SAKURA EXTRACT

Anti-glycation, Whitening, Anti-ageing
Food and Cosmetics Ingredient

- SAKURA EXTRACT-P
  (Water-soluble Powder, Food Grade)
- SAKURA SYRUP
  (Liquid, Food Grade)
- SAKURA EXTRACT-PC
  (Water-soluble Powder, Cosmetic Grade)
- SAKURA EXTRACT-LC
  (Liquid, Cosmetic Grade)

ORYZA OIL & FAT CHEMICAL CO., LTD.

Ver.2.0HS
1. Introduction

Sakura flower (cherry blossom), a symbol of Japan has been favored by Japanese from ancient time. According to the oldest record, it was planted for their beauty and adornment of the ground nobility as early as 794. Japanese people love Sakura flower so much that they enjoy Hanami (a festival to view flowers, probably typical to Japanese custom). Sakura blooms in spring after long and dark winter, so it becomes an omen of good fortune and synonym of spring. People regard Sakura as sic and elegant symbol of Japanese beauty as well as Mount Fuji.

Sakura is full of attractive stories. The etymology of Sakura is said to be based on a goddess in Japanese mythology named Kono Hana no Sakuya Hime (simply Sakuya afterward). Sakuya was symbol of beauty of nature, and got married with Ninigi no Mikoto (simply Ninigi afterward), a grandson of sun goddess. In fact, it was Ninigi that fell in love at first glance of her. Ninigi was a god of agriculture and therefore Sakuya was sued as goddess of rich harvest. Sakura blossom was used for fortune of agriculture of the year in some regeons, implying how Japanese people cared for season when Sakura blooms. Sakuya is deified as a goddess in about 1300 Asama shrines and Sakura is dedicated as a sacred tree there.
Coincidentally, Sakura is classified into *Rosaceae* family (group of rose)—Sakura represents beauty of Japan whereas rose represents beauty of the west. The beauty of Sakura begins to be widely accepted and represented in all manner of consumer goods of Japanese style (*e.g.* kimono, stationery and dishware, *etc.*).

Sakura or cherry blossoms are edible and both are used as food ingredient in Japan. However, the transience of the blossoms (typically one or two weeks) limited its application in food and cosmetics. Oryza Oil & Fat Chemical Co., Ltd. successfully cleared procurement difficulties, enabling the stable production of SAKURA EXTRACT in bulk.

In addition to the emotional values, Sakura was studied by Oryza Oil & Fat Chemical Co., Ltd. and identified caffeoyl glucose (1-caffeoyl-O-β-D-glucopyranoside) and quercetin glucose (quercetin-3-O-β-D-glucopyranoside) as the major functional component. SAKURA EXTRACT has been proven as an anti-glycation agent with promotion of collagen formation in fibroblasts that leads to anti-ageing. It surely satisfies users of your brand, food and cosmetics applications.

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2. Anti-glycation

2-1. Maillard reaction

Maillard reaction is a chemical reaction between amino acids and reducing sugars upon heating, producing brown nitrogenous polymers and melanoidins (Fig. 1). Maillard reaction is useful and important in industries of colors and flavors like caramel. Meanwhile, AGEs (Advanced Glycation End Products) are the result of Maillard reaction.

![Maillard reaction and AGEs production](image)

Fig. 1  Maillard reaction and AGEs production
2-2. Physiological Maillard reaction

Maillard reaction also occurs in human body, which leads to the formation of troublesome AGEs. It often involves sugar binding proteins such as collagen and elastin. Formation of AGEs has been shown to contribute to the progression of age-related diseases and diabetes. Glycation of collagen and elastin in skin causes accumulation of AGEs (Fig. 2), which results in intracellular damage and apoptosis.

![Diagram of Glycation of skin ageing](image)

**Fig 2.** Glycation of skin ageing

2-3. AGEs and ageing skin

Accumulation of AGEs induces damage through cross-link of collagen fibrils, which increases stiffening of collagen network and ultimately leads to apoptosis, or death of fibroblasts. As a result, dermis collagen is damaged and skin becomes wrinkled and dull (Fig. 3).

![Diagram of AGEs by adverse effects on skin](image)

**Fig 3.** AGEs by adverse effects on skin
An investigation conducted by Dyer et al. suggested the contribution of glycation and oxidation reaction to the modification of insoluble collagen in ageing and diabetes. AGE spices such as fructolysine, CML and pentosidin e of diabetic and non-diabetic subjects were measured by collagen-linked fluorescence. There were strong correlation between AGES and ages of subjects of both groups, indicating age-related chemical modification of collagen by Maillard reaction and the process is accelerated in diabetes.

