

SAKURA EXTRACT

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

SAKURA EXTRACT-P

(Water-soluble Powder, Food Grade)

SAKURA SYRUP

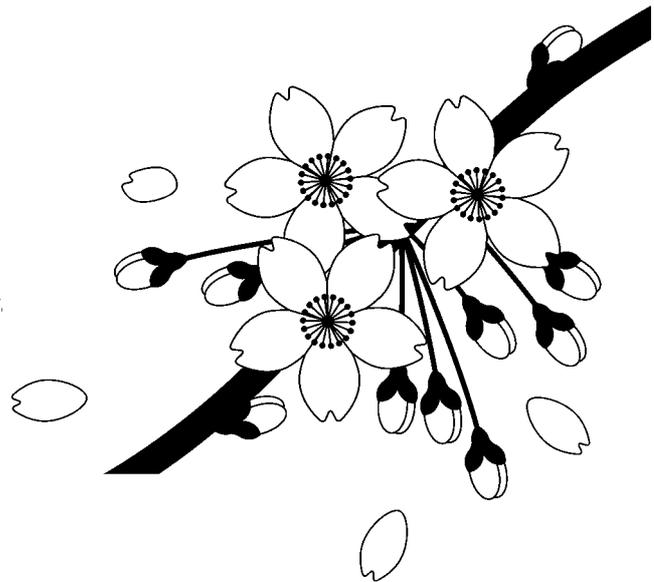
(Liquid, Food Grade)

SAKURA EXTRACT-PC

(Water-soluble Powder, Cosmetic Gr.

SAKURA EXTRACT-LC

(Liquid, Cosmetic Grade)



ORYZA OIL & FAT CHEMICAL CO., LTD.

Ver.4.0HS

SAKURA EXTRACT

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

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 regeons¹⁾, 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.



Doumoto Insyu, *KONO HANA NO SAKUYA HIME* (1929)

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.



Sakura flowers and Mt. Fuji, beautiful spring in Japan.

Reference

- 1) Chikako H., *J. Saitama Junior College.*, **4**, 11-22, 1995.

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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.

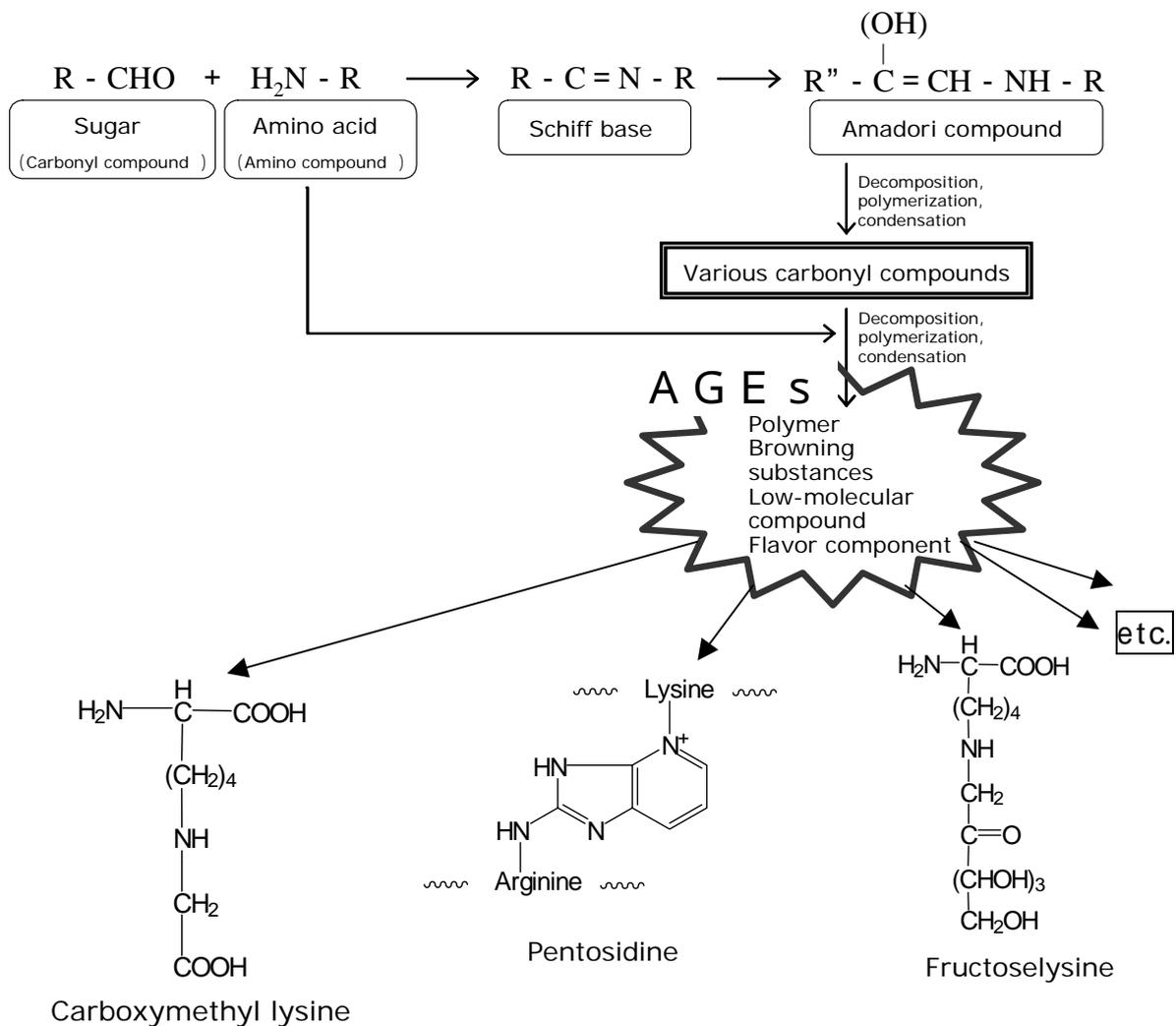


Fig. 1 Maillard reaction and AGEs production

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.

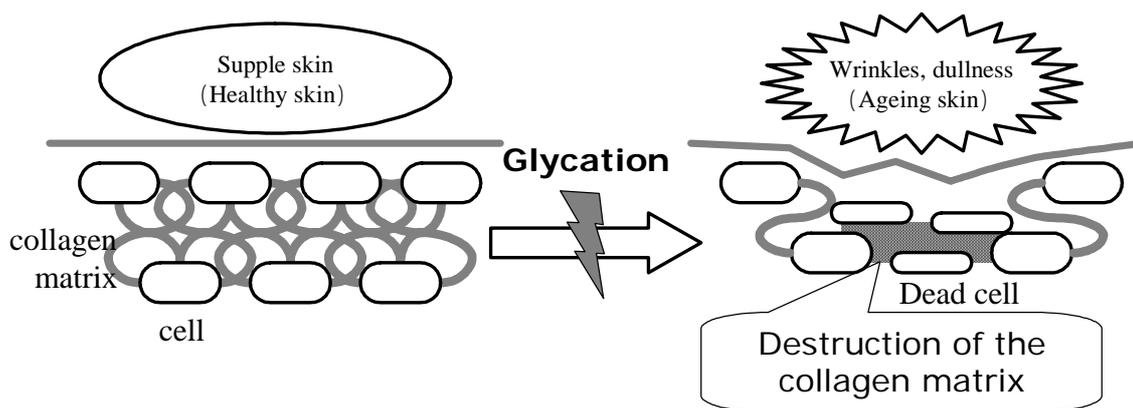


Fig 2. Glycation of skin ageing

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).

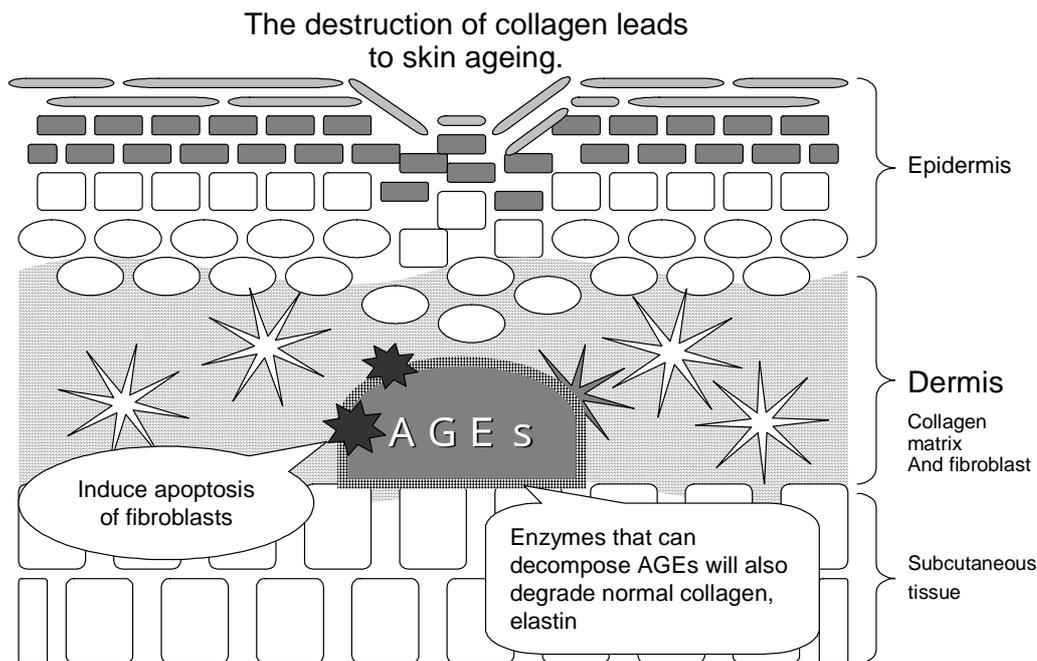


Fig 3. AGEs by adverse effects on skin

An investigation conducted by Dyer *et al.*¹⁾ 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 pentosidine 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.

