Original Article

Anti-allergic efficacy of 80 % methanol extracts from the brown alga, *Sargassum macrocarpum*

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Abstract

Sargassum macrocarpum is a perennial brown macroalga that forms the most important portion of seaweed beds with sufficient biomass. Algae have been proven to be beneficial functional food and pharmaceutical material. There have been a few reports on the bioactivity of algal components. The algal population discovered growing naturally on the coast of Nishinoshima town, Shimane Prefecture, Japan was collected as experimental samples. They were collected from the sea at depths of 1, 5, 10, 15, 20, and 25-m. In this study, an 80% methanol extract of the alga was prepared. Its bioactivity or an anti-allergic effect was demonstrated by reducing ear swelling in Institute of Cancer Research mice, suppressing degranulation in rat basophile leukemia-2H3 cells without inducing cell death, and inhibiting inflammatory-related enzymatic activities (phospholipase A_2, lipoxygenase, cyclooxygenase-2, and hyaluronidase). Thus, *S. macrocarpum* could be utilized as healthy functional food and pharmaceutical material.

Key words

Sargassum macrocarpum, brown algae, anti-allergy, anti-inflammation, lipophilic components

1. Introduction

Sargassum macrocarpum is a large brown macroalga that grows extensively in the Japanese coastal area. The growing area extends from Kanto's Pacific coast to Kyushu and Aomori Prefecture's Sea of Japan coast to Kyushu (Yoshida, 1983; Shimabukuro, 2018). The growing area is perpendicularly oriented and extends from the low-tide line to a depth of approximately 20-m (Shimabukuro, 2018). The alga growing on the coast of the Sea of Japan in the Prefecture of Yamaguchi exhibits a growth phase from February to March and a maturation phase from April to June (Murase and Kito, 1998). *Sargassum macrocarpum* is a perennial macroalga with high biomass production (Murase et al., 2000; Murase, 2000). The brown alga forms *Sargassum* beds, providing habitation and feeding sites for coastal animals and leading to a high biodiversity spot. Therefore, the alga is a naturally abundant source of biomass and is recognized as an important species of coastal fisheries and as resource protection in seaweed beds.

The advantages of *S. macrocarpum* have been well-established in the ecosystem; therefore, we focused on its usage as a healthy functional food and pharmaceutical component. Furthermore, the bioactivity associated with lipophilic components of the alga has been previously acknowledged. For example, Kamei et al. performed a screening study on 200 algae species collected from the coast of Japan for neurite prolonging activity (Sagara et al., 1997). Additionally, extensive research identified active components in lipophilic compounds, including pheophytin a-related compounds, sargaquinoic acid, and sargachromenol (Tsang and Kamei, 2004; Tsang et al., 2005; Ina and Kamei, 2006). Furthermore, researchers found the anti-bacterial activity of sargafuran, a lipophilic compound isolated from the alga (Kamei et al., 2009).

Although many reports have identified the bioactivity of isolated compounds as mentioned earlier, the anti-allergic effect of *S. macrocarpum* is unreported. *Sargassum macrocarpum* grows naturally at a depth of approximately 15-m on the coast of Nishinoshima town (Hayashi et al., 2017). We investigated the anti-allergic effect of the 80 % methanol (80 M) extract from the alga collected off the coast of Nishinoshima. The effect was evaluated using an allergic inflammation mouse model, inflammation-related enzymatic activity inhibition tests, and an anti-degranulation test in the mast cell line. Additionally, trypan blue-exclusion test also determined whether a cell death induction by the 80M extract leads to anti-degranulation. Moreover, samples were collected from various sea depths and examined to investigate the efficacy of the anti-allergic effect in alga growing in perpendicularly-oriented areas.

2. Materials and methods

2.1 Sample preparation

Sargassum macrocarpum was obtained from depths of 1, 5, 10, 15, 20, and 25-m from the coast of Nihshinoshima in December 2017. Tap water was used to wash the algae samples to remove debris, air-dried, and subsequently pulverized with a rotary crusher (NR-04; Sansho Industry Co., Ltd., Higashi-Osaka, Osaka, Japan).

