

ORYZA OIL & FAT CHEMICAL CO., LTD.

## ***JAPANESE BUTTERBUR EXTRACT***

### **Anti-allergy and Hay fever preventive food material**

- **JAPANESE BUTTERBUR EXTRACT-P**  
(Powder, Food Grade)
- **JAPANESE BUTTERBUR EXTRACT-WSP**  
(Water-soluble Powder, Food Grade)
- **JAPANESE BUTTERBUR POWDER**  
(Powder, Food Grade)
- **JAPANESE BUTTERBUR EXTRACT-PC**  
(Powder, Cosmetic Grade)
- **JAPANESE BUTTERBUR EXTRACT-WSPC**  
(Water-soluble Powder, Cosmetic Grade)
- **JAPANESE BUTTERBUR EXTRACT-LC**  
(Liquid, Cosmetic Grade)

ORYZA OIL&FAT CHEMICAL CO., LTD.

ver. 3.1 YF

# JAPANESE BUTTERBUR EXTRACT

## Anti-allergic and pollenosis-preventive food material

### 1. Introduction

Pollenosis is a modern disease that affects 1 in 10 Japanese individuals. There are many who suffer from symptoms such as sniveling, sternutation, and itch of the eyes during the Japanese cedar and cypress pollen-dispersing period from February to May.

A number of anti-allergic food materials derived from plants, such as tiencha, perilla, tomatoes, and roses, have been developed, and data of their mechanisms and functions showing their effectiveness in humans are being collected. However, known anti-allergic materials originate from foreign countries to some extent. We found the effectiveness of Japanese butterbur by studying the literature on the anti-allergic activity of vegetables and fruit grown in Japan and performing screening tests.

As indicated by the name, Japanese butterbur (*Petasites japonicus*) is one of the few vegetables native to Japan. Japanese butterbur has a good flavor, and has long been popular among the Japanese as a food material in the early spring. There are more than 200 kinds of Japanese butterbur, including wild species grown in hills and cultivated vegetables, such as "Aichi wase fuki (*P. japonicus* Maxim.)", "Mizufuki", "Akita buki (*P. japonicus* Maxim. var. *giganteus*)", and "Nobisugidennen". Japanese butterbur is widely cultivated in Aichi, Gunma, and Osaka Prefectures. Among the cultivar species, *P. japonicus* Maxim. is representative due to its rapid growth and high yield. The production of Japanese butterbur is highest in Chita region in Aichi Prefecture, it was 6,800 t in the 2001 fiscal year, accounting for about 90% of that in Aichi Prefecture and about 40% of that in the whole country. Japanese butterbur used to be a synonym for the early spring, but it is now cultivated for a longer time and marketed from October to May.



Japanese butterbur

We examined the anti-allergic activity of extracts of Japanese butterbur grown in Aichi Prefecture, where its production is highest, in cooperation with Japan Agricultural Co-operatives in Aichi Chita and Kyoto Pharmaceutical University, and found inhibitory effects on degranulation from mast cells, release of leukotrienes, and TNF- $\alpha$  production. Furthermore, we confirmed the *in vivo* effectiveness of JAPANESE BUTTERBUR EXTRACT, and evaluated the effectiveness in humans. Additionally, two sesquiterpenes, 6 polyphenols, and 2 triterpene glycosides were identified together with new sesquiterpene glycoside named potassium 2 $\beta$ -hydroxyfukinone 2-*O*- $\beta$ -D-glucopyranoside-6'-sulfate (fukinoside A) as effective constituents. We consider that JAPANESE BUTTERBUR EXTRACT containing these active constituents can be used for products claiming anti-allergic activity for the alleviation of pollenosis and atopic dermatitis.

## 2. Functions and constituents of JAPANESE BUTTERBUR EXTRACT

### (1) Traditional medicinal properties and recent studies

Japanese butterbur, which has another name, Hou-to-sai, has been used for detoxication, ejection of sluggish blood, and the treatment of bruises. Japanese butterbur contains many essential oil components, and there have been studies on sesquiterpenes in Japanese butterbur flower buds and rhizome<sup>1-4)</sup>. The anti-allergic activity of some eremophilane type sesquiterpenes has been reported<sup>5)</sup>.

Researchers in Hokkaido and Osaka Prefecture, where the production of Japanese butterbur has recently been comparable to that in Aichi Prefecture, have studied the edible part of Japanese butterbur. The group of Dr. Tazaki at Obihiro University of Agriculture and Veterinary Medicine and Dr. Iwamoto at the Agricultural, Food and Environmental Sciences Research Center of Osaka Prefecture studied the biosynthetic pathway<sup>6)</sup> and quantification method<sup>7)</sup> of a polyphenol, fukinolic acid, which is characteristic of Japanese butterbur. With regard to the biological activity of Japanese butterbur, antioxidative activity<sup>8)</sup>, collagenolytic activity<sup>9)</sup>, neutrophil elastase inhibitory activity<sup>10)</sup>, estrogenic activity<sup>11)</sup>, vasodilative activity<sup>12)</sup>, and amylase and carboxypeptidase inhibitory activities<sup>13)</sup> have

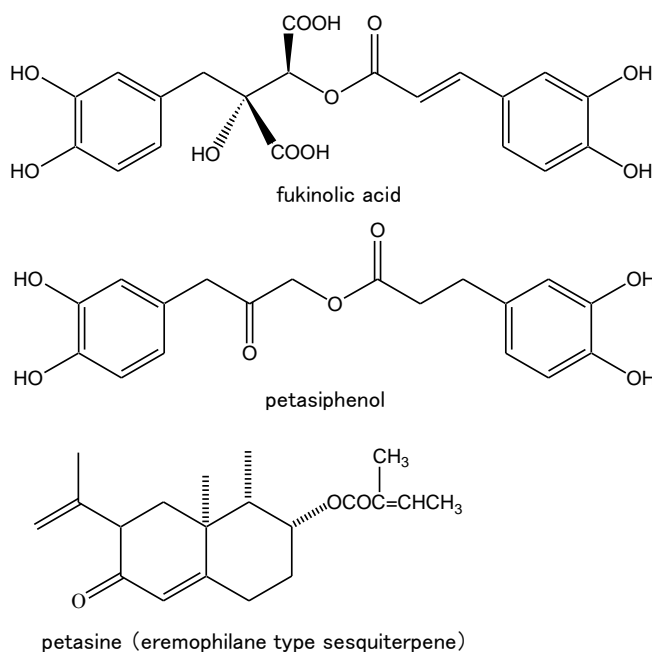


Fig. 1. Known compounds of *P. Japonicus* and *P. hybridus*

been reported. With regard to polyphenols, the DNA polymerase inhibitory activity of a phenylpropanoid, petaciphenol<sup>14,15)</sup>, has also been reported.

Recently, it has been reported that extracts of "European butterbur (*Petasites hybridus*)" roots improved symptoms in patients with allergic rhinitis<sup>16,17)</sup>. These studies indicated that the effects of European butterbur were similar to those of medical antihistamine preparations, and no side effects caused by suppression of the central nervous system were observed. The effective constituent is petasine, which has activities that not only suppress degranulation from mast cells, as observed in flavonoids, but also dilate capillary blood vessels and bronchial tubes and inhibit leukotriene synthesis<sup>18)</sup>. Therefore, unlike conventional anti-allergic materials, JAPANESE BUTTERBUR EXTRACT is expected to be effective at the occurrence of allergic symptoms.

### References

- 1) Naya K., Takagi I. The structure of petasitin, a new sesquiterpene from *Petasites japonicus* Maxim. *Tetrahedron Lett.*, **5**, 629-32 (1968).
- 2) Naya K., Nakagawa M., Hayashi M., Tsuji K., Naito M. The constituents of *Petasites japonicus* Maxim. Rhizomes. *Tetrahedron Lett.*, **31**, 2961-4 (1971).
- 3) Tori M., Kawahara M., Sono M. Novel epoxyremophilanolides, eremopetasitenins A1, A2, B1, and B2, from *Petasites japonicus*. *Tetrahedron Lett.*, **38**, 1965-8 (1997).
- 4) Goto Y., Kojima Y., Nakayama T., Terazawa M. Allelopathic sesquiterpenoids from rhizomes of *Petasites japonicus* ssp. *Giganteus* Kitam. *Phytochemistry*, **57**, 109-13 (2001).
- 5) Tobinaga S., Takeuchi N., Kasama T., Yamashita J., Aida Y., Kaneko T. Anti-histaminic and anti-allergic principles of *Petasites japonicus* Maxim. *Chem. Pharm. Bull.*, **31**, 745-8 (1983).
- 6) Tasaki H., Hildegard F., Namisa Y., Nishimura S., Iwamoto T. Proceeding of general meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry in 2004. pp. 89 (2004).
- 7) Iwamoto T., Tasaki H. Proceeding of general meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry in 2004. pp. 89 (2004).
- 8) Watanabe S., Tasaki H., Misawa N., Sato K., Sakagami H. Proceeding of general meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry in 2004. pp. 87 (2004).
- 9) Kusano A., Seyama Y., Nagai M., Shibano M., Kusano G. Effects of fukinolic acid and cimicifugic acids from *Cimicifuga* species on collagenolytic activity. *Biol. Pharm. Bull.*, **24**, 1198-201 (2001).
- 10) Loser B., Kruse S. O., Melzig M. F., Nahrstedt A. Inhibition of neutrophil elastase activity by cinnamic acid derivatives from *Cimicifuga racemosa*. *Planta Med.*, **66**, 751-3 (2000).
- 11) Kruse S. O., Lohning A., Pauli G. F., Winterhoff H., Nahrstedt A. Fukilic and piscidic acid esters from the rhizome of *Cimicifuga racemosa* and the *in vitro* estrogenic activity of fukinolic acid. *Planta Med.*, **65**, 763-4 (1999).
- 12) Noguchi M., Nagai M., Koeda M., Nakayama S., Sakurai N., Takahira M. Vasoactive effects of cimicifugic acids C and D, and fukinolic acid in cimicifuga rhizome. *Biol. Pharm. Bull.*, **21**, 1163-8 (1998).
- 13) Kusano G., Takahira M., S., Shibano M., Kusano A., Okamoto Y., Tsujibo H., Numata A., Inamori Y. Studies on inhibitory activities of fukilic acid esters on germination,  $\alpha$ -amylase and carboxypeptidase A. *Biol. Pharm. Bull.*, **21**, 997-9 (1998).
- 14) Mizushina Y., Hirota M., Murakami C., Ishidoh T., Kamisuki S., Shimazaki N., Takemura M., Perpelescu M., Suzuki M., Yoshida H., Sugawara F., Koiwai O., Sakaguchi K. Some anti-chronic inflammatory compounds are DNA polymerase  $\lambda$ -specific inhibitors. *Biochem. Pharmacol.*, **66**, 1935-44 (2003).
- 15) Mizushina Y., Kamisuki S., Kasai N., Ishidoh T., Shimazaki N., Takemura M., Asahara H., Linn S., Yoshida S., Koiwai O., Sugawara F., Yoshida H., Sakaguchi K. Petasiphenol: a DNA polymerase  $\lambda$  inhibitor. *Biochemistry*, **41**, 14463-71 (2002).

