

Inhibitory effects of seaweeds on histamine release from rat basophile leukemia cells (RBL-2H3)

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ABSTRACT: The effects of 80% methanol extracts from frozen samples of 41 macroalgae and one sea grass collected in the Ise-Shima region of Japan were investigated on histamine release from rat basophile leukemia cells (RBL-2H3) sensitized with antidinitrophenyl (DNP) IgE and stimulated with DNP-BSA. Of the 21 brown algae, five green and 15 red algae, and one sea grass tested, only extracts from seven brown algae suppressed histamine release from RBL cells, as determined by high-performance liquid chromatography (HPLC) analysis. When the cytotoxic effects of the seven brown algal extracts were investigated by Trypan blue staining, only *Eisenia arborea* and *Sargassum thunbergii* did not show cytotoxic effects. Therefore, we conclude that *E. arborea* and *S. thunbergii* may contain compounds that have antiallergic effects without inducing cell death.

KEY WORDS: brown algae, cytotoxic effect, histamine release, RBL-2H3 cells.

INTRODUCTION

The number of patients with allergies such as atopic dermatitis and asthma has increased recently, particularly in developed countries. This situation has stimulated a search for food components with anti-allergic effects. For example, many reports on compounds such as tea catechin,^{1,2} polyphenols derived from *Perilla frutescens*,³ and ellagitannin derived from *Rubus suavissimus*⁴ have been published. In addition to these compounds, microorganisms^{5,6} such as *Lactobacillus* sp., oligosaccharides such as raffinose⁷ and α -cyclodextrin,⁸ and lipids such as polyunsaturated fatty acids⁹ also are reported to have anti-allergic effects.

As for marine organisms, the sulfated polysaccharide fucoidan¹⁰ and other polysaccharides^{11,12} derived from certain seaweeds also have anti-allergic effects. There are reports of other seaweed compounds besides polysaccharides that may have anti-allergic effects. For example, Shibata *et al.*¹³ reported that phlorotannins derived from the brown alga *Eisenia bicyclis* inhibited hyaluronidase, phospholipase A2, lipoxygenases, and cyclooxygenases,¹⁴ which are involved in the inflammatory response. However, they did not

use either cultured cells or experimental animals in their experiments. Conversely, Ishihara *et al.*¹⁵ reported that polyunsaturated fatty acids derived from *Undaria pinatifida* and *Ulva pertusa*, and tetracosahexanoic acid derived from the brittle star *Ophiura sarsi*¹⁶ inhibited production of histamine and icosanoids by cultured mouse mast MC/9 cells. Further, Na *et al.*¹⁷ reported that the methanol extract of *Sargassum hemiphyllum* inhibits atopic allergic reactions by inhibiting interleukin-8 and tumor necrosis factor (TNF)- α from MHC-1 without reducing the viability of MHC-1, and by decreasing TNF- α induced NF- κ B in 293T cells. Although there are reports on the anti-allergic effects of components of marine products, many marine organisms such as macroalgae remain untested.

In this report, we screened methanol extracts from 41 marine macroalgae and one sea grass collected from the Ise-Shima region of Mie Prefecture, Japan for anti-allergic effects using cultured RBL-2H3 cells.

MATERIALS AND METHODS

Materials

Forty-one macroalgae (5 green, 15 red, and 21 brown) and one sea grass were collected from the coast of the Ise-Shima region of Japan (Mugizaki

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and Zaga Island, Mie Prefecture) in April and May 2001. After washing with tap water, the algae were stored at -40°C until use.

Cell culture

RBL-2H3 cells¹⁸ were provided by the Japanese Cancer Research Resource Bank (JCRB, Ibaraki, Osaka, Japan). Cells were cultured in Eagle's modified essential medium (EMEM, MP Biomedicals, Illkirch, France) supplemented with 20% fetal bovine serum (FBS, Trace Scientific, Melbourne, Australia), 24 mM sodium bicarbonate (Wako Pure Chemical Co, Tokyo, Japan), 2 mM L-glutamine (Invitrogen, Carlsbad, CA, USA), 75 000 IU/L penicillin (Wako), 150 μM streptomycin (Wako), 0.1 mM nonessential amino acid (Invitrogen) and 1 mM sodium pyruvate (Camrex Bio, Walkersville, MD, USA) at 37°C in a humidified atmosphere of 5% CO_2 . The reagents used for cell culture were sterilized by either autoclaving or filtration.