Because a previous report revealed bioactivity in an 80 M extract of the brown alga (Sagara et al., 1997), the algal powder (2 g) was extracted twice with 100 mL of 80 M in 30 min. First, the extract was filtered with a filter paper (No. 2; Toyo Roshi Kaisha, Ltd., Tokyo, Japan), dried using a rotary evaporator (N-1100; Tokyo Rikakikai Co., Ltd., Tokyo, Japan), and concentrated using a centrifugal concentrator (CVE-200D; Tokyo Rikakikai). Then, the resulting algal residues were dissolved in methanol. Finally, the solvent (i.e., 80 M extract) was stored at 4 °C until further use.

2.2 Examination of anti-inflammatory properties based on swelling reduction

An *in vivo* study examined the effect of percutaneously-administered 80 M extracts on swelling in the mouse ear caused by three sensitizers [arachidonic acid (AA), 12-O-tetradecanoylphorbol-13-acetate (TPA), and oxazolone (OXA)], which can cause acute, chronic, or delayed-type allergic inflammation (Young et al., 1984; Xu et al., 1996).

2.2.1 Animals

As a model for ear swelling, the Institute of Cancer Research mice were employed (Kim et al., 2017). The mice (males, four weeks old) were purchased from the KBT Oriental Co., Ltd. (Tosu, Saga, Japan), housed in individual cages, and kept at 23 °C-26 °C under a 12-h light/dark cycle until further experiments. A solid AIN-93G diet (KBT Oriental) and tap water were made available. The mice maintained a good appetite throughout the experimental period, and no diarrhea or abnormal symptoms were observed in any group. Furthermore, no significant differences in food intake or body weight gain were observed among the tested mice. After receiving permission from the Committee for Use and Care of Laboratory Animals of the National Fisheries University, all the animal experiments were performed. They complied with the Guidelines for Animal Experiments in Research Institutes under the Jurisdiction of the Ministry of Agriculture, Forestry and Fisheries (the approval number: 18-7, March 31, 2018).

2.2.2 Arachidonic acid

The AA-induced ear swelling was conducted with little modifications to a previously reported approach by Young et al. (1984). Here, AA (10 µL, 12.5 mg/mL in acetone, stored at −20 °C; FUJIFILM Wako Pure Chemical Co., Osaka, Japan) and the test sample (5 µL) dissolved in methanol were mixed and applied to the mice's ears. After 1 h, the ear swelling was measured using a thickness gauge (547 series, Mitutoyo Corporation, Kawasaki, Kanagawa, Japan).

Using the following formula, the suppression ratio of the ear swelling was determined:

$$\text{Suppression ratio (\%)} = [1 - (T - T_0) / (C - C_0)] \times 100,$$

where C_0 is the ear thickness without the administration of the test sample before AA application, C is the ear thickness without the test sample after AA application, T_0 is the ear thickness with the test sample before AA application, and T is the ear thickness with the test sample after AA application.

2.2.3 12-O-tetradecanoylphorbol-13-acetate

The TPA-induced ear swelling was performed with little modification to a previously reported approach by Young et al. (1984). A stock solution of TPA (800 µg/mL in acetone, stored at −20 °C; FUJIFILM Wako) was diluted ten times with acetone immediately before use. The TPA (10 µL) and the test sample (5 µL) were mixed and applied to the mice's ears. After 4 h, using a thickness gauge, the ear swelling was determined. As explained before, the suppression ratio of the ear swelling was calculated.

2.2.4 Oxazolone

With slight modifications of the previous method reported by Yoshino et al. (2010), the OXA-induced ear swelling was performed. The OXA (Sigma-Aldrich Co., St. Louis, MO, USA) was stored at −20 °C until use. The 1 % OXA (50 µL) dissolved in ethanol was applied to the abdominal region of the mouse. Using an animal shaver (Natsume Seisakusho Co, Ltd., Tokyo, Japan), the abdominal hair was carefully shaved under anesthesia with sevoflurane. Five days later, 0.5 % OXA (10 µL) dissolved in acetone was mixed with the test sample (5 µL) and applied to the mice's ears. Using a thickness gauge, the ear swelling was determined after 24 h, as described before, and the suppression ratio of the ear swelling was calculated accordingly.

