Natural Cosmetic Ingredient

SAKURA Extract B

(Prunus Yedoensis Leaf Extract)

Introducing Traditional Japanese beauty to Global Cosmetic Market

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SAKURA and the Japanese

The sakura (Japanese flowering cherry) is selected along with the chrysanthemum to be Japanese national flowers. The sakura is so familiar to the Japanese and has been for a long time that "the flower means the sakura," and is known as the flower which symbolizes Japan in the world. The history of the sakura is long, and it is said that the beauty of Yamasakura (*Prunus jamasakura* Sieb. ex Koidz) has been enjoyed since before the Heian period. It is recorded in the Nihonshoki that the Emperor Jito went cherry blossom viewing to Mt. Yoshino, a famous place for sakura.

Furthermore, a record of the sakura can be seen in the Kojiki, the most historical book in Japan. It has been thought that the custom of cherry blossom viewing originated from the enjoyment of a wild sakura transferred to the capital and from the holding of a sakura party at the Imperial Palace in the Heian era. Thereafter the custom spread from a patrician to samurai and plebeian and from a capital to the provinces in the Edo era. Various kinds of sakura were produced by improvement in the late Edo era, and Someiyoshino, (now a synonym of the sakura) was also born at this time. The original name of Someiyoshino was Yoshino sakura. To distinguish from the Yoshino sakura from the yamasakura at Mt. Yoshino, however, the Yoshino sakura has been named Someiyoshino, putting the name of Somei mura, (the place where the cultivation of the Yoshino sakura was started) at the beginning.

For Japanese, spring is a season when something important in life occurs. Spring is the season of various turning points in life such as graduation, entrance into a school and getting a job, and it is the sakura that blooms at these scenes. The blooming sakura landscape is written indelibly on our mind, overlapping the memory of the starting of the year or of a new departure. More than three hundred years ago, Basho Matsuo expressed this scene splendidly.

It is the sakura that reminds us of various events (shobun of Oi).

Although the Japanese life-style in the present day is diversified, the custom of the enjoying beautifully blooming cherry blossoms with the excitement about the advent of spring and with forgetting daily stress has been kept.

Sakura extract B was developed with such an image of the raw material that can relive such stress on the skin as inflammation and ultraviolet rays and that can promote admiration of beautiful Japanese skin.

SAKURA

Origin [1) to 14)]

The sakura is a general term of deciduous high trees belonging to *Prunus* subgenus, *Prunus* genus, Rose family (*Rosaceae*) and grows mainly in the Temperate Zones and the subtropical zone of the northern hemisphere. It is said that there are several hundred kinds of wild species and cultivated species with about 10 natural species such as *P. jamasakura* Sieb. ex Koidz, *P. lannesiana* (Carr.) Wilson var. *speciosa* (Koidz) Makino and *P. pendula* Maxim. f. *ascendens* (Makini) Ohwi as the basic species.

Prunus yedoensis Matsum. planted for admiration all over Japan is said to be a natural crossbred of *P. pedula* Maxim. f. *ascendens* (Makini) Ohwi and *P. lannesiana* (Carr.) Wilson var. *Speciosa* (Koidz) Makino. It is reported that an isoflavone, prunetin and its glycoside, Prunetolin, a flavonoid, isoquercitrin and melirhotoside, a coumarin glycoside are contained in the sakura's leaves and sakuranetin and genkanine, flavonoids, and their glycosides, sakuranin and glucogekanine in the bark. The bark is called "Ohhi (bark of the sakura)," and has been used as: detoxication, cough remedy, an expectorant, and to reduce swelling and urticaria as a Japanese folk medicine. The extract prepared from Ohhi has been marketed as an antitussive and expectorant by the trade name of Brocin, and the pharmacological effect has been considered to be caused by the action of a flavonoid glycoside. Also, the salted flowers have been used as an infusion of salted cherry blossoms at a happy event, and the leaves for wrapping a bean paste rice-cake. The heartwood of *Prunus jamasakura* Sieb. ex Koidz, Ooyamasakura have been used for building materials, tools printing blocks, and the bark for handiwork materials.

Introduction

SAKURA Extract B is obtained from the leaf of *Prunus yedoensis* by 1,3-Butylene Glycol.

Efficacy

● Improvement of skin roughness

SAKURA Extract B is observed to inhibit rough skin by surfactant. According to this result, SAKURA Extract B is expected to inhibit rough skin and inflammation caused by rough skin.

● Anti-inflammation effect

SAKURA Extract B is observed to have an inhibition of histamine release, contact dermatitis, carrageenin plantar edema and arachidonic acid induced-edema.

According to this result, SAKURA Extract was shown to have an anti-inflammation effect.

● Whitening effect

SAKURA Extract B is observed to have an inhibition effect for the production of melanin in mouse B16 melanoma cells.

According to this result, SAKURA Extract B was shown to have a whitening effect.

Recommend

- *Cosmetic for people with sensitive skin*
- *Cosmetic for improvement of rough skin*
- *Cleansing*
- *Whitening Cosmetic*
- *Cosmetic having a Japanese Image*
- *Quasi Drug*

Improvement of skin roughness

We investigate the improvement of rough skin (caused by stimulation of surfactant) by visual observation, measurement of erythema value (amount of erythema), measurement of Trans Epidermal Water loss (TEWL) and observation of skin surface by VISIOSCAN.

Test Sample

SAKURA Extract B is used as test sample. 50% 1,3-Butylene Glycol is used as control.

Test Method

Five healthy people (2 males and 3 females), with their written consent, were enrolled as the subjects in this study. A rough skin model was prepared by applying occlusively 5% sodium lauryl sulfate (SDS) on the flexion side of the forearm of each subject for 5 minutes a day for 5 days. Fifty microliters of each test sample was applied on the SDS treated site on each of the right and left arm once a day for 5 days.

The TEWL values at the treated sites were measured before SDS treatment by TEWAMETER TM210 (COURAGE + KHAZAKA Electronic GmbH) from the starting day of the test to day 5. Before SDS treatment on day 5, photos were taken by a digital camera and the erythema values (amount of erythema on the skin) were measured by MEXAMETER MX18 (Courage + Khazaka electronic GmbH). Furthermore, at 24 hours after SDS treatment on day 5, photographing and observation of the skin surface were performed by a visioscan (VISIOSCAN VC 98/COURAGE + KHAZAKA electronic GmbH).