Extraction

As shown in Fig. 1, 10 g (wet weight) of algal tissue was chopped into small pieces and stirred in 100 mL of 80% methanol at room temperature for 30 min. The methanol was decanted and the extraction repeated with a fresh 100-mL aliquot of methanol. The combined extracts were passed through filter paper (No. 2, Toyo, Tokyo, Japan) and concentrated to approximately half the volume (100 mL) by evaporation. Next, the concentrate was fractionated by diethylether to remove pigments and the bottom (aqueous) layer with the water-soluble fraction was collected. The fraction was dried by evaporation and dissolved in Tyrode buffer (pH 7.2).¹ The aqueous solution was stored at -20°C until use.

Cell stimulation¹

RBL cells were collected from a culture dish by trypsinization and suspended at a concentration of 1×10^5 cells/mL in EMEM. Four hundred microliters of cell suspension were inoculated in a 24-well plate. After preculture for 3 days, the cells were sensitized with 1 μL of anti-DNP IgE (Sigma-Aldrich, St. Louis, MO, USA) to final concentration 2.5 $\mu\text{g}/\text{mL}$ for 24 h. After washing with Tyrode buffer (pH 7.2) three times to remove free anti-DNP IgE, 400 μL of Tyrode buffer containing test samples (1 mg/mL) was added to each well. After incubation for 10 min, the cells were stimulated

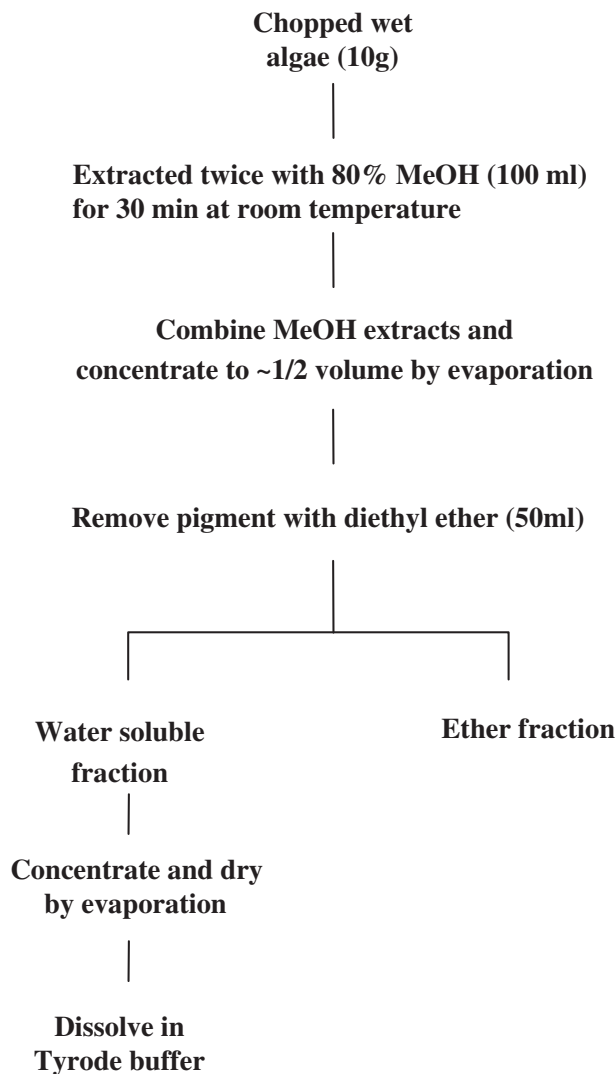


Fig. 1 Methanol extraction scheme.

with DNP-BSA (LSL Co, Tokyo, Japan) to final concentration 0.5 $\mu\text{g}/\text{mL}$ for 35 min and the reaction was stopped on ice for 15 min.

HPLC analysis¹⁹

The supernatants from each well were collected and mixed with perchloric acid to a final concentration of 1.5% v/v. The mixture was centrifuged at 15 000 g at 4°C for 30 min and filtered through a microfilter (0.45 μm , Toyo). The filtrate (10 μL) was applied to a high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan). HPLC analysis was conducted by the on-column method under the following conditions: an ODP-50 4D column (4.6 mm internal diameter $\times$ 150 mm, Showa Denko Co, Tokyo, Japan), mobile

phase of acetonitrile–50 mM sodium tetraborate aqueous solution (18:82, v/v) containing 1 mM *o*-phthalaldehyde (OPA) and 1 mM *N*-acetyl-L-cysteine (NAC), column temperature 40°C, and flow rate of 0.5 mL/min. An RF-550 fluorescence HPLC monitor (Shimadzu) set at excitation and emission wavelengths of 330 and 430 nm, respectively, was used for fluorescence detection.

