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Antiallergic activities of eleven onions (*Allium cepa*) were attributed to quercetin 4'-glucoside using QuEChERS method and Pearson's correlation coefficient

Akihiko Sato ^a, Ting Zhang ^b, Lina Yonekura ^{a,b}, Hirotoshi Tamura ^{a,b,*}

^a The United Graduate School of Agricultural Sciences, Ehime University, 3-5-7 Tarumi, Matsuyama 790-8566, Japan

^b Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa 761-0795, Japan

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ABSTRACT

The antiallergic activity (type I hypersensitivity) of eleven onions (eight cultivars, three different geographical origins) used for daily meals was comparatively determined using RBL-2H3 cells. A large variation of antiallergic activity (IC_{50} = 20.8 to 310.1 μ g/mL) was found in the edible parts. Among the eight cultivars, Satsuki showed the highest activity (IC_{50} = 89.1 μ g/mL), and among the geographical origins Hokkaido was the most effective (20.8 μ g/mL). The correlation coefficient between the antiallergic activities and thirty-four peaks in HPLC chromatograms of eleven onion extracts isolated using the QuEChERS method indicated that quercetin 4'-glucoside has a highly-positive correlation (r = 0.91) with the antiallergic activity. Indeed, quercetin 4'-glucoside was found in high concentration (140.1 mg/kg) in Hokkaido onions, and the isolated compound showed high antiallergic activity (IC_{50} = 3.0 μ g/mL). Therefore, Hokkaido onions were the best inhibitors of type I allergy, possibly due to their high content of quercetin 4'-glucoside.

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1. Introduction

Onion (*Allium cepa*) is one of the major vegetables people consume in their daily life (Griffiths, Trueman, Crownther,

Thomas, & Smith, 2002) and is the second most produced vegetable crop after tomatoes. Furthermore, scientific reports indicate that onion has biological activities, such as antioxidant (Moreno, Corzo-Martinez, Castillo, & Villamiel, 2006; Prakash, Singh, & Upadhyay, 2007; Zill-e-Huma, Fabiano-Tixer,

* Corresponding author. Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa 761-0795, Japan. Tel.: +81 87 891 3104; fax: +81 87 891 3021.

E-mail address: tamura@ag.kagawa-u.ac.jp (H. Tamura).

Abbreviations: RBL-2H3, rat basophilic leukemia cell; DMSO, dimethyl sulfoxide; UV, ultraviolet; HPLC, high performance liquid chromatography; NMR, nuclear magnetic resonance spectroscopy; DEPT, distortionless enhancement by polarization transfer; COSY, correlated spectroscopy; HETCOR, hetero-nuclear correlation; HMBC, hetero-nuclear multiple-bond connectivity; MS, mass spectrometry

Chemical compounds: Quercetin (PubChem CID: 5280343); Quercetin 4'-glucoside (PubChem CID: 12442954); Quercetin 3,4'-diglucoside (PubChem CID: 5320835); Isorhamnetin 4'-glucoside (PubChem CID: 44259381).

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Elmaataoui, Dangles, & Chemat, 2011), anti-inflammatory (Albishi, John, Al-Khalifa, & Shahidi, 2013a; Simin et al., 2013), and antimicrobial (Ye, Dai, & Hu, 2013) properties. Recently, we reported the antiallergic effects of forty-six vegetables, among which the onion was featured as a potent antiallergic vegetable (Sato, Surojanametukul et al., 2014).

Food allergies have become a serious problem for many people and its prevalence is increasing around the world (Sicherer & Sampson, 2007, 2010). Type I allergies including food allergies, atopic dermatitis, and hay fever (Ring, Kramer, Schafer, & Behrendt, 2001) may be partly due to changes in lifestyle, changes in the environment such as planting many of the same varieties of plants and trees, and air-pollution such as NO_x and PM_{2.5} from automobiles and industries. In the development of antiallergic medicines, suppressing β -hexosaminidase release is a common target, because degranulation of rat basophilic leukemia (RBL-2H3) cells is linked to the release of leukotriene and histamine (Chukaew, Ponglimanont, Karalai, & Tewtrakul, 2008; Shimoda et al., 2006; Yoo, Sok, & Kim, 2013). Besides, it is desirable to treat allergies using drugs that do not have side-effects, and potent antiallergic substances from food ingredients may contribute to the decrease in the number of allergy patients without the burden of side-effects.

Japan is a major producer of onion varieties. Consumption of onion may influence the total effective intake of polyphenols (Arabbi, Genovese, & Lajolo, 2004; Harnly et al., 2006; Sampson, Rimm, Hollman, Vries, & Katan, 2002) and other ingredients with various biological activities. So far, new onion varieties have been developed with different features such as longer shelf life and new textures and tastes in response to consumers' demands. As some varieties of onions may contain an abundance of polyphenols, we compared the antiallergic activities of various kinds of onions. The identification of onion varieties with high antiallergic activity may promote the production of specific varieties to help maintain a healthy life. However, the systematic selection of antiallergic active substances in natural plants and food ingredients has not yet been established. Quantitative structure–activity relationship (QSAR) with physicochemical parameters and other statistical methods have been studied to help create new drugs (Fischer, Paulussen, Tollenaere, De Mol, & Janssen, 1998; Zhou, Xu, Wu, & Liu, 1999). However, computational analysis has been applied only in a few cases to examine new chemicals with valuable biological activities in natural products and food ingredients.

In this paper, we applied the Pearson's correlation coefficient between the amount of chemicals in onion extracts and their inhibitory activity on type I allergy, in combination with factor analysis for screening of compounds with antiallergic properties. The most effective cultivar as well as the most probable effector for the inhibition of type I allergy was identified among eight cultivars and three commercially available onions from different geographical origins within Japan.

2. Materials and methods

2.1. Materials

Eight cultivars of onion (Advance, Answer, Momiji No. 3, Momiji no kagayaki, Satsuki, Shippokan 70, Shipposei No. 7, and

Tarzan) were provided from Shippo Co., Ltd. (Kagawa, Japan). Onions whose geographical origins were Kagawa, Hokkaido, and Hyogo prefectures were purchased from a local market in Kagawa, Japan.

Dulbecco's modified Eagle's medium (D-MEM), PBS (–), and Triton X-100 were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Fetal bovine serum (FBS) was obtained from Equitech-bio (Kerrville, TX, USA). Antibiotic-antimycotic 100X was obtained from Invitrogen (Grand Island, NY, USA). Mouse monoclonal anti-dinitrophenyl antibody, albumin dinitrophenyl (HSA), Tyrode's salt solution, and *p*-nitrophenyl-2-acetamido-2-deoxy- β -D-glucopyranoside (*p*-NAG) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Bovine serum albumin (BSA) was obtained from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Quercetin 3,4'-diglucoside, quercetin 4'-glucoside, and quercetin dihydrate were purchased from Extrasynthese (Genay Cedex, France) and Wako Pure Chemical Industries, Ltd. and used without further purification.

2.2. Preparation of the media and samples

DMEM including 10% FBS and 1% antibiotic-antimycotic 100X was used as a base medium. Modified Tyrode (MT) buffer was prepared by dissolving HEPES (4.76 g/L) and BSA (1 g/L) in Tyrode's salt solution. Anti-DNP IgE solution (anti-body) was prepared by diluting mouse monoclonal anti-dinitrophenyl antibody with the base medium (50 ng/mL). HSA solution (antigen) was prepared by dissolving HSA in MT buffer (2.5 μ g/mL). The samples tested were dissolved in dimethyl sulfoxide (DMSO), and the solutions were added to MT buffer (final DMSO concentration was adjusted at 0.1%).