Result and Discussion

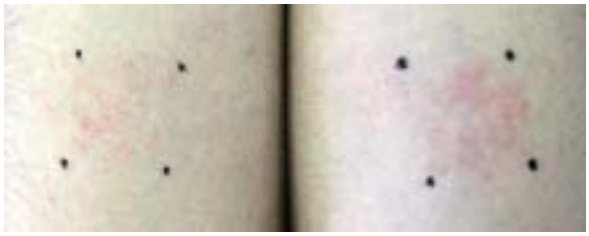
The pictures by the digital camera and the erythema values on day 5 of the rough skin model are shown in Fig. 1, the pictures of the skin surface structure by the visioscan on day 6 in Fig. 2 and the change of the TEWL values in Fig. 3.

The results of the pictures by the digital camera and of the erythema values showed that SDS treatment produced erythema on the skin and that the amount of erythema was decreased by applying Sakura extract B on the SDS treated site.

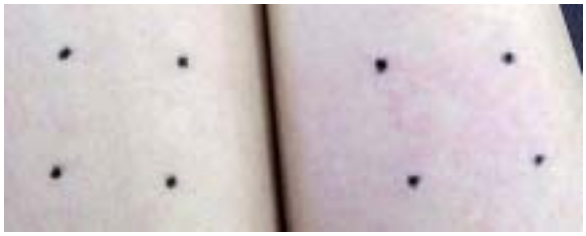
The observation of the skin surface by the visioscan revealed that plenty of scales (a condition where a horny layer is seen coming-up due to dryness of the skin and abnormal exfoliation of the horny layer) were produced, indicating that the skin was rough. At the site treated with Sakura extract B, however, the number of scales was markedly smaller compared with that of the control, indicating that dryness of the skin and exfoliation of the horny layer were improved. The TEWL values were increased in a time-dependent manner, because SDS treatment destroyed the barrier function of the skin. At the site applied with Sakura extract B, however, the increase in the TEWL values tended to be repressed.

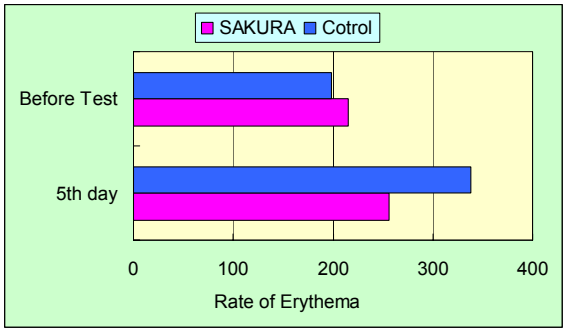
These results indicated that Sakura extract B inhibited inflammation to improve the skin condition in the rough skin produced by SDS treatment. In other words, it is expected that daily usage of a cosmetic containing Sakura extract B can prevent and improve rough skin produced by drying and washing in daily life.

25 years old man



29 years old woman





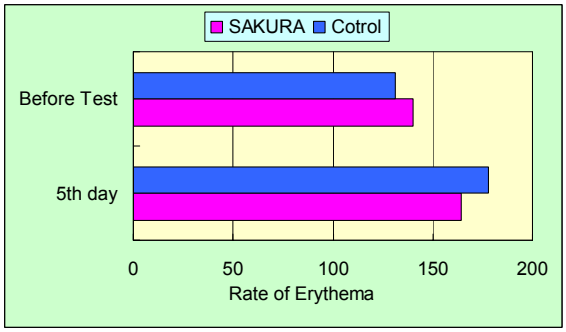
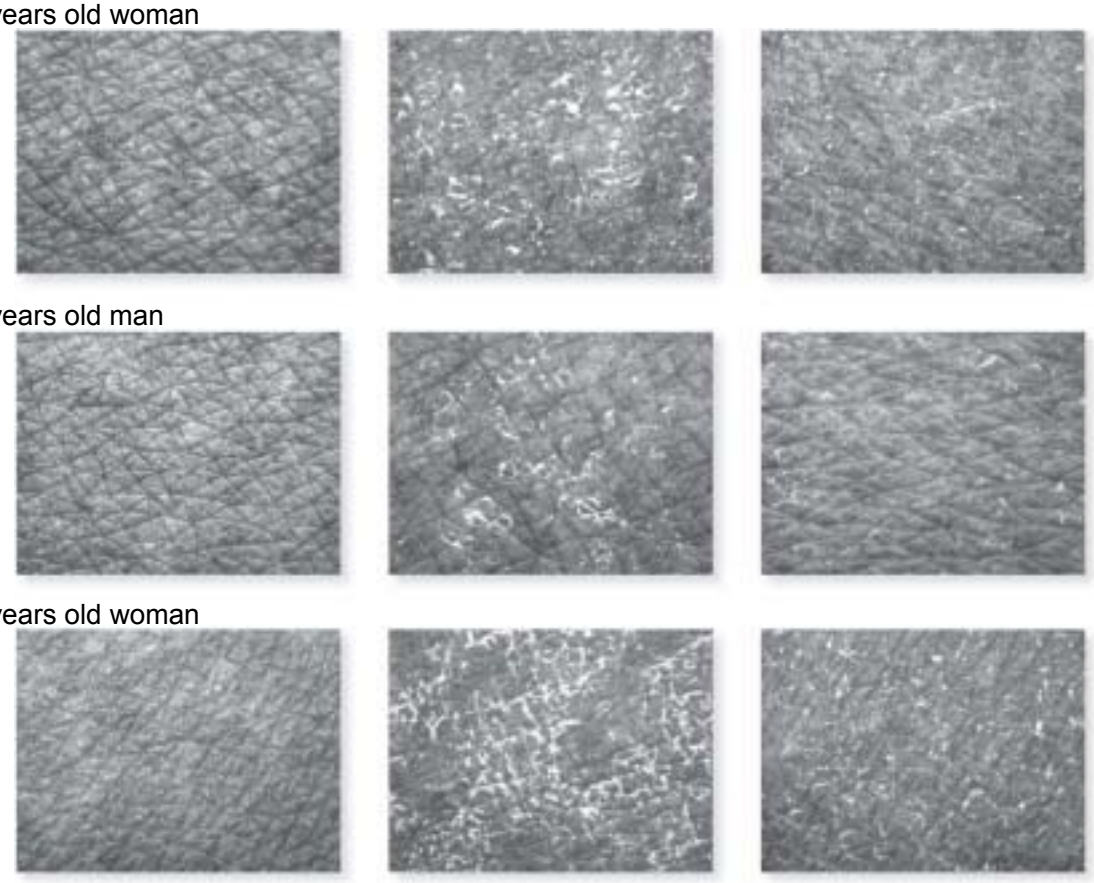