The inhibitory ratio of histamine release from RBL cells was calculated by the formula:

$$\text{Inhibitory ratio (\%)} = 100 - (T - \text{NC}) / (\text{PC} - \text{NC}) \times 100$$

where NC = peak area of the negative control without stimulation, PC = peak area of the positive control that was stimulated, and T = peak area of the test group that was stimulated with the sample extract.

Cell survival

After preculture for 3 days in a 24-well plate, the RBL cells were incubated with 400 µL of each Tyrode buffer including the sample extracts of seven brown algae for 45 min. The concentration of each of the seven extracts was 1 mg/mL. After the culture media were discarded, cells were trypsinized for 5 min. A 190-µL aliquot of the trypsinized cell suspension was mixed with 10 µL of 5% Trypan blue solution, and cell survival in the mixture was immediately checked by the Trypan blue excursion method under a light microscope. Unstained cells were considered to be viable and stained cells non-viable. The survival ratio then was calculated by the formula:

$$\text{Survival ratio (\%)} = S/T \times 100$$

where S = number of unstained cells, and T = total number of cells scored.

Statistics

All data are expressed as means $\pm$ standard deviation (SD) from triplicate measurements ($n = 3$).

RESULTS

Histamine release assay

Methanol extracts (1 mg/mL each) of 41 macroalgae and one sea grass were investigated for inhibitory effects against histamine release from RBL cells stimulated by the antigen–antibody reaction, as analyzed by HPLC. The stimulated cells were

exposed to the extracts for 45 min. As shown in Table 1, inhibitory effects were found in seven brown algal extracts, but extracts of green and red algae, and one sea grass had no effects. Thus, we conclude that among the organisms tested, anti-allergic activity is limited to certain brown macroalgae.

Cell survival assay

Methanol extracts (1 mg/mL each) from the seven brown algae that showed inhibitory effects against histamine release by RBL cells were tested for cytotoxicity by Trypan blue staining. As shown in Fig. 2, only *E. arborea* ($88.6 \pm 4.5\%$) and *S. thunbergii* ($88.7 \pm 10.7\%$) showed limited cytotoxic effects against RBL cells, but the other five brown algae induced cell death. The survival ratios for cells exposed to the extract from the other five brown algae were: *Ecklonia cava* $55.1 \pm 7.0\%$, *Ishige foliacea* $11.7 \pm 10.2\%$, *I. okamurai* $14.7 \pm 11.6\%$, *Sargassum micracanthum* $19.3 \pm 13.3\%$, and *S. ringgoldianum* $12.2 \pm 10.2\%$.

From these data, it appears that *E. arborea* and *S. thunbergii* have antihistamine release effects without cell death.

DISCUSSION

Of the 41 seaweeds and one sea grass tested, only the methanol extracts of seven brown algae

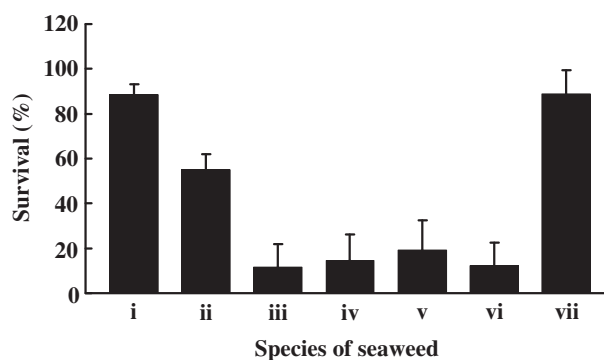


Fig. 2 Cytotoxic effects of 80% methanol extracts from brown algae on RBL-2H3 cells exposed to the extracts for 45 min. After trypsinization, cells were stained with Trypan blue. Concentrations of all extracts were 1 mg/mL. The survival ratio was calculated as the ratio of the number of unstained (live) cells to the total number of cells scored. Values are means $\pm$ standard deviation, $n = 3$. i, *Eisenia arobrea*; ii, *Ecklonia cava*; iii, *Ishige foliacea*; iv, *Ishige okamurai*; v, *Sargassum micracanthum*; vi, *Sargassum ringgoldianum*; vii, *Sargassum thunbergii*.

