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Anti Type I Allergic Property of Japanese Butterbur Extract and Its Mast Cell Degranulation Inhibitory Ingredients

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Pollenosis is a disease that affects 1 in 10 of the Japanese population. During the season of cedar pollen dispersal, many patients suffer from symptoms such as sniffling, sternutation, and itching of the eyes. Japanese butterbur is a popular vegetable and is one of the few domestic vegetables in Japan. The anti type I allergic effects of an aqueous ethanol extract from aerial parts of Japanese butterbur (JBE) were evaluated in rats and RBL-2H3 mast cells. In the passive cutaneous anaphylaxis reaction in rats, a single oral treatment of JBE (1000 mg/kg) was found to suppress the reaction. In IgE-sensitized RBL-2H3 cells, JBE (10−100 μg/mL) inhibited β-hexosaminidase release, leukotriene $C_4/D_4/E_4$ synthesis, and TNF-α production. Moreover, a high concentration of JBE (1000 μg/mL) suppressed smooth muscle constriction induced by histamine (10 μM) and leukotriene D_4 (10 nM) in a guinea pig trachea strip. The search for components in JBE with an inhibitory activity on mast cell degranulation was guided by inhibition of β-hexsosaminidase release. Two eremophilane-type sesquiterpenes, six polyphenolic compounds, and two triterpene glycosides were isolated. Of these compounds, fukinolic acid, a principal polyphenol constituent, showed potent inhibitory activity (IC_{50} value = 2.1 μg/mL). Consequently, On the basis of its inhibition of mast cell activation and direct smooth muscle reaction induced by released mediators, JBE was found to suppress the type I allergic reaction.

KEYWORDS: Japanese butterbur; fukinolic acid; sesquiterpene; mast cell; degranulation

INTRODUCTION

Japanese butterbur (*Petasites japonicus*, Compositae) is a common domestic Japanese vegetable. It has a good flavor and has long been used as a food source. The well-baked flower bud is used in traditional medicine as an expectorant or in the treatment of asthma. Juice squeezed from the leaves has been used to treat bee stings. Japanese butterbur contains several essential oil components, and chemical studies on sesquiterpenes in the flower buds and the rhizome have been reported (*1*−*4*). Flower buds of *P. japonicus* have been reported to contain hepatotoxic and carcinogenic pyrrolizidine alkaloids (*5*). The contents of the alkaloids are low, and boiled or processed flower buds have been consumed widely in Japan. Recently, petaslignolide A (*6*), a furfuran lignan with neuroprotective activity, was isolated from the leaves of *P. japonicus*. However, biological reports about Japanese butterbur are limited to areas such as its urokinase inhibitory activity (*7*), antioxidative activity (*8*, *9*), and DNA polymerase inhibitory activities (*10*, *11*). Recently, it was reported that the root extract of the European butterbur (*Petasites hybridus*) improved symptoms in patients with allergic rhinitis (*12*, *13*). These studies indicated that the effects of the European butterbur were similar to those of antihistaminic drugs, and no side effects due to central nervous system suppression were observed. The effective constituent in the extract is petasine, an eremophilane-type sesquiterpene with inhibitory effects on leukotriene synthesis (*14*) and bronchoconstriction (*15*). Therefore, Japanese butterbur was expected to be effective for the treatment of type I allergic symptoms. In this study, we prepared an aqueous ethanol-soluble extract from the aerial parts (leaves and stems) of the plant and evaluated its effect on the type I allergic reaction. Furthermore, some constituents with mast cell degranulation inhibitory activity were isolated.

MATERIALS AND METHODS

Animals and Cells. Male Wistar rats (8 weeks old) and female guinea pigs (Hartley, 250−300 g) were purchased from Japan SLC, Inc. (Shizuoka, Japan). The animals were housed in an air-conditioned room (23 ± 1 °C). They were fed a standard nonpurified diet (CE-2)

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(Clea Japan, Inc., Shizuoka, Japan) or RC4 (Oriental Yeast, Tokyo, Japan) and tap water ad libitum. The experiments were performed in accordance with the Guidelines for Animal Experimentation (Japan Association for Laboratory Animal Science, 1987). The basophilic leukemia cells, RBL-2H3 (cell no. JCRB0023), were obtained from the Japan Health Science Foundation (Osaka, Japan).