- 16) Lee D. K., Carstairs I. J., Haggart K., Jacson C. M., Currie G. P., Lipworth B. J. Butterbur, a herbal remedy, attenuates adenosine monophosphate induced nasal responsiveness in seasonal allergic rhinitis. *Clin. Exp. Allergy*, **33**, 882-6 (2003).
- 17) Schapowal A. Randomised controlled trial of butterbur and cetirizine for treating seasonal allergic rhinitis. *B. M. J.*, **328**, 1-4 (2002).
- 18) Bickel D., Rder T., Bestmann H. J., Brune K. Identification and characterization of inhibitors of peptide-leukotriene-synthesis from *Petasites hybridus*. *Plant Med.*, **60**, 318-22 (1994).

## (2) Anti-allergic constituents of JAPANESE BUTTERBUR EXTRACT

Japanese butterbur contains characteristic flavor constituents, monoterpenes and sesquiterpenes, and many polyphenols. However, as described above, it has been reported that sesquiterpenes are contained in rhizomes and flower buds, but there have been few studies on the edible aerial part. To investigate hydrophobic fraction of extracts of Japanese butterbur aerial parts, we have studied the anti-allergic active components using a criterion of the suppressive activity of degranulation of rat basophilic mast cells (RBL-2H3), and isolated bellow constituents.<sup>1)</sup> Additionally, we have isolated a novel sesquiterpene glycoside, potassium 2 $\beta$ -hydroxyfukinone 2-O- $\beta$ -D-glucopyranoside-6'-sulfate (fukinocide A).<sup>2)</sup> This compound has been patented in Japan.

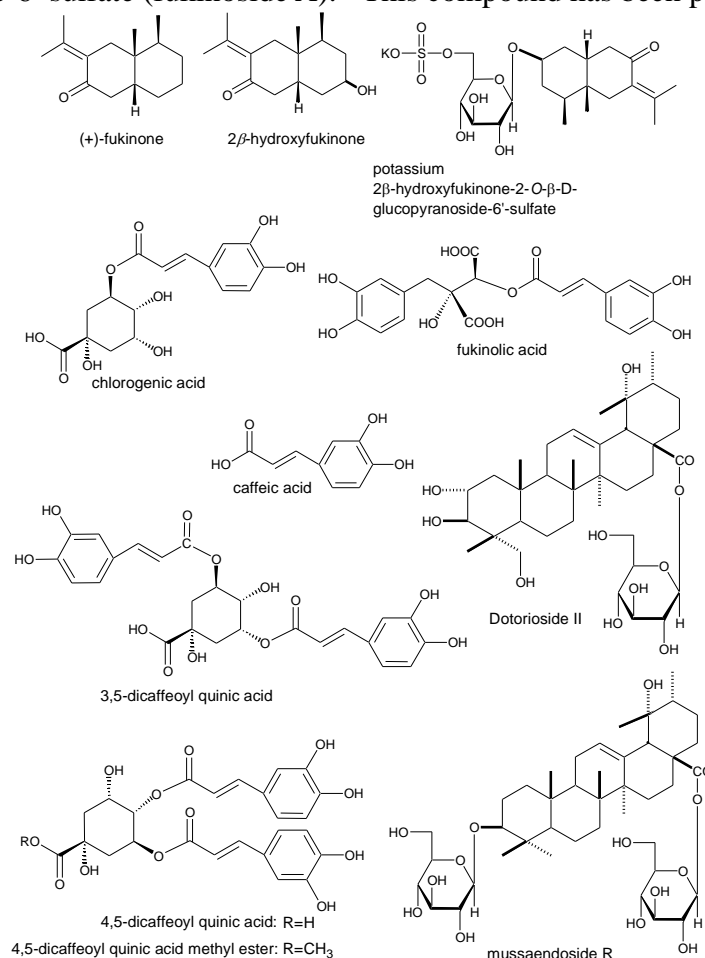
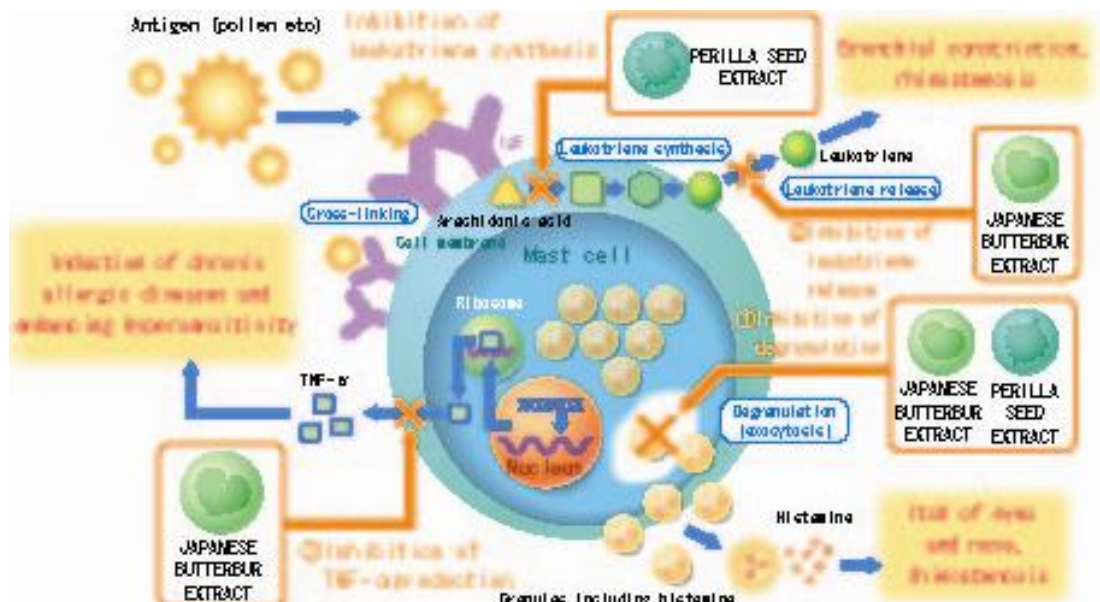


Fig. 2. Constituents in aerial part of Japanese butterbur

- 1) Shimoda H., Tanaka J., Yamada E., Morikawa T., Kasajima N., Yoshikawa M. Anti type I allergic property of Japanese butterbur extract and its mast cell degranulation inhibitory ingredients. *J. Agric. Food Chem.* 54, 2915-2920 (2006).
- 2) Yoshikawa M., Morikawa T., Tanaka J., Shimoda H. Medicinal food stuffs. XXXII. Novel sesquiterpene glycoside sulfate, fukinoside A, with antiallergic activity from Japanese butterbur (*Petasites Japonicus*) *Heterocycles*, 68, 2335-2342 (2006).

IgE antibody binding to specific antigens, such as pollens, exists on the surface of mast cells in patients with allergic diseases. The binding of antigens that have invaded the body to IgE and its cross-linking by antigens induce phosphorylation of specific proteins, such as Lyn and Syk, and elevation of the concentration of intracellular calcium ion and diacylglycerol by inflow of calcium ions. Consequently, exocytosis of granules containing histamine (degranulation), production of leukotrienes by activation of 5-lipoxygenase, and production of TNF- $\alpha$  by activation of intranuclear cytokine transcription factors are induced. These substances produced by mast cells cause itch of eyes and nose and increased sniveling in pollenosis and bronchial constriction and nasal congestion in asthma. TNF- $\alpha$  is involved in inducing chronic allergic diseases and enhancing hypersensitivity (Fig. 3).



5

## 2. Synergic effect of JAPANESE BUTTERBUR EXTRACT and PERILLA SEED EXTRACT

We have placed "PERILLA SEED EXTRACT" on the market as an anti-allergic material. The extracts not only have effects of suppression of degranulation and inhibition of leukotriene synthesis but also show anti-inflammatory effects, and have been used in many anti-allergic food products.

In addition to suppressive effects of degranulation, JAPANESE BUTTERBUR EXTRACT have suppressive effects of leukotriene release and TNF- $\alpha$  production, which are not observed in PERILLA SEED EXTRACT, indicating that JAPANESE BUTTERBUR EXTRACT can alleviate various allergic symptoms.

The combination of JAPANESE BUTTERBUR EXTRACT and PERILLA SEED EXTRACT may have anti-allergic effects, as shown in Table 1. Fig. 4 shows the effective functions of these extracts in allergic reactions in which mast cells are involved.

Table 1. Synergic effect of JAPANESE BUTTERBUR EXTRACT and PERILLA SEED EXTRACT

1	Enhancement of degranulation inhibitory activity	JAPANESE BUTTERBUR EXTRACT & PERILLA SEED EXTRACT	Enhancement of relief on itch of eyes and nose, and nasal drip
2	Inhibition of leukotriene synthesis and release	Inhibition of synthesis : PERILLA SEED EXTRACT Inhibition of release : JAPANESE BUTTERBUR EXTRACT	Enhancement of suppressive effect on rhinostenosis and bronchoconstriction
3	Suppression of TNF- $\alpha$ production	JAPANESE BUTTERBUR EXTRACT	Suppression of inducing chronic allergic diseases and enhancing hypersensitivity of eyes and nasal mucosa

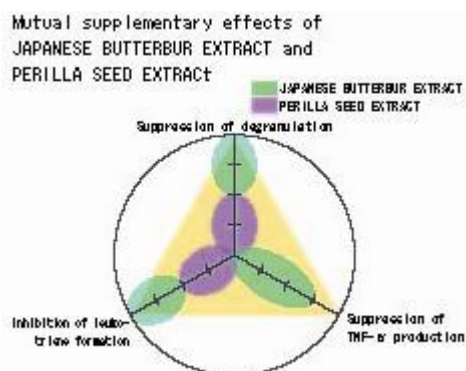


Fig. 4 Outlines of the activities of our materials against allergic reactions in mast cells

### 3. Anti-type I allergic effect of JAPANESE BUTTERBUR EXTRACT

#### (1) Suppressive effect on degranulation (*in vitro*)

Suppressive effects of JAPANESE BUTTERBUR EXTRACT and conventional anti-allergic materials on the degranulation from mast cells were examined in rat basophilic mast cells (RBL-2H3) using the release of hexosaminidase as a parameter. It was found that JAPANESE BUTTERBUR EXTRACT had suppressive effects on degranulation at a level equivalent to that of tiencha extracts (Fig. 5).