2.3. Extraction using the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method

Extraction from the edible part of eleven onion samples (eight cultivars and three onions from different geographical origins described in the "Materials" section) and from the skin of the Satsuki cultivar was performed using the QuEChERS method that is commonly used in the analysis of residual agricultural chemicals (Lehotay et al., 2010; Paya et al., 2007). Onion sample (100 g) was chopped in a food processor, then 10 g of chopped sample was placed in a polypropylene centrifuge tube (50 mL) and 10 mL acetonitrile was added. The mixture was homogenized for 3 min. Sodium chloride (1 g), trisodium citrate dehydrate (1 g), disodium hydrogen citrate sesquihydrate (0.5 g), and anhydrous magnesium sulfate (4 g) were added to the solution, and the mixture was immediately hand-shaken for 1 min. Finally, the mixture was centrifuged at 3000 rpm (1670 $\times$ g) for 5 min. The acetonitrile extract obtained was dried using an evaporator with a vacuum pump. The weight of each dried extract is shown in Table 1.

2.4. Extraction of antiallergic flavonoids from the Satsuki cultivar

The edible part of the Satsuki cultivar (300 g) was crushed using a hand mixer. The crushed onion was soaked in 100% methanol (300 mL) three times. The methanol was then removed

Table 1 – Correlation coefficients between antiallergic activity and amount of compounds (peaks 6, 13, 15, 19, 22, 26, 29, and 34).

Sample	QuEChERS extract	Antiallergic activity ^a		Peak area ^b							
	[mg/10 g of onion sample]	IC ₅₀ [μg/mL]	100/IC ₅₀	Peak 6	Peak 13	Peak 15	Peak 19	Peak 22	Peak 26	Peak 29	Peak 34
Shipposei No. 7	14.3	310.1 ± 13.3	0.32	ND	ND	976121	73079	800525	77772	31790	ND
Momiji No. 3	26.6	293.6 ± 32.8	0.34	ND	19631	1008072	53528	1785277	234848	35488	ND
Shippokan 70	11.3	227.9 ± 7.9	0.43	ND	12049	985650	57984	778879	58936	15045	ND
Answer	17.4	168.7 ± 9.9	0.59	ND	18109	1527816	78552	2559064	201804	29910	ND
Advance	17.8	148.4 ± 9.0	0.67	29185	31853	2719146	102968	2243776	380453	60918	ND
Tarzan	16.0	136.1 ± 0.3	0.73	15015	29769	2113066	67462	2200391	263871	30746	ND
Momiji no kagayaki	21.7	102.5 ± 6.0	0.97	ND	27245	1294059	94176	3189134	363017	158111	13073
Satsuki	19.8	89.1 ± 8.2	1.12	116155	35163	3535323	149428	4705759	718793	145828	ND
Hyogo	20.1	61.4 ± 2.1	1.62	ND	38033	2894313	53645	3037734	372961	66798	ND
Kagawa	24.4	38.1 ± 1.6	2.62	69667	37687	4488444	137569	4156602	350788	51604	ND
Hokkaido	23.5	20.8 ± 1.7	4.80	88486	54401	3948521	215380	7997698	531211	264785	18637
Correlation coefficient ^c			1.00	0.62*	0.81**	0.75**	0.84**	0.91**	0.52	0.76**	0.69*

^a IC₅₀ values of antiallergic activity were calculated from the results of β -hexosaminidase release inhibitory activity assay.

^b Peak areas were calculated from HPLC chromatograms (λ = 290 nm, ND = not detected).

^c Pearson's correlation coefficient of each peak between the differences in antiallergic activity and peak area depending on cultivar.

Identified peaks: peak 15, quercetin 3,4'-diglucoside; peak 22, quercetin 4'-glucoside; peak 26, isorhamnetin 4'-glucoside; peak 29, quercetin.

Each IC₅₀ and peak area represents the mean (n = 3).

Asterisks show that correlation is significantly different: *p < 0.05, **p < 0.01, respectively.

under vacuum. A water-soluble impurity separated from the methanol extracts was recovered again from water extracts by partition with butanol and water. Finally, 28.8 g of methanol extract were obtained from the methanol solution, and then 0.32 g of butanol extract and 28.3 g of water extract were obtained from the methanol extract (Supplementary Fig. S1).

2.5. Isolation of quercetin 3,4'-diglucoside (15), quercetin 4'-glucoside (22), and isorhamnetin 4'-glucoside (26) from Satsuki butanol extract

To isolate quercetin 3,4'-diglucoside (15), quercetin 4'-glucoside (22), and isorhamnetin 4'-glucoside (26), Satsuki butanol extract (300 mg) was purified using an HPLC 5 μ -ODS column with 40% methanol aqueous solution at a flow rate of 1 mL/min and at a UV wavelength of 360 nm. Finally, 23.7 mg of pure quercetin 3,4'-diglucoside (15) with 94.9% purity, 24.1 mg of pure quercetin 4'-glucoside (22) with 99.1% purity, and 4.6 mg of pure isorhamnetin 4'-glucoside (26) with 98.3% purity were isolated after drying under vacuum (Supplementary Fig. S1 for the isolation scheme and Fig. S2 for chemical structure of compounds, 15, 22, 26, and 29).

2.6. Analytical HPLC

HPLC analysis was performed according to Asada and Tamura (2012) and Sato, Shinozaki, and Tamura (2014). The JASCO HPLC system (Tokyo, Japan) comprised a 250 $\times$ 4.6 mm COSMOSIL 5C₁₈-ARII column (Nacalai Tesque Inc., Kyoto, Japan) coupled to a JASCO MD 2010 Plus photodiode array detector and double JASCO PU-980 pumps. Chromatograms were obtained from UV absorbance at 290 nm. The column temperature was set at 40 °C.

The flow rate was 1 mL/min; the mobile phase used was a mixture of 2% acetic acid, 2.5% acetonitrile, and 1.5% phosphoric acid in water (A), and a mixture of 20% acetic acid, 25% acetonitrile, and 1.5% phosphoric acid in water (B). The total analytical time was 41 min and the gradient was done as follows: 100% A to 0% A for 35 min and 0% A was kept for 5 min. For preparative HPLC, the HPLC system consisted of a 20 $\times$ 250 mm DEVELOSIL Ph-UG column (Nomura Chemical Co., Ltd., Aichi, Japan) coupled to a JASCO 875-UV detector (360 nm) with a JASCO PU-980 pump at 1 mL/min, and 40% methanol aqueous solution was used as the mobile phase. Quercetin 3,4'-diglucoside, quercetin 4'-glucoside, and quercetin were dissolved in DMSO and then diluted to prepare a standard solution (0.067 μ g/mL to 0.5 μ g/mL) to make the calibration curve of each chemical.