2.3 Inhibitory effects on enzymatic activity

In an allergic reaction, phospholipase A_2 (PLA_2), lipoxygenase (LOX), cyclooxygenase (COX)-2, and hyaluronidase (HA) are inflammation-related enzymes (Sakamoto et al., 1980; Funk, 2001). Additionally, their enzymatic activities are involved in mouse ear swelling (Meurer et al., 1988; Kujubu et al., 1991; Rao et al., 1993). Consequently, the following analysis was done to determine the extracts' inhibitory effects on enzymatic activities.

2.3.1 Phospholipase A_2

With a little modification of the method reported by Sugiura et al. (2009), a PLA_2 inhibition assay was performed. Diheptanoyl thio-PC (1.66 mM; Cayman Chemical Co., Ann Arbor, MI, USA) was dissolved in dimethyl sulfoxide (DMSO) and diluted with 25 mM Tris-HCl buffer (pH 7.5). The diheptanoyl thio-PC solution (100 µL) and either the test sample (5 µL) or DMSO (5 µL; solvent control) were mixed, and 5 µL of 5,5′-dithio-bis-(2-nitrobenzoic acid) (50 mM; Sigma-Aldrich) dissolved in Tris-HCl buffer was added. Background absorbance at 415 nm (OD_{415}) was measured using a 96-well microplate reader (Infinite F50, TECAN Austria GmbH, Grodig, Austria), after incubating the reaction mixture at room temperature for 5 min. Porcine pancreatic PLA_2 (5 µL; 25 units; Sigma-Aldrich) diluted with Tris-HCl buffer was immediately added to induce the enzymatic reaction. After 75 min of adding PLA_2, the increase in OD_{415} from 5-thio-2-nitrobenzoic acid yielded by the reaction was measured. Using the following formula, the inhibition ratio of the PLA_2 activity was calculated:

$$\text{Inhibition ratio (\%)} = [1 - (T_{75} - T_0) / (C_{75} - C_0)] \times 100,$$

where C_0 is the OD_{415} without the test sample before adding the PLA_2, C_{75} is the OD_{415} without the test sample at 75 min after adding the PLA_2, T_0 is the OD_{415} with the test sample before adding the PLA_2, and T_{75} is the OD_{415} with the test sample in 75 min after adding the PLA_2.

2.3.2 Lipoxygenase

Soybean LOX (SBL, Type I-B, Sigma-Aldrich) was used as an alternative to human 5-LOX (Komoda et al., 1995). The inhibitory effects of the test samples on the SBL activity were investigated using a method previously described by Komoda et al. (1995). A mixture of SBL (2 mL; 100 units) in 0.2 M borate buffer (pH 9.0) and either the test sample (20 µL) or methanol (20 µL; solvent control) was incubated at room temperature for 5 min. After that, 0.4 mM linoleic acid (50 µL) diluted with ethanol was added as a substrate, and the optical density at 234 nm (OD_{234}) was measured. After 20 min, an increase in OD_{234} due to conjugated diene production by SBL activity from the linoleic acid was obtained. The following formula was employed to calculate the inhibition ratio of SBL activity:

$$\text{Inhibition ratio (\%)} = [1 - (T_{20} - T_0) / (C_{20} - C_0)] \times 100,$$

where C_0 is the OD_{234} without the test sample when the linoleic acid was added, C_{20} is the OD_{234} without the test sample at 20 min after adding the linoleic acid, T_0 is the OD_{234} with the test sample when the linoleic acid was added, and T_{20} is the OD_{234} with the test sample in 20 min after adding the linoleic acid.