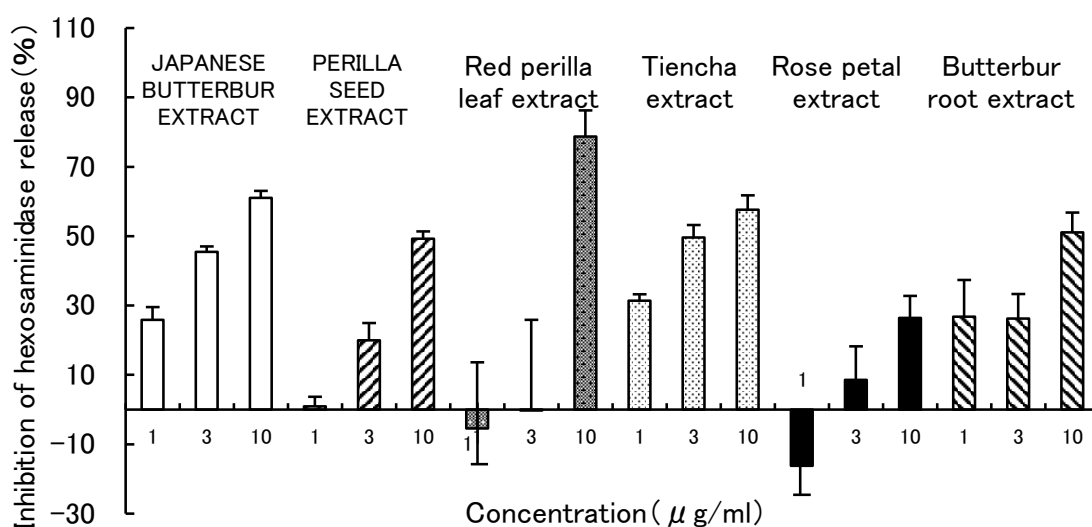


Fig. 5. Suppressive effects of JAPANESE BUTTERBUR EXTRACT and conventional anti-allergic materials on antigen-inducing hexosaminidase release from RBL-2H3 cells. (n=4-6, mean  $\pm$  S.E.)

Table 2. IC<sub>50</sub> value of JAPANESE BUTTERBUR EXTRACT and conventional anti-allergic materials on antigen-inducing hexosaminidase release from RBL-2H3 cells.

	IC <sub>50</sub> (µg/ml)
JAPANESE BUTTERBUR EXTRACT	5.0
PERILLA SEED EXTRACT	15.2
Red perilla leaf extract	8.6
Tiencha extract	4.2
Rose petal extract	59.9
Butterbur root extract	6.8

The suppressive effects of JAPANESE BUTTERBUR EXTRACT components on degranulation from mast cells were examined in rat basophilic mast cells (RBL-2H3) using the release of hexosaminidase as a parameter. It was found all compounds except mussaendoside R had suppressive effects on degranulation (Fig. 6, Table 3).



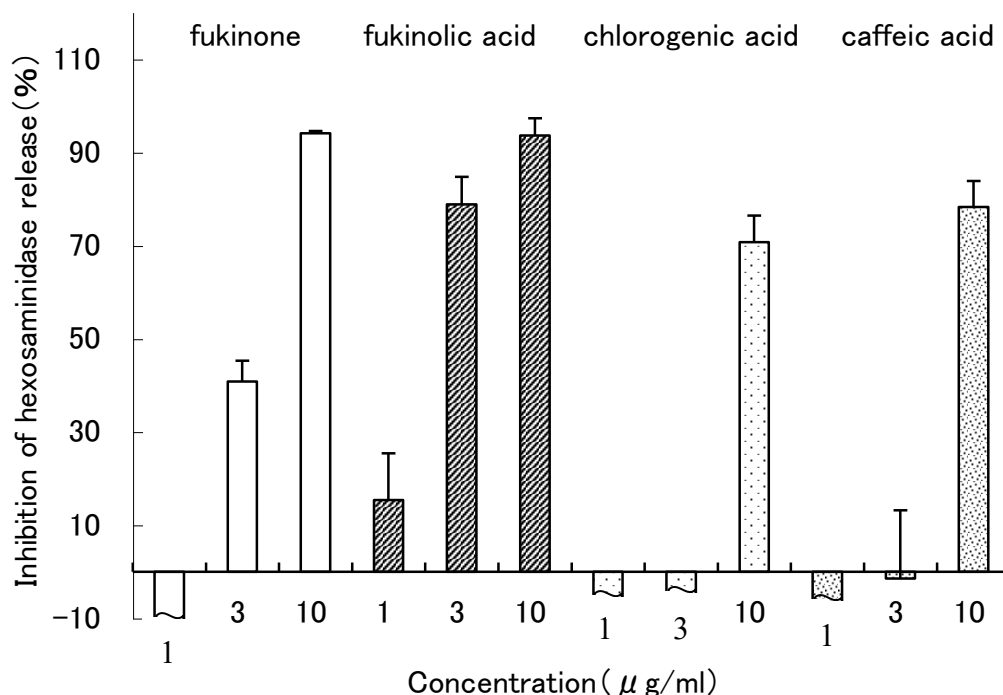


Fig. 6. Suppressive effects of constituents in JAPANESE BUTTERBUR EXTRACT on antigen-inducing hexosaminidase release from RBL-2H3 cells (n=4, mean  $\pm$  S.E.)

Table3. IC<sub>50</sub> value of constituents in JAPANESE BUTTERBUR EXTRACT on antigen-inducing hexosaminidase release from RBL-2H3 cells.

Constituents	IC <sub>50</sub> ( $\mu$ g/ml)	Constituents	IC <sub>50</sub> ( $\mu$ g/ml)
(+)-Fukinone	4.2	2 $\beta$ -Hydroxyfukinone	4.2
Chlorogenic acid	9.5	Fukinolic acid	2.1
Caffeic acid	8.6	3,5-Dicaffeoyl quinic acid	2.9
4,5-Dicaffeoyl quinic acid	3.3	4,5-Dicaffeoyl quinic acid methyl ester	4.0
Dotorioside II	4.0	Mussaendoside R	>100
Constituents		IC <sub>50</sub> ( $\mu$ g/ml)	
Potassium 2 $\beta$ -hydroxyfukinone-2-O- $\beta$ -D-glucopyranoside-6'-sulfate		8.6	

#### 【Method】

RBL-2H3 cells were sub-cultured in Minimum Essential Medium (MEM medium, Sigma Inc.) containing fetal bovine serum (10%), penicillin (100 units/ml), and streptomycin (100  $\mu$  g/ml) at 37°C with 5% CO<sub>2</sub>. RBL-2H3 cells were inoculated in flat-bottom 24-well microplates (2.0 $\times$ 10<sup>5</sup> cells in 400  $\mu$  l/well) on the day before reaction. After incubation for 1 hour, rat anti-dinitrophenyl (DNP) monoclonal IgE antibody (Sigma Inc.) was added to the final concentration of 0.45  $\mu$  g protein/ml, and the cells were incubated in the medium for 24 hours for sensitization. On the day of

reaction, the cells in each well were rinsed twice with 500  $\mu$ l of Siraganian buffer (pH 7.2, 119 mM NaCl, 5 mM KCl, 0.4 mM  $MgCl_2$ , 25 mM PIPES), and preincubated in 160  $\mu$ l Siraganian buffer containing 5.6 mM glucose, 1 mM  $CaCl_2$ , and 0.1% bovine serum albumin (BSA) at 37°C for 10 min. Subsequently, 20  $\mu$ l of the test solution was added, and 10 min later, DNP-BSA (final concentration: 10  $\mu$ g/ml) was added as an antigen. After incubation for 30 min, the reaction was terminated by chilling the plate with ice for 10 min, and 50  $\mu$ l of the supernatant was transferred to a flat-bottom 96-well microplate. To each well, 50  $\mu$ l of 1 mM *p*-nitrophenyl-*N*-acetyl- $\beta$ -glucosaminide (PNAG) in 0.1 M citrate buffer was added, mixed, and incubated at 37°C for 1 hour.

After the reaction, 200  $\mu$ l of reaction stopping solution (0.1 M  $NaHCO_3/Na_2CO_3$ , pH 10.0) was added, and the rate of hexosaminidase release was determined by measuring the absorbance at 410 nm using a microplate reader.

## (2) Suppressive effect on leukotriene release (*in vitro*)

Leukotrienes are inflammation mediators that are synthesized from the cell membrane phospholipid of mast cells by antigen stimulation, and are involved in airway constriction in bronchial asthma. We examined JAPANESE BUTTERBUR EXTRACT and other known anti-allergic materials for their effects on the release of leukotrienes upon antigen stimulation. We found suppressive effects with JAPANESE BUTTERBUR EXTRACT but not with tiencha extract or PERILLA SEED EXTRACT (Fig. 7).

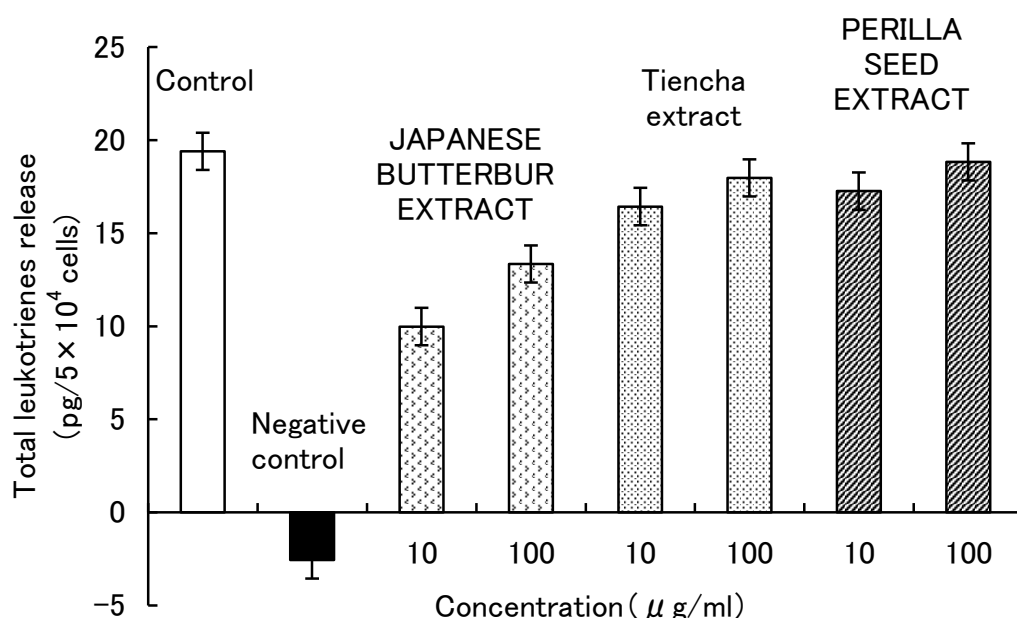


Fig. 7. Inhibitory effects of JAPANESE BUTTERBUR EXTRACT and conventional anti-allergic materials on antigen-inducing leukotriene release from RBL-2H3 cells (n=6, mean  $\pm$  S.E.)