![Graph 1](image1.png)

![Graph 2](image2.png)

Fig. 4. Accumulation of AGES in skin collagen of aged and diabetic subjects.

As mentioned earlier, accumulation of AGES due to glycation of collagen may induce apoptosis in fibroblasts creating a negative influence on hyaluronic acid, collagen and elastin. “Anti-glycation” risen as one of the latest approach in preventing ageing and maintenance of healthy youthful skin in cosmetic science.

Studies conducted in the R&D of Oryza Oil & Fat Chemical Co. Ltd. revealed the inhibitory effect of SAKURA EXTRACT on AGES production, CML-collagen and suppression of apoptosis induction in fibroblasts.

Reference
3. Bioactive components of SAKURA EXTRACT

3-1. Bioactive components

A joint study by Oryza Oil & Fat Chemical CO., Ltd. and Kyoto Pharmaceutical University successfully determined caffeoyl glucose (1-caffeoyl-\(\beta\)-D-glucopyranoside) and quercetin glucoside (quercetin 3-\(\beta\)-D-glucopyranoside) as bioactive components of SAKURA EXTRACT for the first time (Fig. 5).

![Chemical Structures]

Fig. 5. Bioactive components of SAKURA EXTRACT
3-2. Inhibition of AGEs production

Bioactive components of SAKURA EXTRACT and crude SAKURA EXTRACT were added to buffer solution containing D-glucose and bovine serum albumin at 60°C and left to stand for 2 days. Crude SAKURA EXTRACT (100µg/mL) significantly inhibited the production of AGEs. Meanwhile, the major bioactive component, caffeoyl glucose significantly inhibited the production of AGEs at concentration as low as 10µg/mL (Table 1, Fig. 6.) There were trace amount of hydroxyl caffeoyl glucoses in SAKURA EXTRACT, cinnamoyl glucose and coumaroyl glucose, but the inhibitory potency in AGEs production was weak. Although flavonoid glycosides were minor components, they showed strong inhibitory potency. Quercetin glucoside exerted inhibitory effect of two-fold stronger than that of kaempferol glucoside in IC50.

Table 1: The effect of SAKURA EXTRACT and its bioactive components on the production of AGEs

<table>
<thead>
<tr>
<th>Inhibition of AGEs production ratio (%)</th>
<th>IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 (µg/mL)</td>
</tr>
<tr>
<td>Crude SAKURA EXTRACT</td>
<td></td>
</tr>
<tr>
<td>Caffeoyl glucose</td>
<td>-14.6±0.7</td>
</tr>
<tr>
<td>Caffeoyl glucose</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Coumaroyl glucose</td>
<td>-8.2±0.1**</td>
</tr>
<tr>
<td>Cinnamoyl glucose</td>
<td>-10.4±0.1</td>
</tr>
<tr>
<td>Kaempferol glucoside</td>
<td>-9.1±0.2**</td>
</tr>
<tr>
<td>Quercetin glucoside</td>
<td>6.5±0.1*</td>
</tr>
<tr>
<td>Kaempferol malonyl glucoside</td>
<td>-8.5±0.1</td>
</tr>
<tr>
<td>Quercetin malonyl glucoside</td>
<td>1.9±0.1</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>6.0±0.1</td>
</tr>
<tr>
<td>Coumaric acid</td>
<td>-19.7±0.5</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>-4.9±0.3</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>6.9±0.5*</td>
</tr>
<tr>
<td>Quercetin</td>
<td>-22.2±1.0</td>
</tr>
<tr>
<td>†Aminoguanidine hydrochloride (positive control)</td>
<td>-</td>
</tr>
</tbody>
</table>

† Aminoguanidine hydrochloride suppresses glycation by many amide groups.
Each value was shown in the mean and standard error of three cases. With asterisks were significantly different from untreated samples Dunnet's multiple comparison test. *: p <0.05, **: p <0.01
Fig. 6. Effect of SAKURA EXTRACT and its bioactive components on the production of AGEs

References
3-3. Inhibition of fibroblasts apoptosis

Accumulation of AGEs in skin triggers skin damage and apoptosis of fibroblasts. The effect of Crude SAKURA EXTRACT and its bioactive components on carboxymethyl lysine (CML)-collagen induced fibroblasts apoptosis was examined. SAKURA EXTRACT, caffeoyl glucose and quercetin glucose decreased caspase activity, meaning fibroblasts apoptosis were suppressed. The effect of caffeoyl glucose was outstanding in the inhibition. SAKURA EXTRACT is potentially beneficial as an anti-ageing agent (Table 2, Fig. 7).