2.3.3 Cyclooxygenase-2

To investigate the effect of the test samples on COX-2 activity, enzymatic inhibition assays and enzyme-linked immunosorbent assay (ELISA) were used; the amount of prostaglandin E_2 produced by the COX-2 enzymatic reaction was measured using a COX inhibitor screening kit (Cayman Chemical). Enzymatic inhibition assays and ELISA for prostaglandin E_2 were performed as per the manufacturer's protocol.

2.3.4 Hyaluronidase

The test samples' inhibitory effect on HA activity was examined following Kakegawa et al. (1985). First, the HA (from bovine testes, Sigma-Aldrich), compound 48/80 (Sigma-Aldrich), and sodium hyaluronate (Nacalai Tesque Inc., Kyoto, Japan) were dissolved in 0.1 M acetate buffer (pH 4.0). After that, the HA (12.5 µL; 0.8 mg/mL) was mixed with the test sample (25 µL) and then incubated at 37 °C for 20 min. Then, after adding compound 48/80 (25 µL; 0.1 mg/mL) and incubating at 37 °C for 20 min to activate the HA, sodium hyaluronate (62.5 µL; 0.16 mg/mL) was added and the mixture was incubated at 37 °C for 40 min. Next, 25 µL of 0.4 M sodium hydrate and 0.8 M potassium borate were added, and the reaction mixture was boiled for 3 min and cooled with tap water to stop the enzymatic reaction. Finally, p-dimethylaminobenzaldehyde (750 µL; 10 mg/mL; FUJIFILM Wako) dissolved in acetate was added, and the OD_{585} was measured. Then, the relative ratio of HA activity inhibition was calculated as described below:

$$\text{Inhibition (\%)} = [1 - (T - T_B) / (C - C_B)] \times 100,$$

where C is the OD_{585} without the test sample, C_B is the OD_{585} without the test sample when the enzyme was not added, T is the OD_{585} with the test sample, and T_B is the OD_{585} with the test sample when the enzyme was not added.

2.4 Assay for anti-degranulation

2.4.1 Anti-degranulation activity

The extract was tested for its ability to suppress degranulation in rat basophile leukemia (RBL)-2H3 cells (JCRB0023, Health Science Research Resources Bank, Tokyo, Japan) (Barsumian et al., 1981). First, the RBL cells were cultured as described in a previous report (Sugiura et al., 2012). Then, because the amount of degranulation in mast cells is proportional to the activity of β-hexosaminidase (β-Hex; equivalent to histamine) (Schwartz et al., 1981), a degranulation assay was performed in stimulated RBL cells by measuring the β-Hex activity as described in a previous report (Sugiura et al., 2012). The experimental conditions of the cell stimulation and exposure to test samples were as follows: Cells (2×10^5 cells/well) were pre-cultured and sensitized overnight with 0.2 µg of anti-dinitrophenyl (DNP) IgE (Sigma-Aldrich) in a 24-well plate. After removing the anti-DNP IgE, the test samples

were added, and the cells were incubated for 10 min. Subsequently, the cells were stimulated with 4 μg of DNP-bovine serum albumin (LSL Co., Ltd., Tokyo, Japan) for 30 min. In this study, no inhibition of the β-Hex activity was observed in the tested samples.

2.4.2 Cell survival

Trypan blue-exclusion test was performed at a concentration of 100 μg/mL to determine whether the anti-degranulation activity was attributable to cell death by the 80M extracts. The RBL cell test protocol was based on the Sugiura et al. (2006) approach.

2.5 Statistical analysis

The data were represented as mean ± standard deviation. Statistical analyses were performed using the Tukey–Kramer test using the Excel Statistics 2020 software (Social Survey Research Information Co., Ltd., Tokyo, Japan). A $p < 0.05$ was considered statistically significant.