### 【Method】

The same experimental procedures used in the degranulation tests were used in this test. The amount of leukotrienes released into the medium 30 min after antigen stimulation was determined by ELISA (Leukotriene C<sub>4</sub>/D<sub>4</sub>/E<sub>4</sub> enzyme immunoassay system, Amersham-Pharmacia Inc.).

### (3) Suppressive effect on TNF- $\alpha$ production (*in vitro*)

TNF- $\alpha$  is an inflammatory cytokine produced several hours after antigen stimulation (delayed phase of immediate type allergy), which is involved in the aggravation and chronicity of allergic diseases. JAPANESE BUTTERBUR EXTRACT showed dose-dependent suppressive effects on TNF- $\alpha$  production (Fig. 8). However, rose petal extract at low doses (1 and 10  $\mu$ g/ml) and PERILLA SEED EXTRACT did not show suppressive effects.

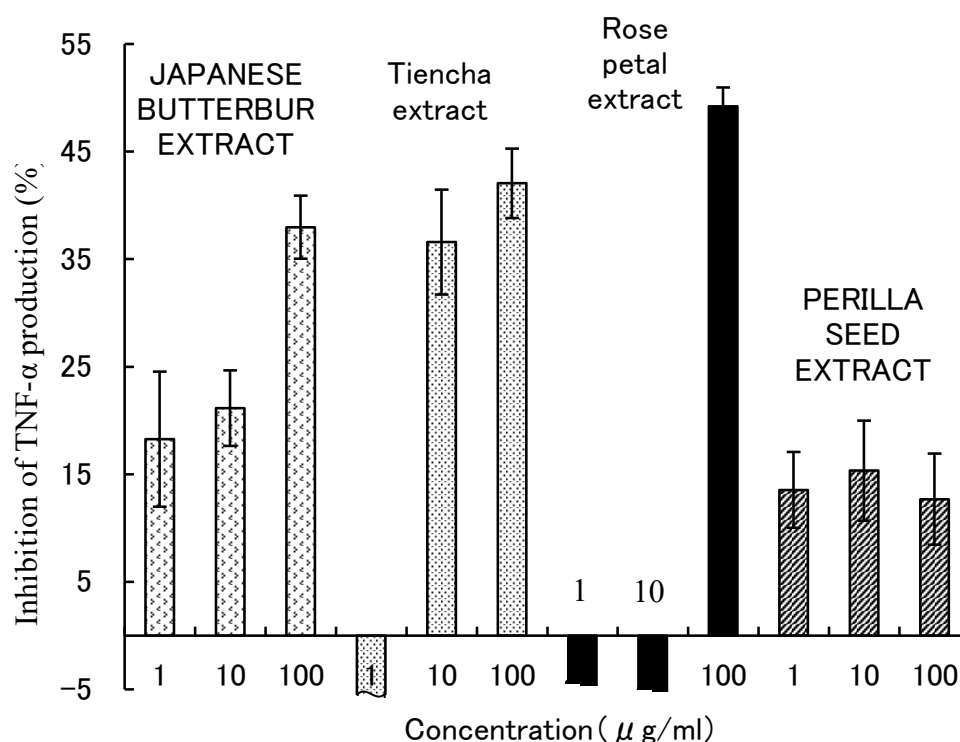


Fig. 8. Suppressive effect JAPANESE BUTTERBUR EXTRACT and conventional anti-allergic materials on antigen-inducing TNF- $\alpha$  production from RBL-2H3 cells (n=4, mean  $\pm$  S.E.)

### 【Method】

The same experimental procedures used in the degranulation tests were used in this test. The amount of TNF- $\alpha$  produced 4 hours after antigen stimulation was determined by ELISA (Tumor Necrosis Factor Alpha [( $\gamma$ )TNF $\alpha$ ], Rat, Biotrak ELISA system, Amersham-Pharmacia Inc.).

#### (4) Passive cutaneous anaphylaxis (PCA) reaction in rats (*in vivo*)

The anti-allergic activity *in vivo* of JAPANESE BUTTERBUR EXTRACT was tested using the rat passive cutaneous anaphylaxis (PCA) reaction, which is a model of type I allergy. It was found that oral administration of JAPANESE BUTTERBUR EXTRACT 2 hours prior to the induction of reaction suppressed leakage of the dye into the skin due to inflammation (Fig. 9).

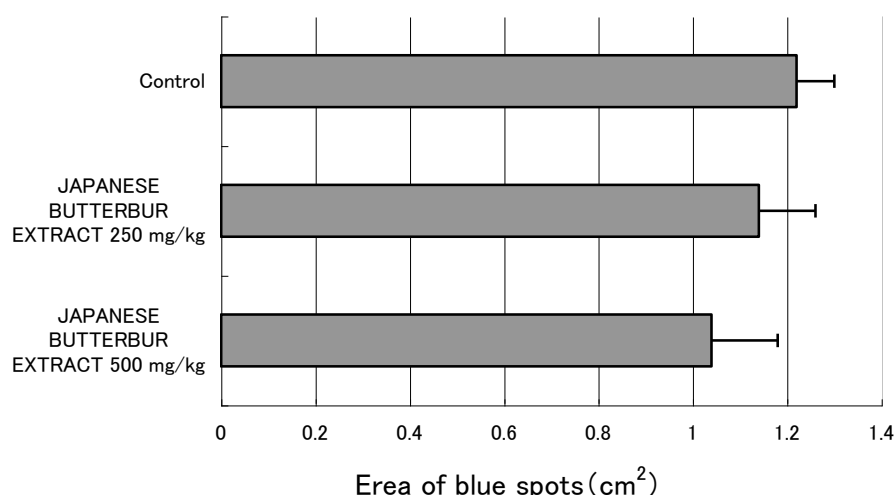


Fig. 9. Effect of JAPANESE BUTTERBUR EXTRACT on PCA reaction (n=6, mean ± S.E.)

#### 【Method】

The back of male 7 weeks old Wistar rats was shaven, and anti-DNP-IgE diluted 12,500-fold in PBS (-) was subcutaneously administered (100  $\mu$ l/site). After 2 days (after fasting for 20 hours), JAPANESE BUTTERBUR EXTRACT suspended in 5% acacia were orally administered, and 2 hours later, 0.5 ml of 1% Evans blue solution containing 1.5 mg/ml DNP-BSA was intravenously administered. The animals were sacrificed 30 min after administration, and the area of Evans blue spot on the skin was measured using a digital planimeter.

#### (5) Suppressive effect on leukotriene-inducing inflammation (*in vivo*)

The anti-inflammatory activity of JAPANESE BUTTERBUR EXTRACT was evaluated using inflammation models with subcutaneous administration of leukotriene D<sub>4</sub>. It was found that administration of 500 mg/kg of JAPANESE BUTTERBUR EXTRACT had anti-inflammatory effects.

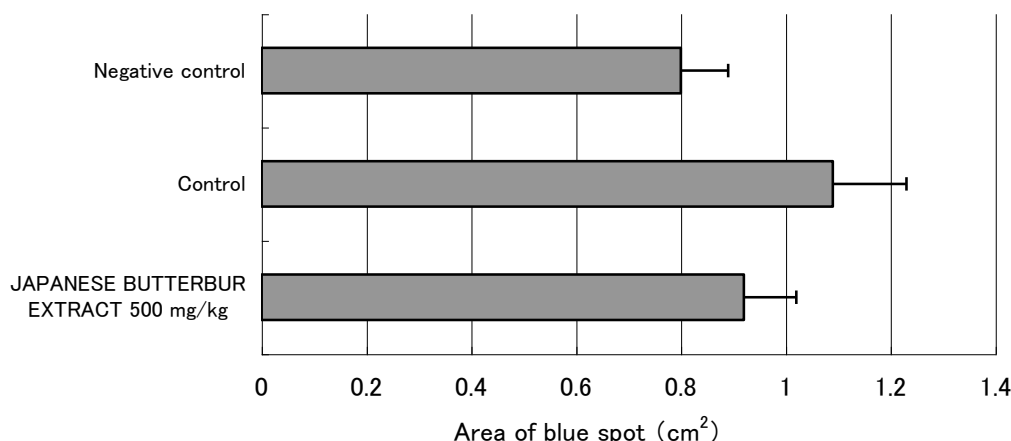


Fig. 10. Effect of JAPANESE BUTTERBUR EXTRACT on dermal inflammation induced by leukotriene D<sub>4</sub> (n=4, mean ± S.E.)

#### 【Method】

JAPANESE BUTTERBUR EXTRACT suspended in 5% acacia were orally administered to rats after fasting for 20 hours. After 2 hours, 0.5 ml of 1% Evans blue solution in PBS (-) was intravenously administered. The back of the rats was shaven, and 30 min after administration of Evans blue, 100  $\mu$ l/site of 1  $\mu$ g/ml leukotriene D<sub>4</sub> solution or PBS (-) as a negative control was subcutaneously administered at 2 sites on either side of the median line. The animals were sacrificed 30 min after this administration, and the area of Evans blue spot on the skin was measured using a digital planimeter.

#### (6) Suppressive effect on vasoconstriction and bronchoconstriction (*in vitro*)

To suppress allergic symptoms that have already been onset, direct action to targeting tissue is required. For example, for congested nose in pollinosis, the blood vessel of nasal mucosa is contracted by histamine that was released from mast cell. On the other hand, airway contraction is occurred in bronchial asthma due to bronchial smooth muscle constriction induced by mast cell-derived leukotrienes. We examined the effects of JAPANESE BUTTERBUR EXTRACT and conventional anti-allergic materials on KCl- or norepinephrine-induced vasoconstriction and histamine- or leukotriene D<sub>4</sub>-induced bronchoconstriction by using guinea pig trachea and rat thoracic aorta. As a result, JAPANESE BUTTERBUR EXTRACT and Tiencha extract relaxed vasoconstriction that was contracted by KCl (Fig. 11). Tiencha extract also suppressed vasoconstriction induced by norepinephrine, however JAPANESE BUTTERBUR EXTRACT was not suppressed. Hence, JAPANESE BUTTERBUR EXTRACT was found to possess non-specific suppressive activity against vasoconstriction. On the other hand, on bronchoconstriction, JAPANESE BUTTERBUR EXTRACT (1000  $\mu$ g/mL) significantly suppressed histamine-induced bronchoconstriction. JAPANESE BUTTERBUR EXTRACT and PERILLA SEED EXTRACT significantly suppressed

bronchoconstriction induced by leukotriene D<sub>4</sub>.