Table 2. The Effect of SAKURA EXTRACT and its bioactive components on fibroblasts apoptosis

<table>
<thead>
<tr>
<th></th>
<th>1 (µg/mL)</th>
<th>3</th>
<th>10</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SAKURA EXTRACT</strong></td>
<td>-</td>
<td>-</td>
<td>61.8±2.6</td>
<td>77.1±4.2*</td>
</tr>
<tr>
<td>Caffeoyl glucose</td>
<td>26.2±0.5*</td>
<td>37.6±1.2</td>
<td>72.2±2.7*</td>
<td>-</td>
</tr>
<tr>
<td>Coumaroyl glucose</td>
<td>17.2±0.5</td>
<td>7.1±0.2</td>
<td>51.1±1.9</td>
<td>-</td>
</tr>
<tr>
<td>Cinnamoyl glucose</td>
<td>-11.8±0.3</td>
<td>19.7±0.9</td>
<td>48.6±2.9</td>
<td>-</td>
</tr>
<tr>
<td>Kaempferol glucoside</td>
<td>-0.7±0.1</td>
<td>27.9±1.1</td>
<td>100.7±4.2</td>
<td>-</td>
</tr>
<tr>
<td><strong>Quercetin glucoside</strong></td>
<td>44.2±1.5*</td>
<td>39.0±1.1*</td>
<td>121.5±5.4**</td>
<td>-</td>
</tr>
<tr>
<td>Kaempferol malonyl glucoside</td>
<td>-18.9±0.6</td>
<td>-17.3±0.6</td>
<td>10.5±0.5</td>
<td>-</td>
</tr>
<tr>
<td>Quercetin malonyl glucoside</td>
<td>21.8±0.7</td>
<td>36.6±1.4</td>
<td>98.4±4.4*</td>
<td>-</td>
</tr>
</tbody>
</table>

† Aminoguanidine hydrochloride (positive control)

† Aminoguanidine hydrochloride suppresses glycation by many amide groups.

Each value was shown in the mean and standard error of three cases. With asterisks were significantly different from untreated samples Dunnett's Dunnett's multiple comparison test. *: p <0.05, **: p <0.01

References
Fig. 7. Effect of SAKURA EXTRACT and its bioactive components on fibroblasts apoptosis

3-4. Promotion of collagen formation in fibroblasts

Fibroblasts were cultured in collagen containing medium and treated with glyoxal, a glycation inducer. Regularly, fibroblasts produces collagen lattice when cultured in collagen containing medium. As illustrated in Figs. 8 and 9, growth of fibroblasts and collagen lattice formation were enhanced in the presence of SAKURA EXTRACT of 100µg/mL and 1000µg/mL. SAKURA EXTRACT was suggested to prevent glycation of fibroblasts and to be effective in maintaining extracellular collagen matrix.

References
Collagen lattice and the process of fibroblast spreading white haze

**Fig 8.** Macroscopic images of wells after 24-hour co-incubation

Compared to the Normal culture, Control shows fewer fibroblasts.

Addition of Sakura extract to the Control, formation of the protrusion of fibroblasts (▼) was observed

**Fig 9.** Macroscopic images of wells after 24-hour co-incubation
3-5. Inhibition of AGEs production in fibroblasts

Normal human fibroblasts were treated with glyoxal (glycated intermediate) and incubated for 5 days, then skin glycation was analyzed by Western-blotting method\textsuperscript{7,8}. As illustrated in Fig. 10, SAKURA EXTRACT at 10 µg/mL significantly inhibited the production of AGEs by glyoxal (indicated as thinner band), compared with control group. Caffeoyl glucose inhibited the production of AGEs at 1 and 10 µg/mL, but quercetin glucose acted less potent. It was suggested that caffeoyl glucose plays the major role in the inhibitory effect of SAKURA EXTRACT on the production of AGEs.

![Thinner bands of AGEs compared with that of control.](image)

**SAKURA EXTRACT**

- Glyoxal

**Caffeoyl glucose**

+ Glyoxal

Band density is similar to that of Control. Caffeoyl Glucose exerted stronger inhibition on AGEs production.