Preparation of Japanese Butterbur Extract. Dried Japanese butterbur (3.3 kg), cultivated in Aichi prefecture in Japan, was extracted (70 °C, 2 h) with 70% ethanol (20 L), and the solvent was evaporated. The extract was powdered by spray-drying. The yield of Japanese butterbur extract (JBE) was 12.4%.

Reagents. Anti-dinitrophenyl (DNP) IgE was purchased from Seikagaku Industry (Tokyo, Japan). Dinitrophenylated bovine serum albumin (DNP-BSA) was obtained from Cosmo Bio (Tokyo, Japan). Evans blue was purchased from Tokyo Kasei Industry (Tokyo, Japan). Eagle's minimum essential medium (MEM), fetal calf serum (FCS), penicillin and streptomycin mixture solution, piperazine *N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, histamine HCl, leukotriene D_4, indomethacin, dimethyl sulfoxide (DMSO), and luteolin were obtained from Sigma-Aldrich (St. Louis, MO). A leukotriene $C_4/D_4/E_4$ enzyme immunoassay system and a rat tumor necrosis factor α [(r)TNFα] ELISA system were purchased from GE Healthcare (Pittsburgh, PA).

Passive Cutaneous Anaphylaxis (PCA) Reaction in Rats. The back of the rat was shaved, and 100 μL of anti-DNP IgE diluted with PBS (PCA titer: ×4) was injected into the skin. After 1 day, the rats were fasted for 20 h. JBE suspended with 5% w/v of gum arabic in water was orally administered to the rat. The antigen solution (0.5 mL) containing DNB-BSA (1.5 mg/mL) and Evans blue (10 mg/mL) was injected into the tail vein. Thirty minutes later, the rats were sacrificed under ether anesthesia and the back skins removed. The blue spot area was measured by using a digital planimeter (Tech-Jam, Osaka, Japan).

Antigen-Induced β-Hexosaminidase Release in Sensitized RBL-2H3 Cells. RBL-2H3 cells in MEM (500 μL) containing FCS (10%), penicillin (100 units/mL), streptomycin (100 μg/mL), and 0.45 μg/mL of anti-DNP IgE were dispensed into 24-well plates at a cell density of 2×10^5 cells/well, and cells were incubated overnight at 37 °C in 5% CO_2 for sensitization of the cells. The cells were then washed twice with 500 μL of Siraganian buffer [119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM PIPES, and 40 mM NaOH, pH 7.2] and incubated in 160 μL of Siraganian buffer containing 5.6 mM glucose, 1 mM $CaCl_2$, and 0.1% BSA for 10 min at 37 °C. Aliquots (20 μL) of the test sample solution were added to each well and incubated for 10 min. This was followed by the addition of 20 μL of antigen (DNP-BSA, final concentration = 10 μg/mL) for 10 min to evoke allergic reactions (degranulation). The reaction was stopped by cooling in an ice bath for 10 min. The supernatant (50 μL) was transferred into a 96-well microplate and incubated with 50 μL of substrate (1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37 °C for 1 h. The reaction was stopped by adding 200 μL of stop solution (0.1 M $Na_2CO_3/NaHCO_3$, pH 10.0). The test sample was dissolved in DMSO, and the solution was added to Siraganian buffer (final DMSO concentration = 0.1%). Luteolin was used as a positive control. The absorbance (OD) was measured using a microplate reader at 405 nm. The gaining ratio of OD reflects the β-hexosaminidase release. The calculation was performed by the following equations. To obtain the valid value, the factors that are not typically induced by samples need to be excluded. In "spontaneous", neither DNP-BSA nor sample was added to cells to confirm the spontaneous β-hexosaminidase release from cells. In "control", DNP-BSA but not samples was added to cells to confirm the β-hexosaminidase release from cells in conditions without sample. In "total", cells were sonicated to confirm the total amount of β-hexosaminidase contained in the cells. In "sample", both DNP-BSA and samples were added to cells to confirm the β-hexosaminidase release from cells in these conditions.