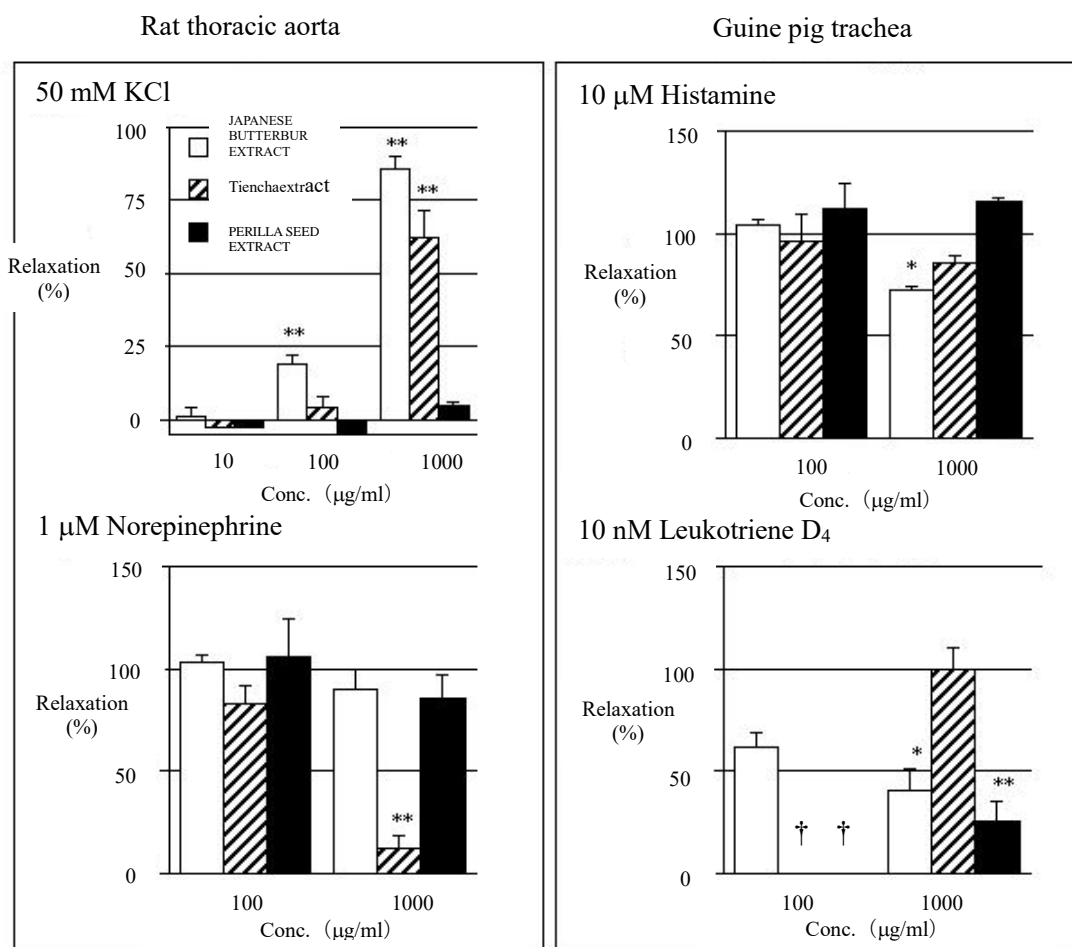


Fig. 11. Effects of JAPANESE BUTTERBUR EXTRACT and conventional anti-allergic materials on vasoconstriction and bronchoconstriction. (n=3-6, mean ± S. E., \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , † : not tested)

These results suggest that JAPANESE BUTTERBUR EXTRACT has an inhibitory action against smooth muscle contraction of blood vessel and trachea, and it can expect healing effects against the allergic symptom (rhinostenosis and bronchocontraction) which has already occurred. Furthermore, as a result of examination of (+)-fukinone, a sesquiterpene in JAPANESE BUTTERBUR EXTRACT, on smooth muscle constriction, it suppressed KCl-induced vasoconstriction and histamine- and leukotriene D<sub>4</sub>-induced bronchoconstriction (Tables 4-6).

Table 4. Effect of (+)-fukinone on vasoconstriction in rat thoracic aorta induced by KCl.

	n	Relaxation (%)		
		1 (μg/ml)	3	10
Control	4	3.1 ± 2.3	5.0 ± 2.0	6.3 ± 1.9
(+)-fukinone	4	1.6 ± 0.3	10.9 ± 0.7**	49.2 ± 2.2**

mean ± S.E., \*\*:  $p < 0.01$

Table 5. Effect of (+)-fukinone on bronchoconstriction in guinea pig trachea induced by histamine.

	Conc. (μg/ml)	n	Constriction (%)		
			His 1 (μM)	3	10
Control	-	5	22.0 ± 4.7	45.8 ± 4.5	97.0 ± 8.5
(+) -fukinone	10	3	15.7 ± 4.3	34.6 ± 10.5	92.9 ± 7.5
	30	3	25.0 ± 2.2	54.4 ± 6.4	102.1 ± 6.4
	100	3	11.5 ± 5.3	24.7 ± 11.1	54.1 ± 19.2*

mean ± S.E., \*:  $p < 0.05$

Table 6. Effect of (+)-fukinone on bronchoconstriction in guinea pig trachea induced by leukotriene D<sub>4</sub> (LT)

	Conc. (μg/ml)	n	Constriction (%)
			LT 10 (nM)
Control	-	3	104.5 ± 20.1
(+) -fukinone	10	3	92.7 ± 9.5
	100	3	51.6 ± 15.3

mean ± S.E

**【Method】****1) Constriction of rat thoracic aorta strips induced by KCl and norepinephrine.**

A rat was anesthetized with ether and the thoracic aorta was removed. The aorta was cut helical like, and prepared 15 mm length specimen. The aorta preparation was attached to a Magnus apparatus and placed in an organ-bath filled with Krebs-Henseleit (118 mM NaCl, 2.7 mM KCl, 1.2 mM  $\text{KH}_2\text{PO}_4$ , 1.2 mM  $\text{MgSO}_4$ , 2.5 mM  $\text{CaCl}_2$ , 25 mM  $\text{NaHCO}_3$ , 10 mM glucose, equilibrated with 95% $\text{N}_2$ /5% $\text{O}_2$  gas) solution (6 ml). The organ bath was warmed at 37 °C and the solution was bubbled gently with 95% $\text{N}_2$ /5% $\text{O}_2$  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, 3M KCl solution (100  $\mu\text{l}$ ) was added to the bath (final concentration: KCl 50 mM) and the change in tension was recorded by the extent of constriction plateaued. The specimen was washed 3 times with Krebs-Henseleit solution at 10-min intervals. This procedure was repeated again and the test sample diluted in DMSO (3  $\mu\text{l}$ ) was added accumulatively at 30-min intervals. The change in tension was recorded.

On the other hand, norepinephrine-induced aorta constriction was performed as follows. The aorta specimen was attached to the organ-bath filled with Krebs-Henseleit solution containing EDTA (37.2 mg/l) and tension was stabilized. A 1 mM nifedipine diluted with DMSO (6  $\mu\text{l}$ ) was added to organ-bath, and 1 mM norepinephrine (6  $\mu\text{l}$ ) was added 10 minutes later. After recording the contraction for 25 minutes, the specimen was washed 3 times at 10 min interval. Then, 1 mM nifedipine (6  $\mu\text{l}$ ) was added again, and a sample dissolved in DMSO (6  $\mu\text{l}$ ) was added 10 min later. After 10 min, norepinephrine was added accumulatively to become 1 nM~10  $\mu\text{M}$ , and tension was recorded.

**3) Constriction of guinea pig trachea strips induced by histamine and leukotriene D<sub>4</sub>.**

A guinea pig was anesthetized with ether and the trachea was removed. The trachea was cut into 3 to 4 mm length and its cartilage part was opened in Krebs-Henseleit solution containing 1  $\mu\text{M}$  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% $\text{N}_2$ /5% $\text{O}_2$  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<sub>4</sub> solution (6  $\mu\text{L}$ ) was added to the bath (final concentration: histamine, 10  $\mu\text{M}$ ; leukotriene D<sub>4</sub>, 10 nM) and the change in tension was recorded by the extent of constriction plateaued. The specimen was washed 3 times with Krebs-Henseleit solution at 10-min intervals. This procedure was repeated again and the test sample diluted in DMSO (6  $\mu\text{L}$ ) was added (final DMSO concentration, 0.1%). Ten minutes later, 1 to 10  $\mu\text{M}$  histamine or 10 nM leukotriene D<sub>4</sub> was added accumulatively and the change in tension was recorded.



## 6. Effect of JAPANESE BUTTERBUR EXTRACT on patients with pollenosis

We asked 10 patients with Japanese cedar pollenosis, of whom 3 patients were evaluated using a questionnaire, to freely take JAPANESE BUTTERBUR EXTRACT (250 mg) for 4 weeks between the times before and during pollen dispersing, and examined allergy-related parameters in the blood immediately and 4 weeks (recovery period) after the completion of intake of JAPANESE BUTTERBUR EXTRACT. We also investigated the pollenosis symptoms during the intake period and over 4 weeks after the completion of intake (recovery period) using a questionnaire.