3. Results

3.1 Suppressive effect on mouse ear swelling

The 80 M extract obtained from all sea depths significantly reduced the ear swelling in mice induced by AA, TPA, and OXA (Table 1). The suppressive effect of the extracts obtained at depths of 1, 5, 10, and 15-m was significantly higher and/or tended to be higher than those obtained from depths of 20 and 25-m in AA-induced ear swelling. The extracts from a depth of 5 m had the highest suppressive effect, with a suppression ratio of 71.6 %. However, in the TPA-induced ear swelling, the suppression ratio in the extracts obtained from depths of 1, 10, 20, and 25-m was more than 50 %, and their suppressive effects were significantly higher and/or tended to be higher than the extracts obtained from depths of 5 and 15-m. The suppression ratio in the extract obtained from a depth of 1-m was the highest (74.4 %). Conversely, the extract obtained from a depth of 15-m had the lowest suppression ratio (14.5 %). Furthermore, the suppression ratio of the 80 M extracts in OXA-induced ear swelling was approximately 20 %-40 %. There was no significance in the suppressive effect among the test groups, regardless of the depth of the sea.

3.2 Inhibition of inflammation-related enzymatic activities

The corresponding enzyme activities related to inflammation were all suppressed by the 80 M extracts from all water depths (Table 2). The extract from a depth of 10 m had the greatest inhibitory effect on the PLA_2 activity (IC_{50} value, 135.9 μg/mL). In contrast, the inhibitory effect of the extract obtained from depths of 1, 5, 20, and 25-m was comparable. The inhibitory effect of the extract obtained from a depth of 15-m was reduced because the IC_{50} value (807.0 μg/mL) was significantly higher than that of the other extracts. Furthermore, the inhibition of the COX-2 activity by the effect obtained from a depth of 20-m was the highest (8.9 μg/mL). In contrast, the other extracts demonstrated a comparable inhibition of approximately 30-40 μg/mL. The inhibition of the LOX and HA activities by the extract collected from a depth of 5-m was greater than those of other extracts (LOX, 9.9 μg/mL; HA, 33.4 μg/mL), and no significant differences in the effect were observed among the other samples.

Table 1: The suppressive effect of 80 % methanol (80 M) extracts from *Sargassum macrocarpum* on mouse ear swelling

Depth of the sea		1 m	5 m	10 m	15 m	20 m	25 m
Suppression ratio (%)	AA	$60.5^{a} \pm 7.0$	$71.6^{a} \pm 14.7$	$55.6^{a} \pm 12.8$	$54.3^{ac} \pm 13.0$	$29.6^{bc} \pm 11.7$	$28.4^{b} \pm 2.9$
	TPA	$74.4^{a} \pm 4.2$	$39.7^{b} \pm 10.6$	$51.2^{bd} \pm 8.9$	$14.5^{c} \pm 12.9$	$61.5^{ad} \pm 3.0$	$52.6^{b} \pm 7.7$
	OXA	30.9 ± 1.7	38.2 ± 6.4	22.1 ± 6.1	34.6 ± 8.8	27.9 ± 12.1	27.9 ± 3.8

Notes: The sample dose was 0.1 mg/mouse. The data obtained resulted from four different experiments ($n = 4$). The values are represented as mean ± standard deviation. The differences between the samples with different letters of the alphabet are statistically significant ($p < 0.05$). The samples did not observe significant differences in the OXA-induced ear swelling test. Abbreviations: AA, arachidonic acid; TPA, 12-O-tetradecanoylphorbol-13-acetate; OXA, oxazolone.

Table 2: The inhibitory effect of 80 M extract obtained from *S. macrocarpum* on inflammation-related enzymatic activities

Depth of the sea		1 m	5 m	10 m	15 m	20 m	25 m
IC_{50} value (μg/mL)	PLA_2	$246.3^{a} \pm 36.0$	$200.1^{ac} \pm 24.7$	$135.9^{bc} \pm 22.3$	$807.0^{d} \pm 53.4$	$159.0^{a} \pm 22.6$	$185.1^{a} \pm 33.9$
	LOX	19.4 ± 1.1	9.9 ± 1.8	17.4 ± 2.3	47.3 ± 20.1	33.3 ± 19.3	23.4 ± 8.3
	COX-2	$29.5^{ab} \pm 15.1$	$33.7^{a} \pm 11.8$	$41.6^{a} \pm 2.2$	$40.9^{a} \pm 5.7$	$8.9^{b} \pm 1.1$	$31.2^{ab} \pm 6.4$
	HA	90.7 ± 43.6	33.4 ± 0.8	59.1 ± 1.8	41.6 ± 2.9	74.5 ± 13.8	53.5 ± 30.7