Fig.1 Improvement effect SAKURA Extract B against of erythema caused by SDS treatment

26 years old woman

29 years old man

33 years old woman



Non-Application by SDS　　SDS + Control　　SDS + SAKURA Extract B

Fig.2 Improvement effect of SAKURA Extract B against skin surface damage caused by SDS treatment

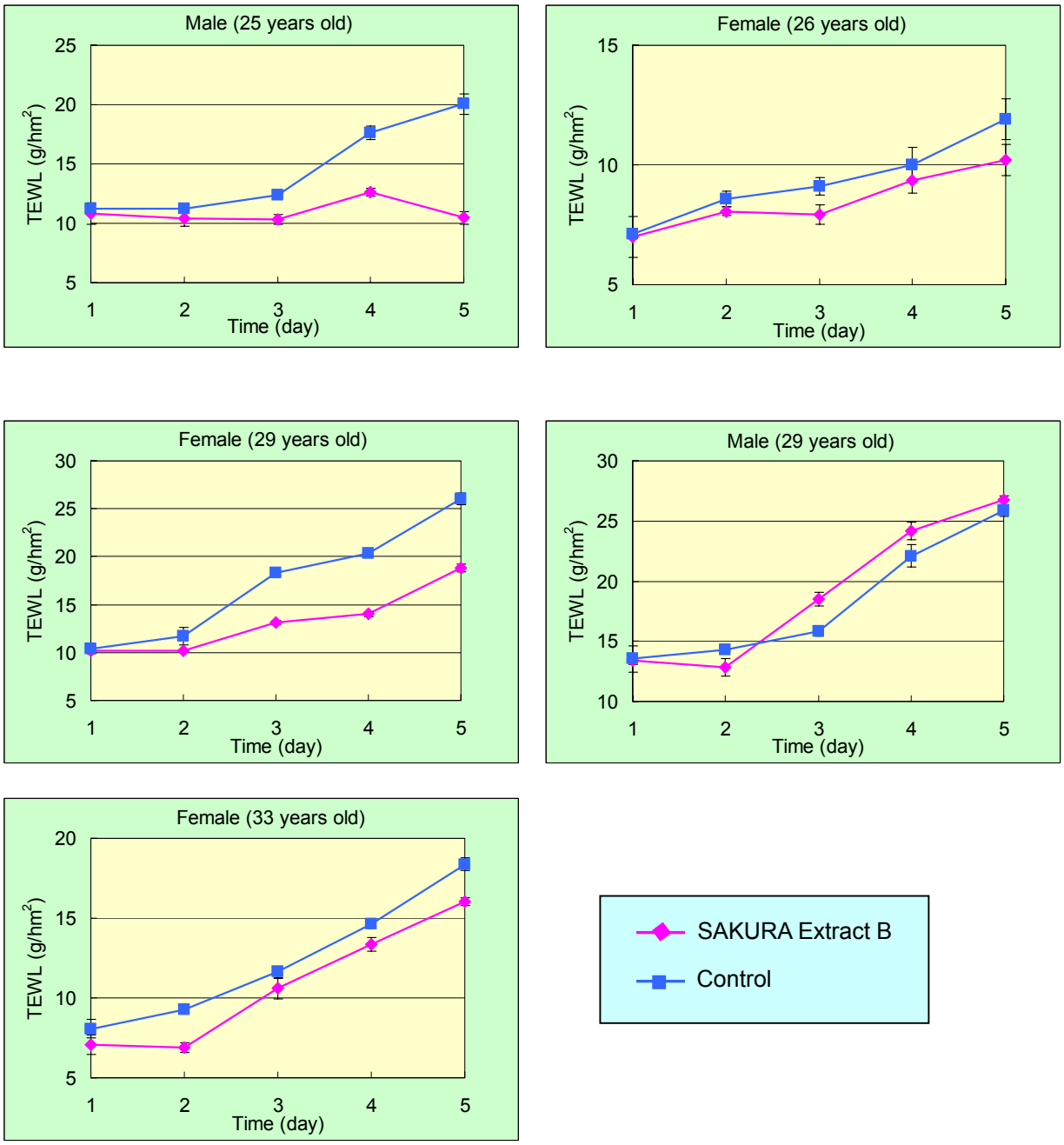


Fig.3: Improvement of SAKURA Extract B
against the disturbance of barrier function caused by SDS treatment

Inhibition Effect of Histamine Release

Histamine is released from mast cells and can cause allergic diseases such as eczema, asthma and itching.

We investigated the inhibition effect of histamine release of SAKURA Extract B.

Test Sample

SAKURA Extract B is adjusted to 135 μ g/mL and 67.5 μ g/mL solid matter by purified water. (Product concentration is 0.675% and 0.338%.)

Test Method [15 16)]

Mast cells thus obtained were suspended in 2% FT solution to make about 1.0×10^5 cells/mL. After adding test substance into the cell suspension and keeping at stand for 10 min at 37°C, add histamine-releasing agent compound 48/80 (Sigma) (final concentration: 1 μg/mL) and keep at stand for 15 min at 37°C. The reaction was stopped by cooling on ice, and the reaction mixture was centrifuged at 100 ×g for 10 min at 4°C to determine histamine in the supernatant. Briefly, purified water, 1 mol/mL of NaOH solution and 1% o-phthaldialdehyde-methanol solution were added to the supernatant. After keeping at stand for 5 min, the reaction was stopped by adding 3mol/L of HCl solution. At 10 min after terminating the reaction, the reaction mixture was centrifuged at 1,900 rpm for 25 min at 5°C to obtain the supernatant and sediment. Histamine in the supernatant was determined on the calibration curve of histamine using the fluorescence values at 360 nm of excitation wavelength and 450 nm of emission wavelength. Furthermore, histamine remained in the mast cells was determined by the same way as that described above in the ultrasonically treated sediment in 2% FT solution after 1-day storage at freezing. Then histamine-release ratio and the inhibition rate of histamine-release were obtained.

$$\text{Histamine Release Ratio} = \frac{\text{Histamine amount released from cell}}{\text{Total histamine amount in cell}}$$

$$\text{Inhibition rate of Histamine Release (\%)} = [1-(A-C / B-C)] \text{ X } 100$$

A : Histamine release ratio; which histamine release agent is added in what mast cell is added in test sample.