$$\text{ratio of } \beta\text{-hexosaminidase release (\%)} = \left(\frac{\text{OD control or OD sample} - \text{OD spontaneous}}{\text{OD total} - \text{OD spontaneous}}\right) \times 100$$

$$\text{inhibition of } \beta\text{-hexosaminidase release (\%)} = \left[\frac{(\text{OD sample} - \text{OD spontaneous})}{(\text{OD control} - \text{OD spontaneous})}\right] \times 100$$

IC_{50} values were determined graphically. The ratio of β-hexsosaminidase release of control groups ranged from 25 to 55%.

Antigen-Induced Leukotriene Release and TNF-α Production in Sensitized RBL-2H3 Cells. RBL-2H3 cells were incubated with JBE and stimulated by antigen according to the above method. The supernatant was collected 30 min or 4 h after antigen stimulation for the determination of leukotrienes or TNF-α, respectively. Determinations of leukotrienes C_4, D_4, and E_4 and of TNF-α were carried out using commercial kits.

Constriction of Guinea Pig Trachea Strips Induced by Histamine and Leukotriene D_4. A guinea pig was anesthetized with ether, and the trachea was removed. The trachea was cut into 3−4 mm length, and its cartilage part was opened in Krebs−Henseleit solution (118 mM NaCl, 2.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 10 mM glucose, equilibrated with 95%N_2/5%O_2 gas) containing 1 μM indomethacin. The tracheal preparation was attached to a Magnus apparatus and placed in an organ bath filled with Krebs−Henseleit solution (6 mL). The organ bath was warmed at 37 °C, and the solution was bubbled gently with 95%N_2/5%O_2 gas. The specimen was pulled gently with a tension of 1 g, and the change in tension was recorded by MacLab 8e (AD Instruments, Tokyo, Japan) via an isotonic transducer. After the tension was balanced, histamine or leukotriene D_4 solution (6 μL) was added to the bath (final concentrations: histamine, 10 μM; leukotriene D_4, 10 nM), and the change in tension was recorded by the extent of constriction plateaued. The specimen was washed three times with Krebs−Henseleit solution at 10-min intervals. This procedure was repeated again, and the test sample diluted in DMSO (6 μL) was added (final DMSO concentration = 0.1%). Ten minutes later, 1−10 μM histamine or 10 nM leukotriene D_4 was added accumulatively, and the change in tension was recorded.

Statistics. The results were expressed as means ± standard error (SE). Significance of the differences was examined by one-way ANOVA followed by Dunnett's test. Differences with $p < 0.05$ were considered to be significant.