Table 1 Inhibitory effects of seaweeds on histamine release of RBL-2H3 cells

Classification	Taxonomic name	% Inhibition [†]	Classification	Taxonomic name	% Inhibition [†]
Brown algae	<i>Colpomenia sinnosa</i>	–	Green algae	<i>Cladophora</i> sp.	–
	<i>Cystophyllum turneri</i>	–		<i>Codium fragile</i>	–
	<i>Dictyopteris undulata</i>	–		<i>Monostroma nitidum</i>	–
	<i>Dictyota dichotoma</i>	–		<i>Ulva pertusa</i>	–
	<i>Ecklonia cava</i>	91.9 ± 10.5		<i>Urothrix flacca</i>	–
	<i>Eisenia arobreia</i>	74.3 ± 33.8	Red algae	<i>Amphiroa zonata</i>	–
	<i>Hizikia fusiformis</i>	–		<i>Gelidium amansii</i>	–
	<i>Hydroclathras clathratus</i>	–		<i>Gloiopeltis complanata</i>	–
	<i>Ishige foliacea</i>	63.3 ± 5.9		<i>Gloiopeltis furcata</i>	–
	<i>Ishige okamurai</i>	63.2 ± 28.6		<i>Gracilaria compressa</i>	–
	<i>Padina arborescens</i>	–		<i>Gracilaria textorii</i>	–
	<i>Pterocladia tenuis</i>	–		<i>Gracilaria verrucosa</i>	–
	<i>Sargassum micracanthum</i>	20.0 ± 7.1		<i>Grateloupia filitina</i>	–
	<i>Sargassum muticum</i>	–		<i>Grateloupia turuturu</i>	–
	<i>Sargassum patens</i>	–		<i>Ahnfeltiopsis flabeliformis</i>	–
	<i>Sargassum piluliferum</i>	–		<i>Hypnea variabilis</i>	–
	<i>Sargassum racemosum</i>	–		<i>Laurencia intermedia</i>	–
	<i>Sargassum ringgoldianum</i>	70.0 ± 14.1		<i>Laurencia okamurai</i>	–
	<i>Sargassum thunbergii</i>	40.7 ± 14.0		<i>Marginisporum aberrans</i>	–
	<i>Tinocladia crassa</i>	–		<i>Marginisporum crassissima</i>	–
	<i>Undaria pinnatifida</i>	–	Sea grass	<i>Phyllospadix japonicus</i>	–

RBL cells stimulated by antigen-antibody reaction were exposed to the samples for 45 min. Concentration of all extracts were 1 mg/mL. Values are means ± SD, $n = 3$.

[†]no inhibition.

inhibited histamine release from RBL-2H3 cells. Further, only the extracts of *E. arborea* and *S. thunbergii* did not show cytotoxicity. Therefore, these two brown algae appear to be the best candidates for development as anti-allergic foodstuffs.

Methanol extracts prepared in this study may include phlorotannins as the main phenolic component. We detected phenolic compounds in the *E. arborea* extract (10.5 ± 0.1 mg/g, $n = 3$) by the Folin–Denis method. Because polysaccharides such as alginate and fucoidan are poorly extracted in alcohols such as methanol, the antihistamine effects found in the brown algal extracts are unlikely to be attributed to polysaccharides.

For the anti-allergic effects of phlorotannins, Shibata *et al.*¹³ reported that phlorotannins derived from a chloroform–methanol extract of *E. bicyclis* inhibits hyaluronidase, phospholipase A2, lipoxygenases, and cyclooxygenase¹⁴ related to inflammatory reactions, although they did not use either cultured cells or experimental animals in their study. Tea catechin, an important terrestrial anti-allergic polyphenol, inhibits hyaluronidase²⁰ and the degranulation of RBL-2H3 cells¹ or human basophile leukemia (KU812) cells²¹ that cause type I allergic reactions such as asthma, allergic rhinitis, atopic dermatitis, and anaphylaxis. Therefore,

together with these reports,^{1,13,14,20,21} we conclude that the extracts from *E. arborea* have inhibitory effects on type I allergies.

Conversely, since phenolic compounds were not detected in the methanol extract of *S. thunbergii*, the active components are not phlorotannins. Since there is a report that sulfoquinovosyldiacylglycerides (SQDG) and sulfoquinovosylmonoacylglycerides (SQMG) from the methanol extract of *S. hemiphyllum* inhibited histamine release from rat peritoneal mast cells,¹⁷ further studies are needed to determine the active components in the extract of *S. thunbergii*.

Other than anti-inflammatory or anti-allergic effects, algal phlorotannins have inhibitory activities against oxidation,²² tumors,^{23,24} diabetes complications,^{25,26} α 2-macroglobulin and plasmin,²⁷ and HIV.²⁸ They also have bactericidal activity,²⁹ aid improvement of memory,³⁰ and have deodorizing effects.³¹ Based on this research, phlorotannins are promising compounds not only for controlling allergic reactions, but many other diseases.

This study is the first report on methanol extracts of *E. arborea* and *S. thunbergii* inhibiting histamine release from cultured RBL-2H3 cells, and suggests that the components of certain brown algae may contain beneficial material for human

health that can be used as functional foodstuffs. Further studies on the determination of active compounds are now in progress.

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