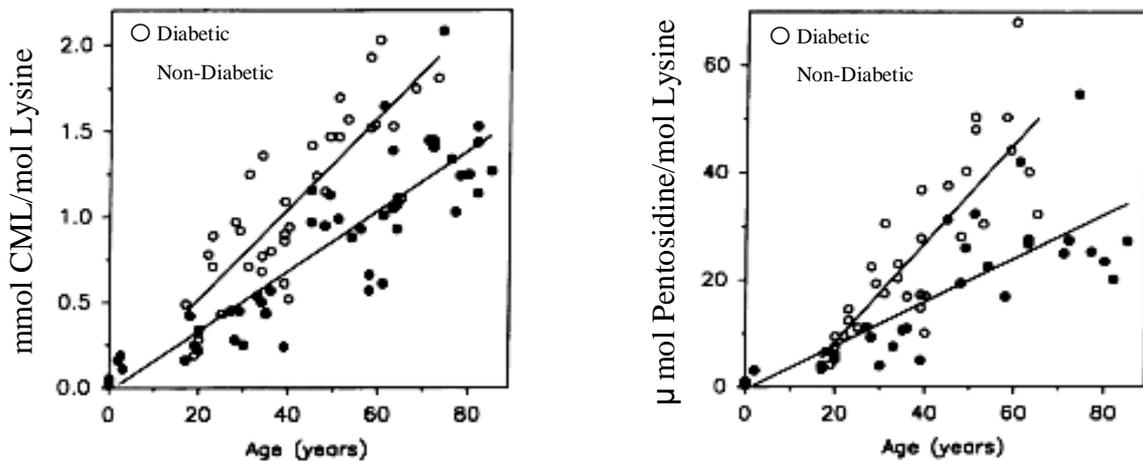


Fig. 4. Accumulation of AGEs in skin collagen of aged and diabetic subjects.

As mentioned earlier, accumulation of AGEs due to glycation of collagen may induce apoptosis in fibroblasts creating a negative impact 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

2) Dyer D.G. *et al.*, Accumulation of maillard reaction products in skin collagen in diabetes and aging. *J. Clin. Invest.*, **91**, 2463-2469, 1993.

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-*O*- β -D-glucopyranoside) and quercetin glucoside (quercetin 3-*O*- β -D-glucopyranoside) as bioactive components of SAKURA EXTRACT for the first time (Fig. 5)

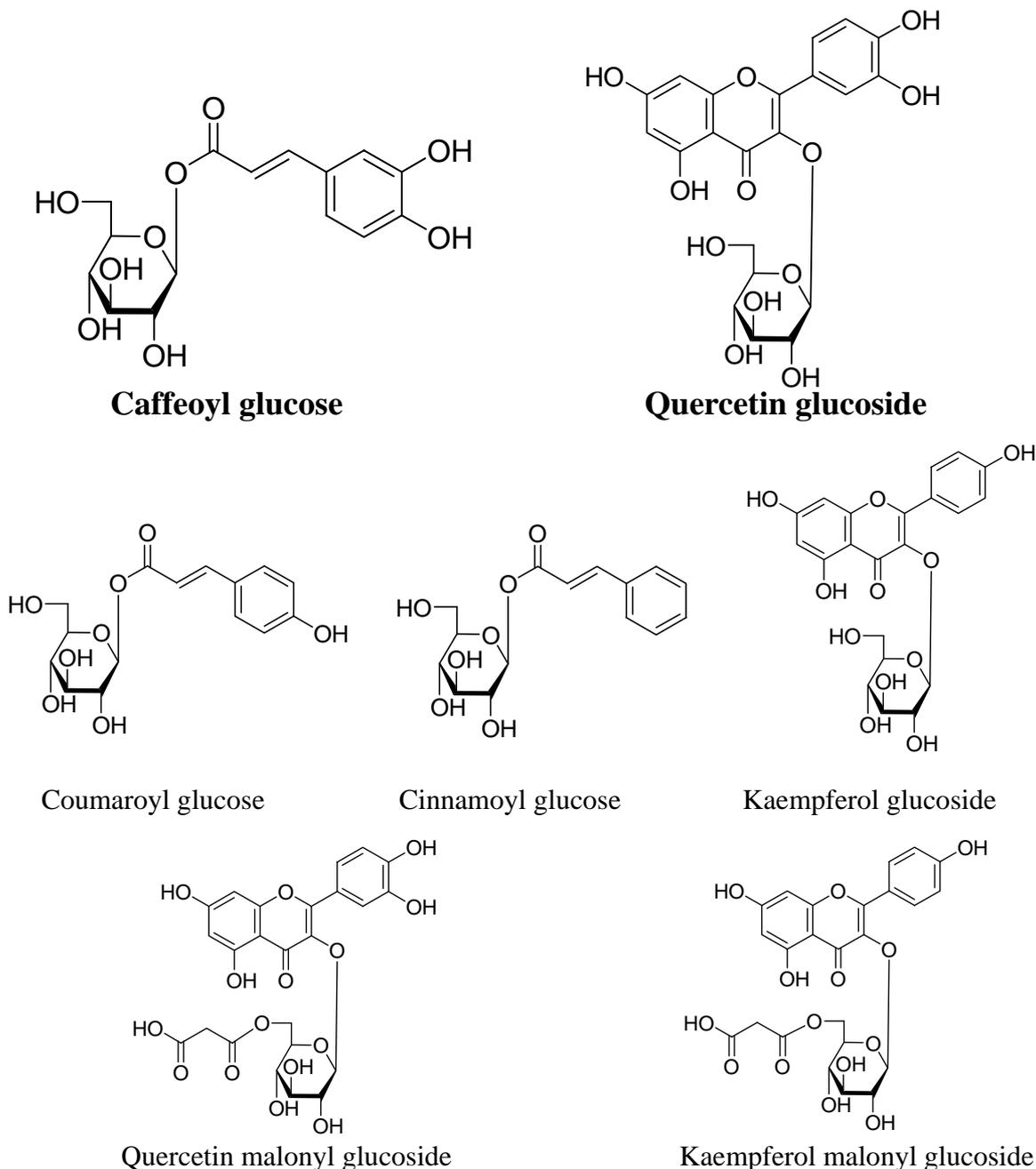


Fig. 5. Bioactive components of SAKURA EXTRACT

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.

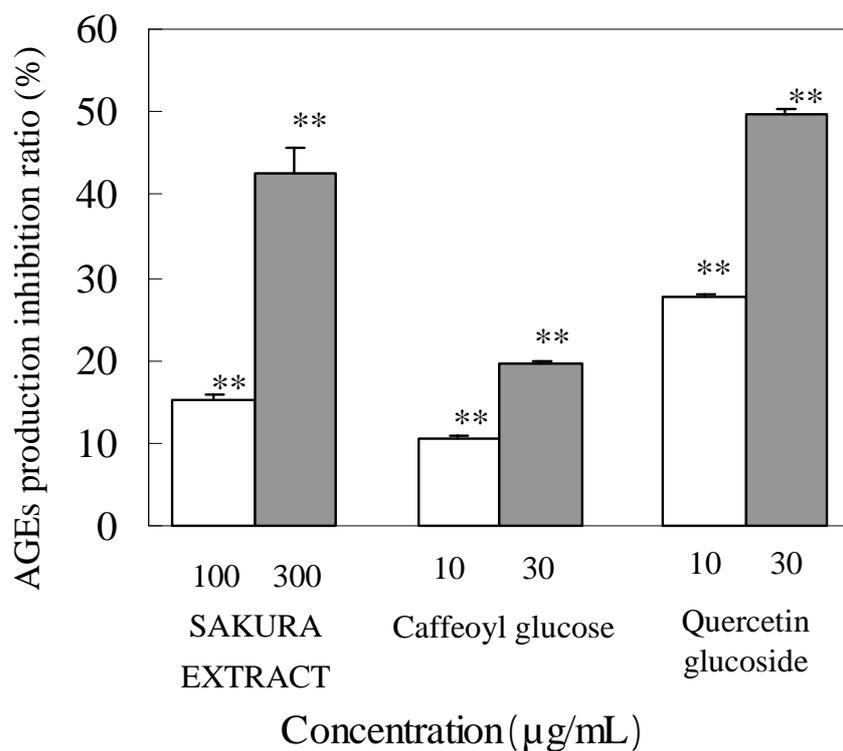
Table 1: The effect of SAKURA EXTRACT and its bioactive components on the production of AGEs