![Band density is similar to that of Control.](image)

**Quercetin glucoside**

- Glyoxal

+ Glyoxal

Fig. 10. Effect of SAKURA EXTRACT and its components on AGEs production in normal human fibroblasts

References


3-6. Inhibition of melanin formation in B16 melanoma cells

The skin whitening effect of SAKURA EXTRACT was examined in B16 melanoma cells. SAKURA EXTRACT was added to cell culture and incubated for 3 days. Then cells were crushed by hypersonication followed by absorbance measurement. As illustrated in Table 3, SAKURA EXTRACT demonstrated inhibition of melanin formation in a dose-dependent manner, similar potency with ascorbic acid glucoside (vitamin C).

Table 3. Effect of SAKURA EXTRACT on melanin formation in B16 melanoma cells

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>10 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAKURA EXTRACT</td>
<td>100±1.7</td>
<td>96.2±2.8</td>
<td>94.9±1.2</td>
<td>90.0±3.5</td>
</tr>
<tr>
<td>β-arbutin (positive control)</td>
<td>100±3.7</td>
<td>95.5±1.5</td>
<td>87.9±1.3</td>
<td>84.6±0.5</td>
</tr>
<tr>
<td>ascorbic acid glucoside</td>
<td>100±3.2</td>
<td>97.9±0.3</td>
<td>94.6±1.0</td>
<td>90.2±0.5</td>
</tr>
</tbody>
</table>

* Melanin production ratio (% of control)
3-7. Inhibition of tyrosinase activity

Further experiment was prompted to examine the effect of SAKURA EXTRACT on tyrosinase activity which is directly involved in melanogenesis. As illustrated in Fig. 11, SAKURA EXTRACT showed inhibitory effect on tyrosinase activity in a dose-dependent manner, suggesting its skin whitening effect.

Fig 11. Effect of SAKURA EXTRACT on tyrosinase activity (mean±S.D., n=5, **: p<0.01)
3-8. Collagen production and matrix formation

We added SAKURA EXTRACT (30, 100 µg/mL) to human normal diploid fibroblasts and cultured for 3 days. Then released and accumulated type I collagen was detected by Western blotting. In addition, cellular type I collagen was evaluated by Western blotting and RT-PCR. As a result, excreted type I collagen was increased by the treatment of SAKURA EXTRACT (30, 100 µg/mL, Fig. 12 upper). Cellular type I collagen was also increased (Fig. 12, lower).

Fig. 12 Enhancement of type I collagen production in fibroblasts by SAKURA EXTRACT (CBE).
Upper: protein of type I collagen, Lower: mRNA of type I collagen (mean ± SE, n=4)
In dermis, extracellular components including collagen forms extracellular matrix. By addition of collagen to fibroblast, ling shape gel matrix was formed (Fig. 13). On the other hand, by addition of collagen to fibroblasts cultured with SAKURA EXTRACT, accumulation of gel matrix was observed. This phenomena was clearly observe when cafferoyl glucose was added.

We have reported recovery effect of the extract on matrix formation formed by glycated fibroblasts and collagen. From this experiment, SAKURA EXTRACT was found to enhance matrix formation in normal fibroblasts.

Fig. 13 Enhancement of matrix (collagen lattice) formation by SAKURA EXTRACT and caffeoyl glucose.
3-9. Anti-inflammatory effect

We evaluated anti-inflammatory effect of SAKURA EXTRACT on nitric oxide (NO) production from RAW264 cells. As a result, SAKURA EXTRACT (10 to 100 µg/mL) suppressed NO production induced by LPS. Caffeoyl glucose also suppressed NO production at 1 to 100 µg/mL (Fig. 14).

Fig. 14 Suppressive effect of SAKURA EXTRACT on NO production

### : vs Cont., p<0.001
** : vs LPS, p<0.01
*** : vs LPS, p<0.001
Indo : Indomethacin (8.9 µg/mL), n = 5-6
3-9. Clinical trial

We conducted double blind placebo controlled clinical study of SAKURA EXTRACT and evaluated the effect on skin condition. Twenty Japanese female aged 30 to 60 years old with skin conscious were chosen as subjects. Subjects were divided into 2 groups (10 each). For SAKURA EXTRACT group, SAKURA EXTRACT (150 mg/day) was given and dextrin (150 mg/day) was given to placebo group for 8 weeks. Each test sample was ingested after every meal.

As a result, SAKURA EXTRACT exhibited (1) reduction of skin AGEs, (2) suppression of skin elasticity reduction, (3) reduction of pigmentation and reddish area, (4) suppression of increase in pores area, (5) suppression of dry skin and (6) improvement of skin smoothness.