B : Histamine release ratio; which histamine release agent is added in mast cell.

C : Histamine release ratio; which is naturally released from the mast cell.

Result

Inhibition rate of histamine release is shown in Fig.4. 0.675% and 0.338% of SAKURA Extract B is observed inhibition effect as 70.0% and 33.0%.

According to this result, SAKURA Extract B is expected to have an anti-inflammation effect and an anti-allergic effect.

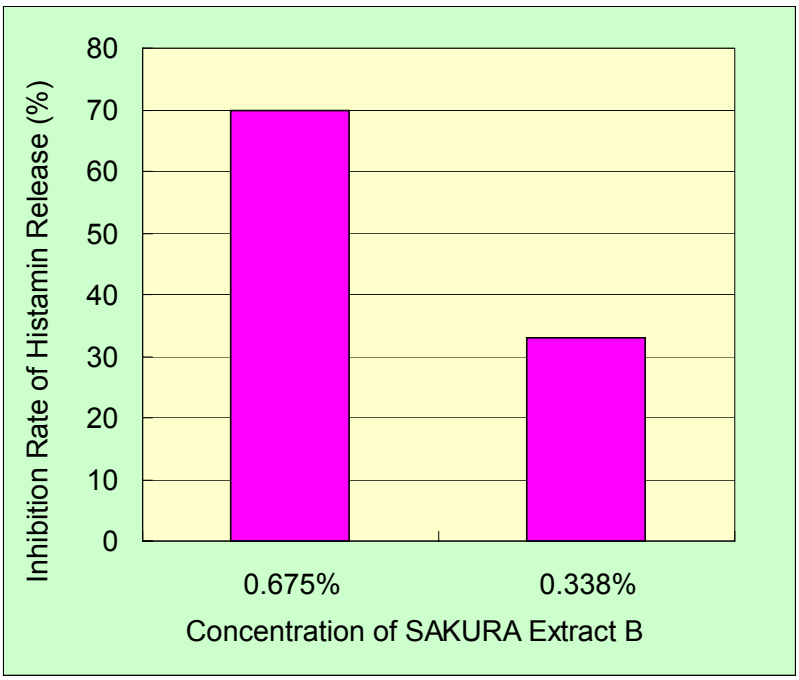


Fig.4, Inhibition rate of histamine release by SAKURA Extract B

Inhibition Effect of Contact Dermatitis

The problem is, cosmetics can, when in direct contact with skin, caused contact dermatitis. We investigated the inhibition effect contact dermatitis of SAKURA Extract B caused by *p*-1,4-phenylendiamine.

Test Sample

Hydrophilic Vaseline containing 10% of SAKURA Extract B was adjusted to use for the test sample. Control was adjusted using same amount of purified water instead of SAKURA Extract B.

Test Method [17)]

Abdominal hair of female mice(BALB/c, 8 weeks) were shaved. In order to sensitize, 2.5% *p*-1,4-phenylendiamine /acetone : olive oil = 4 : 1 (PPD) applied every day for three days on the abdominal area of the mice. 5 days later, test sample applied three times on one side of ear. After 1 hour it is applied for the last time, test sample was wiped enough off and PPD was applied, and 17 hours later test sample was applied two times again. 24 hours later both ears were cut off by punch of a certain amount of area, and they were measured. Regarding the judgment, measure the different weight between the ears applied with test sample and the other ear non-applied, and calculated the inhibition rate by means of comparing with control. 8 mice were used for each group.

$$\text{Swelling Rate (\%)} = \frac{\text{Weight of ears applied with test sample} - \text{Weight of ears non-applied}}{\text{Weight of ears non-applied}} \times 100$$

Result

Swelling rate of SAKURA Extract B and control were shown Fig. 5. SAKURA Extract B inhibited swelling by sensitization of PPD solution.

According to this result, SAKURA Extract B is expected to inhibit contact dermatitis.

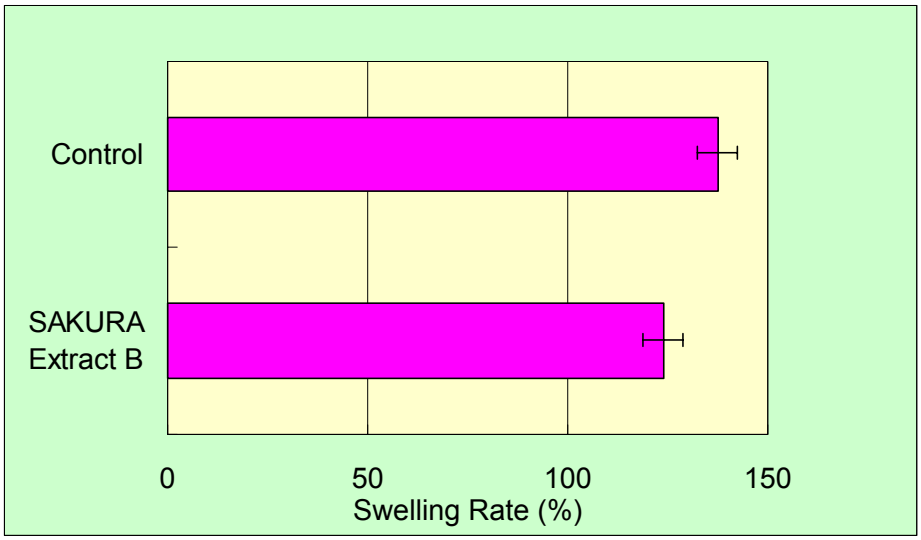


Fig. 5, Inhibition Effect of Contact Dermatitis by SAKURA Extract B

Inhibition Effect of Arachidonic acid-induced Edema

Arachidonic acid is released from a cell membrane as an allergic reaction or as a response to exogenic stimulation, and released Arachidonic acid is metabolized into prostaglandin. Inflammation is induced during this cascade. A substance which inhibits this inflammation pathway is expected to show an anti-allergic effect.