	Inhibition of AGEs production ratio (%)					IC50 (µg/mL)
	3 (µg/mL)	10	30	100	300	
Crude SAKURA EXTRACT	-14.6±0.7	-10.8±0.4	-9.9±0.6	15.1±0.7**	42.6±3.2**	>300
Caffeoyl glucose	0.9±0.1	10.7±0.1**	19.5±0.3**	25.0±0.3**	30.0±0.4**	>300
Coumaroyl glucose	-8.2±0.1**	-8.6±0.1**	-8.9±0.1**	-3.7±0.1**	11.6±0.1**	>300
Cinnamoyl glucose	-10.4±0.1	-10.9±0.4*	-7.8±0.1	5.7±0.1	23.3±0.4**	>300
Kaempferol glucoside	-9.1±0.2**	-2.0±0.1	19.4±0.1**	45.0±0.5**	80.3±0.7**	102
Quercetin glucoside	6.5±0.1*	27.6±0.5**	49.8±0.7**	74.2±1.1**	100.8±0.6**	30
Kaempferol malonyl glucoside	-8.5±0.1	0.2±0.1	20.5±0.3**	50.8±0.4**	91.7±1.7**	78
Quercetin malonyl glucoside	1.9±0.1	20.4±0.3**	43.7±0.7**	74.6±0.7**	103.9±3.6**	36
Caffeic acid	6.0±0.1	17.9±0.3**	19.3±0.5**	0.4±0.1	-5.7±0.1**	-
Coumaric acid	-19.7±0.5	-7.4±0.5	-14.5±0.1	29.9±0.7*	91.8±27.3*	165
Cinnamic acid	-4.9±0.3	1.9±0.1	7.2±0.6	28.2±2.2*	52.7±4.1**	259
Kaempferol	6.9±0.5*	30.4±2.8**	61.5±7.5**	87.5±7.2**	98.2±7.3**	21
Quercetin	-22.2±1.0	4.5±0.1*	38.9±1.4**	96.1±4.3**	-	32
† Aminoguanidine hydrochloride (positive control)	-	1.4±0.1	18.1±1.1	42.6±1.7**	67.7±1.6**	138

† 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 multiple comparison test.

*: $p < 0.05$, **: $p < 0.01$



Mean \pm SD ($n=3$), **: $p < 0.01$ represent.

Fig. 6. Effect of SAKURA EXTRACT and its bioactive components on the production of AGEs

References

- 3) Lee E.H. *et al.*, Inhibitory effect of the compounds isolated from *Rhus verniciflua* on aldose reductase and advanced glycation endproducts. *Biol. Pharm. Bull.*, **31**, 1626-1630, 2008.
- 4) Nakamura K. *et al.*, Acid-stable fluorescent advanced glycation end products: Vesperlysines A, B, and C are formed as crosslinked products in the Maillard reaction between lysine or proteins with glucose. *Biochem. Biophys. Res. Commun.*, **232**, 227-230, 1997.

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

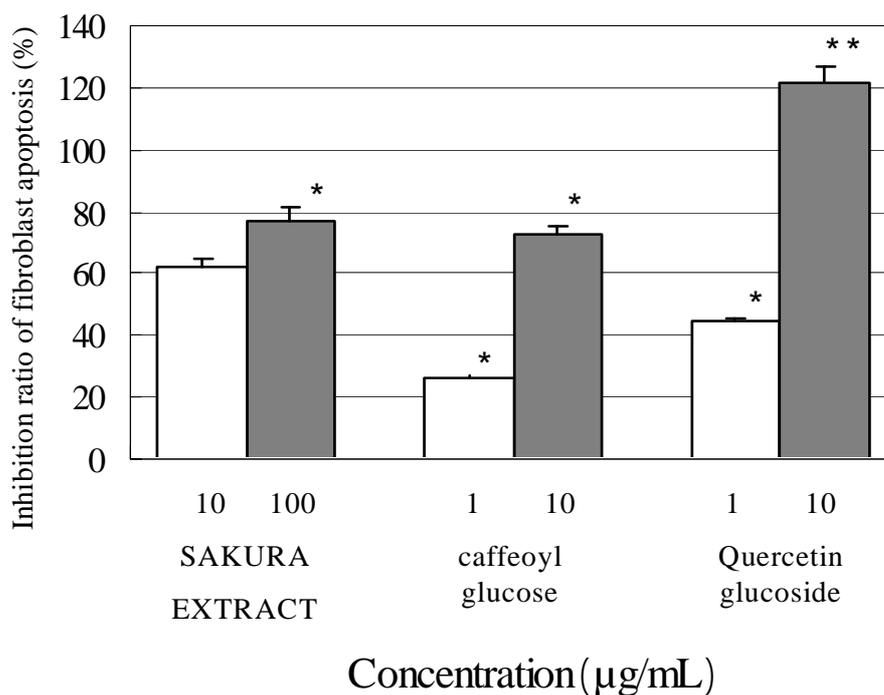
	Inhibition rate of apoptosis (%)			
	1 ($\mu\text{g/mL}$)	3	10	100
SAKURA EXTRACT	-	-	61.8 \pm 2.6	77.1 \pm 4.2*
Caffeoyl glucose	26.2 \pm 0.5*	37.6 \pm 1.2	72.2 \pm 2.7*	-
Coumaroyl glucose	17.2 \pm 0.5	7.1 \pm 0.2	51.1 \pm 1.9	-
Cinnamoyl glucose	-11.8 \pm 0.3	19.7 \pm 0.9	48.6 \pm 2.9	-
Kaempferol glucoside	-0.7 \pm 0.1	27.9 \pm 1.1	100.7 \pm 4.2	-
Quercetin glucoside	44.2 \pm 1.5*	39.0 \pm 1.1*	121.5 \pm 5.4**	-
Kaempferol malonyl glucoside	-18.9 \pm 0.6	-17.3 \pm 0.6	10.5 \pm 0.5	-
Quercetin malonyl glucoside	21.8 \pm 0.7	36.6 \pm 1.4	98.4 \pm 4.4*	-
† Aminoguanidine hydrochloride (positive control)	-	-	-	104.8 \pm 34*

† 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

- 5) Alikhani Z. *et al.*, Advanced glycation end products enhance expression of pro-apoptotic genes and stimulate fibroblast apoptosis through cytoplasmic and mitochondrial pathways. *J. Biol. Chem.*, **280**, 12087-12095, 2005.



Mean \pm SD ($n=3$), **: $p < 0.01$

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 maintaing extracellular collagen matrix.

References

- 6) Kueper T. *et al.*, Vimentin is the specific target in skin glycation. *J. Biol. Chem.*, **282**, 23427-23436, 2007.