Isolation and Identification of Constituents in JBE. JBE (333 g) was suspended in H_2O (2 L) and partitioned successively with EtOAc (2 L × 3) and *n*-BuOH (2 L × 3). Each solvent was evaporated to obtain the EtOAc portion (25.3 g), *n*-BuOH portion (17.2 g), and H_2O portion (250 g). The EtOAc portion (24 g) was fractionated by normal-phase silica gel chromatography (500 g, *n*-hexane/EtOAc, 2:1→1:1→1:2→MeOH) to obtain fractions 1 (0.34 g), 2 (0.63 g), 3 (2.69 g), 4 (1.62 g), 5 (1.00 g), 6 (3.10 g), 7 (1.52 g), 8 (10.4 g), and 9 (6.31 g). Fraction 2 (0.55 g) was purified successively by normal-phase silica gel chromatography (20 g, *n*-hexane/EtOAc, 10:1→1:1→0:1), preparative TLC (*n*-hexane/EtOAc, 9:1), and normal-phase HPLC on a 250 × 20 mm i.d. Chromatorex Si column (Fuji Silysia Chemical, Aichi, Japan) with *n*-hexane/EtOAc, 10:1, at 10 mL/min to obtain (+)-fukinone (**1**, 87 mg) (*16*). Fraction 7 (1.0 g) was partitioned by reversed-phase column chromatography on an ODS column (30 g) (Fuji Silysia Chemical) with 30% MeOH→100% MeOH to obtain fractions 7-1 (344 mg), 7-2 (89 mg), 7-3 (173 mg), 7-4 (95 mg), 7-5 (90 mg), 7-6 (73 mg), 7-7 (90 mg), 7-8 (131 mg), 7-9 (114 mg), and 7-10 (64 mg). Fraction 7-1 (150 mg) was subjected to reversed-phase HPLC on a 250 × 10 mm i.d. ODS column (GL Science, Tokyo, Japan) in 50% MeOH at 4 mL/min to obtain caffeic acid (**2**, 60.0 mg). Fraction 7-4 (95 mg) was subjected to normal-phase HPLC on a 250 × 20 mm i.d. Chromatorex Si column (Fuji Silysia Chemical) in EtOAc at 10 mL/min to obtain 2β-hydroxyfukinone (**3**, 13 mg) (*17*). The *n*-BuOH portion (17 g) was fractionated by ODS reversed-phase column chromatography (300 g, 15% MeOH→70% MeOH) to obtain fractions 1 (3.47 g), 2 (1.85 g), 3 (0.90 g), 4 (0.95 g), 5 (0.41 g), 6 (0.25 g), 7 (0.39 g), 8 (0.48 g), 9 (0.33 g), 10 (0.40 g), 11 (0.27 g), 12 (0.26 g), 13 (0.30 g), 14 (0.29 g), 15 (0.06 g), and 16 (0.26 g). Fraction 3 (900 mg) was subjected to reversed-phase HPLC on a 250 × 10 mm i.d. ODS column (GL Science) in 30% MeOH to obtain chlorogenic acid (**4**, 2 mg) (*18*) and fukinolic acid (**5**, 10 mg) (*19*). Fraction 7 (386 mg) was purified by reversed-phase HPLC on a 250 × 10 mm i.d. ODS column (GL

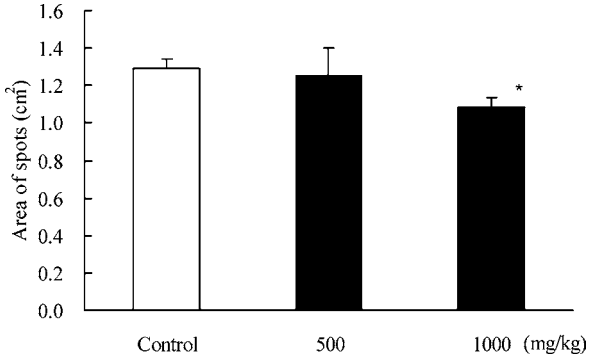

Figure 1. Effect of JBE on passive cutaneous anaphylaxis reaction in rats. Each column represents mean ± SE of six animals. An asterisk denotes significant difference from the control at $p < 0.05$.

Science) in 40% MeOH to obtain 4,5-dicaffeoylquinic acid (**6**, 31 mg) (*20*) and 3,5-dicaffeoylquinic acid (**7**, 11 mg) (*20*). Fraction 9 (325 mg) was separated by reversed-phase HPLC on a 250 × 10 mm i.d. ODS column (GL Science) in 30% MeOH to yield compound **6** (36 mg) and 4,5-dicaffeoylquinic acid methyl ester (**8**, 52 mg) (*21*). Fraction 12 (265 mg) was purified by reversed-phase HPLC on a 250 × 10 mm i.d. ODS column (GL Science) in 60% MeOH to obtain dotorioside II (**9**, 13 mg) (*22*). Fraction 14 (294 mg) was purified by reversed-phase HPLC on a 250 × 10 mm i.d. ODS column (GL Science) in 70% MeOH to yield mussaendoside R (**10**, 9.7 mg) (*23*). The H_2O portion (250 g) was desalted by 1 N HCl and treated with DMT1020 resin (Fuji Silysia Chemical), 400 × 30 mm i.d., eluted with H_2O→20% MeOH→40% MeOH. Forty percent of the MeOH eluent was evaporated and purified by reversed-phase HPLC on a 250 × 20 mm i.d., UG-80 ODS column (Shiseido, Tokyo, Japan) with 10 mM phosphate buffer (pH 2.6)/acetonitrile, 80:20, to obtain compound **5** (209 mg). With the exception of compound **2**, these compounds were identified by comparing their physical data with the reported values. Caffeic acid (**2**) was identified by comparing its ^{13}C and ^{1}H NMR spectra with those of an authentic sample (Nacalai Tesque, Kyoto, Japan).

