binding specificity analysis revealed that the lectin isolated from *K. striatus* demonstrated affinity for D-(+)-galactose, GalNac,

GlcNAc, sialic acid, mannose, and fucose. This broad carbohydrate-binding spectrum is particularly significant, as these glycans are known to be major components of the SARS-CoV-2 spike protein glycosylation [18]. The inhibition of hemagglutination in the presence of these sugars suggests that the lectin binds effectively to these carbohydrates, supporting its potential antiviral mechanism through glycan interaction. This finding aligns with research on *K. alvarezii* lectin, which similarly binds to viral glycoproteins. *Kappaphycus alvarezii* lectin demonstrated effective binding to the spike protein of SARS-CoV-2 through these sugar molecules, as evidenced by HI assays [7]. Hence, by reviewing thermostability, pH resistance and cation-independent activity of *K. striatus* lectin, this allows their biological potency to be standardized based on consistent activity across these conditions. Moreover, its defined carbohydrate-binding specificity towards these sugars also provides a measurable biochemical parameter for dose normalization and quality control in therapeutic applications.

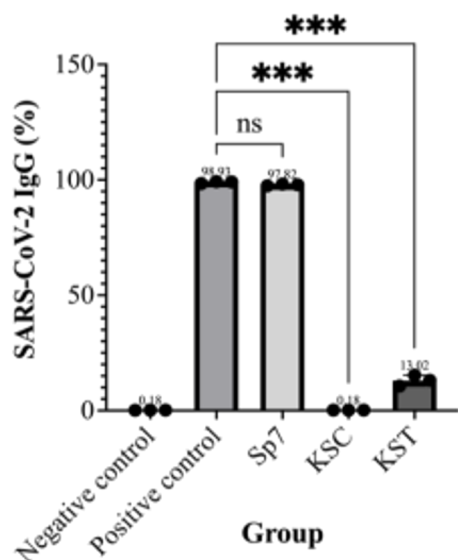
**Table 1.** Lectin from *Kappaphycus striatus* hemagglutination activity using different sugars and glycoproteins.

Carbohydrates	Lectin Activity
D-(+)-galactose	++
D-(-)-fructose	-
D-mannitol	-
Lactose	-
Sucrose	-
D-(+)-maltose	-
N-acetyl-D-galactosamine	++
N-acetyl-D-glucosamine	++
Sialic acid	++
Mannose	++
Fucose	++

### 3.2 SARS-CoV-2 IgG levels in mice

From Figure 3, ELISA results indicated that the mice group challenged with the SARS-CoV-2 spike protein for seven days (Sp7) showed a significant elevation in IgG levels, with an average percentage of 97.82%, comparable with the positive control provided with the

ELISA kit (98.93%). Mice that received both lectin treatment with spike protein challenge exhibited higher IgG levels (13.02%) compared to those that received lectin treatment alone (0.18%). This suggests lectin treatment can potentiate the immune response, potentially enhancing the production of antibodies specific to SARS-CoV-2 antigens. Conversely, despite the heightened IgG levels in the lectin-treated and spike protein-challenged group, the antibody production was lower compared to mice exposed solely to the S protein without lectin treatment. This suggests that treatment with *K. striatus* lectin may have mitigated viral infection or reduced viral load, leading to a weaker IgG-mediated immune response. This supports the idea that lectins can alter how the immune system interacts with viral antigens. In addition, B cells are possibly less stimulated to produce antibodies in lectin-treated mice, resulting in lower IgG levels. These findings are consistent with the broader role of lectins in modulating immune responses, as discussed in receptor interaction studies like those in Lempp et al. [19]. Lectins can also interact with glycan structures on immune cells or pathogens, thereby influencing immune cell signaling and function. In this context, *K. striatus* lectins are capable of agglutinating the sugar moieties present on the SARS-CoV-2 spike protein, further supporting their immunomodulatory potential. The HI assay results also showed that *K. striatus* lectins are thermostable, pH tolerant, and cation independent. These traits are essential for their potential effectiveness against SARS-CoV-2, as they allow the lectins to stay active under various physiological conditions.



**Fig. 3.** SARS-CoV-2 IgG levels in mice detected using the SARS-CoV-2 Spike Protein IgG ELISA Kit (Elabscience). The y-axis represents the SARS-CoV-2 IgG percentage level, and the x-axis represents different treatment groups. Significant differences between groups are indicated by \*\*\* ( $p < 0.0001$ ), and non-significant differences are denoted as “ns” ( $p > 0.05$ ). Abbreviation: Sp7, mice challenged with SARS-CoV-2 spike protein for 7 days; KSC, mice treated with *Kappaphycus striatus* lectin; KST, mice challenged with SARS-CoV-2 spike protein for 7 days treated with *K. striatus* lectin.

## 4 Conclusion

This study demonstrated that *K. striatus* lectin possesses strong carbohydrate-binding properties, thermostability, and pH tolerance, making it a robust candidate for biomedical applications. In addition, lectin administration in spike protein-challenged mice resulted in moderated SARS-CoV-2-specific IgG levels, suggesting that the lectin may reduce antigenic load or alter immune activation through glycan interactions. These findings emphasize the prospective use of *K. striatus* lectin as a therapeutic agent and provide preliminary *in vivo* evidence supporting the development of seaweed-derived lectins as sustainable therapeutics against coronavirus infections.

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