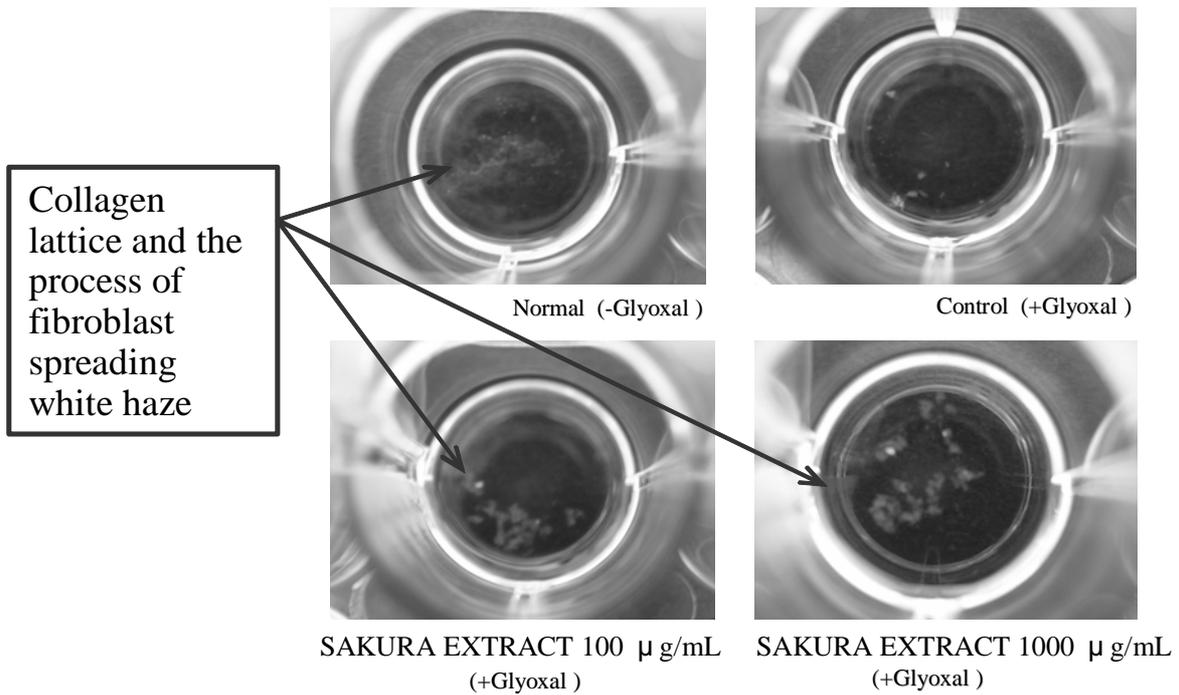


Fig 8. Macroscopic images of wells after 24-hour co-incubation

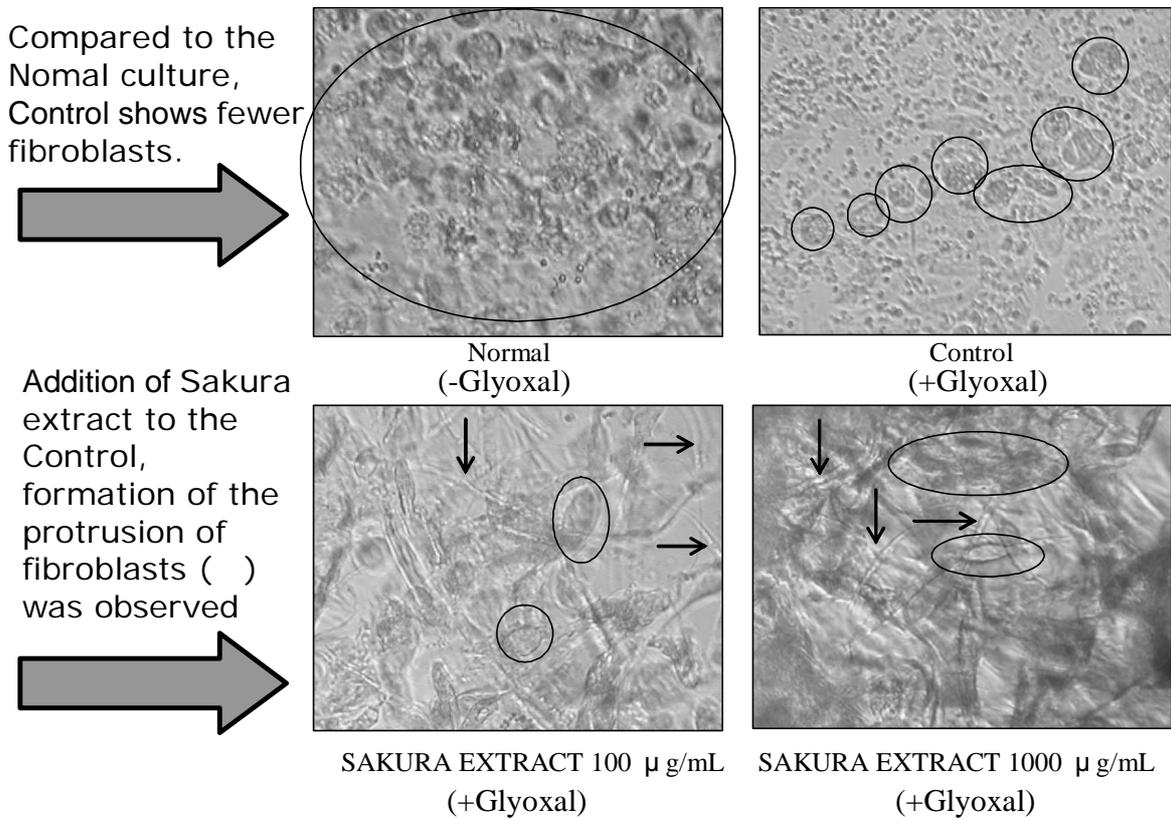


Fig 9. Macroscopic images of wells after 24-hour co-incubation

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^{7, 8}. As illustrated in Fig. 10, SAKURA EXTRACT at 10 μ g/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 μ g/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.

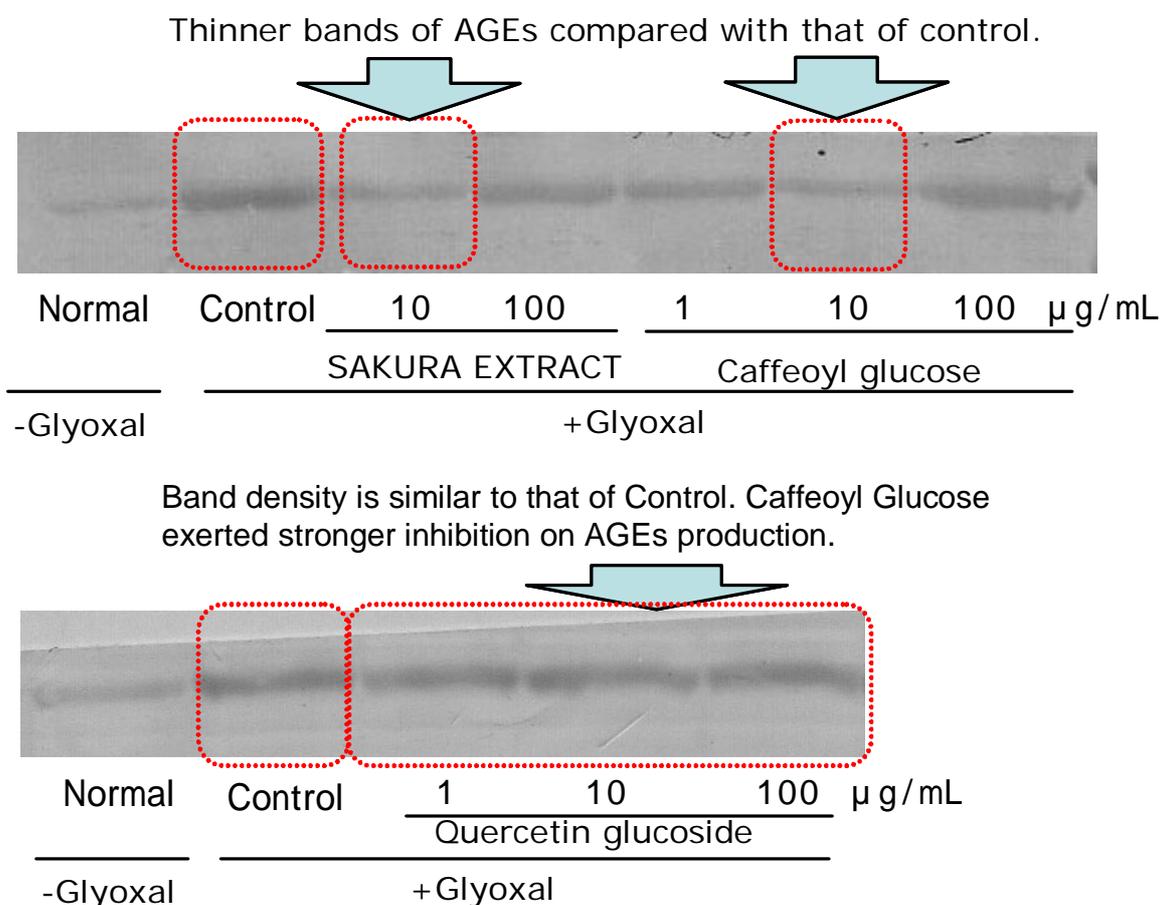


Fig. 10. Effect of SAKURA EXTRACT and its components on AGEs production in normal human fibroblasts

References

- 7) Kueper T. *et al.*, Vimentin is the specific target in skin glycation. *J. Biol. Chem.*, **282**, 23427-23436, 2007.
- 8) Cantero A.V. *et al.*, Methylglyoxal induces advanced glycation end product (AGEs) formation and dysfunction of PDGF receptor- β : implications for diabetic atherosclerosis. *FASEB J.*, **21**, 3096-3106, 2007.

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

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

* 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.