2.7. β -Hexosaminidase release assay of RBL-2H3 cells

The amount of β -hexosaminidase release by basophilic leukemia cells (RBL-2H3) was determined using a previous method with some modifications (Sato, Shinozaki et al., 2014; Sato, Yamagami, & Tamura, 2012; Sugiura, Torii, Matsuda, & Yamada, 2009). RBL-2H3 was obtained from RIKEN BioResource Center Cell Bank (Ibaraki, Japan). The RBL-2H3 cells in the base medium (D-MEM containing 10% FBS and 1% antibiotic-antimycotic 100X) were dispensed into a 24-well plate (2.5 $\times$ 10⁵ cells/well) and were incubated overnight at 37 °C under a humidified 5% CO₂ atmosphere. After washing with 1 mL PBS, the cells were incubated with 500 μ L antibody solution (mouse monoclonal anti-dinitrophenyl antibody, 50 ng/mL dissolved in base medium) for 2 h sensitization. A test sample solution of 490 μ L was added to the well after washing twice with 500 μ L of modified Tyrode's

buffer (MT buffer, Tyrode's salt solution containing 1 g/L bovine serum albumin (BSA) and 4.76 g/L HEPES). The MT buffer was used as control instead of the sample. The test sample was dissolved in DMSO and diluted with MT buffer (the final concentration of DMSO was set at 0.1%). After 10 min incubation, albumin dinitrophenyl (10 μ L, final concentration = 50 ng/mL) was added to each well and the cells were incubated for 30 min to evoke allergic reactions (degranulation). The reaction was stopped by cooling in an ice bath for 10 min. The supernatant (50 μ L) was transferred to a 96-well plate and incubated with 100 μ L substrate (3.3 mM *p*-nitrophenyl-2-acetamide-2-deoxy- β -D-glucopyranoside) in 0.1 M citrate buffer (pH 4.5) at 37 °C for 25 min. The reaction was terminated by adding 100 μ L of stop solution (2 M glycine buffer, pH 10.0). The absorbance (OD) was measured at 405 nm using a microplate reader (Thermo Scientific Multiskan FC, Yokohama, Japan). The gain in OD reflects β -hexosaminidase release. The calculation was performed using Equations (1) and (2) below. To obtain a valid value, the factors that are not typically induced by samples need to be excluded. In "blank", neither antibody solution nor sample was added to the cells to confirm spontaneous β -hexosaminidase release from the cells. In "control", MT buffer instead of samples was added to the cells to confirm β -hexosaminidase release from the cells in conditions without a sample. In "total", the cells were lysed in 0.1% Triton X-100 in MT buffer to confirm the total amount of β -hexosaminidase contained in the cells. In "sample", both antibody solution and samples were added to the cells to confirm β -hexosaminidase release from the cells in these conditions.

$$\text{ratio of } \beta\text{-hexosaminidase release (\%)} = \frac{(\text{OD}_{\text{control or sample}} - \text{OD}_{\text{blank}})}{(\text{OD}_{\text{total}} - \text{OD}_{\text{blank}})} \times 100 \quad (1)$$

$$\beta\text{-hexosaminidase release (\%)} = \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}}{\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}} \times 100 \quad (2)$$

The ratio of β -hexosaminidase release of "control" should be greater than 25%.

2.8. β -Hexosaminidase inhibitory activity assay

β -Hexosaminidase inhibitory activity was measured according to previous methods with some modifications (Matsuda, Morikawa, Managi, & Yoshikawa, 2003; Matsuda, Morikawa, Ueda, Managi, & Yoshikawa, 2002; Matsuda, Tewtrakul, Morikawa, Nakamura, & Yoshikawa, 2004). RBL-2H3 cells were grown in base medium. The cells were lysed in 0.1% Triton X-100 (5.0×10^5 cells/mL). The cell lysate (25 μ L) was transferred to a 96-well plate. The tested solutions were diluted in different concentrations with MT buffer and then added to the sample well (25 μ L); MT buffer was added to the control well (25 μ L). Following that, 100 μ L of 0.1 M citrate buffer (pH 4.5) containing 3.3 mM *p*-NAG (substrate) was added to each well and incubated for 25 min at 37 °C. The reaction was stopped by adding 100 μ L of 2 M glycine buffer (the stop buffer solution) at pH 10.0 to each well. The absorbance (OD) of each reaction was measured at 405 nm using a microplate reader (Thermo Scientific Multiskan FC). The inhibitory activity of β -hexosaminidase was calculated using the following equation.

$$\beta\text{-hexosaminidase inhibitory (\%)} = \left(1 - \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}}\right) \times 100 \quad (3)$$

2.9. NMR analysis

^1H , ^{13}C , DEPT-45, DEPT-90, DEPT-135, COSY, HETCOR, and HMBC NMR spectra of isorhamnetin 4'-glucoside were recorded with a 600 MHz JNM-ECA spectrometer (JEOL Ltd., Tokyo, Japan), dissolving samples in deuterated methanol with TMS (Isotec, Taiyo Nippon Sanso Corp. Ltd., Tokyo, Japan) as an internal standard.

2.10. MS analysis

The mass spectrum of isorhamnetin 4'-glucoside was measured using fast atom bombardment mass spectrometry (FAB-MS) with a JMS-SX102AAQ Hybrid Mass Spectrometer (JEOL Ltd., Tokyo, Japan). Xenon gas was used as ionized gas and glycerol was used as matrix.

2.11. Statistical analysis

Factor analysis was carried out using IBM SPSS Statistics version 20. The values represent means $\pm$ standard deviation (SD) in triplicate at least. Significant differences were considered at $p < 0.05$ and $p < 0.01$.

3. Results and discussion

3.1. Antiallergic activity of QuEChERS extracts from the flesh of eleven onion samples

Fig. 1 shows the β -hexosaminidase release inhibitory activities of eight cultivars of onions and three different geographical origins. All QuEChERS extracts had β -hexosaminidase release below 50% at 400 μ g/mL of sample concentration thus confirming that onions generally have strong suppressing effect on type I allergy (Sato, Surojanametakul et al., 2014). Of the eight cultivars, the Satsuki cultivar exhibited the highest activity ($44.3 \pm 4.0\%$) at 100 μ g/mL, followed by Momiji no kagayaki ($50.5 \pm 1.3\%$). Among three kinds of commercially available onions, the Hokkaido onion interestingly exhibited the highest activity ($10.0 \pm 0.4\%$), followed by the Kagawa onion ($20.8 \pm 0.6\%$) at 100 μ g/mL. The top five IC_{50} values of antiallergic activity among all eleven samples of onions were 20.8 μ g/mL for Hokkaido, 38.1 μ g/mL for Kagawa, 61.4 μ g/mL for Hyogo, 89.1 μ g/mL for Satsuki cultivar, 102.5 μ g/mL for Momiji no kagayaki cultivar as shown in Table 1.

Fig. 2 shows HPLC chromatograms of extracts of the Hokkaido onion and the Satsuki cultivar obtained from the edible parts (flesh) by the QuEChERS method. Thirty-four peaks in the HPLC chromatograms of eleven onions were compared with respect to retention time and concentration. Quercetin 4'-glucoside (22; 99.1% purity), quercetin 3,4'-diglucoside (15; 94.9% purity), and isorhamnetin 4'-glucoside (26; 98.3% purity), were identified (Fig. S2) as the three most concentrated compounds, comprising 3.5, 3.0, and 0.9% of the QuEChERS dry extract (19.8 mg dry extract/10 g sample) of the Satsuki cultivar when acquired at 290 nm (Fig. 2). Onion contains quercetin

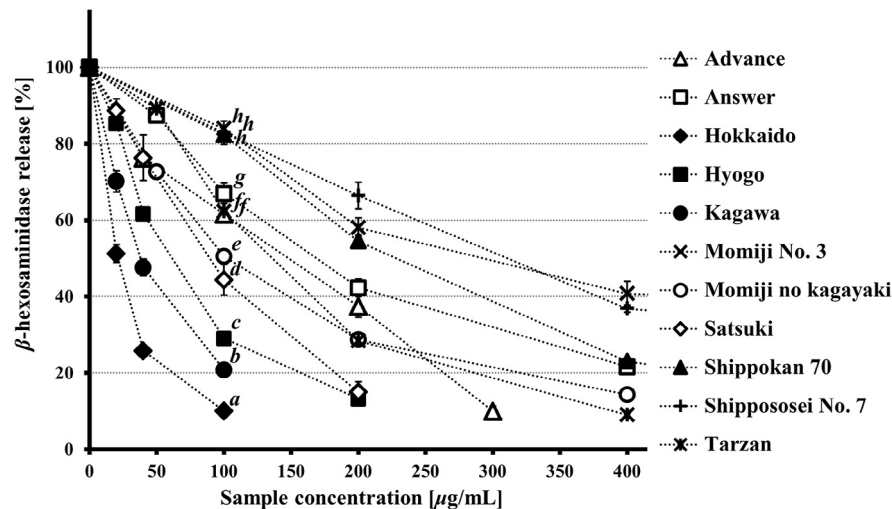


Fig. 1 – Antiallergic activities of eleven onion samples. Each value is mean $\pm$ SD ($n = 4$). Means with different letters are significantly different ($p < 0.05$).