RESULTS AND DISCUSSION

The antiallergic activity of JBE in vivo was examined using the PCA reaction, which is a basic model of type I allergy. **Figure 1** shows the mean blue spot area of three groups. Oral treatment with JBE (1000 mg/kg) 2 h prior to the antigen challenge slightly but significantly suppressed leakage of dye into the skin due to an antigen−antibody reaction. To elucidate the suppressive mechanism of JBE on type I allergic reaction, its effect on antigen-induced mast cell degranulation was evaluated using sensitized RBL-2H3 cells guided by release of β-hexosaminidase as a parameter. JBE was found to suppress β-hexosaminidase release from 1 to 100 μg/mL in a concentration-dependent manner (**Figure 2A**). It was found that JBE potently suppressed the degranulation in a concentration-dependent manner with an IC_{50} value of 5.8 μg/mL. The potency of JBE is quite similar to that of tien-cha (*Rubus suavissimus*) (IC_{50} = 4.2 μg/mL) and perilla leaf (*Perilla frutescens*) extracts (IC_{50} = 8.6 μg/mL) (data not shown). These plant extracts are popular in the Japanese market for the prevention of pollenosis (*24*, *25*).

Leukotrienes C_4, D_4, and E_4 are inflammatory mediators that are synthesized from cell membrane phospholipids by antigen stimulation and are involved in airway constriction in bronchial asthma. JBE (10 and 100 μg/mL) significantly suppressed leukotriene release, but this suppression was not concentration dependent (**Figure 2B**). In dietary materials and edible plant extracts, α-linoleic acid in perilla seed oil (*26*) and caffeic acid (**2**) in coffee bean (*27*) have been reported to inhibit leukotriene release or its production in mast cells. JBE contained compound **2** and its related compounds (**4** and **6**−**8**). These

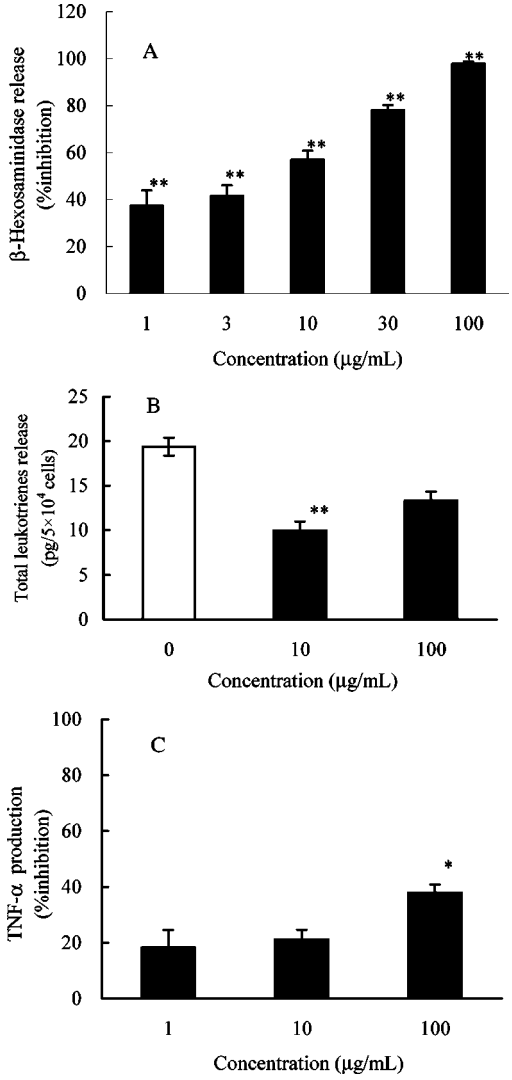

Figure 2. Inhibitory effect of JBE on (**A**) β-hexosaminidase release, (**B**) leukotriene release, and (**C**) TNF-α production in sensitized RBL-2H3 cells induced by antigen–antibody reaction. Each column represents mean ± SE of four experiments. Asterisks denote significant differences from the control at *, $p < 0.05$, and **, $p < 0.01$, respectively.

compounds were suggested to be involved in the inhibitory activity of JBE on leukotriene production in mast cells.