Notes: The data were collected from the results of four experiments ($n = 3$). The values are represented as mean ± standard deviation. Repeated runs confirmed the reproducibility. The differences between the samples with different letters of the alphabet are statistically significant ($p < 0.05$). No significant differences in LOX and HA inhibition were observed among the samples. Abbreviations: PLA_2, phospholipase A_2; LOX, lipoxygenase; COX-2, cyclooxygenase-2; HA, hyaluronidase.

3.3 Anti-degranulation

The degranulation was suppressed in RBL cells by the 80 M extracts obtained at all depths (Figure 1). The suppressive effect was the highest in the extract collected from a depth of 25-m (IC_{50} value, 1.5 µg/mL), and the suppressive effect of the extracts collected from depths of 1, 5, 15, and 20-m was comparable (1-m, 11.4; 5-m, 9.7; 15-m, 11.6; 20-m, 8.5 µg/mL). The suppressive effect was lowest in the extract obtained from a depth of 10-m, with an IC_{50} value of 14.7 µg/mL.

The possible induction of cell death with the 80 M extracts was tested by trypan blue exclusion. The survival rate of the control group without samples was 94.9 %. The cell viability was over 90 % in all the extract-treated cells, similar to the control group (Figure 2). The test groups showed no significance. Thus, no cell death was observed.

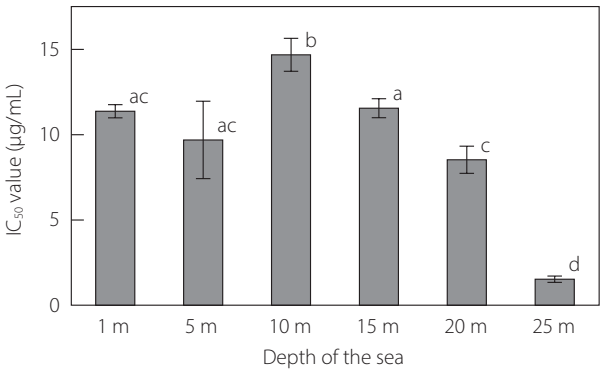

Figure 1: Suppressive effects of 80 % methanol (80 M) extracts obtained from *S. macrocarpum* on degranulation in RBL-2H3 cells

Note: Data were collected from the results of three experiments (n = 3). Values are represented as mean ± standard deviation. Repeated runs confirmed the reproducibility. The differences between the samples with different letters of the alphabet are statistically significant ($p < 0.05$).

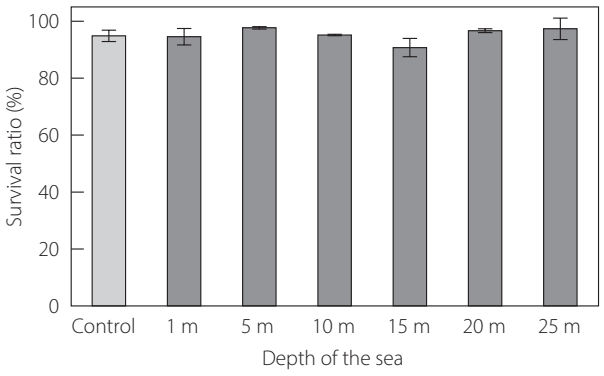

Figure 2: Survival ratio of RBL cells exposed to the 80 M extracts.

Note: Data were obtained from the results of two experiments (n = 2). Values are represented as mean ± standard deviation. Repeated runs confirmed reproducibility. Control, without sample.