and quercetin derivatives as major components, as previously reported (Bonaccorsi, Caristi, Gargiulli, & Leuzzi, 2005; Perez-Gregorio, Garcia-Falcon, Simal-Gandara, Rodrigues, & Almeida, 2010). Among the eleven cultivars, these three compounds were the majority in HPLC chromatograms acquired at 290 nm (Table 2). The major components are probably the most important effectors for the total antiallergic activity.

3.2. Prediction of antiallergic substances in crude extracts from eleven onion samples using the Pearson's correlation coefficient between the relative peak areas and antiallergic activity

The biological activity of certain chemicals in natural food ingredients is generally determined by biological testing of isolated compounds. However, isolating all components in crude

samples is time-consuming and requires preparative HPLC and purification of certain compounds (Tewtrakul & Itharat, 2006; Yan, Murphy, Hammond, Vinson, & Neto, 2002). To avoid such tedious procedures, the QuEChERS method, commonly used to quantitatively analyze food residues and agrochemicals (Lehotay et al., 2010; Paya et al., 2007), was applied to obtain crude extracts from eleven onion cultivars in this experiment. The correlation coefficients between the concentration of thirty-four peaks in the QuEChERS extracts and their antiallergic activities are listed in Table 1 and supplementary Tables S1–S3. The correlation coefficients of quercetin 4'-glucoside (22), quercetin 3,4'-diglucoside (15), and isorhamnetin 4'-glucoside (26), three major peaks, were 0.91, 0.75, and 0.52, respectively. The correlation coefficient of quercetin 4'-glucoside (22, $r = 0.91$) was the highest among the thirty-four peaks found in eleven crude extracts, followed by those of peaks 19 ($r = 0.84$),

Table 2 – Amount of antiallergic flavonoids in onion.

Onion sample	Amount ^a					
	Quercetin 4'-glucoside (22)		Isorhamnetin 4'-glucoside (26)		Quercetin (29)	
	mg/100 mg of extract	mg/kg of onion ^b	mg/100 mg of extract	mg/kg of onion ^b	mg/100 mg of extract	mg/kg of onion ^b
Shipposei No. 7	0.59 $\pm$ 0.01	8.5 $\pm$ 0.1	0.10 $\pm$ 0.01	1.4 $\pm$ 0.1	0.02 $\pm$ 0.01	0.3 $\pm$ 0.1
Momiji No. 3	1.33 $\pm$ 0.03	35.3 $\pm$ 0.8	0.30 $\pm$ 0.01	8.2 $\pm$ 0.1	0.02 $\pm$ 0.01	0.6 $\pm$ 0.1
Shippokan 70	0.58 $\pm$ 0.01	6.5 $\pm$ 0.1	0.07 $\pm$ 0.01	0.8 $\pm$ 0.1	0.01 $\pm$ 0.01	0.1 $\pm$ 0.1
Answer	1.90 $\pm$ 0.01	33.1 $\pm$ 0.2	0.26 $\pm$ 0.01	4.6 $\pm$ 0.1	0.02 $\pm$ 0.01	0.3 $\pm$ 0.1
Advance	1.67 $\pm$ 0.02	29.7 $\pm$ 0.4	0.50 $\pm$ 0.01	8.9 $\pm$ 0.2	0.04 $\pm$ 0.01	0.7 $\pm$ 0.1
Tarzan	1.64 $\pm$ 0.02	26.2 $\pm$ 0.3	0.34 $\pm$ 0.01	5.5 $\pm$ 0.2	0.02 $\pm$ 0.01	0.3 $\pm$ 0.1
Momiji no kagayaki	2.37 $\pm$ 0.03	51.5 $\pm$ 0.7	0.47 $\pm$ 0.01	10.3 $\pm$ 0.2	0.11 $\pm$ 0.01	2.4 $\pm$ 0.1
Satsuki	3.50 $\pm$ 0.05	69.4 $\pm$ 1.1	0.94 $\pm$ 0.01	18.7 $\pm$ 0.3	0.10 $\pm$ 0.01	2.0 $\pm$ 0.1
Hyogo	2.26 $\pm$ 0.03	45.5 $\pm$ 0.6	0.49 $\pm$ 0.01	9.8 $\pm$ 0.1	0.04 $\pm$ 0.01	0.9 $\pm$ 0.1
Kagawa	3.09 $\pm$ 0.04	75.5 $\pm$ 1.1	0.46 $\pm$ 0.01	11.2 $\pm$ 0.1	0.03 $\pm$ 0.01	0.8 $\pm$ 0.1
Hokkaido	5.96 $\pm$ 0.10	140.1 $\pm$ 2.4	0.69 $\pm$ 0.01	16.4 $\pm$ 0.4	0.18 $\pm$ 0.01	4.3 $\pm$ 0.1
Skin of Satsuki	6.12 $\pm$ 0.16	1578.6 $\pm$ 41.7	2.83 $\pm$ 0.40	787.4 $\pm$ 45.9	4.97 $\pm$ 0.21	1281.4 $\pm$ 54.5

^a Amount of three flavonols was calculated from HPLC chromatograms of QuEChERS extracts ($\lambda = 290$ nm). Each value represents the mean $\pm$ SD ($n = 3$).

^b In the case of the skin of Satsuki cultivar, the amount was shown by "mg/kg of skin".