TNF-α is an inflammatory cytokine produced several hours after antigenic stimulation and is involved in the aggravation and chronicity of allergic diseases. JBE (1−100 μg/mL) showed concentration-dependent suppressive effects on TNF-α production (**Figure 2C**). Luteolin, which is found in a high concentration in perilla leaves, has also been reported to suppress TNF-α production in mast cells (*28*). Japanese butterbur is a dioecious plant, and the male plant has flower buds. As pollen in the flower buds contains luteolin-related flavonols and their glycosides (*8*, *10*), it was suspected that these flavonols in JBE inhibited TNF-α production. However, only female Japanese butterburs are cultivated in Aichi prefecture (*29*); hence, in our study, flavonols were not detected in JBE. In previous papers, black cohosh extract was reported to suppress TNF-α production in mast cells (*30*) and to contain fukinolic acid (**5**) that was also present in JBE (*31*). Therefore, compound **5** is suspected to be a principal JBE component with the ability to inhibit TNF-α production.

Furthermore, the effect of JBE on smooth muscle constriction induced by histamine and leukotriene D_4 was also investigated.

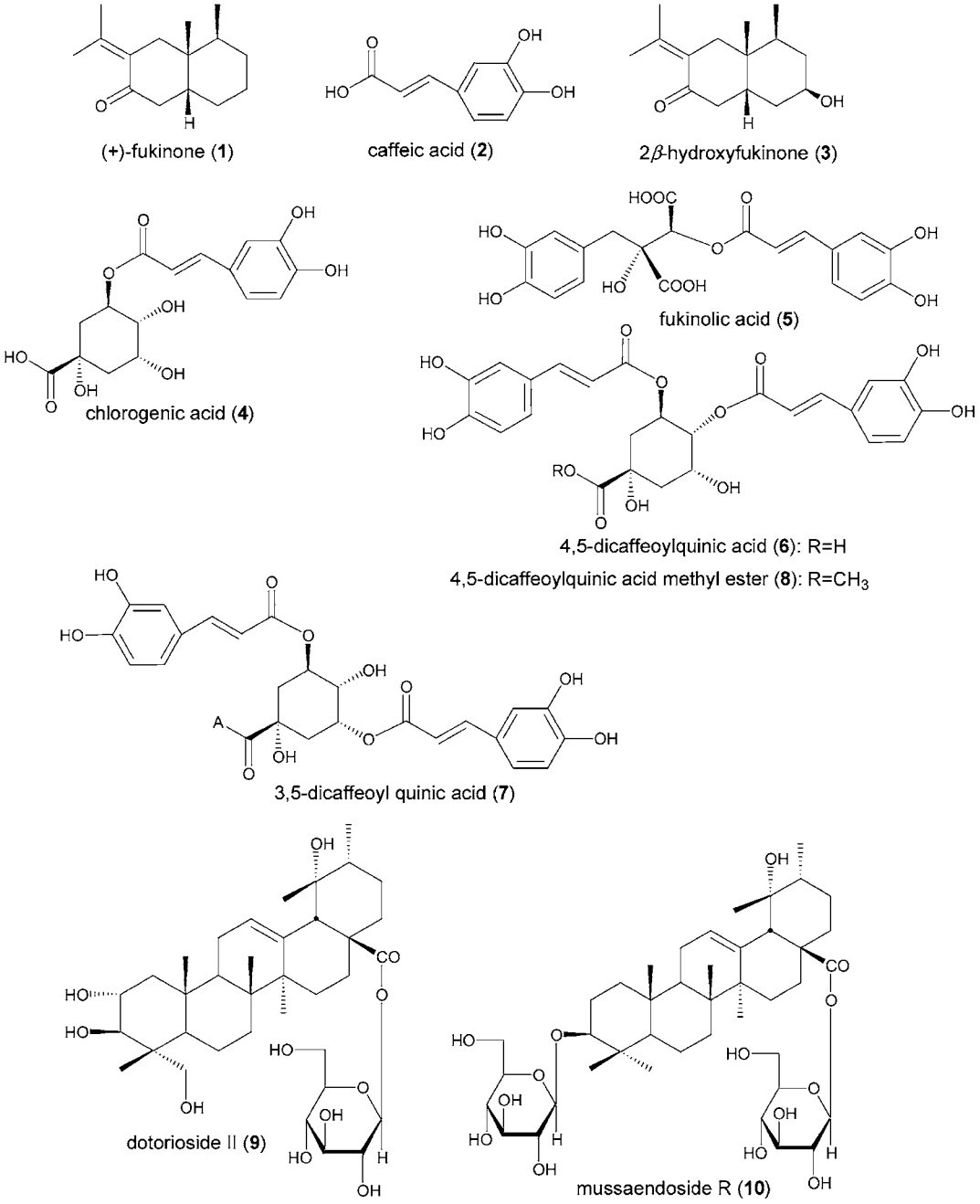

Figure 3. Structures of compounds isolated from Japanese butterbur extract.

Table 1. Effect of JBE on Constriction of Guinea Pig Trachea Induced by Histamine and Leukotriene D_4[a]

			constriction (%)			
			histamine			leukotriene D_4
	concn (μg/mL)	*N*	1 μM	3 μM	10 μM	10 nM
control		4	22.0 ± 9.5	53.7 ± 10.3	103.1 ± 4.4	104.5 ± 20.1
JBE	100	4	30.3 ± 4.8	70.8 ± 2.5	104.0 ± 2.9	61.8 ± 6.7
	1000	4	13.7 ± 3.9	30.9 ± 6.1	72.6 ± 1.5*	40.3 ± 11.1**