We investigated the anti-inflammation effect of SAKURA Extract B caused by the application of arachidonic acid.

Test Sample

Hydrophilic Vaseline containing 10% of SAKURA Extract B was adjusted to use for the test sample. Control was adjusted using the same amount of purified water instead of SAKURA Extract B.

Test Method [18)]

Test sample was applied three times on the right ear of ICR female mice which were 6 weeks old each one hour later. After that, the test sample was wiped off and 5% Arachidonic acid dissolved by acetone was applied. The dose was 20 μ l, 1 hour later, the ear was cut off by paunch. (ears applied with test sample and ears non-applied) Regarding the judgment, measure the different ear edema expansion rate between the ear applied with the test sample and the other non-applied ear, and calculate the inhibition rate by means of comparing with control subject. 8 mice per group were used in this test.

$$\text{Swelling Rate (\%)} = \frac{\text{Weight of ears applied with test sample} - \text{Weight of ears non-applied}}{\text{Weight of ears non-applied}} \times 100$$

Result and Discussion

Swelling rate of SAKURA Extract B and control were shown Fig. 6. SAKURA Extract B inhibited swelling by application of arachidonic acid.

According to this result, SAKURA Extract B is expected to inhibit contact dermatitis.

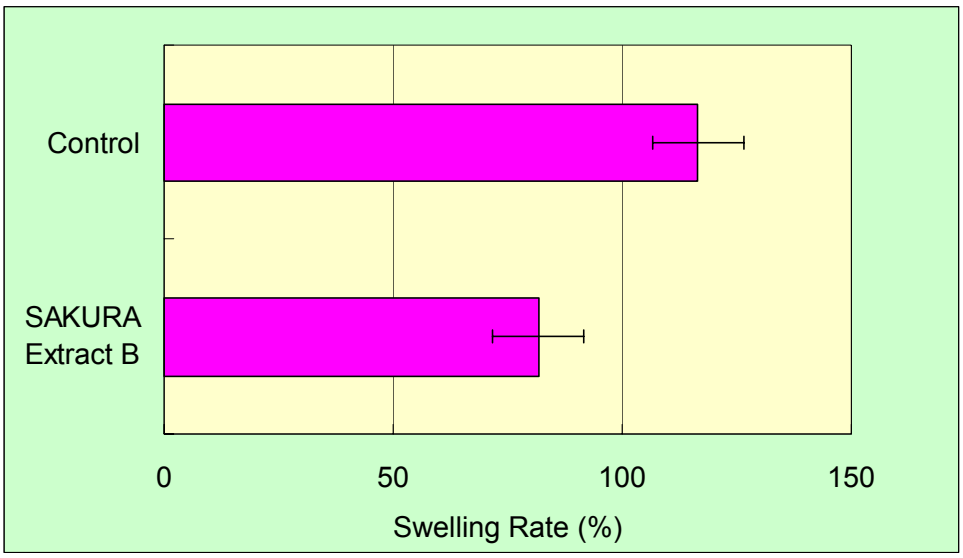


Fig. 6, Inhibition Effect of Arachidonic acid-induced Edema by SAKURA Extract B

Inhibition Effect of Carrageenin Plantar Edema

Carrageenin plantar edema inhibition test is a known screening test of anti-inflammation and is said to have relativity to human efficacy. A substance which inhibits this inflammation pathway is expected to show an anti-allergic effect

We investigated anti-inflammation effect of SAKURA Extract B caused by carrageenin plantar edema.

Test Sample

Hydrophilic Vaseline containing 10% of SAKURA Extract B was adjusted to use for the test sample. Control was adjusted using same amount of purified water instead of SAKURA Extract B.

Test Method [19)]

Determine the thickness of the plantar of ICR mice (6 week females) in advance. Apply the test sample to the right plantar twice before 1-2 hours from the test on the test day.

After 1 hour interval, apply physiological saline contained 2% of λ-carrageenin to right plantar. Determine the thickness of the plantar every 1 hour for 5 hours after application, determine the difference of thickness of the plantar between before and after application, and calculate the inhibition ratio comparing control group. 8 mice were used per group.

Result and Discussion

Swelling rate of SAKURA Extract B and control were shown Fig. 7. SAKURA Extract B inhibited swelling by application of carrageenin.

According to this result, SAKURA Extract B is expected to have an anti-inflammation effect and an anti-allergic effect.

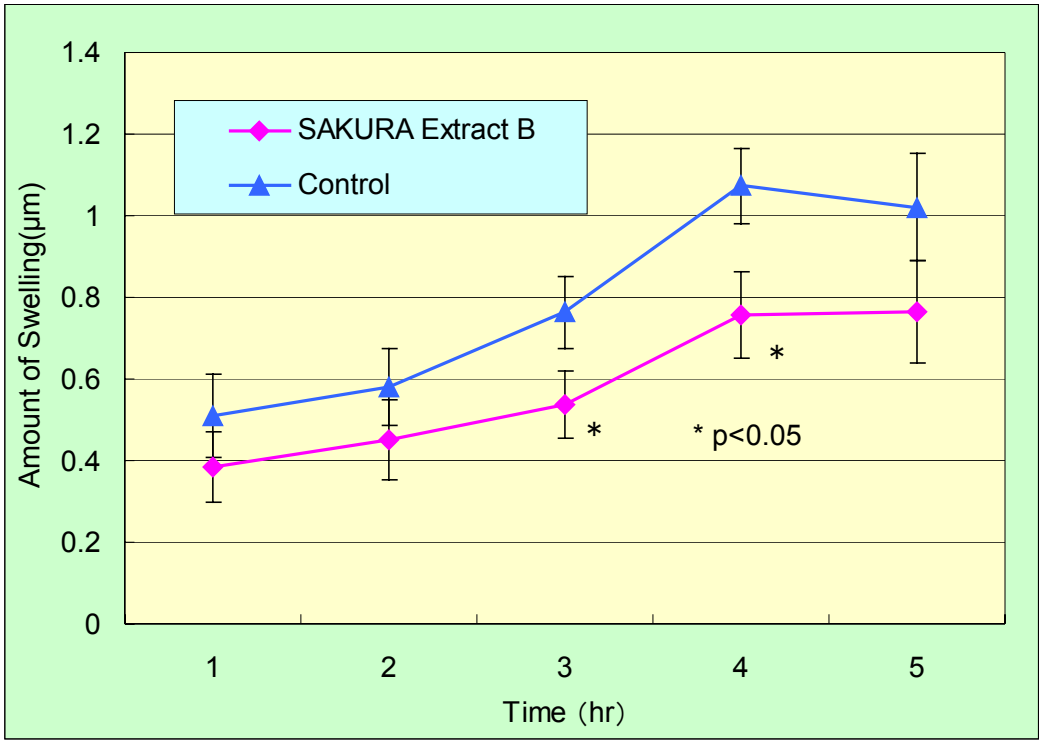


Fig.7, Inhibition Effect of Carrageenin Plantar Edema by SAKURA Extract B

B16 Melanoma cell Inhibition Effect

We investigated melanin production inhibition test on B16 melanoma cells on SAKURA Extract B.

