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Anti-allergic activity of an ethanol extract of bunching onion (*Allium fistulosum*), a traditional vegetable from Osaka

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ABSTRACT

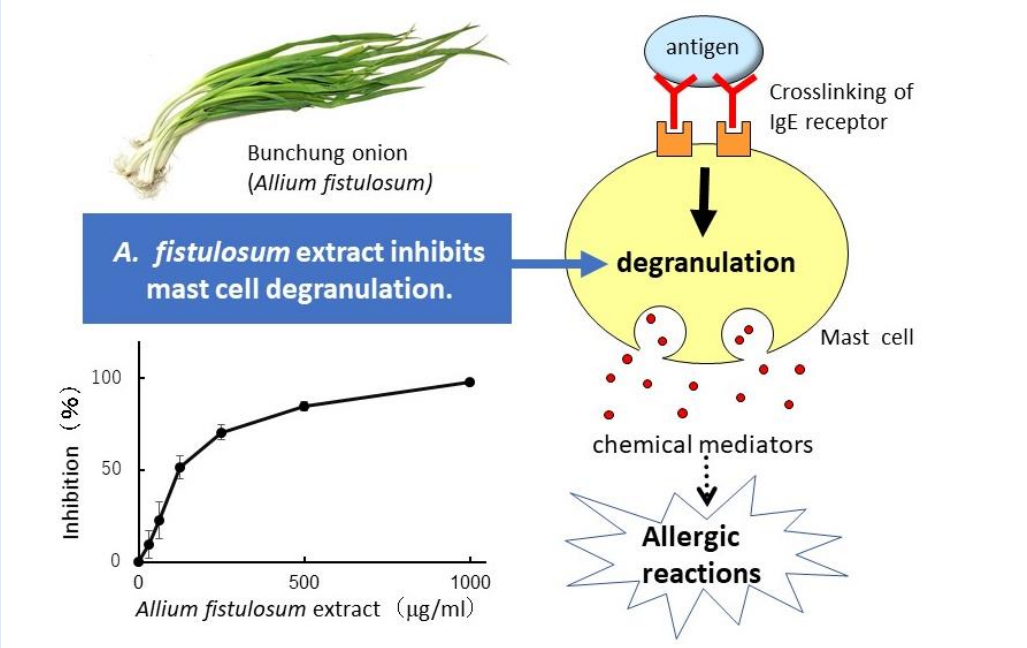
Introduction: The incidences of allergic diseases such as allergic rhinitis, atopic dermatitis, asthma, and food allergies are rising in developed countries. Mast cells play critical roles in various biologic processes related to allergic diseases, including expressing the high-affinity receptor for immunoglobulin (Ig) E on their surface. The interaction of multivalent antigens with surface-bound IgE causes the secretion of granule-stored mediators, as well as the de novo synthesis of cytokines. These mediators and cytokines precede the development of allergic diseases. In Osaka Prefecture, Japan, certain locally cultivated crops have received 'Naniwa-yasai', a designation that certifies the significance of traditional vegetables in supporting Osaka's agriculture and cuisine for over a century. In this study, we investigated the anti-allergic effects of four traditional vegetables from Osaka.

Methods: Vegetables were obtained from Research Institute of Environment, Agriculture and Fisheries, Osaka Prefecture. Vegetables (100g) were extracted using 60% aqueous ethanol (1litter) for 1h. After cooling and filtration, the filtrate was concentrated in vacuo and lyophilized to give an extract. Rat basophilic leukemia RBL-2H3 cells, which are used as a mast cell model, were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). The degranulation of mast cells was determined using a β-hexosaminidase release assay.

Results: The anti-allergic activity of extracts made from Tanabe radish, Moriguchi radish, bunching onion (Allium fistulosum), and mizu eggplant were examined. Various concentrations of each extract were added to RBL-2H3 mast cells prior to stimulation with the calcium ionophore A23187. β-hexosaminidase release was markedly and dose dependently decreased in mast cells following the addition of A. fistulosum, demonstrating its anti-allergic activity. None of the other vegetables had an anti-allergic effect on RBL-2H3 cells.

Conclusion: An Osakan traditional vegetable, bunching onion (Allium fistulosum), exhibited anti-allergic activity, and should be explored further as a possible treatment for allergic diseases.

Keywords: mast cell, anti-allergy, traditional vegetables, bunching onion (Allium fistulosum)



INTRODUCTION

The incidences of allergic diseases such as allergic rhinitis, atopic dermatitis, asthma, and food allergies have increased in several countries [1]. Mast cells and basophils play critical roles in various biologic processes related to allergic diseases [2, 3], and express the high-affinity receptor for immunoglobulin (Ig) E on their surface. The interaction of multivalent antigens with surface-bound IgE causes the secretion of granule-stored mediators, as well as the *de novo* synthesis of cytokines [4]. These mediators and cytokines activate the migration of neutrophils and macrophages, and the reactions brought about by these cells cause tissue inflammation [5] and an allergic reaction.