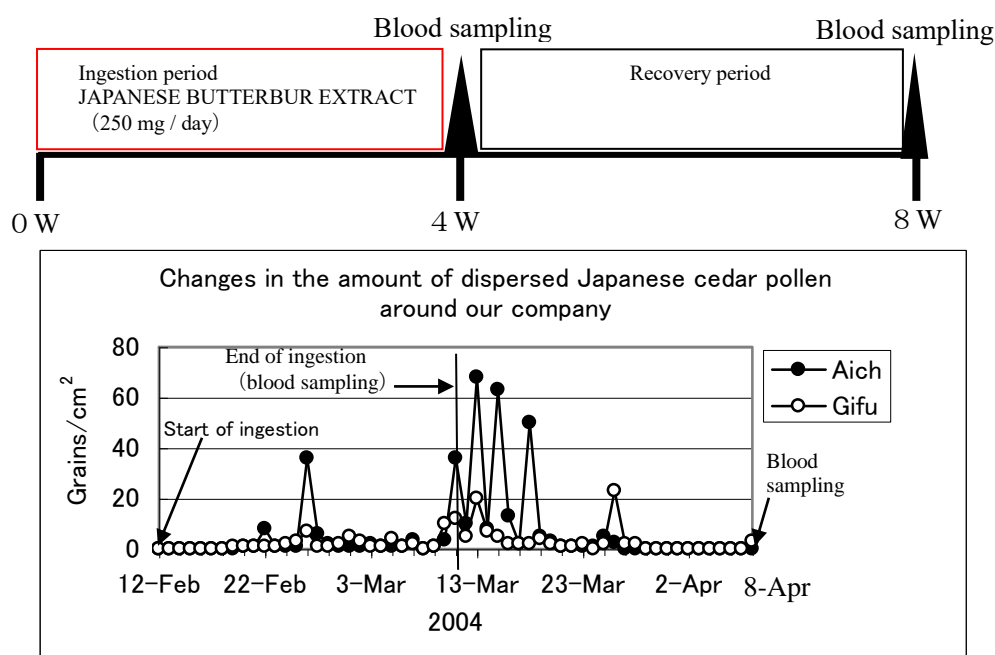


Fig. 12. Experimental protocol and change in amount of dispersed Japanese cedar pollen

Although the amount of dispersed pollen was small in 2004, on March 11, when the intake of JAPANESE BUTTERBUR EXTRACT was completed, a large amount of pollen was observed in Nagoya City in Aichi Prefecture (36 pollen grains/cm<sup>2</sup>) and in Gifu City in Gifu Prefecture (12 pollen grains/cm<sup>2</sup>). The investigation using the questionnaire demonstrated that the symptoms, such as sternutation, olfaction, and itch of the eyes, were slightly milder during the period of intake of JAPANESE BUTTERBUR EXTRACT than during the recovery period (Fig. 13). The rate of basophils in the leukocytes was lower immediately after intake of JAPANESE BUTTERBUR EXTRACT than after the recovery period (Fig. 14). Since basophils exist in peripheral tissues as mast cells, suppression of the increase in the rate of basophils in the leukocytes may contribute to the prevention of type I allergy, in which mast cells are involved. The blood concentration of eosinophilic basicity protein (ECP) produced by eosinophils was lower immediately after intake of JAPANESE

BUTTERBUR EXTRACT than after the recovery period, suggesting that the activity of eosinophils was suppressed during the intake period of JAPANESE BUTTERBUR EXTRACT.

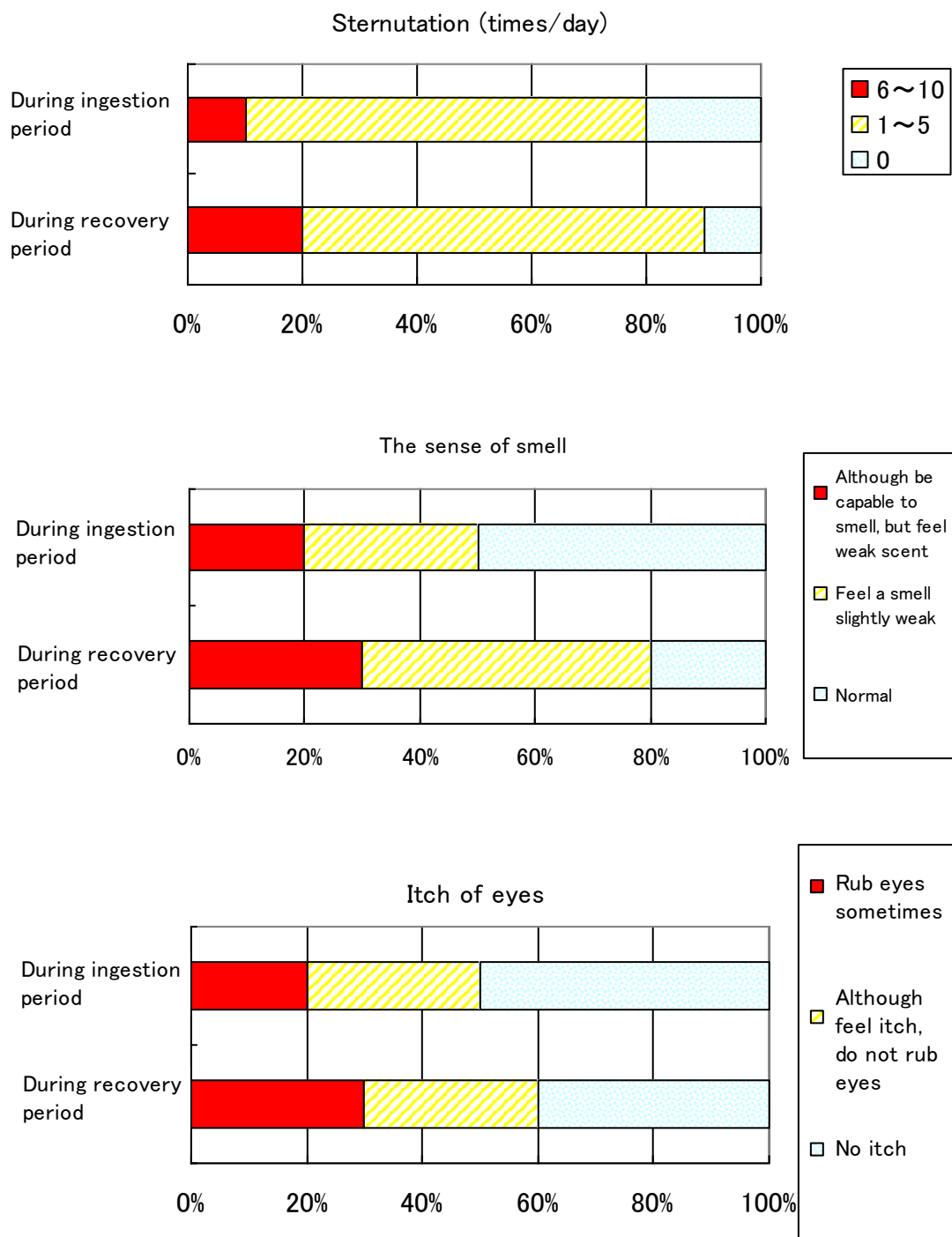


Fig. 13. Changes in symptoms during ingestion period and recovery period

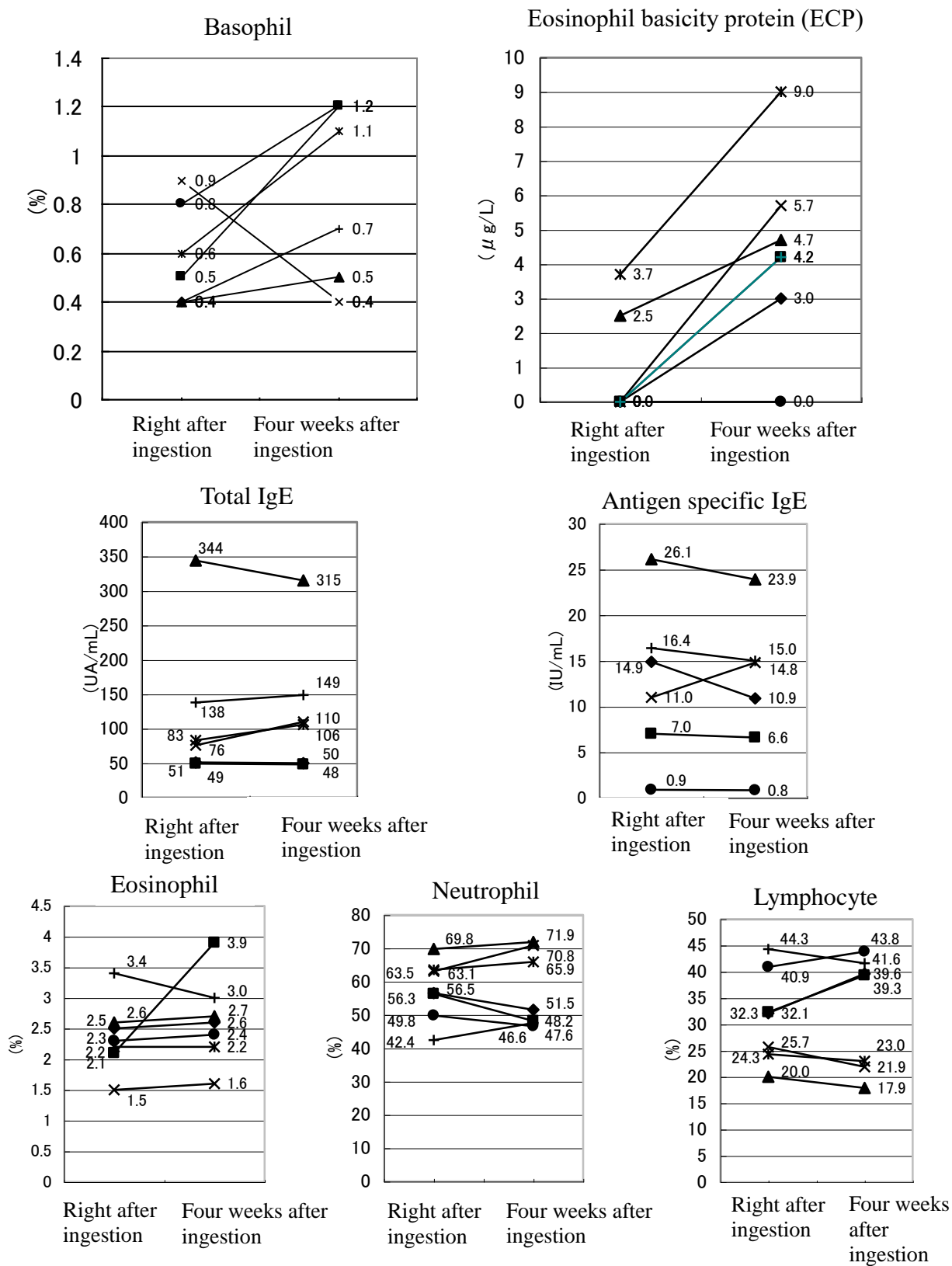


Fig. 14. Changes in blood parameter at right after ingestion and 4 weeks after ingestion

## 7. Stability of JAPANESE BUTTERBUR EXTRACT-P (in non-excipient form)

### (1) Thermal resistance

Evaluation of the heat stability of JAPANESE BUTTERBUR EXTRACT-P (in non-excipient form) showed no changes in the fukinone and total polyphenols contents even after heating at 120°C for 1 hour.