.preprocessing AGEs

Skin AGEs was determined using fluorescence AGE leader. This apparatus can determine fluorescent AGE without invasion and can be used for study of cardiovascular failures, diabetes mellitus and renal failures. Skin AGEs were decreased approx. 7% in 8 weeks by SAKURA EXTRACT with significance. On the other hand, AGEs were decreased approx. 3% in placebo group without significance (Fig. 15).

AGEs are reported to increase 1.5 to 2.5 toward aging 20 to 80 years old, and indicate 2.0 at 45 years old9). Hence, we performed classification analysis for AGEs divided into 2 group by AGEs value (2). As a result, in subjects with more than 2 AGEs, AGEs were decreased approx. 8% with significance. On the other hand, in subject with less than 2 AGEs, no change in AGEs was detected (Fig. 16). Hence, SAKURA EXTRACT was found to decrease skin AGEs, and the effect was clearly in subjects with high AGEs value.
Fig. 16 Classified analysis of change in skin fluorescent AGEs (**: p<0.01)

Skin Elasticity (R0)

Elasticity in placebo group was decrease approx. 13% with significance. SAKURA EXTRACT also reduced approx. 6%, however the effect was without significance (Fig. 17). SAKURA EXTRACT was found to suppress reduction of skin elasticity caused by seasonally change.

Fig. 17 Change in elasticity (**: p<0.01)

Pigmentaion and reddish area

Using VISIA Evolution, we evaluated facial pigmentation and reddish area. As a result, SAKURA EXTRACT reduced 7% pigmentation and 15% flea area with significance. No changes were observed in placebo group (Fig. 18 and 19).

Fig. 18 Change in pimentated area (*: p<0.05)
Using VISIA evolution, we evaluated the change in pores. As a result, no significant change was observed in SAKURA EXTRACT group. On the other hand, placebo group exhibited 20% increase in pores area with significance (Fig. 20). Hence, SAKURA EXTRACT was found to suppress increase in pores area.

-### Moisture

Significant decreases in skin moisture were observed in both groups (Fig. 21). Decrease in moisture in SAKURA EXTRACT group was 13% and that of placebo was 16%.

The season (From October to December), when performed the study, was dry season in a year (Fig. 22). The significant decrease in moisture seems to be affected by the humidity. However, the decrease in humidity of SAKURA EXTRACT group was less than placebo. Hence, SAKURA EXTRACT is thought to be possessed moisturizing effect.
Smoothness of skin
Smoothness scores are significantly improved in both groups. Increase ratio in SAKURA EXTRACT group is superior than placebo group.

9) H.L. Lutgers et. al., Diabetes Care, December 2006
4. Stability

4-1. Heat stability

Heat stability of SAKURA EXTRACT-P (water soluble powder) was examined by quercetin glucoside and caffeoyl glucoside, bioactive components. As illustrated in Fig. 24, the bioactive components remained stable upon heating at 120°C for 1 hour. SAKURA EXTRACT is highly stable upon heating in food processing.

![Fig 24. Heat stability of SAKURA EXTRACT-P](image)

4-2. Water solubility

Water solubility of SAKURA EXTRACT-P at different concentrations was evaluated. SAKURA EXTRACT-P was dissolved in water and stored for 3 days at room temperature or 5°C. As shown in Table 4, precipitation and turbidity were observed in higher concentration of SAKURA EXTRACT-P. There is no problem at all to use SAKURA EXTRACT-P of daily recommended dosage of 50mg-150mg in very small volume as 30mL.

<table>
<thead>
<tr>
<th>Concentration of SAKURA EXTRACT-P</th>
<th>Neutral (pH 6 - 7)</th>
<th>Acidic (pH 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Room Temperature</td>
<td>5°C</td>
</tr>
<tr>
<td>1%</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>2%</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>5%</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>10%</td>
<td>Fair</td>
<td>No Good</td>
</tr>
<tr>
<td>20%</td>
<td>No Good</td>
<td>No Good</td>
</tr>
</tbody>
</table>

Good: Transparent, no sediment, Fair: slight haze, with settling, No Good: turbidity, sedimentation
4-3. pH Stability

pH stability of SAKURA EXTRACT was examined by dissolving SAKURA EXTRACT-P in distilled water with different pH, and stored at room temperature in darkness for one week. As illustrated in Fig. 25, bioactive component caffeoyl glucose was highly stable at acidic condition but began to disintegrate at alkaline condition.

Fig 25. pH stability of caffeoyl glucose

4-4. Stability of aqueous solution

Aqueous solution of SAKURA EXTRACT-P of 0.5% at pH 3.5 was prepared and stored at room temperature under light condition, at 5°C, 25°C and 40°C for 4 months. As illustrated below, neither precipitation nor turbidity was observed at all storage temperature examined, hence SAKURA EXTRACT was considered suitable for beverage use.