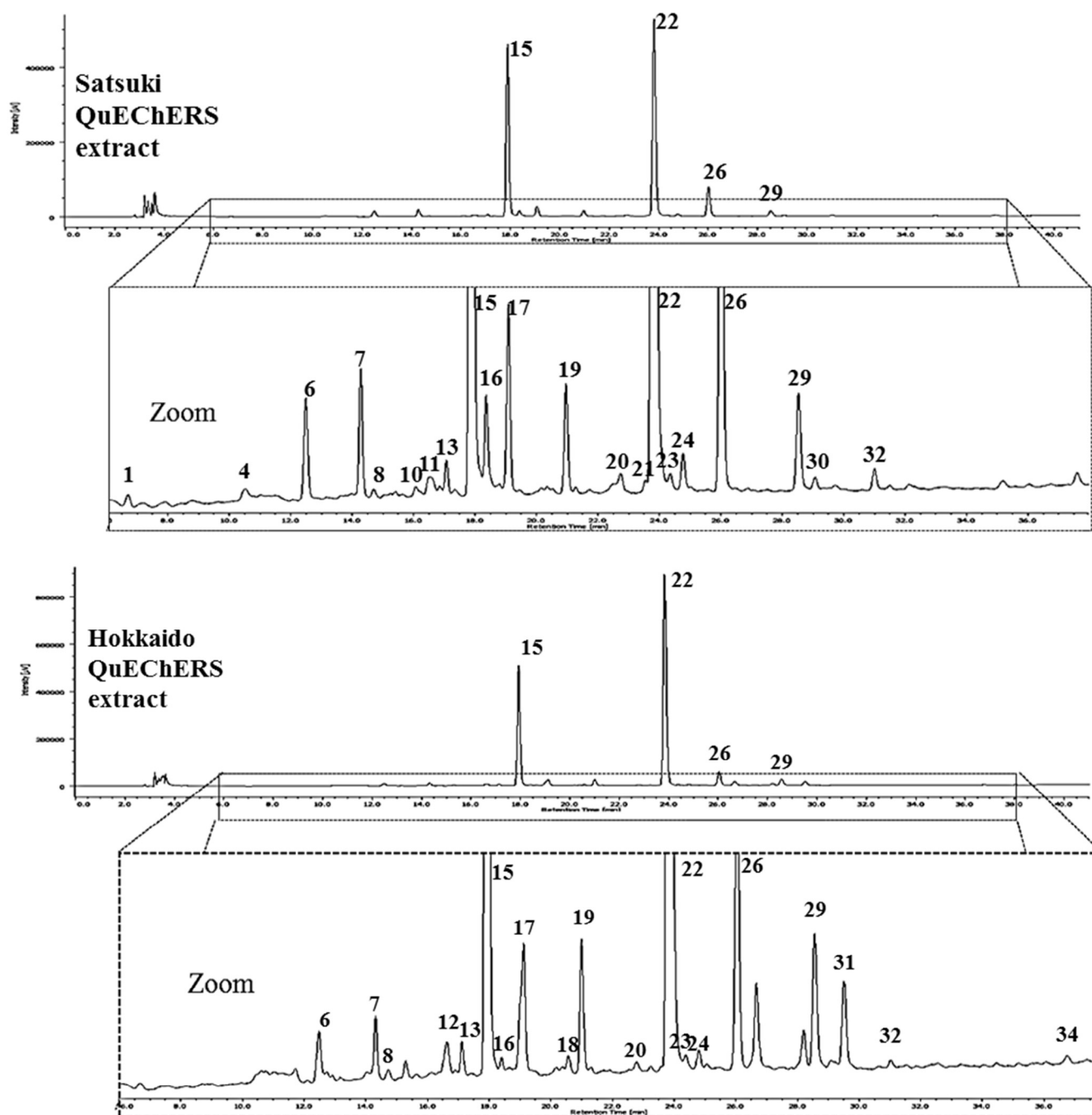


Fig. 2 – HPLC chromatograms of Satsuki and Hokkaido QuEChERS extracts ($\lambda = 290$ nm). Compounds 15, 22, and 29 were identified as quercetin 3,4'-diglucoside, quercetin 4'-glucoside, and quercetin, respectively, by comparing their retention times and UV spectra of each peak with those of authentic compounds. Compound 26 was identified as isorhamnetin 4'-glucoside by NMR and MS analysis.

13 ($r = 0.81$), 29 ($r = 0.76$), and 15 ($r = 0.75$), all of those found to be significantly correlated with their activities at $p < 0.01$. Peaks 13, 17, 19, 21, 23, 24, 29, and 32 were minor components in the extracts. These minor components were assigned to be quercetin derivatives determined by a specific UV absorption band I at ca. 300 nm (Takeda & Hayashi, 1980).

Target peaks with high concentration and high biological activities should be determined by the β -hexosaminidase release inhibitory activity after isolation and purification.

3.3. Antiallergic activities of quercetin 3,4'-diglucoside (15), quercetin 4'-glucoside (22), and isorhamnetin 4'-glucoside (26), major components in eleven onions

Quercetin 3,4'-diglucoside (15) is one of main compounds present in all eleven onions but its IC_{50} value for antiallergic activity was higher than 1.27 mM. Therefore, the contribution of compound 15 for the antiallergic activity was not so important. Actually, it is well-known that the 3- or 7-glycoside

of flavonoids have about ten times lower antiallergic activity compared to its aglycones (Itoh et al., 2011; Matsuda et al., 2002; Murata, Takano, Masuda, Iinuma, & Matsuda, 2013; Sato et al., 2012). However, the antiallergic activity of quercetin 4'-glucoside (22, $IC_{50} = 6.5 \pm 0.5 \mu M$) and isorhamnetin 4'-glucoside (26, $IC_{50} = 17.5 \pm 0.3 \mu M$) was comparable with that of quercetin (29, $IC_{50} = 3.2 \pm 0.1 \mu M$), a typical flavonol aglycone without enzyme inhibition (Table S4). Thus, the antiallergic activity of quercetin 4'-glucoside (22) is nearly equal to well-known aglycones such as luteolin ($IC_{50} = 2.6 \pm 0.1 \mu M$), apigenin ($IC_{50} = 2.9 \pm 0.2 \mu M$), and quercetin ($IC_{50} = 3.2 \pm 0.1 \mu M$), even though it is a glucoside and is water-soluble. Quercetin 4'-glucoside (22) was not only highly active but also soluble in polar solvents. Moreover, quercetin 4'-glucoside is highly bioavailable in humans (Murota & Terao, 2003; Olthof, Hollman, Vree, & Katan, 2000). On the other hand, quercetin 4'-glucoside (22) and isorhamnetin 4'-glucoside (26) were also major components in all eleven onions, especially in Hokkaido, Satsuki cultivar, Kagawa, and Hyogo onions. The average amount of quercetin 4'-glucoside (22) in eleven cultivars was 47.3 mg/kg of the edible part of onion as shown in Table 2. Therefore, it has advantages as food ingredients, additives and supplements over quercetin.

The Satsuki extract showed an IC_{50} of 89.1 $\mu g/mL$ of solution (Table 1) and contained 3.5 mg of quercetin 4'-glucoside per 100 mg of Satsuki dry extract (Table 2). Therefore, the total amount of quercetin 4'-glucoside in the 89.1 $\mu g/mL$ solution should be 3.1 $\mu g/mL$. As the IC_{50} of quercetin 4'-glucoside was 3.0 $\mu g/mL$ (Table S4), almost 100% of the antiallergic activity of the Satsuki cultivar came from quercetin 4'-glucoside. The contribution factor of compounds 22, 26, and 29 to the overall antiallergic activity was calculated with the following Equation (4) and listed in Table 3.

$$\text{Contribution to antiallergic activity (\%)} = \frac{\sum_{22,26,29} IC_{50}}{\times \text{relative concentration (\%)}}_{22,26 \text{ or } 29} \times 100 / IC_{50 \text{ 22,26 or 29}} \quad (4)$$

Table 3 – Comprehensive antiallergic activity of compounds 22, 26, and 29 in crude onion extracts.

Onion sample	Antiallergic activity contribution % ^a			
	Quercetin 4'-glucoside (22)	Isorhamnetin 4'-glucoside (26)	Quercetin (29)	Total
Satsuki	104.2	10.2	10.1	124.4
Advance	82.7	9.0	7.0	98.7
Shipposei No. 7	61.7	3.8	7.7	73.2
Shippokan 70	44.1	2.1	2.7	48.9
Momiji no kagayaki	81.2	5.9	12.6	99.7
Momiji No. 3	130.2	10.9	8.1	149.3
Answer	107.3	5.4	3.9	116.6
Tarzan	74.4	5.7	3.3	83.4
Hokkaido	41.3	1.8	4.3	47.4
Hyogo	46.3	3.6	3.2	53.2
Kagawa	39.3	2.1	1.5	43.0
Satsuki skin	30.6	5.1	82.8	118.6

^a Antiallergic activity contribution % = (IC_{50} of onion extract) $\times$ (content % of compound in the extract) $\times 100 / (IC_{50}$ of compound).