Test Sample

Adjust SAKURA Extract B to 100 μg/mL and 50 μg/mL as final solid concentration (0.5% and 0.25% as product concentration) by 50% 1,3-Butylene Glycol. 2% Arbutin and 50% 1,3-Butylene Glycol was adjusted to 50 μg/mL as final concentration and used as positive control. Also, 50% 1,3-Butylene Glycol was used as control.

Test Method

a. Cells and the culture condition

B16 mouse melanoma cells were used and were cultured in an MEM culture medium including 5% Fetal Bovine Serum (made by GIBCO BRL). The culture conditions were 37 ° C, 5% CO_2.

b. Measurement [20)]

2 x 10^5 B16 melanoma cells were cultured in a 60 mm plastic culture dish and were pre-cultured 24 hours. Then they were transferred to a fresh culture medium and test materials were added to be 50 μL to culture medium. The cells were collected by processing with trypsin after culturing for three days. they were dissolved in 1N NaOH and 10% DMSO and then absorbance was measured at 420nm. At the same time the activity of the cells which were supplemented with various materials was measured by the MTT reduction method.

Results

Amount of melanin in medium added SAKURA Extract B in Fig. 8. SAKURA Extract B has inhibition effect of melanin production the same as Arbutin. Also cell toxicity on SAKURA Extract B was not observed in MTT Test at same time.

According to results, SAKURA Extract B is expected to have an inhibition effect of melanin production.

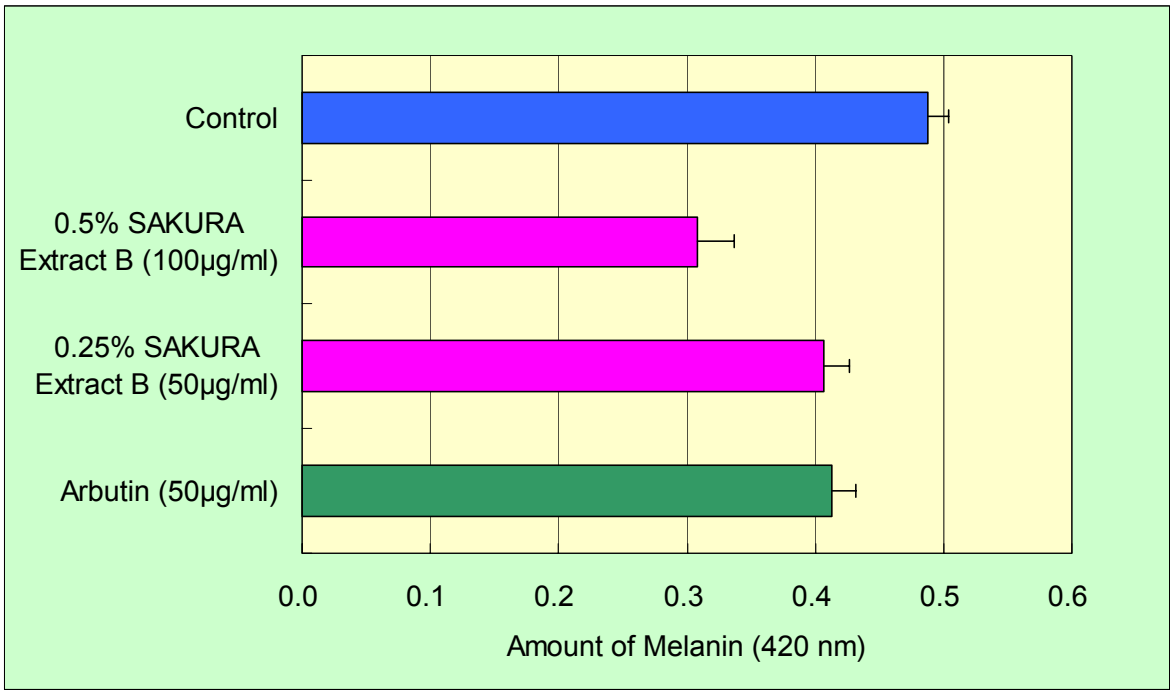


Fig. 8, B16 Melanoma cell Inhibition Effect by SAKURA Extract B

Stability Test

We investigate the stability of SAKURA Extract B.

1.Long term Stability

Store SAKURA Extract B in a cool dark place(4℃), room temperature (10 to 20℃), window side, at 40℃ and at 50℃. Visual observation and absorbance value (1 -> 10) at 470nm were determined.

Result and Discussion

Change of Absorbance value is shown in Fig. 9. The color turned dark at 40℃ and 50℃ with time, but color was almost not changed in a cool dark place, room temperature and window side.

According to this result, SAKURA Extract B is preserved in a place away from high temperature.