<table>
<thead>
<tr>
<th>Stability of Aqueous Solution (0.5% aqueous solution, pH 3.5)</th>
<th>Room Temperature (light)</th>
<th>5°C</th>
<th>25°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitation</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>Turbidity</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
</tbody>
</table>

4-5. Synergistic combination with collagen

Precipitation and turbidity are principle problem in development of collagen drink with polyphenols which exhibit strong anti-oxidative properties. Although SAKURA EXTRACT is rich in polyphenol, neither precipitation nor turbidity was observed in the aqueous mixture of 1% collagen and 0.2% SAKURA EXTRACT. Synergism between collagen and SAKURA EXTRACT is expected in beverage applications for healthy skin maintenance.
5. Safety Profile

5-1. Residual agricultural chemicals

Sakura flower (raw material) underwent screening analysis of residual agricultural chemicals (518 items) under the Food Sanitation Act and Pesticides Control Act, presence of the test items was lower than the allowed limits.

Test Trustee: Masis Co., Ltd.; Center for Food Safety Evaluation and Analysis
Date: November 9, 2009
Report No. 34234

5-2. Mutagenicity (Ames test)

Ames test was conducted to evaluate the mutagenicity of SAKURA EXTRACT (no binder extract) using Salmonella typhimurium TA98 and TA100. There was no increase in the number of colonies in both direct method and metabolism activation method. SAKURA EXTRACT was considered as non-mutagenic.

5-3. Skin irritation test, alternative method (EpiSkin method)

Aqueous solution of SAKURA EXTRACT of 1% (no binder extract) was applied to the EpiSkin skin model to examine the skin irritation potential of SAKURA EXTRACT. As quantified by MTT assay, the cell survival ratio of IL-1α and EVCAM was lower than the standard Risk Phase R38. SAKURA EXTRACT was considered as non-skin-irritating.

5-4. Eye irritation test, alternative method (HCE method)

Aqueous Solution of SAKURA EXTRACT of 1% (no binder extract) was applied to SkinEthic™ HCE and exposed for one hour. Eye irritation was determined by MTT assay. Results showed that SAKURA EXTRACT was considered as non-eye-irritating.

5-5. Patch test

Patch test using solution of SAKURA EXTRACT of 1% (no binder extract) was conducted among 18 healthy Japanese men and women (2 men, 18 women) aged between 20 to 60. A patch test unit with moistened samples was applied to each subject in their back (para vertebral part) for 24 hours then skin irritation was observed. Skin irritation index of SAKURA EXTRACT was considered as very good.
5-6. Repeated insult patch test (RIPT)

Repeated insult patch test using SAKURA EXTRACT (no binder extract) was conducted among 30 panelists of either sex, without visible skin diseases or known hypersensitivity.

The test substance was applied to the skin of the panelist via an occlusive patch at a suitable concentration. The patch limited contact of the panelist’s skin with the test substance to a local area and exposure was exaggerated due to the occlusive conditions. The skin was checked 3 times within 7 weeks each time 24, 48 and 72 hours after patch application.

As a result, SAKURA EXTRACT (no binder extract) was considered the rating of very good. This product did not lead to toxic-irritative intolerance reactions in repeated patch testing carried out in accordance with international guidelines.

6. Nutritional Profile

<table>
<thead>
<tr>
<th>Description</th>
<th>SAKURA EXTRACT-P</th>
<th>Sakura Syrup</th>
<th>Remark</th>
<th>Analysis Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>5.0 g/100g</td>
<td>43.4g/100g</td>
<td></td>
<td>Heat drying at atmospheric pressure</td>
</tr>
<tr>
<td>Protein</td>
<td>4.5 g/100g</td>
<td>0.0 g/100g</td>
<td>1</td>
<td>Combustion method</td>
</tr>
<tr>
<td>Fat</td>
<td>1.5 g/100g</td>
<td>0.0 g/100g</td>
<td>Acid degradation</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>2.5 g/100g</td>
<td>0.0 g/100g</td>
<td>Direct incineration</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>86.5 g/100g</td>
<td>56.4 g/100g</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Energy</td>
<td>378 kcal/100g</td>
<td>138 kcal/100g</td>
<td>3</td>
<td>Modified Atwater method</td>
</tr>
<tr>
<td>Food Fiber</td>
<td>0.0 g/100g</td>
<td>0.0 g/100g</td>
<td>Prosky method</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>50 mg/100g</td>
<td>0.4 mg/100g</td>
<td>Atomic absorption spectrophotometry</td>
<td></td>
</tr>
<tr>
<td>NaCl equiv.</td>
<td>0.1 g/100g</td>
<td>0.0 g/100g</td>
<td>Sodium equiv. value</td>
<td></td>
</tr>
</tbody>
</table>