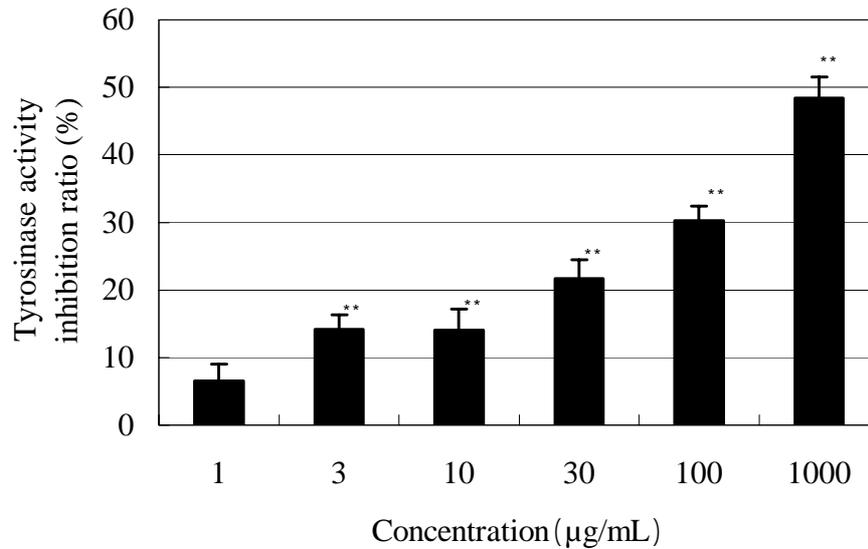


Fig 11. Effect of SAKURA EXTRACT on tyrosinase activity (mean±S.D., $n=5$, **: $p<0.01$)

3-8. Collagen production and matrix formation

We added SAKURA EXTRACT (30, 100 $\mu\text{g/mL}$) 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 $\mu\text{g/mL}$, Fig. 12 upper). Cellular type I collagen was also increased (Fig. 12, lower).

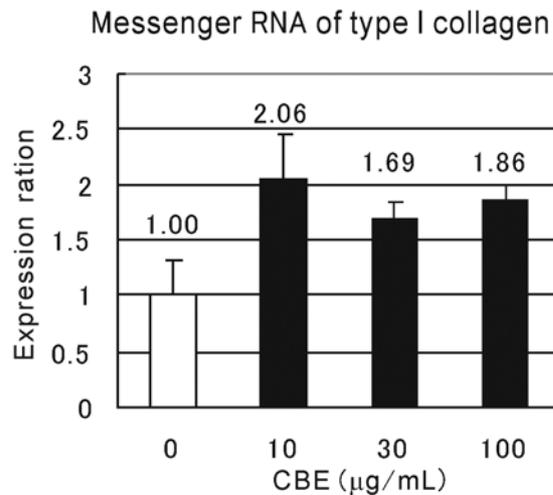
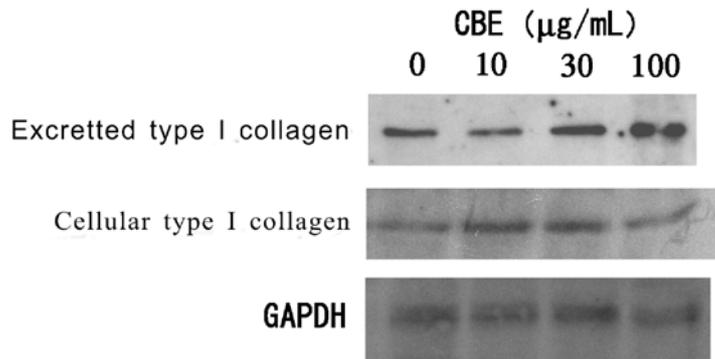


Fig. 12 Enhancement of type I collagen production in fibroblasts by SAKURA EXTRACT (CBE).

Upper: protein of type I collagen, Lower: mRNA of type I collagen (mean \pm SE, n=4)

In dermis, extracellular components including collagen forms extracellular matrix. By addition of collagen to fibroblast, ring 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.

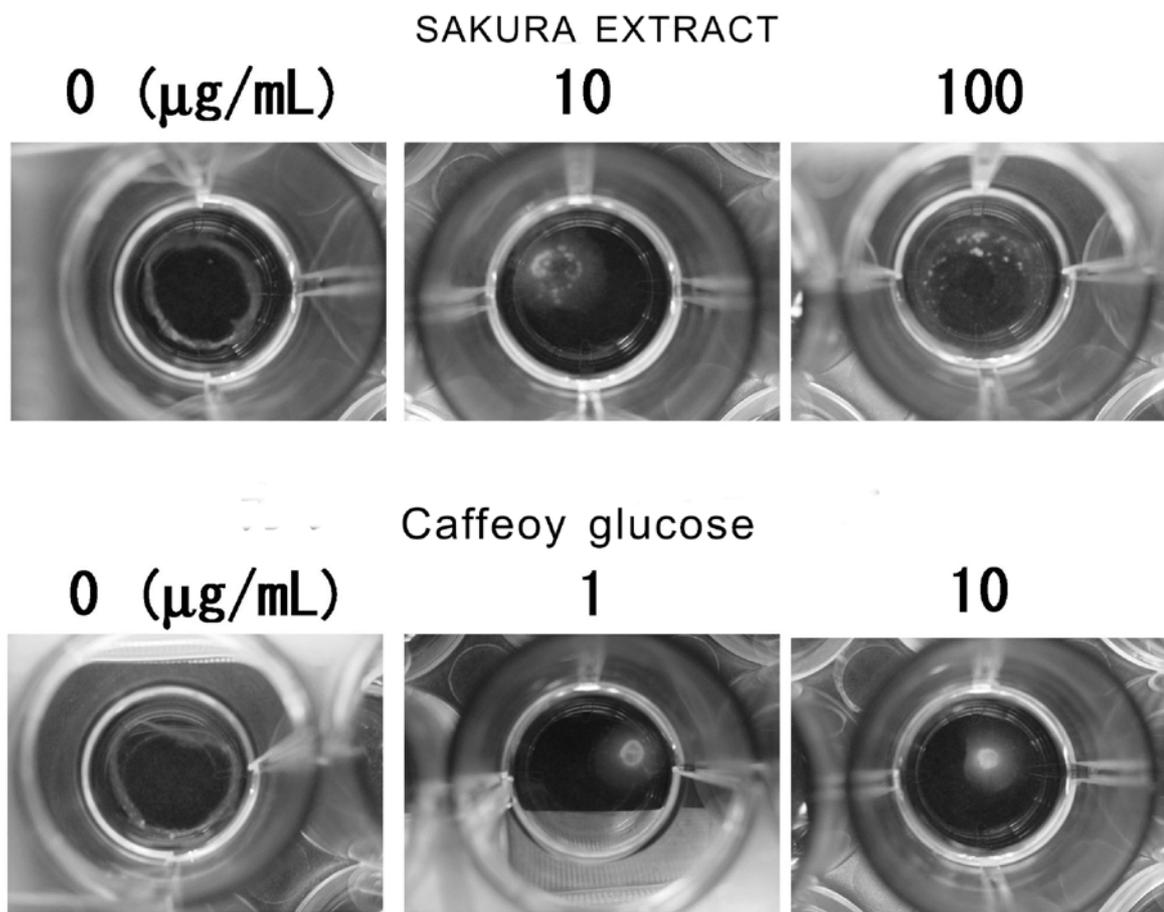


Fig. 13 Enhancement of matrix (collagen lattice) formation by SAKURA EXTRACT and caffeoyl glucose.

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 ~ 100 $\mu\text{g}/\text{mL}$) suppressed NO production induced by LPS. Caffeoyl glucose also suppressed NO production at 1 to 100 $\mu\text{g}/\text{mL}$ (Fig. 14).

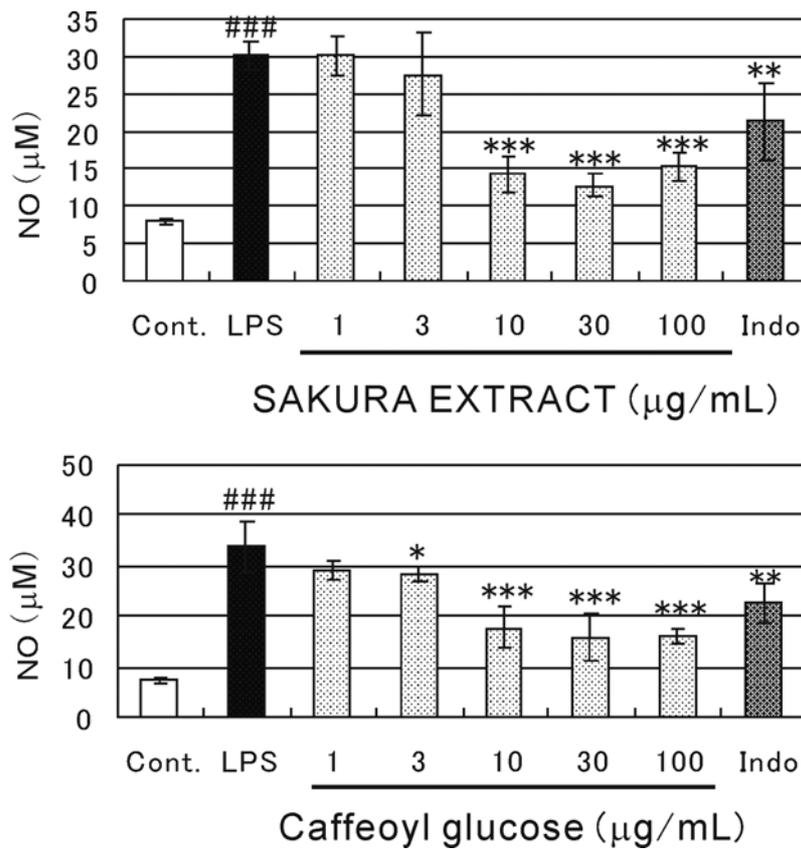


Fig. 14 Suppressive effect of SAKURA EXTRACT on NO production

- ### : vs Cont., $p < 0.001$
- ** : vs LPS, $p < 0.01$
- *** : vs LPS, $p < 0.001$
- Indo : Indomethacin (8.9 $\mu\text{g}/\text{mL}$), $n = 5-6$

3-10. 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.