In Osaka Prefecture, Japan, locally cultivated crops of traditional vegetables are granted a designation of 'Naniwa-yasai', which certifies their significance in supporting Osaka's agriculture and cuisine for over a century. The criteria for this designation are that the vegetable is clearly distinguishable from other vegetables of the same type, and that its seeds and seedlings are

commercially available for cultivation [6].

In the present study, we evaluated the anti-allergic activity of four Osakan vegetables that have been traditionally consumed for relief from allergic symptoms.

METHODS

Reagents: Dulbecco's modified Eagle's medium (DMEM), an antibiotic solution (100 ×), *p*-nitrophenyl-*N*-acetyl-β-D-glucopyranoside (PNAG), and calcium ionophore A23187 were obtained from Sigma-Aldrich Japan (Tokyo, Japan). Fetal bovine serum (FBS) was purchased from Biowest (Nuaillé, France).

Vegetable extract preparation: Vegetables were obtained from Research Institute of Environment, Agriculture and Fisheries, Osaka Prefecture. Vegetables (100g) were extracted using 60% aqueous ethanol (1litter) for 1h. After cooling and filtration, the filtrate was concentrated *in vacuo* and lyophilized to give an extract. Dried extracts were dissolved in 10% dimethyl sulfoxide and diluted with Tyrode's buffer (137 mM NaCl, 5.6 mM glucose, 11.9 mM $NaHCO_3$, 2.7 mM KCl and 0.32 mM NaH_2PO_4) before use.

Cell culture: Rat basophilic leukemia RBL-2H3 cells, which are used as a mast cell model, were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). RBL-2H3 cells were maintained in DMEM supplemented with 10% heat-inactivated FBS.

Degranulation of mast cells: The release of β-hexosaminidase has been well correlated with that of histamine, a major component of mast cell granules [7], so the degranulation of mast cells was determined using a β-hexosaminidase release assay as previously described [8]. Briefly, RBL-2H3 mast cells were washed with Tyrode's buffer containing 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$, and then various concentrations of vegetable extracts (0–1000 μg/mL) were added. The cells (9.6×10^4) were treated with 10 μM A23187 for 30 min at 37°C immediately after adding the extracts. Cell supernatants and total cell lysates dissolved in 2% Triton X-100 were collected separately, and the β-hexosaminidase activity in each was quantified by spectrophotometric measurement of the hydrolysis of PNAG in 0.1 M sodium citrate buffer (pH 4.5). Reactions were terminated after 90 min by adding 0.2 M glycine (pH 11.0).

RESULTS

Osakan traditional vegetables with potential anti-allergic activity: The four Osakan traditional vegetables that were investigated for anti-allergic activity are listed in Table 1. Extracts were produced differently for each vegetables: the leaves and roots of Tanabe radish and Moriguchi radish, the leaves of bunching onion (*Allium fistulosum*), and the fruit of mizu eggplant.

Table 1. Osakan traditional vegetables tested for anti-allergic activity

English name	Japanese name	Part(s) used for extract preparation
Tanabe radish	Tanabe daikon	leaves, root
Moriguchi radish	Moriguchi daikon	leaves, root
Bunching onion (*Allium fistulosum*)	Nanba negi	leaves
Mizu eggplant	Mizu nasu	fruit

Effects of vegetable extracts on mast cell degranulation: Various concentrations of each vegetable extract were

added to RBL-2H3 mast cells prior to stimulation with A23187. β-hexosaminidase release was markedly and dose dependently decreased (Fig. 1) by the addition of *A. fistulosum* extract, indicating a suppressive effect of *A. fistulosum* on the degranulation of RBL-2H3 cells induced by A23187. In contrast, Tanabe radish, Moriguchi radish and mizu eggplant extracts did not inhibit β-hexosaminidase release in RBL-2H3 mast cells (data not shown), indicating that they do not have anti-allergic activity. *A. fistulosum* extract had no cytotoxic effect by the trypan blue exclusion method (data not shown).