1) Protein conversion factor: 6.25
2) Calculation: 100 g – (water + protein + fat + ash)
3) Conversion factor: Protein 4, fat 9, sugar 4 – (sorbitol + maltitol + mannitol + maltotriitol + maltotetraotiol), dietary fiber 2, sorbitol 3, maltitol 2, mannitol 2, maltotriitol 2, maltotetraotiol 3

Test trustee: SRL, Inc
Date of analysis: Feb 22, 2010
Test No.: 201002030028

7. Recommended Dosage

Recommended daily dosage of SAKURA EXTRACT-P is 50 to 150 mg.
8. Applications

<table>
<thead>
<tr>
<th>Foods</th>
<th>Applications</th>
<th>Claims</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beauty Food</td>
<td>1) Anti-glycation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-glycation</td>
<td>2) Anti-ageing</td>
<td>Beverages (soft drinks etc), hard and soft capsules, tablets, candies, chewing gum, cookies, chocolate wafers, jelly, etc.</td>
</tr>
</tbody>
</table>

| Cosmetics | Beauty | Anti-glycation | Sunscreen, toner, lotion, body gel, shampoo, conditioner and bath salts, etc. |

9. Packaging

SAKURA EXTRACT-P (water soluble powder, food grade)
5kg  Interior Packaging: Aluminium bag
     Exterior Packaging: Cardboard

Sakura Syrup (liquid, food grade)
20kg  Interior Packaging: Cubic polyethylene container
     Exterior Packaging: Cardboard

SAKURA EXTRACT-PC (water soluble powder, cosmetic grade)
5kg  Interior Packaging: Aluminium bag
     Exterior Packaging: Cardboard

SAKURA EXTRACT-LC (liquid, cosmetic grade)
5kg  Interior Packaging: Cubic polyethylene container
     Exterior Packaging: Cardboard

10. Storage

SAKURA EXTRACT-P, -PC:  Store in cool, dry and dark place. Avoid places with high humidity and direct heat.
SAKURA SYRUP:  Store in cool, dry and dark place.
SAKURA EXTRACT-LC:  Store in cool, dry and dark place.
11. Expression

[Food]  Please follow regulations in your country.

SAKURA EXTRACT-P
Expression: Cherry Blossom Extract Powder
or
[starch degradation product/dextrin]
[Cherry blossom extract/Cherry Extract/SAKURA EXTRACT]
[Ascorbic acid], [Malic Acid]
* [] If you have multiple representation, please select one.

SAKURA SYRUP
Expression: Cherry Blossom Extract Liquid
or
[Reduced syrup / syrup]
[Cherry Blossom Extract / Extract of cherry blossom/Cherry Extract/ 
SAKURA EXTRACT]
[Ascorbic acid], [Malic acid]
* [] If you have multiple representation, please select one.

[Cosmetic]

SAKURA EXTRACT-PC
INCI name : Dextrin (and) Prunus Lannesiana Flower Extract (and) Ascorbic
Acid (and) Malic Acid

SAKURA EXTRACT-LC
INCI name : Water (and) Butylene Glycol (and) Prunus Lannesiana Flower
Extract (and) Ascorbic Acid (and) Malic Acid
This product is extracted from cherry blossom (*Prunus lannesiana*) flower with aqueous ethanol. It guarantees minimum of 2.0 % caffeoyl glucose and 0.05 % quercetin glucoside. This product is water-soluble.

**Appearance**
Pale pink to pale red brown powder with light unique smell.

**Caffeoyl glucose**
Min. 2.0 % (HPLC)

**Quercetin glucoside**
Min. 0.05 % (HPLC)

**Loss on Drying**
Max. 10 % (Analysis for Hygienic Chemist, 1 g, 105°C, 2 h)

**Purity Test**
(1) Heavy Metals (as Pb) Max. 20 ppm (Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As₂O₃) Max. 1 ppm (Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)

**Standard Plate Counts**
Max. 3×10³ cfu/g (Analysis for Hygienic Chemists)

**Moulds and Yeasts**
Max. 1×10³ cfu/g (Analysis for Hygienic Chemists)

**Coliforms**
Negative (Analysis for Hygienic Chemists)

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrin</td>
<td>69 %</td>
</tr>
<tr>
<td>Cherry blossom flower extract</td>
<td>25 %</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>3 %</td>
</tr>
<tr>
<td>Malic acid</td>
<td>3 %</td>
</tr>
<tr>
<td>Total</td>
<td>100 %</td>
</tr>
</tbody>
</table>
This product is extracted from cherry blossom (*Prunus lannesiana*) flower with aqueous ethanol and is dissolved in aqueous glutinous starch syrup. This product is water-soluble.