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.

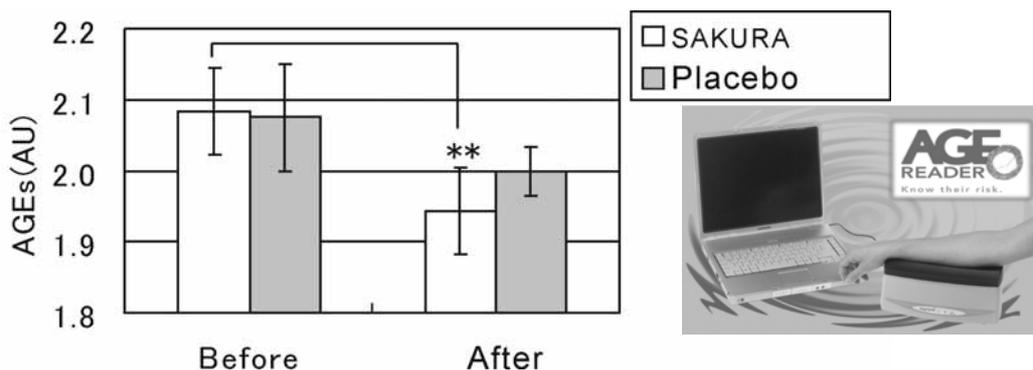


Fig. 15 Change in skin fluorescent AGEs (**: $p < 0.01$)

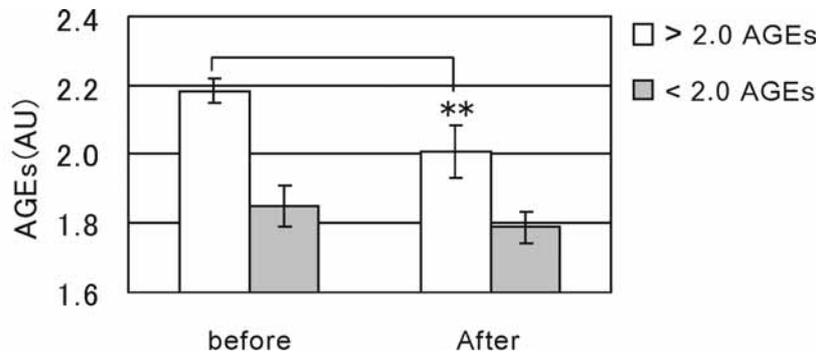


Fig. 16 Classified analysis of change in skin fluorescent AGEs (**: $p < 0.01$)

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.

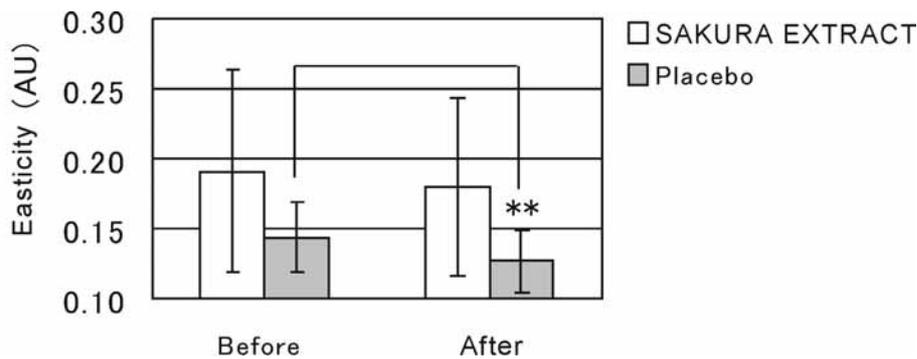


Fig. 17 Change in elasticity (**: $p < 0.01$)

Pigmentaion and reddish are

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) .

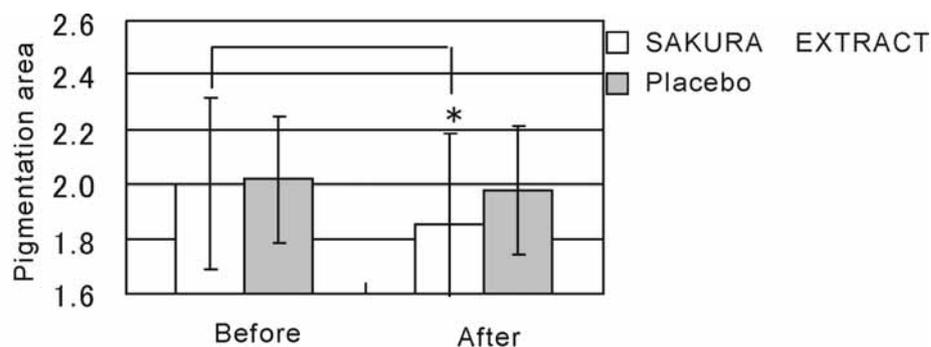


Fig. 18 Change in pimentated area (*: $p < 0.05$)

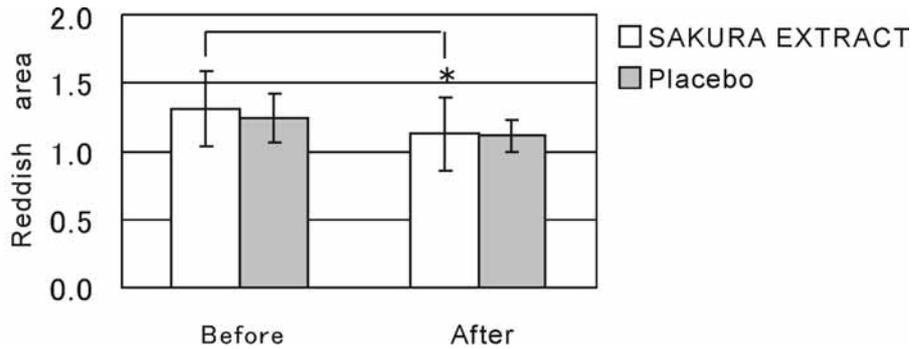


Fig. 19 Change in reddish area (*: $p < 0.05$)

Pores area

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.

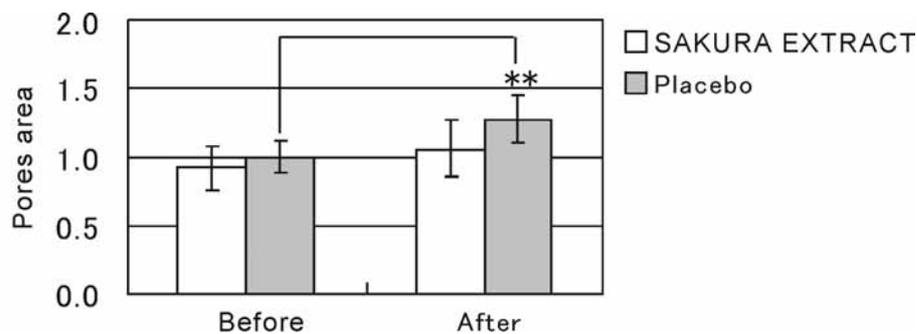


Fig. 20 Change in pores area (**: $p < 0.01$)

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.