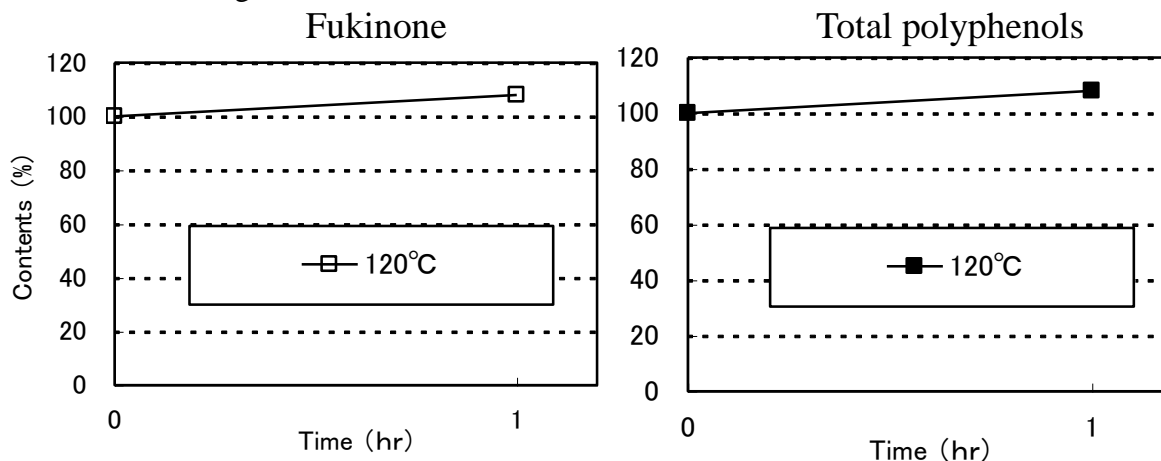


Fig.15. Thermal resistance of JAPANESE BUTTERBUR EXTRACT-P  
(In non-excipient form, 100% as initial value)

### (2) pH stability

After storing of H<sub>2</sub>O solution of JAPANESE BUTTERBUR EXTRACT-P (in non-excipient form) without light shielding at room temperature for 1 week, fukinone and total polyphenols contents were measured. Both contents in JAPANESE BUTTERBUR EXTRACT-P (in non-excipient form) was found to be stable from acid to alkali range of pH.

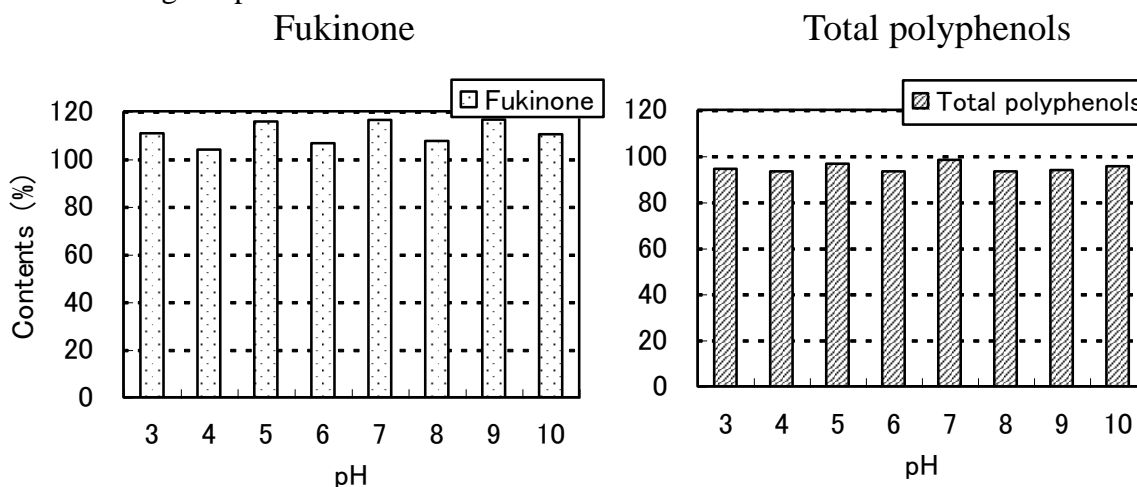


Fig.16. pH stability of JAPANESE BUTTERBUR EXTRACT-P  
(In non-excipient form, 100% as initial value)

### (3) Solubility

JAPANESE BUTTERBUR EXTRACT-WSP (in non-excipient form) was dissolved in water (pH 3.5) and stored at room temperature, 40°C and 4°C for 12 weeks. The presence or absence of precipitation, turbid and versicolor was observed. JAPANESE BUTTERBUR EXTRACT-WSP (in non-excipient form) was highly soluble in water in the acid range.

		Water solubility (0.5% solution)		
		Room temperature (light shielding)	40°C (without light shielding)	4°C (without light shielding)
Acid (pH3.5)	Precipitation and Turbid	Neither precipitation nor turbid were observed for 12 weeks.	Neither precipitation nor turbid were observed for 12 weeks.	Neither precipitation nor turbid were observed for 12 weeks.
	Versicolor	No versicolor was observed for 12 weeks.	No versicolor was observed for 12 weeks.	No versicolor was observed for 12 weeks.

## 8. Nutrition facts of JAPANESE BUTTERBUR EXTRACT-P (in non-excipient form)

	Results	Method
Moisture	0.8 g/100g	Heat drying method under reduced pressure
Protein <sup>*1</sup>	6.2 g/100g	Kjeldahl method
Fat	3.6 g/100g	Acid fat dissolution method
Ash	20.9 g/100g	Direct ashing method
Carbohydrate <sup>*2</sup>	68.5 g/100g	
Energy <sup>*3</sup>	331 kcal/100g	
Dietary fiber	0.3 g/100g	Prosky method
Sodium	1200 mg/100g	Atomic absorption spectrophotometry

\*1) N=6.25

\*2) 100 – (moisture + protein + fat + ash)

\*3) Factors for calculating the energy value : protein, 4; fat, 9; carbohydrate, 4; dietary fiber, 2

Test trustee : SRL, Inc.

Date of issue of the test result report : April 19, 2004

Research result issue number : No. 200404060017

## 9. Safety profile of JAPANESE BUTTERBUR EXTRACT-P (in non-excipient form, 100% as initial value)

### (1) Residual agricultural chemicals

The 33 agrochemicals, of which the standard levels of residues are established by the Food Sanitation Law (Ministry of Health, Labour and Welfare), were examined.

		Result	Detectable limit	Method
1	Acephate	Not detected	1 ppm	GC-MS
2	Iprodione	Not detected	0.1 ppm	GC-MS
3	Etofenprox	Not detected	0.1 ppm	GC-MS
4	Etrimfos	Not detected	0.1 ppm	GC-MS
5	Chlorpyrifos	Not detected	0.05 ppm	GC-MS
6	Diethofencarb	Not detected	0.1 ppm	GC-MS
7	Dichlorvos	Not detected	0.1 ppm	GC-MS
8	Cyhalothrin	Not detected	0.1 ppm	GC-MS
9	Cyfluthrin	Not detected	1 ppm	GC-MS
10	Cypermethrin	Not detected	0.1 ppm	GC-MS
11	Thiobencarb	Not detected	0.1 ppm	GC-MS
12	Thiometon	Not detected	0.1 ppm	GC-MS
13	Trichlormide	Not detected	0.1 ppm	GC-MS
14	Trifluralin	Not detected	0.05 ppm	GC-MS
15	Tolclofos-Methyl	Not detected	0.1 ppm	GC-MS
16	Parathion-Methyl	Not detected	0.1 ppm	GC-MS
17	Bioresmethrin	Not detected	0.1 ppm	GC-MS
18	Pyridaben	Not detected	0.1 ppm	GC-MS
19	Pirimiphos-Methyl	Not detected	0.1 ppm	GC-MS
20	Pyrethrins	Not detected	1 ppm	GC-MS
21	Fenarimol	Not detected	0.1 ppm	GC-MS
22	Fenitrothion	Not detected	0.1 ppm	GC-MS
23	Fenobucarb	Not detected	0.5 ppm	GC-MS
24	Fenvalerate	Not detected	0.1 ppm	GC-MS
25	Flucythrinate	Not detected	0.1 ppm	GC-MS
26	Flutolanil	Not detected	0.1 ppm	GC-MS
27	Procymidone	Not detected	0.1 ppm	GC-MS
28	Permethrin	Not detected	0.1 ppm	GC-MS
29	Pendimethalin	Not detected	0.05 ppm	GC-MS
30	Malathion	Not detected	0.1 ppm	GC-MS
31	Myclobutanil	Not detected	0.1 ppm	GC-MS
32	Methiocarb	Not detected	0.05 ppm	GC-MS
33	Lenacil	Not detected	0.1 ppm	GC-MS

Test trustee : Kyusai analysis institute Co., LTD.

Date of issue of the test result report : April 12, 2004

Research result issue number : No. 20040405-1

## (2) Acute toxicity (LD<sub>50</sub>)

JAPANESE BUTTERBUR EXTRACT (in non-excipient form, 2,000 mg/kg) was orally administered to male and female ICR mice aged 5 weeks under fasting, and the mice were maintained and observed for 14 days. Neither deaths nor abnormal in body weight compared with the controls were observed. Autopsy performed after the discontinuation of the experiment showed no macroscopic abnormalities in organs. Therefore, in male and female mice, the LD<sub>50</sub> of JAPANESE BUTTERBUR EXTRACT (in non-excipient form) by oral administration is  $\geq 2,000$  mg/kg.

## 10. Recommended daily dosage

It is recommended to take 250 mg/day of JAPANESE BUTTERBUR EXTRACT-P.

## 11. Applications

	Application	Health claim	Examples
Foods	Anti-allergic foods	1) Prevention of pollenosis 2) Anti-atopic dermatitis, <i>etc.</i>	Drinks (beverage, juice, <i>etc.</i> ), hard and soft gel capsule, tablet, candy, chewing, gum, cookies, chocolate, wafers, jelly, <i>etc.</i>
Cosmetics	Anti-allergic cosmetics		Lotion, cream, facial pack, body gel, <i>etc.</i>

JAPANESE BUTTERBUR EXTRACT-WSP, which is highly water-soluble, can be widely applied such as drinks among foods and lotions among cosmetics.