Compounds 22, 26, and 29 in seven cultivars (Satsuki, Advance, Shipposei No. 7, Momiji no kagayaki, Momiji No. 3, Answer, and Tarzan) contributed to more than 73% antiallergic activity, as shown in Table 3. Particularly, the antiallergic activity of Satsuki, Momiji No. 3, and Advance cultivars would be due to quercetin 4'-glucoside. Therefore, quercetin 4'-glucoside (22) is a major antiallergic substance in the edible part of those onions. Furthermore, in case of Shippokan 70, Hokkaido, Hyogo, and Kagawa, compounds 22, 26, and 29 explained approximately 45% of the antiallergic activity. Thus, the components 22, 26, and 29 can explain 45% or more of the antiallergic activity of the eleven onion samples. Other minor components might influence around 50% of the total antiallergic activity of Shippokan 70, Hokkaido, Hyogo, and Kagawa onion cultivars. Moreover, onion skin which also had higher antiallergic activity and high concentration of compounds 22 and 29 (6.12 mg/100 mg for 22 and 4.97 mg/100 mg for 29, as shown in Table 2) might be used in the future as a source of antiallergic and antioxidant compounds from unused parts of onions (Albishi, John, Al-Khalifa, & Shahidi, 2013b).

3.4. Factor analysis of eleven onions for characterization of quercetin 4'-glucoside (22) with the relationship to antiallergic activity

Factor analysis (Fig. 3) based on thirty-four compounds using quantitative data from eight onion cultivars and three local products from different geographical origins revealed 31.0% variance of Factor 1 and 19.0% variance of Factor 2. Factor 1 can be explained by the amount of quercetin, quercetin glucosides and the related flavonols. The vector for compound 22 (quercetin 4'-glucoside), with high correlation coefficient and high antiallergic activity, showed strong contribution to Factor 1, which in turn explained 31.0% of the total variance. The Hokkaido ($IC_{50} = 20.8 \mu g/mL$), Kagawa, Hyogo, and Satsuki ($IC_{50} = 89.1 \mu g/mL$) cultivars were also located at the positive direction of Factor 1. So, the high antiallergic activities of Hokkaido and Satsuki onions may be related to compound 22 and other quercetin derivatives which were also plotted within the close area with compound 22 (Fig. 3, enclosed by the dotted line). As compounds 15 (quercetin 3,4'-diglucoside), 26 (isorhamnetin 4'-glucoside), 22 (quercetin 4'-glucoside) and other minor quercetin derivatives (-) were located at the similar area as shown in Fig. 3, it indicates that Hokkaido and Satsuki onions have a great gene expression for several enzymes for quercetin and quercetin glucosides biosynthesis.

4. Conclusion

Quercetin 4'-glucoside (22) was the most effective substance for the suppression of type I allergy, among the compounds found in the edible part of eleven onion samples from different cultivars and geographical origins within Japan. Its efficacy corresponded well to the aglycone. Target compounds for antiallergic active substances can be screened by correlation coefficients between activity and peak areas of chromatograms from QuChERS extracts combined with factor analysis without conducting biological tests of pure chemicals,

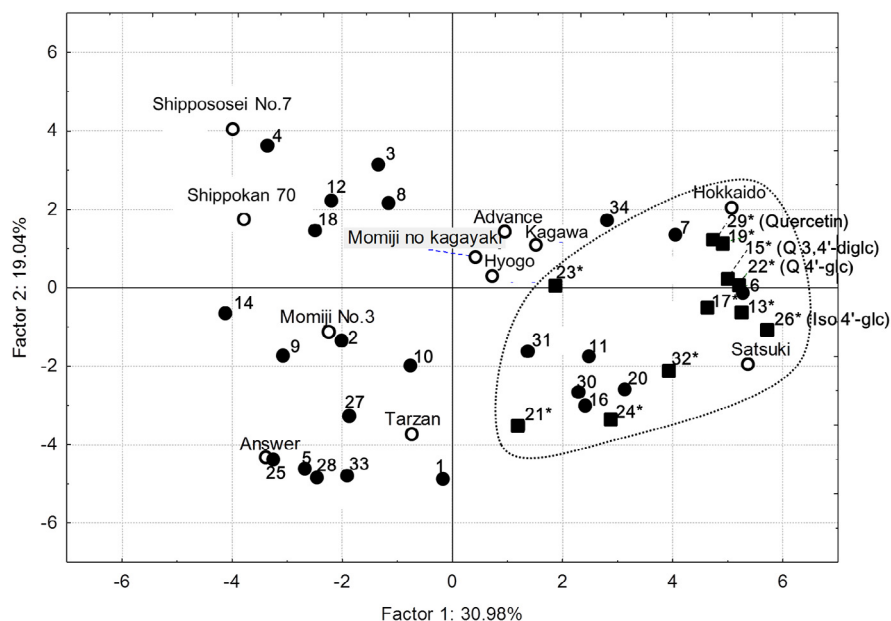


Fig. 3 – Factor analysis of onion cultivars and the amount of compounds. White circle (○) means onion cultivar, and black circle (●) and square (◻) mean individual compounds in the HPLC chromatograms. *Flavonol group confirmed by each UV spectrum is indicated as square (◻).

directly. Quercetin and its derivatives were the main effectors that contributed to the overall antiallergic activity. We provide initial evidence that meals containing onions could be an approach for preventing type I allergies, as quercetin 4'-glucoside is highly bioavailable. Onions from the markets in Hokkaido and Kagawa prefectures are an excellent source of compounds with antiallergic activities.

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Appendix: Supplementary material

Supplementary data to this article can be found online at [doi:10.1016/j.jff.2015.02.029](https://doi.org/10.1016/j.jff.2015.02.029).

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Supplementary data

Table S1 – Correlation coefficients between antiallergic activity and amount of compounds (peaks **1–13**).

		peak area ^b													
		retention time	6.7	7.8	8.7	10.5	10.8	12.5	14.3	14.7	15.8	16.1	16.6	16.8	17.1
sample	IC ₅₀ ^a	100 / IC ₅₀	peak 1	peak 2	peak 3	peak 4	peak 5	peak 6	peak 7	peak 8	peak 9	peak 10	peak 11	peak 12	peak 13
Shipposei No. 7	310.1	0.32	ND	643757	24579	283952	ND	ND	78921	26884	ND	ND	ND	100675	ND
Momiji No. 3	293.6	0.34	19880	90172	ND	54782	166618	ND	46970	11223	ND	ND	ND	67384	19631
Shippokan 70	227.9	0.43	ND	ND	ND	162647	ND	ND	59393	18472	22616	27747	ND	79653	12049
Answer	168.7	0.59	41994	110824	ND	ND	212709	ND	42488	14336	29899	13668	15937	34544	18109
Advance	148.4	0.67	20610	ND	18385	24047	ND	29185	67985	ND	ND	ND	30646	ND	31853
Tarzan	136.1	0.73	27731	1122939	ND	ND	136490	15015	48087	11245	ND	23815	ND	90618	29769
Momiji no kagayaki	102.5	0.97	29177	57390	21902	ND	ND	ND	119615	16598	12930	13518	ND	75662	27245
Satsuki	89.1	1.12	26847	ND	ND	28007	ND	116155	146339	12117	ND	15516	44091	ND	35163
Hyogo	61.4	1.62	21836	ND	ND	ND	ND	ND	47827	ND	ND	ND	ND	74007	38033
Kagawa	38.1	2.62	ND	ND	ND	41869	ND	69667	59010	26108	ND	20651	ND	50762	37687
Hokkaido	20.8	4.80	ND	ND	ND	ND	ND	88486	101428	18206	ND	ND	ND	92130	54401
correlation coefficient ^c		1.00	−0.42	−0.29	−0.30	−0.35	−0.34	0.62 [*]	0.24	0.18	−0.30	−0.18	−0.18	0.17	0.81 ^{**}

^aIC₅₀ values of antiallergic activity were calculated from the results of β -hexosaminidase release inhibitory activity assay.