[a] Each value represents mean ± SE. Asterisks denote significant differences from the control group at *, $p < 0.05$, and **, $p < 0.01$, respectively.

Table 1 shows the effects of JBE against tracheal smooth muscle constriction induced by histamine and leukotriene D_4. The high concentration of JBE (1000 μg/mL) significantly suppressed tracheal constriction induced by histamine (10 μM) and leukotriene D_4 (10 nM). Fukinolic acid (**5**) in JBE has been reported to suppress the constriction of the rat isolated aorta strip induced by norepinephrine (*32*). The inhibition of Ca^{2+} influx was suggested to be involved in its suppressive mechanism. This nonspecific inhibition of smooth muscle constriction by compound **5** appears to be partially involved in the suppressive mechanism of JBE on tracheal constriction induced by histamine and leukotriene D_4. Moreover, eremophilenolides isolated from flower buds have been reported to suppress the constriction of isolated guinea pig trachea induced by histamine (*33*). Hence, it is suspected that eremophilane-type sesquiterpenes in JBE, (+)-fukinone (**1**) and 2β-hydroxyfukinone (**3**), may contribute to the suppressive effect of JBE on tracheal constriction. In conclusion, it was clarified that nonspecific inhibition of smooth muscle constriction and inhibition of degranulation, leukotriene release, and TNF-α production from mast cells were involved in the anti type I allergic activity of JBE. Both polyphenolic compounds (**2** and **4**−**8**) and eremophilane-type sesquiterpenes

Table 2. Effects of Constituents Isolated from JBE on Degranulation in RBL-2H3 Induced by Antigen–Antibody Reaction