## 12. Packaging

JAPANESE BUTTERBUR EXTRACT-P, WSP (Powder, food grade)

JAPANESE BUTTERBUR POWDER (Powder, food grade)

JAPANESE BUTTERBUR EXTRACT-PC, WSPC (Powder, cosmetic grade)

5kg Interior packaging : aluminum-coated plastic bag

Exterior packaging : cardboard box

JAPANESE BUTTERBUR EXTRACT –LC (Liquid, cosmetic grade)

5kg Interior packaging : cubic polyethylene container

Exterior packaging : cardboard box

## 13. Storage

Store in cool, dry and dark place. Avoid places with high humidity and direct heat.

## Expression

### <Food>

JAPANESE BUTTERBUR EXTRACT-P, JAPANESE BUTTERBUR EXTRACT-WSP

Expression : Japanese butterbur extract

JAPANESE BUTTERBUR POWDER

Expression : Japanese butterbur

### <Cosmetics>

JAPANESE BUTTERBUR EXTRACT-PC

INCI name : Dextrin, Petasites Japonicus Leaf/Stem Extract

JAPANESE BUTTERBUR EXTRACT-WSPC

INCI name : Dextrin, Petasites Japonicus Leaf/Stem Extract

JAPANESE BUTTERBUR POWDER

INCI name : Petasites Japonicus Powder

JAPANESE BUTTERBUR EXTRACT-LC

INCI name : Butylene glycol, Water, Petasites Japonicus Leaf/Stem Extract



PRODUCT STANDARD  
PRODUCT NAME

**JAPANESE BUTTERBUR EXTRACT-P**

FOOD

This product is extracted from Japanese butterbur, the stems and the leaves of *Petasites japonicus* (Compositae), with aqueous ethanol.

Appearance Slight yellowish powder with slight unique aroma.

Certification Test

Fukinolic acid A peak is detected. (HPLC)

Fukinone A peak is detected. (HPLC)

Total polyphenols Min. 1.0 % (Folin-Denis method)

Total terpenoids [In non-excipient form (JAPANESE BUTTERBUR EXTRACT)]

Min. 300 µl/100 g (Japanese Pharmacopoeia,  
Essential oil content)

(Theoretical value in JAPANESE BUTTERBUR EXTRACT-P : Min. 60 µl/100g)

Loss on Drying Max. 10.0 % (Analysis for Hygienic Chemists, 1g, 105°C, 2h)

Purity Test

(1) Heavy Metals (as Pb<sub>2</sub>) Max. 10 ppm (Sodium sulfide colorimetric Method)

(2) Arsenic (as As<sub>2</sub>O<sub>3</sub>) Max. 1 ppm (Standard Methods Analysis in Food Safety  
Regulation, The Third Method, Apparatus B)

Standard Plate Counts Max. 3 × 10<sup>3</sup> cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max. 1 × 10<sup>3</sup> cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

Composition

<u>Ingredients</u>	<u>Contents</u>
Dextrin	80 %
Japanese Butterbur Extract	20 %
Total	100 %

PRODUCT STANDARD  
PRODUCT NAME

**JAPANESE BUTTERBUR EXTRACT-WSP**

FOOD

This product is extracted with aqueous ethanol from Japanese butterbur, the stems and the leaves of *Petasites japonicus* (Compositae). This product is water-soluble.

Appearance Slight yellowish powder with slightly unique smell.

Certification Test

Fukinolic acid A peak is detected. (HPLC)

Total polyphenols Min. 1.0 % (Folin-Denis method)

Loss on Drying Max. 10.0 % (Analysis for Hygienic Chemists, 1g, 105°C, 2h)

Purity Test

(1) Heavy Metals (as Pb<sub>2</sub>) Max. 10 ppm (Sodium Sulfide Colorimetric Method)  
(2) Arsenic (as As<sub>2</sub>O<sub>2</sub>) Max. 1 ppm (Standard Methods Analysis in Food Safety Regulation, The Third Method, Apparatus B)

Standard Plate Counts Max.  $3 \times 10^3$  cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max.  $1 \times 10^3$  cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

Composition

<u>Ingredients</u>	<u>Contents</u>
Dextrin	80 %
Japanese Butterbur Extract	20 %
Total	100 %

PRODUCT STANDARD  
PRODUCT NAME

**JAPANESE BUTTERBUR POWDER**

FOOD

This product is dried and grounded Japanese butterbur, the stems and the leaves of *Petasites japonicus* (Compositae).

Appearance

Green powder with slightly unique smell.

Certification Test

Fukinolic acid

A peak is detected. (HPLC)

Fukinone

A peak is detected. (HPLC)

Loss on Drying

Max. 10.0 % (Analysis for Hygienic Chemists, 1g, 105°C, 2h)

Purity Test

(1) Heavy Metals (as Pb<sub>2</sub>)

Max. 30 ppm (Sodium Sulfide Colorimetric Method)

(2) Arsenic (as As<sub>2</sub>O<sub>3</sub>)

Max. 1 ppm (Standard Methods Analysis in Food Safety Regulation, The Third Method, Apparatus B)

Standard Plate Counts

Max.  $3 \times 10^3$  cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts

Max.  $1 \times 10^3$  cfu/g (Analysis for Hygienic Chemists)

Coliforms

Negative (Analysis for Hygienic Chemists)

Composition

Ingredients

Japanese Butterbur Powder

Contents

100 %

PRODUCT STANDARD  
PRODUCT NAME

**JAPANESE BUTTERBUR EXTRACT-PC**

COSMETIC

This product is extracted with aqueous ethanol from Japanese butterbur, the stems and the leaves of *Petasites japonicus* (Compositae).

Appearance Slight yellowish powder with slightly unique smell.

Certification Test

Fukinolic acid A peak is detected. (HPLC)

Fukinone A peak is detected. (HPLC)

Total polyphenols Min. 1.0 % (Folin-Denis method)

Total terpenoids [In non-excipient form (JAPANESE BUTTERBUR EXTRACT)]

Min. 300 µl/100 g (Japanese Pharmacopoeia,  
Essential oil content)

(Theoretical value in JAPANESE BUTTERBUR EXTRACT-P : Min. 60 µl/100g)

Loss on Drying Max. 10.0 % (1g,105°C,2h)

Purity Test

(1)Heavy Metals (as Pb<sub>2</sub>) Max. 10 ppm (The Second Method of The Japanese Standards of Quasi-Drug Ingredients)

(2)Arsenic (as As<sub>2</sub>O<sub>3</sub>) Max. 1 ppm (The Third Method of The Japanese Standards of Quasi-Drug Ingredients)

Standard Plate Counts Max. 1 × 10<sup>2</sup> cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max. 1 × 10<sup>2</sup> cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Dextrin	80 %
<u>Petasites Japonicus Leaf/Stem Extract</u>	20 %
Total	100 %

PRODUCT STANDARD  
PRODUCT NAME

**JAPANESE BUTTERBUR EXTRACT-WSPC**

COSMETIC

This product is extracted with aqueous ethanol from Japanese butterbur, the stems and the leaves of *Petasites japonicus* (Compositae). This product is water soluble.

Appearance Slight yellowish powder with slightly unique smell.

Certification Test

Fukinolic acid A peak is detected. (HPLC)

Total polyphenols Min. 1.0 % (Folin-Denis method)

Loss on Drying Max. 10.0 % (1g, 105°C, 2h)

Purity Test

(1) Heavy Metals (as Pb<sub>2</sub>) Max. 10 ppm (The Second Method of The Japanese Standards of Quasi-Drug Ingredient)

(2) Arsenic (as As<sub>2</sub>O<sub>3</sub>) Max. 1 ppm (The Third Method of The Japanese Standards of Quasi-Drug Ingredient)

Standard Plate Counts Max. 1 × 10<sup>2</sup> cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max. 1 × 10<sup>2</sup> cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

Composition

<u>Ingredients</u>	<u>Contents</u>
Dextrin	80 %
Petasites Japonicus Leaf/Stem Extract	20 %
Total	100 %

PRODUCT STANDARD  
PRODUCT NAME

**JAPANESE BUTTERBUR EXTRACT-LC**

COSMETIC

This product is extracted with aqueous 1,3-butyleneglycol from Japanese butterbur, the stems and the leaves of *Petasites japonicus* (Compositae).

Appearance Brown color liquid with scentless or slightly unique smell.

Certification Test

Polyphenol Mix this product (30  $\mu$ l) with water (3.5 ml), and Folin-Denis reagent (0.2 ml) and saturated  $\text{Na}_2\text{CO}_3$  solution (0.4 ml) are added. The solution reveals blue color.

Purity Test

(1)Heavy Metals (as  $\text{Pb}_2$ ) Max. 10 ppm (The Second Method of The Japanese Standards of Quasi-Drug Ingredient)

(2)Arsenic (as  $\text{As}_2\text{O}_3$ ) Max. 1 ppm (The Third Method of The Japanese Standards of Quasi-Drug Ingredient)

Standard Plate Counts Max.  $1 \times 10^2$  cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max.  $1 \times 10^2$  cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Butylene Glycol	69 %
Water	30 %
<u>Petasites Japonicus Leaf/Stem Extract</u>	<u>1 %</u>
Total	100 %

**ORYZA OIL & FAT CHEMICAL CO.,LTD.**, striving for the development of the new functional food materials to promote your health.

- **From product planning to OEM** – For any additional information or assistance, please contact :

**ORYZA OIL & FAT CHEMICAL CO.,LTD.**

**No.1, Numata kitagata-cho, Ichinomiya-city, Aichi-pref.,**

**493-8001 JAPAN**

**TEL : +81(0)586 86 5141**

**FAX : +81(0)586 86 6191**

**URL/http : //www.oryza.co.jp/**

**E-mail : info@oryza.co.jp**

**Tokyo sales office:**

5F Diamant-building 1-5 Kanda-suda-cho,  
Chiyoda-ku, Tokyo, 101-0041 Japan

TEL: +81 (0) 3 5209 9150

FAX: +81 (0) 3 5209 9151

E-mail: tokyo@oryza.co.jp

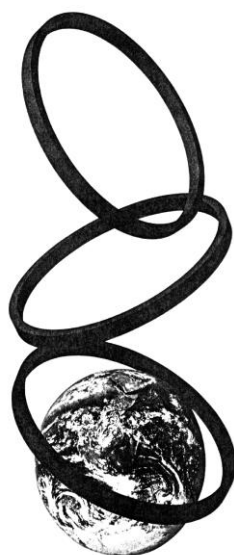


\*The unapproved copy of this catalogue and appropriation are forbidden except for the exception on the Copyright Act.

\*The contents of catalogue may be changed without prior notice.

Established Date : June 11, 2004

Revised Date: May 30, 2019



ORYZA OIL & FAT CHEMICAL CO., LTD.