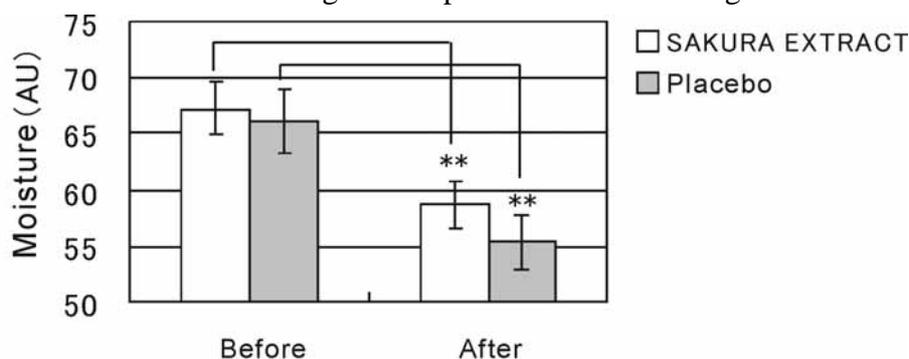


Fig. 21 Change in skin moisture (**: $p < 0.01$)

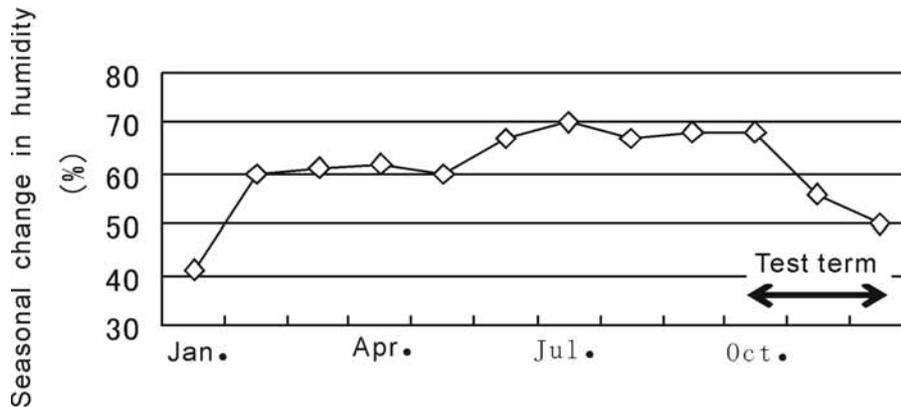


Fig. 22 Change in humidity (Tokyo 2010)

Smoothness of skin

Smoothness scores are significantly improved in both groups. Increase ratio in SAKURA EXTRACT group is superior than placebo group.

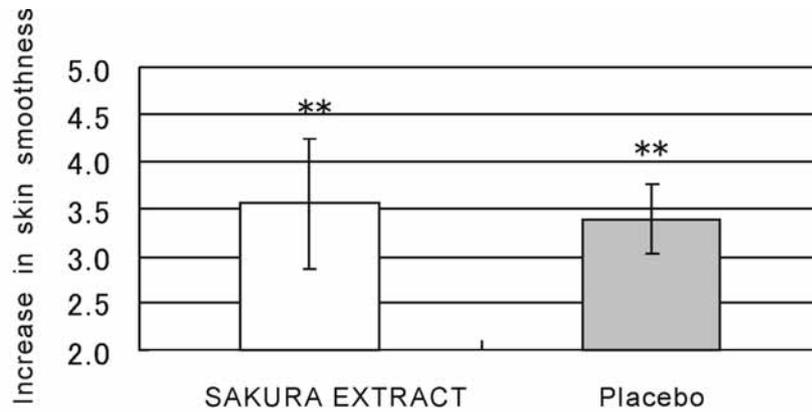


Fig. 23 Change in skin smoothness (**: $p < 0.01$)

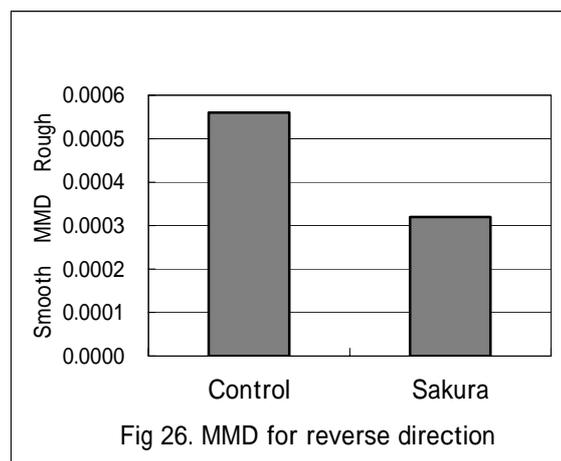
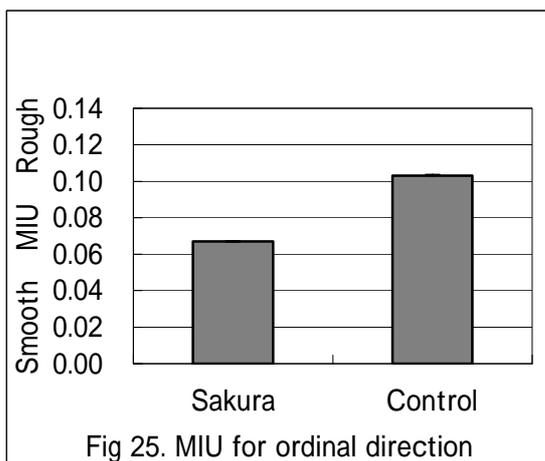
9) H.L. Lutgers et. al., Diabetes Care, December 2006

3-11. Hair Care

We evaluated the hair care effect of SAKURA EXTRACT as following procedure. Human hair was treated with 2% surfactant solution (40°C, 30 min) and washed with running water. The hair was dried to be the normal hair. The normal hair was treated with 1% ammonia solution and 3% H₂O₂ for 40 min at 30°C. The procedure was repeated for 3 times to prepare the damaged hair. The damaged hair was soaked in 1% SAKURA EXTRACT solution for 10 min at 40°C and washed followed by dried with a towel and a drier. The procedure was repeated for 10 times and appearance of hairs was compared.

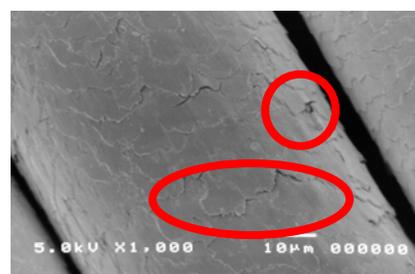
Sense of touch

The 20 hairs were fixed onto a glass plate and the mean of coefficient of friction (MIU) and the change of MIU on the hair surface was measured. As a result, as shown in Fig. 25 and 26, SAKURA EXTRACT improved MIU and MMD. Thus, SAKURA EXTRACT was found to suppress increase in roughness of hair surface based on less damage of cuticle.

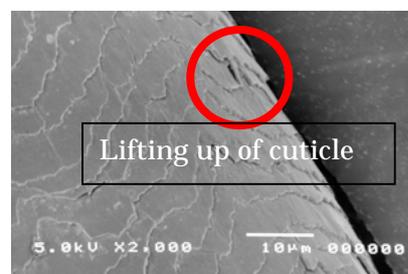


Scanning electron microscopic (SEM) analysis

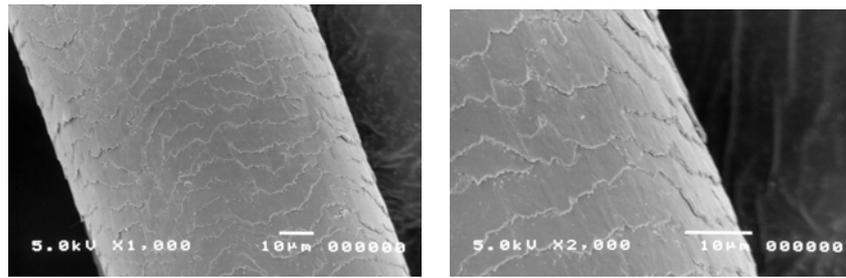
SEM analysis was performed to check the effect on cuticle. As a result, as shown in Fig. 27, the lifting up of the cuticle was confirmed by the treatment of SAKURA EXTRACT.



Control × 1000



Control × 2000



Sakura × 1000

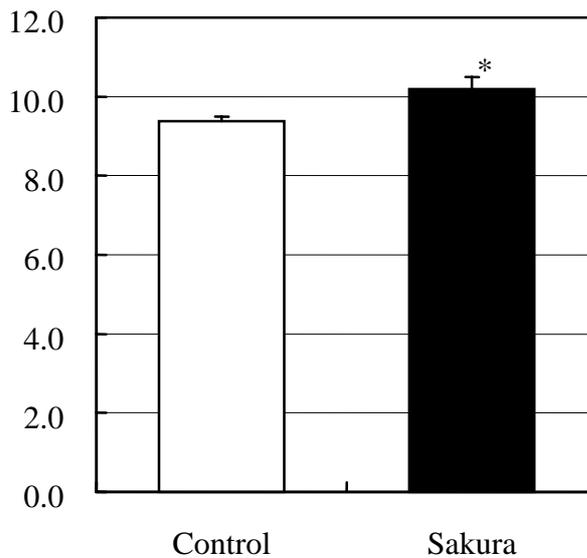
Sakura × 2000

Fig. 27. SEM analysis

Moisture

We measured the secondary evaporating water loss of the hairs. The value indicates the retained water in hair. As a result, the water loss of the hair was significantly improved by the treatment of SAKURA EXTRACT (Fig. 28). Thus, the moisturizing effect of SAKURA EXTRACT was confirmed.