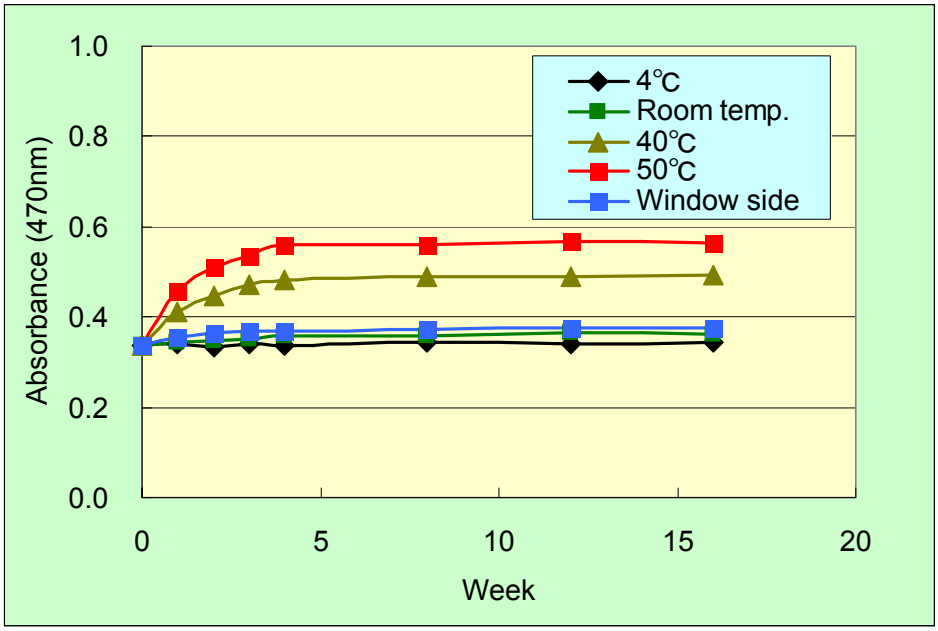


Fig.9, Long Term Stability

2.pH Stability

pH of SAKURA Extract B is adjusted from 2 to 12 by 1 mol/L HCl and 2mol/L NaOH, and . Absorbance value is measured at 470nm.

Result and Discussion

Absorbance value of SAKURA Extract B is shown in Fig.10. After adjusting pH, absorbance value is a little down at acid range but absorbance value is up at alkali range. Also, after heating, absorbance value is a little up at acid range.

A little turbid was observed from pH 2 to 3 in visual but no precipitate was observed at other pH. Also, any turbid and precipitate was not observed by heat treatment.

The change of absorbance and turbid caused by pH adjustment was returned to neutral pH of original (6.2) by re-adjustment.

According to this result, SAKURA Extract B is recommended to add neutral pH range and it is necessary to take care at alkali range.

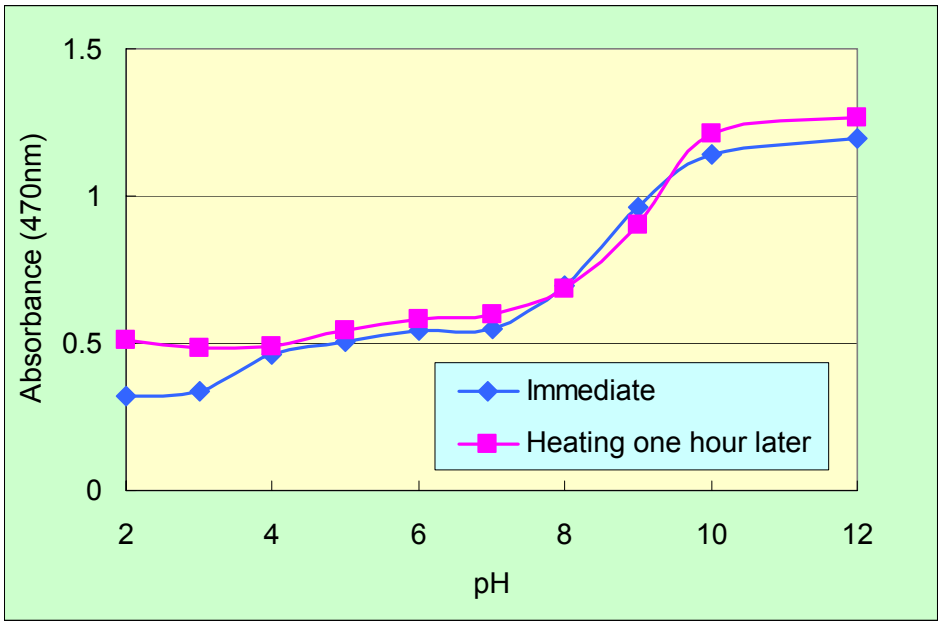


Fig.10, pH Stability

3. Thermal Stability

Store SAKURA Extract; which has been diluted 10 times by purified water in water bath of 90℃. Absorbance value at 470nm and were determined.

Result

Change of absorbance value is shown in Fig.10. Although the tone of color is stable until 5 hours, the color became dark after more than 6 hours. Turbid, precipitate and change of odor were not observed.

According to this result, SAKURA Extract B is stable for heating, but it is careful for long time heating.