^bPeak areas were calculated from HPLC chromatograms (λ = 290 nm, ND = not detected).

^cPearson's correlation coefficient of each peak between the differences in antiallergic activity and peak area depending on cultivar.

Identified peaks: peak **15**, quercetin 3,4'-diglucoside; peak **22**, quercetin 4'-glucoside; peak **26**, isorhamnetin 4'-glucoside; peak **29**, quercetin.

Each IC₅₀ and peak area represents the mean (n = 3).

Asterisks show correlation is significantly different: ^{*}, $p < 0.05$, ^{**}, $p < 0.01$, respectively.

Table S2 – Correlation coefficients between antiallergic activity and amount of compounds (peaks **14–25**).

		peak area ^b												
		retention time	17.5	17.9	18.3	19.1	20.4	20.9	22.7	23.5	23.8	24.3	24.7	25.0
sample	IC ₅₀ ^a	100 / IC ₅₀	peak 14	peak 15	peak 16	peak 17	peak 18	peak 19	peak 20	peak 21	peak 22	peak 23	peak 24	peak 25
Shipposei No. 7	310.1	0.32	13555	976121	19075	55662	19125	73079	ND	ND	800525	ND	ND	ND
Momiji No. 3	293.6	0.34	ND	1008072	61273	146853	12179	53528	ND	ND	1785277	19616	38277	26941
Shippokan 70	227.9	0.43	20774	985650	41445	65593	25862	57984	ND	ND	778879	ND	20008	12142
Answer	168.7	0.59	27971	1527816	42513	77079	ND	78552	ND	14242	2559064	15744	36898	46252
Advance	148.4	0.67	ND	2719146	54372	243867	ND	102968	ND	ND	2243776	19640	13896	ND
Tarzan	136.1	0.73	ND	2113066	126253	253842	22648	67462	55795	ND	2200391	11585	29021	21995
Momiji no kagayaki	102.5	0.97	ND	1294059	36277	191423	ND	94176	ND	ND	3189134	112498	46313	11125
Satsuki	89.1	1.12	ND	3535323	123541	212650	ND	149428	48159	16566	4705759	29311	51527	ND
Hyogo	61.4	1.62	ND	2894313	112559	286966	ND	53645	ND	ND	3037734	12393	40724	ND
Kagawa	38.1	2.62	ND	4488444	99801	167878	11823	137569	27655	ND	4156602	16572	24662	ND
Hokkaido	20.8	4.80	ND	3948521	30006	300685	13022	215380	19964	ND	7997698	30035	32512	ND
correlation coefficient ^c		1.00	−0.36	0.75**	−0.01	0.57	−0.02	0.84**	0.23	−0.15	0.91**	0.10	0.15	−0.41

^aIC₅₀ values of antiallergic activity were calculated from the results of β -hexosaminidase release inhibitory activity assay.

^bPeak areas were calculated from HPLC chromatograms (λ = 290 nm, ND = not detected).

^cPearson's correlation coefficient of each peak between the differences in antiallergic activity and peak area depending on cultivar.

Identified peaks: peak **15**, quercetin 3,4'-diglucoside; peak **22**, quercetin 4'-glucoside; peak **26**, isorhamnetin 4'-glucoside; peak **29**, quercetin.

Each IC₅₀ and peak area represents the mean (n = 3).

Asterisks show correlation is significantly different: *, $p < 0.05$, **, $p < 0.01$, respectively.

Table S3 – Correlation coefficients between antiallergic activity and amount of compounds (peaks **26–34**).

		peak area ^b									
		retention time	26.0	26.5	27.4	28.5	29.1	29.3	31.0	32.5	36.7
sample	IC ₅₀ ^a	100 / IC ₅₀	peak 26	peak 27	peak 28	peak 29	peak 30	peak 31	peak 32	peak 33	peak 34
Shipposei No. 7	310.1	0.32	77772	ND	ND	31790	ND	ND	ND	ND	ND
Momiji No. 3	293.6	0.34	234848	25993	18718	35488	ND	63769	ND	ND	ND
Shippokan 70	227.9	0.43	58936	ND	ND	15045	ND	ND	ND	ND	ND
Answer	168.7	0.59	201804	19770	21987	29910	ND	71420	ND	16882	ND
Advance	148.4	0.67	380453	ND	ND	60918	ND	ND	ND	ND	ND
Tarzan	136.1	0.73	263871	21084	20430	30746	21383	90159	19056	15248	ND
Momiji no kagayaki	102.5	0.97	363017	29477	ND	158111	ND	ND	ND	ND	13073
Satsuki	89.1	1.12	718793	ND	ND	145828	18771	ND	29736	ND	ND
Hyogo	61.4	1.62	372961	ND	ND	66798	ND	ND	ND	ND	ND
Kagawa	38.1	2.62	350788	ND	ND	51604	14574	ND	ND	ND	ND
Hokkaido	20.8	4.80	531211	ND	ND	264785	ND	169701	16211	ND	18637
correlation coefficient ^c	1.00		0.52	−0.36	−0.34	0.76 ^{**}	0.03	0.57	0.28	−0.23	0.69 [*]

^aIC₅₀ values of antiallergic activity were calculated from the results of β -hexosaminidase release inhibitory activity assay.

^bPeak areas were calculated from HPLC chromatograms (λ = 290 nm, ND = not detected).

^cPearson's correlation coefficient of each peak between the differences in antiallergic activity and peak area depending on cultivar.

Identified peaks: peak **15**, quercetin 3,4'-diglucoside; peak **22**, quercetin 4'-glucoside; peak **26**, isorhamnetin 4'-glucoside; peak **29**, quercetin.

Each IC₅₀ and peak area represents the mean (n = 3).

Asterisks show correlation is significantly different: *, $p < 0.05$, **, $p < 0.01$, respectively.

Table S4 – Antiallergic activities of chemicals found in onion extract.

crude samples and chemicals	IC ₅₀ [μ g/mL]	IC ₅₀ [μ M]	enzyme inhibition (%) ^a
fresh extract by methanol	> 800	-	-
skin extract by QuEChERS	15.7 $\pm$ 0.1	-	-
quercetin 3,4'-diglucoside (15)	> 800	-	-
quercetin 4'-glucoside (22)	3.0 $\pm$ 0.2	6.5 $\pm$ 0.5	0.6 $\pm$ 3.3
isorhamnetin 4'-glucoside (26)	8.3 $\pm$ 0.1	17.5 $\pm$ 0.3	0.6 $\pm$ 2.8
quercetin (29)	0.9 $\pm$ 0.1	3.2 $\pm$ 0.1	1.3 $\pm$ 2.7

^aValues indicate enzyme inhibition (%) against β -hexosaminidase at 100 μ M.

Each value represents the mean $\pm$ SD (n = 4).