**Appearance**
Pale pink to pale red liquid with light unique smell.

**Purity test**

1. **Heavy Metals (as Pb)** Max. 20 ppm (The Japanese Standards for Food Additives)
2. **Arsenic (as As₂O₃)** Max. 1 ppm (Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)

**Standard Plate Counts**
Max. $1 \times 10^3$ 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**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutinous starch syrup</td>
<td>99.4 %</td>
</tr>
<tr>
<td>Cherry blossom flower extract</td>
<td>0.2 %</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.2 %</td>
</tr>
<tr>
<td>Malic acid</td>
<td>0.2 %</td>
</tr>
<tr>
<td>Total</td>
<td>100 %</td>
</tr>
</tbody>
</table>
SAKURA EXTRACT CATALOG Ver. 2.0HS

PRODUCT STANDARD
PRODUCT NAME

SAKURA EXTRACT-PC
COSMETIC

This product is extracted from cherry blossom (*Prunus lannesiana*) flower with aqueous ethanol. It guarantees minimum of 2.0 % caffeoyl glucose and 0.05 % quercetin glucoside. This product is water-soluble.

**Appearance**
Pale pink to pale red brown powder with light unique smell.

**Caffeoyl glucose**
Min. 2.0 % (HPLC)

**Quercetin glucoside**
Min. 0.05 % (HPLC)

**Loss on Drying**
Max. 10 % (1 g, 105°C, 2 h)

**Purity Test**
1. Heavy Metals (as Pb) Max. 20 ppm (The Second method of The Japanese Standards of Quasi-Drug Ingredients)
2. Arsenic (as As₂O₃) Max. 1 ppm (The Third method of The Japanese Standards of Quasi-Drug Ingredients)

**Standard Plate Counts**
Max.1×10^2 cfu/g (Analysis for Hygienic Chemists)

**Moulds and Yeasts**
Max.1×10^2 cfu/g (Analysis for Hygienic Chemists)

**Coliforms**
Negative (Analysis for Hygienic Chemists)

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrin</td>
<td>69 %</td>
</tr>
<tr>
<td>Prunus lannesiana flower extract</td>
<td>25 %</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>3 %</td>
</tr>
<tr>
<td>Malic acid</td>
<td>3 %</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100 %</td>
</tr>
</tbody>
</table>
SAKURA EXTRACT-LC
COSMETIC

This product is extracted from cherry blossom (*Prunus lannesiana*) flower with aqueous ethanol and is dissolved in aqueous 1,3-butylene glycol. This product is water-soluble.

**Appearance**
Brown to red brown liquid with unique smell.

**Certification**

**Polyphenols**
Dissolve 30 µl of this product in 3.5 ml water. Add 0.2 ml Folin-Denis reagent into the solution followed by 0.4 ml saturated Na₂CO₃. The solution will change into blue.

**Purity Test**

1. **Heavy Metals (as Pb)**
   Max. 10 ppm  
   (The Second method of The Japanese Standards of Quasi-Drug Ingredients)

2. **Arsenic (as As₂O₃)**
   Max. 1 ppm  
   (The Third method of The Japanese Standards of Quasi-Drug Ingredients)

**Standard Plate Counts**
Max. 1×10² cfu/g  
(Analysis for Hygienic Chemists)

**Moulds and Yeasts**
Max. 1×10² cfu/g  
(Analysis for Hygienic Chemists)

**Coliforms**
Negative  
(Analysis for Hygienic Chemists)

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>68.76 %</td>
</tr>
<tr>
<td>Butylene glycol</td>
<td>30.00 %</td>
</tr>
<tr>
<td><em>Prunus lannesiana</em> flower extract</td>
<td>1.00 %</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.12 %</td>
</tr>
<tr>
<td>Malic acid</td>
<td>0.12 %</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.00 %</strong></td>
</tr>
</tbody>
</table>
ORYZA OIL & FAT CHEMICAL CO., LTD. striving for the development of the new functional food materials to promote health and general well-being.

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

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