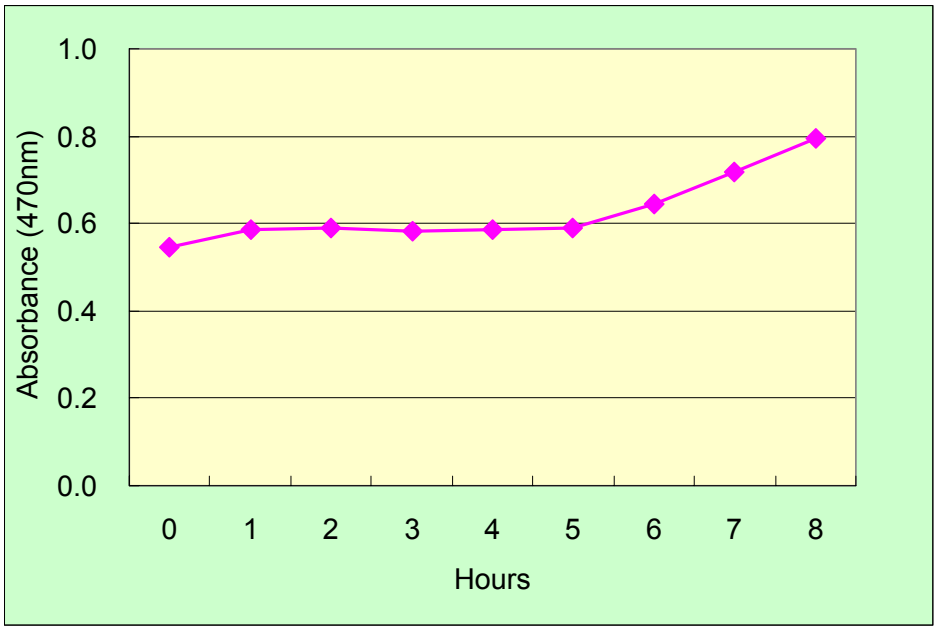


Fig.11, Thermal Stability

Compatibility

1.Compatibility of SAKURA Extract B with Surfactant

	%	Ingredients	10%	2%
Cation	10	Stearyl Trimethyl Ammonium Chloride	○	N.P.
	10	Cetyltrimehylammonium Chloride	○	N.P.
	10	Lauryltrimethylammonium Chloride	△	△
Anion	25	Triethanolamine Lauryl Sulfate	×	○
	25	Sodium Laureth Sulfate	×	○
	25	Triethanolamine Laureth Sulfate	○	N.P.
	10	Potassium N-Cocoyl Glycinate	△	○
	25	Sodium Lauroyl Methylaminopropionate	○	N.P.
	25	Laureth-6 Carboxylic Acid	○	N.P.
	25	Sodium Tetradecenesulfonate	×	△
Nonion	10	Polyethylene Glycol (50) Oleyl Ether	○	N.P.
	10	Coconut Tatty Acid Diethanolamide	○	N.P.
	10	Sorbeth-60 Tetraoleate	○	N.P.
	10	Polyoxyethylene Sorbitan Monooleate (20E.O.)	○	N.P.
Silicone	10	Polyoxyethylene・Methylpolysiloxane Copolymer	○	N.P.
Ampholytic	10	Lauryl Dimethylaminoacetic Acid Betaine	×	×
	10	Lauroyl Amide Propylhydroxysulfobetaine	△	○
	10	Sodium N-Cocoyl-N-Carboxymethyl-N-Hydroxyethyl Ethylenediamide	○	N.P.

○: Good, △: Slight Turbidity, ×: Precipitate, N.P. : Not Performed

2.Compatibility of SAKURA Extract B with other ingredients

	%	Ingredients	10%	2%
Solvent	50	Glycerin	○	N.P.
	50	1,3-Butylene Glycol	○	N.P.
	50	Propylene Glycol	○	N.P.
	50	Isopropyl Alcohol	×	○
	50	Ethanol	×	○
Synthetic polymer	0.1	Carboxyvinyl polymer	○	N.P.
	1	Polyvinylpyrrolidone	○	N.P.
	1	Polyvinyl Alcohol	○	N.P.
	1	Polyethylene glycol (6000)	△	○
Natural polymer	1	Carboxymethyl cellulose	○	N.P.
	1	Cationic cellulose	×	○
	1	Hydroxypropyl cellulose	○	N.P.
	1	Sodium alginate	○	N.P.
Phospholipid	1	Lipidure-PMB	○	N.P.
Vitamin-C derivative	2	Magnesium ascorbyl-2-phosphate	○	N.P.
	2	Ascorbyl Glucoside	×	○

○: Good, △: Slight Turbidity, ×: Precipitate, N.P. : Not Performed

3.Compatibility of SAKURA Extract B with other our products.

%	Product name	INCI Name	10%	2%
10	FM Extract LA-B	Lactobacillus / Milk Ferment Filtrate	×	×
10	AQUACRUSTAR	Hydroxyethyl Chitosan	×	△
10	YEAST Liquid ZB	Yeast Extrcat	○	N.P.
10	CHITIN Liquid (N)	Carboxymethyl Chitin	○	N.P.
10	SUPER Hair Coat	Hydroxypropyl Chitosan	△	N.P.
10	MARINWORT IPC-14 SBW	Algae Extract	○	N.P.
10	SILKGEN G Soluble	Hydrolyzed Silk	○	N.P.
10	SILKGEN G Soluble-S	Hydrolyzed Silk	×	×
10	TREHALOSE 30	Trehalose	○	N.P.
10	NEEM Leaf Liquid B	Melia Azadirachta Leaf Extract	○	N.P.
10	Bio-PGA Solution HB	Polyglutamic Acid	○	N.P.
10	Bio-PGA Solution LB	Polyglutamic Acid	○	N.P.
10	Bio antiage B	Pueraria Lobata Root Extract Chlorella Vulgaris Extract Aloe Barbadensis Leaf Extract	○	N.P.
0.1	Bio Sodium Hyaluronate	Sodium Hyaluronate	○	N.P.
10	Biocellact ALOE VERA B	Aloe Barbadensis Leaf Extract	○	N.P.
10	Fermentage Chardonnary B	Lactobacillus/Grape Juice Ferment (under applying)	○	N.P.
10	Fermentage Pear B	Lactobacillus/Pyrus Communis (Pear) Fruit Juice Ferment (under applying)	○	N.P.
10	Pharconix CTP-F (BG)	Hydrolyzed Collagen	×	×
10	JIOU Liquid	Rehmannia Chinensis Root Extract	○	N.P.
10	HIOUGI Liquid	Belamcanda Chinensis Root Extract	○	N.P.
10	BOTANPI Liquid E	Paeonia Suffruticosa Root Extract	○	N.P.
10	ROOIBOS Liquid B(N)	Aspalathus Linearis Extract	○	N.P.
10	LEMONGRASS Liquid B	Cymbopogon Schoenanthus Extract	○	N.P.
10	Phyto COLLAGE (N)	Natto Gum	○	N.P.
10	ALPROTECTOR	Paeonia Suffruticosa Root Extract Tilia Cordata Flower Extract Althaea Officinalis Root Extract Arnica Montana Flower Extract	○	N.P.
10	Phyto HYALURON B	Hibiscus Esculentus Fruit Extract	○	N.P.
10	FLAVOSTERONE SB	Glycine Soja (Soybean) Protein	○	N.P.
10	YUZU Ceramide B	Citrus Junos Fruit Extract	○	N.P.
10	LACTOFERRIN-S	Lactoferrin	△	○
0.5	LEXSOD-P	Tannic Acid	○	N.P.

○: Good, △: Slight Turbidity, ×: Precipitate, N.P. : Not Performed

Specification

Subject	Specification
Appearance	Brown to reddish brown liquid, having characteristic odor
Identification Flavonoid Coumarin derivative	 Positive Positive
Purity Heavy metals Arsenic	 20 ppm max. 2 ppm max.
Residue on Evaporation	1.0 to 3.0 w/v%
INCI Name	Water Butylene Glycol Prunus Yedoensis Leaf Extract
CAS Number	None
EINECS Number	None

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