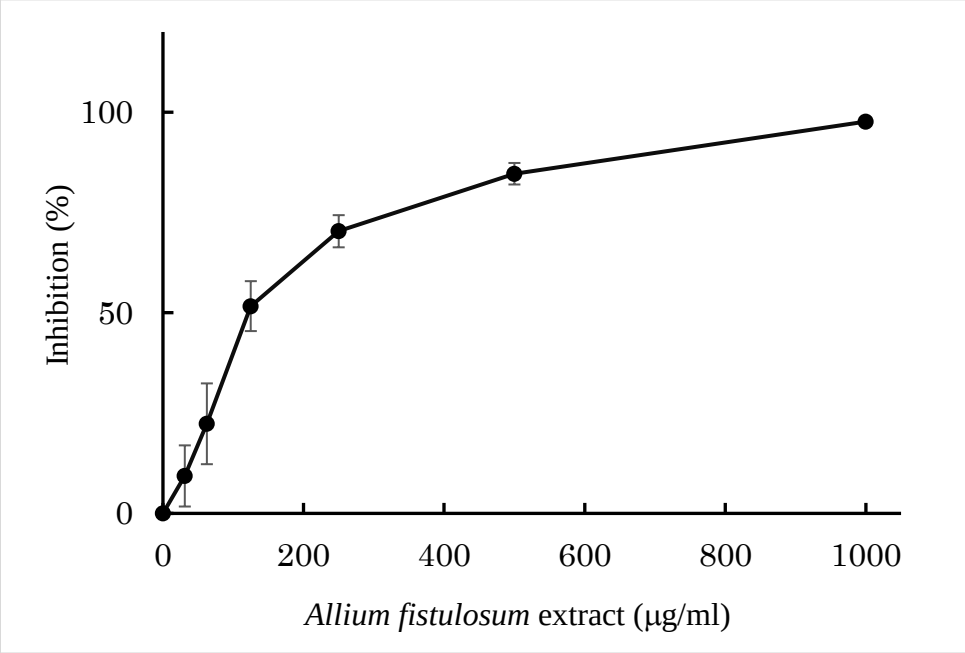


Fig. 1. Effect of bunching onion (*Allium fistulosum*) extract on mast cell degranulation. Effect of bunching onion extract on RBL-2H3 mast cell degranulation as measured by a β-hexosaminidase release assay. Each value represents the mean ± standard error of three experiments.

DISCUSSION

We tested extracts of four Osakan traditional vegetables for anti-allergic activity, and found that one, bunching onion *A. fistulosum*, inhibited A23187-induced β-hexosaminidase release in RBL-2H3 mast cells. The amount of β-hexosaminidase released from mast cells is commonly used to evaluate the extent of degranulation. The release of histamine and other chemical mediators by mast cells is important initiatory process in immediate types of anaphylactic reactions. Therefore, our results indicated that *A. fistulosum* extract may exert its anti-allergic effects by inhibiting the chemical mediators from mast cells. It has been reported that leek (*Allium porrum*) extract decreased the degranulation of mast cells and may have potential anti-allergic effects [9]. Therefore, this inhibitory effect is not a characteristic of bunching onion.

A. fistulosum has a variety of physiological effects, including the inhibition of platelet aggregation, modulation of aortic vascular tone, and lowering of blood pressure and hyperglycemia [10-13]. An aqueous extract of *A. fistulosum* reportedly attenuates anti-inflammatory effects in mice [14]. Furthermore, an oral administration of *A. fistulosum* leaves was shown to promote the activation of immune responses and suppress tumor cell proliferation in mice [15].

The traditional vegetables described in this study offer

unique flavors that can only be eaten and enjoyed in the Osaka region. Another Osakan traditional vegetable, kintoki carrot, has been used for Osechi (Japanese New Year) dishes, while Kotsuma pumpkin is served at local events such as pumpkin festivals [6]. For a time, traditional vegetables unique to the Osaka region were not cultivated because they were considered difficult to prepare and the traditional cooking methods had been forgotten [6]. However, because these vegetables are not only tasty, but are also believed to help prevent illnesses, including modern lifestyle-related diseases, they are likely to become more popular than ordinary vegetables in supermarkets. Amidst the challenging circumstances created by the COVID-19 pandemic, a reintroduction of traditional vegetables, together with recipes and guidance on their preparation, could help to revitalize local communities and businesses. Public exposure through restaurant menus and increased availability in retail outlets might further promote the brand of health-promoting traditional vegetables. This would also support the achievement of several of the Sustainable Development Goals for Food and Nutrition for 2020–2025 [16]; namely, good health and well-being (no. 3), industry, innovation, and infrastructure (no. 9), responsible consumption and production (no. 12), and life on land (no. 15).

CONCLUSION

An extract of *A. fistulosum* suppressed the degranulation of RBL-2H3 mast cells, suggesting that *A. fistulosum* may have an inhibitory effect on the allergic reaction. The anti-allergic effect of this traditional vegetable may have therapeutic applications for inflammatory allergic diseases.

Competing Interests: The authors have no financial interests or conflicts of interests.

Authors' Contributions: TJ and YK conceived, designed, and performed the experiments, and analyzed the data. KK and KK contributed to the preparation of materials. TJ and YK wrote the manuscript.

Abbreviations: Ig: immunoglobulin, DMEM: Dulbecco's modified Eagle's medium, PNAG: *p*-nitrophenyl-N-acetyl-β-D-glucopyranoside, FBS: fetal bovine serum

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