4. Discussion

Acute, chronic, and delayed-type (type IV) allergic inflammation is caused by compounds such as AA, TPA, and OXA (Young et al., 1984; Xu et al., 1996). The efficacy of algal extracts on various allergic inflammation reactions is demonstrated in Table 1 after obtaining positive results of 80 M extract suppressing mouse ear swelling induced by sensitizers. LOX and COX-2 enzymatic activity were involved in the allergic inflammatory reaction induced by the sensitizers (Meurer et al., 1988; Kujubu et al., 1991; Rao et al., 1993), and LOX, COX-2, and PLA_2 activities were involved in the AA cascade (Funk, 2001). Furthermore, degranulation in mast cells was involved in the mouse ear swelling (Kerdel et al., 1987). A strong correlation existed between degranulation and HA activity (Sakamoto et al., 1980). In this study, we discovered that inflammation-related enzymatic activities (PLA_2, LOX, COX-2, and HA) were inhibited by the 80 M extracts (Table 2), and degranulation in RBL cells was also suppressed (Figure 1). Therefore, a suppressive mechanism in ear swelling would lead to attenuation of the AA cascade, anti-degranulation, and inhibition of HA activity.

Because brown algal polyphenols (phlorotannins) have anti-allergic properties (Sugiura et al., 2021), the active components in the *S. macrocarpum* 80 M extract were initially thought to be phlorotannins. The phlorotannin content was estimated using the Folin–Denis method (Folin and Denis, 1915) in the 80 M extracts from 1, 5, 10, 15, 20, and 25-m, and was found to be 1.6 %, 1.4 %, 0.4 %, 0.3 %, 1.5 %, and 1.1 %, respectively. The content was lower than that of *Ecklonia* sp. and *Eisenia* sp. (approximately 2 %-14 %) (Sugiura et al., 2011). Moreover, no correlation existed between the phlorotannin amount and the anti-allergic effect of the 80 M extracts (Figure 1, Tables 1 and 2). Many reports have indicated that lipophilic compounds found in the 80 M extracts have bioactive components (Tsang and Kamei, 2004; Tsang et al., 2005; Ina and Kamei, 2006; Kamei et al., 2009). When the constituent of the 80 M extract was analyzed using reversed-phase high-performance liquid chromatography (HPLC), most components were hydrophobic (data not shown). Therefore, as mentioned before, the anti-allergic components included in the 80 M extract may be lipophilic compounds. Since the difference in the constituent ratio among the 80 M extracts was observed from the HPLC analyses (data not shown), a significant difference in the anti-allergic effects of the 80 M extracts may be attributed to the difference in the active component amount among the 80 M extracts. Further studies are required for the identification of the active compounds.

The 80 M extracts were discovered to exert an anti-allergic effect despite the depth of the sea from which the samples were acquired (Figure 1, Tables 1 and 2). Although the suppressive effect on persistent OXA-induced inflammation is less than that on AA and TPA-induced inflammation, a stable

and comparable effect was observed in this study among the 80 M extracts (approximate inhibition ratios, 20 %-40 %; Table 1). However, an eating experience of *S. macrocarpum* was not observed. In contrast, the alga has beneficial bioactivities. Furthermore, in this study, the 80 M extracts obtained at all depths did not induce cell death in RBL cells (Figure 2). Since 80 M extract of the edible brown alga, *Eisenia nipponica*, also showed anti-degranulation activity without inducing cell death (Sugiura et al., 2006), there is a probability that an extract of *S. macrocarpum* may be used as a food material. Thus, *S. macrocarpum* containing active components suppresses allergic inflammation and is hypothesized to be a beneficial raw material for the production of healthy functional food and pharmaceutical components.

5. Conclusion

In this study, the anti-allergic efficacy of *S. macrocarpum* was indicated after obtaining positive results, including suppressed mouse ear swelling, degranulation in RBL cells, and inhibited inflammation-related enzymatic activities. Furthermore, regardless of the depth of the sea (1, 5, 10, 15, 20, and 25-m), the 80 M extracts exhibited efficacy in all experiments. Hence, *S. macrocarpum* is a possible source material for yielding healthy functional food and pharmaceutical components. Therefore, further in-depth identification of the active compounds is important.

Acknowledgments

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