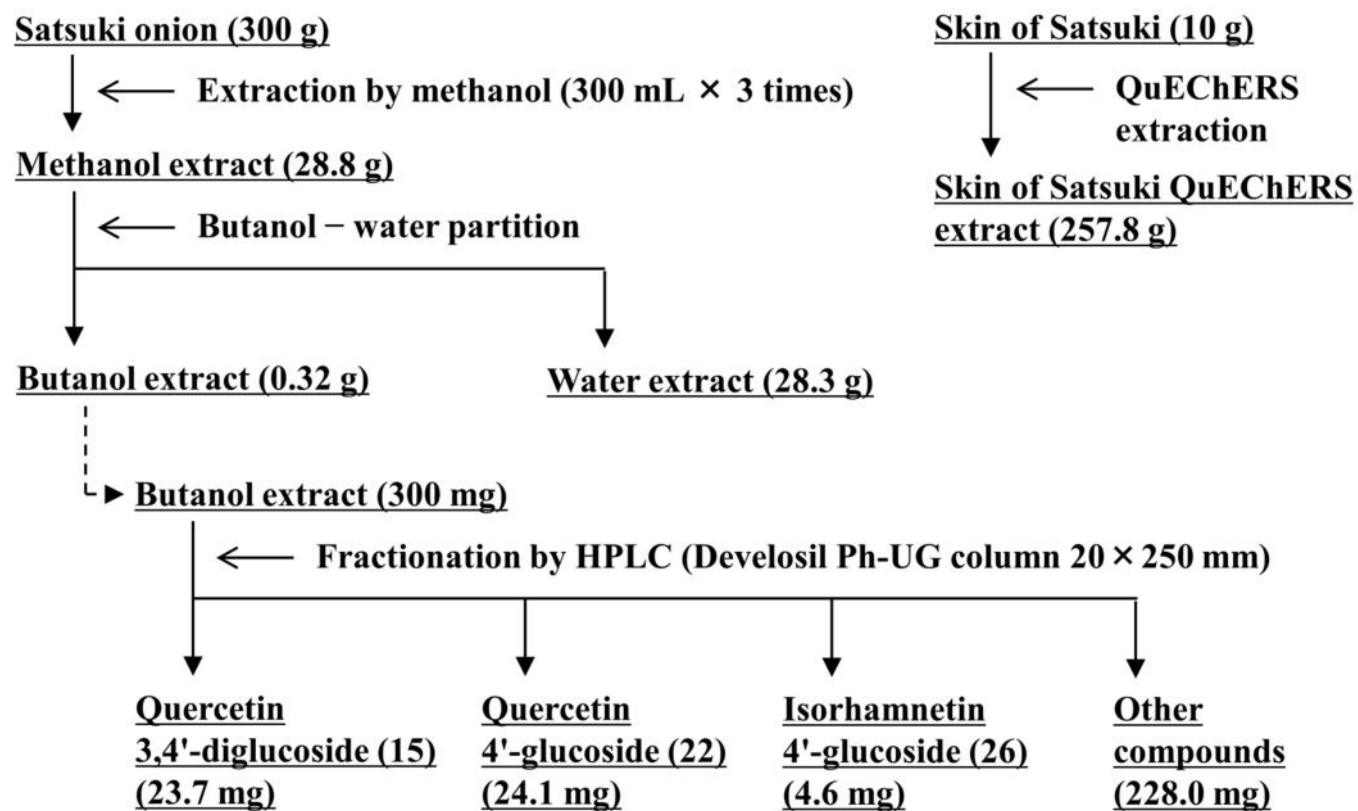


Fig. S1 – Extraction and isolation of flavonol glucosides from Satsuki cultivar.

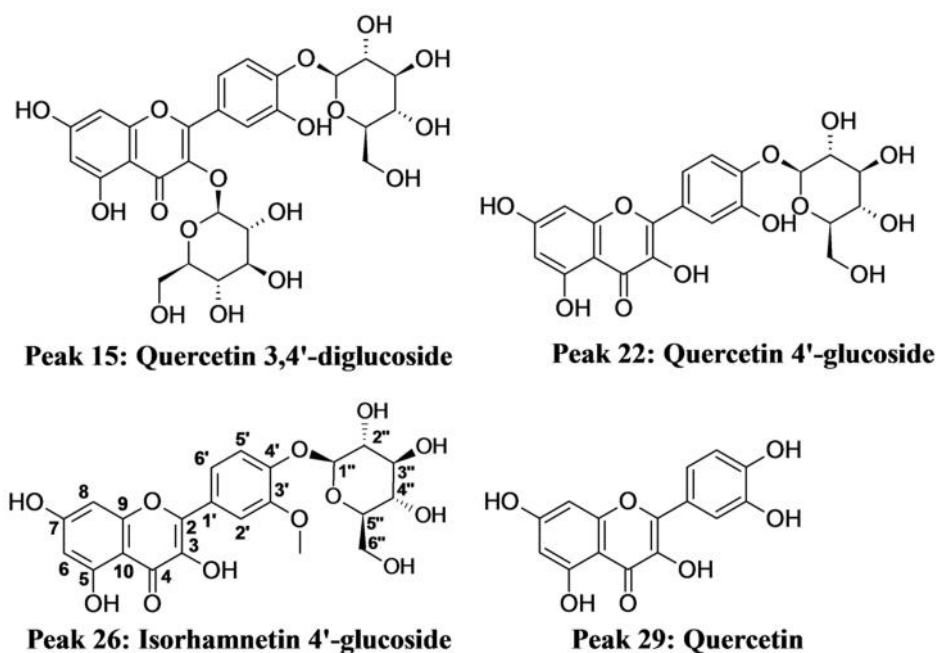


Fig. S2 – Chemical structure and physical properties of compounds **15**, **22**, **26**, and **29**. Quercetin 3,4'-diglucoside (**15**), quercetin 4'-glucoside (**22**), and quercetin (**29**) were identified by comparing retention time and $\lambda_{\max}$ of UV spectrum with commercially available chemicals ($\lambda_{\max}$ of each compound is as follows: compound **15** at 239, 263, and 343 nm; compound **22** at 239 and 363 nm; compound **29** at 239 and 367 nm).

Compound 26: $C_{22}H_{22}O_{12}$; FAB-MS m/z : 479 $[M+H]^+$; ^1H NMR (600 MHz, methanol- d_4) δ : 6.17 (1H, d, $J = 1.38$ Hz, H-6), 6.40 (1H, s, H-8), 7.89 (1H, d, $J = 1.38$ Hz, H-2'), 7.27 (1H, d, $J = 8.22$ Hz, H-5'), 7.79 (1H, dd, $J = 1.38, 8.22$ Hz, H-6'), 3.92 (3H, s, 3'-OCH $_3$), 5.01 (1H, d, $J = 7.56$ Hz, H-1''), 3.52 (1H, dd, $J = 7.50, 8.94$ Hz, H-2''), 3.47 (1H, d, $J = 8.22$ Hz, H-3''), 3.40 (1H, dd, $J = 8.94, 9.60$ Hz, H-4''), 3.45 (1H, dd, $J = 2.10$ Hz, H-5''), 3.68 (1H, dd, $J = 5.46, 11.64$ Hz, H-6''a), 3.88 (1H, dd, $J = 2.04, 12.36$ Hz, H-6''b); ^{13}C -NMR (150 MHz, methanol- d_4) δ : 145.57 (C-2), 136.61 (C-3), 176.17 (C-4), 161.25 (C-5), 98.03 (C-6), 164.49 (C-7), 93.20 (C-8), 156.99 (C-9), 103.27 (C-10), 125.72 (C-1'), 111.89 (C-2'), 149.08 (C-3'), 148.12 (C-4'), 115.73 (C-5'), 121.14 (C-6'), 55.98 (3'-OCH $_3$), 100.98 (C-1''), 73.50 (C-2''), 76.56 (C-3''), 69.98 (C-4''), 76.99 (C-5''), 61.16 (C-6'').