	IC_{50} [μg/mL (μM)]
JBE	5.8
(+)-fukinone (**1**)	4.2 (19.1)
caffeic acid (**2**)	8.6 (47.8)
2β-hydroxyfukinone (**3**)	4.0 (16.9)
chlorogenic acid (**4**)	9.5 (26.8)
fukinolic acid (**5**)	2.1 (4.8)
4,5-dicaffeoylquinic acid (**6**)	3.3 (6.4)
3,5-dicaffeoylquinic acid (**7**)	2.9 (5.6)
4,5-dicaffeoylquinic acid methyl ester (**8**)	4.0 (7.5)
dotorioside II (**9**)	4.0 (6.0)
mussaendoside R (**10**)	>100 (>126)
luteolin	1.0 (3.5)

[a] Sensitized RBL-2H3 cells were incubated with samples for 10 min followed by stimulation by DNP–BSA for 10 min. Released β-hexosaminidase activity in supernatant was determined enzymatically.

(**1** and **3**), appear to be involved in the anti type I allergic mechanism of JBE.

To identify the compounds with inhibitory effect on mast cell degranulation, bioassay-guided separation was performed. **Table 2** shows the effects of sesquiterpenes, triterpene glycosides, and phenolic compounds isolated from JBE (**Figure 3**) on antigen-induced β-hexosaminidase release from sensitized RBL-2H3 cells. All compounds, except mussaendoside R (**10**), showed inhibitory activity against mast cell degranulation. The inhibitory activity of fukinolic acid (**5**) was the most potent among the constituents of JBE. The structure of compound **5** is quite similar to that of rosmarinic acid present in Labiatae plant with anti type I allergic effect (*34*). In the course of our experiments using sensitized RBL-2H3 cells, rosmarinic acid potently inhibited degranulation with an IC_{50} value of 2.9 μg/mL (data not shown). The IC_{50} value (2.1 μg/mL) of compound **5** is similar to that of rosmarinic acid. On the other hand, 50 mg/day of rosmarinic acid is reported to be effective for the treatment of seasonal allergic rhinitis (*35*). Furthermore, it is hoped that the elucidation of compound **5** will be helpful in the treatment of pollenosis. The other polyphenolic compounds (**2**, **4**, and **6**−**8**) also inhibited degranulation. Chlorogenic acid (**4**) has been reported to exert a weak inhibitory effect (5% inhibition at 100 μg/mL) on degranulation in RBL-2H3 cells (*36*). Caffeic acid (**2**) is also reported to inhibit histamine release from mast cells (*37*). However, the activities of compounds **6**−**8** rich in green coffee bean have not been elucidated; this is the first study to report these activities. On the other hand, the eremophilane-type sesquiterpenes, namely, fukinones (**1** and **3**), showed suppressive activities. Morikawa (*38*) reported that eudesmane-type sesquiterpenes isolated from *Alpinia* plants, namely, nootkatone and oxyphyllol A, suppressed degranulation from RBL-2H3. On the other hand, eremophilane-type sesquiterpenes, namely, oxyphyllols B and C, did not suppress the degranulation. Although bioactive sesquiterpenes originating from natural resources, such as thapsigargin and cytochalasin D, have been used for studying the signaling system in mast cells, few papers describing the antiallergic effect of sesquiterpene from natural resources exist. Further studies on the structure−activity relationship of sesquiterpenes with regard to antiallergic activity are required. Moreover, we isolated two triterpene glycosides (**9** and **10**) from JBE. Dotorioside II (**9**), a triterpene monoglycoside, showed inhibitory activity on degranulation. On the other hand, mussaendoside R (**10**) with two sugars in its structure did not suppress degranulation. Glycosylation of triterpenes appeared to reduce their inhibitory effect on mast cell degranulation.

In conclusion, we found anti type I allergic activities of Japanese butterbur such as inhibition of degranulation, leukotriene production, and TNF-α production from mast cells. Additionally, some compounds with inhibitory activity on mast cell degranulation were isolated.

SAFETY

Aerial parts of *Petasites japonicus* except flower buds were used to prepare JBE. In the course of study of *P. japonicus*, we performed LC-MS analysis on JBE. As a result of scanning at ES^+ 335, 351, 365, 381, and 408, they were the MW of pyrrolizidine alkaloids, and no peak originated from alkaloids was detected. Therefore, we have concluded that JBE is a safe plant extract.

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