Ratio of water loss (%)



*p < 0.05 (paired t-test)

Fig. 28. Moisturizing effect of SAKURA EXTRACT

Settlement appearance of hair bundle

After brushing of hair bundle (47% R.H., 20°C), we evaluated the settlement appearance. By the treatment of SAKURA EXTRACT, the appearance was improved.

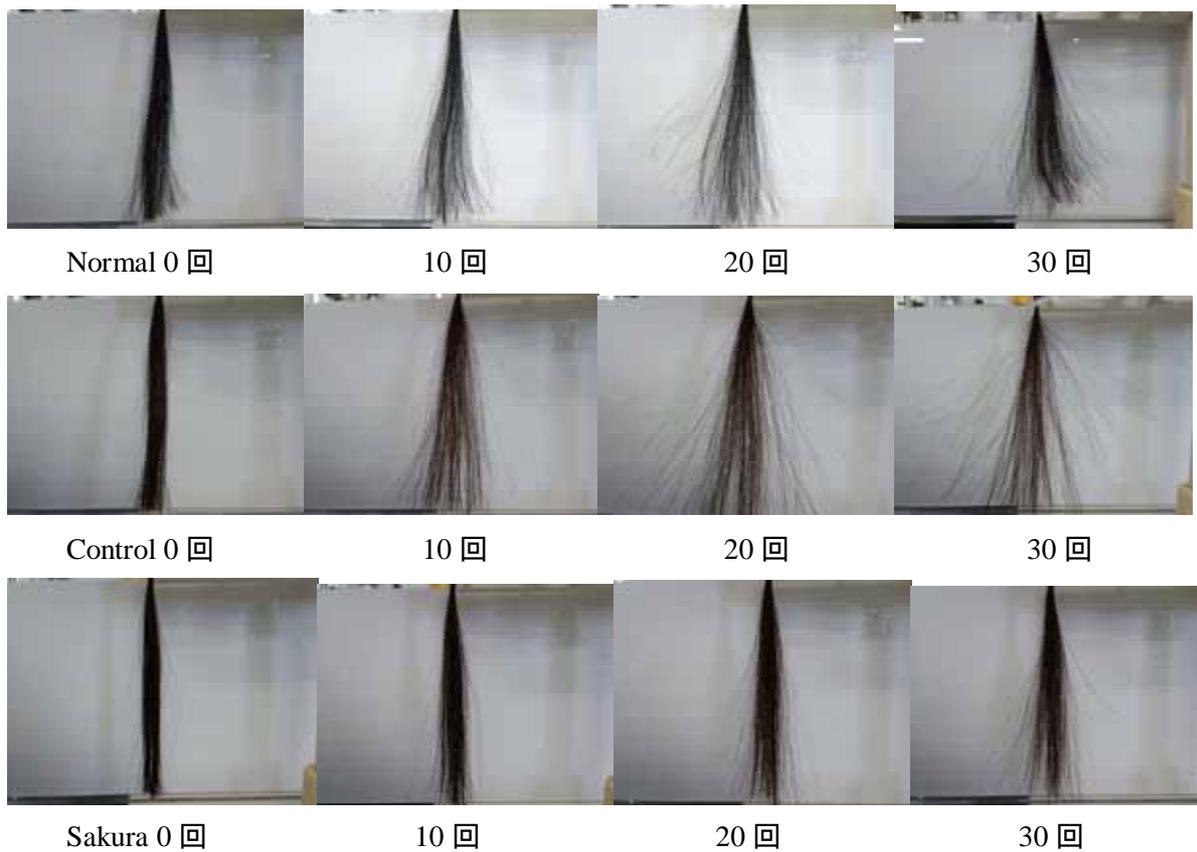


Fig. 29 Appearance of the hair bundle after brushing (回 : brushing times)

Fig. 30 shows the wide of hair bundle. By the treatment of SAKURA EXTRACT, the looseness of the bundle was improved.

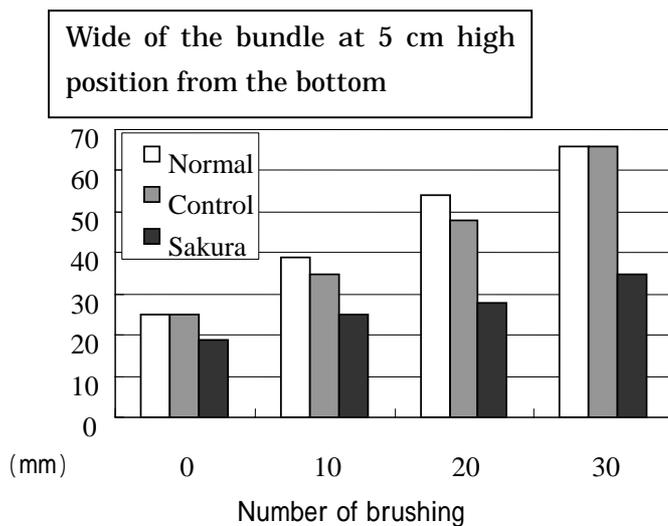


Fig. 30. Wide of hair bundle

3-12. Visualized Data of Anti-glycative Effect

To visualize the anti-glycative effect of SAKURA EXTRACT, we performed additional experiments. Fig. 31 illustrated suppressive effect of the extract on fluorescent AGE production described in Table 1 (page 8). By addition of SAKURA EXTRACT, the intensity of fluorescence was found to be suppressed.



Fig. 31. Anti-glycative effect of SAKURA EXTRACT
 Left: non-glycated, middle: glycated, right: glycated with SAKURA EXTRACT

Dermal AGEs increase toward aging and are thought to cause loss of skin elasticity and yellowish change. AGEs also exist in epidermis and are thought to induce roughness of surface skin and stiff skin. Therefore, study about epidermal AGEs is important to clarify the action of AGEs in aging skin (Fig. 12).

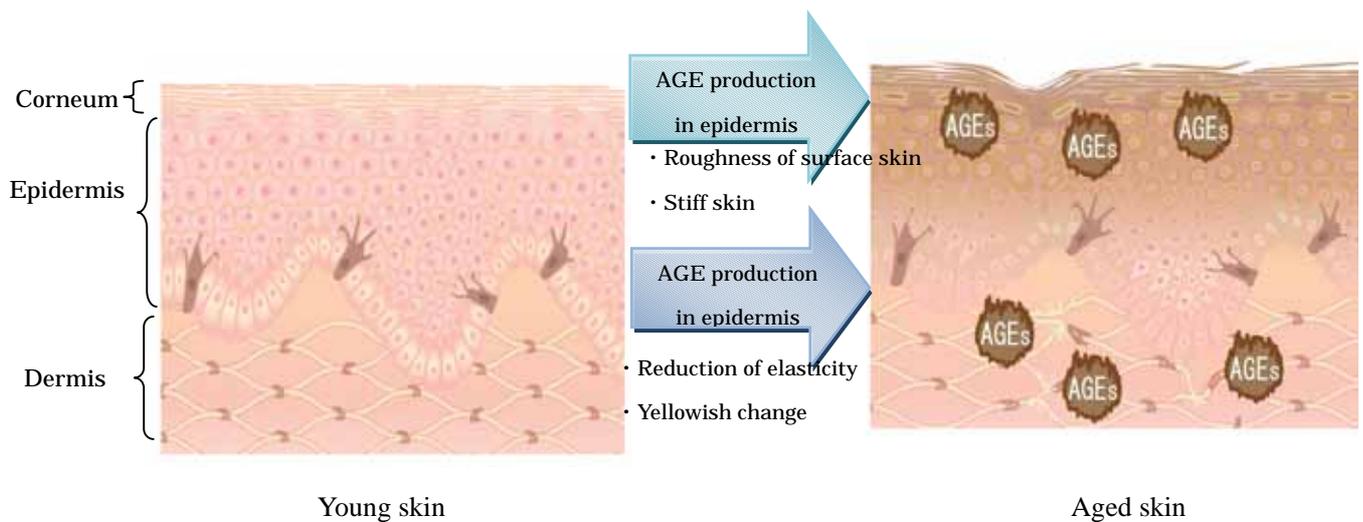


Fig. 12. Skin AGEs in young skin and aged skin

We collected corneum from male subject aged 60 years old and evaluated the effect of SAKURA EXTRACT on accumulated AGEs. The corneum was soaked in the solution of SAKURA EXTRACT and AGEs were detected by anti-AGEs antibody. As a result, the amount of AGEs was found to be decreased by the treatment of SAKURA EXTRACT (Fig. 33).

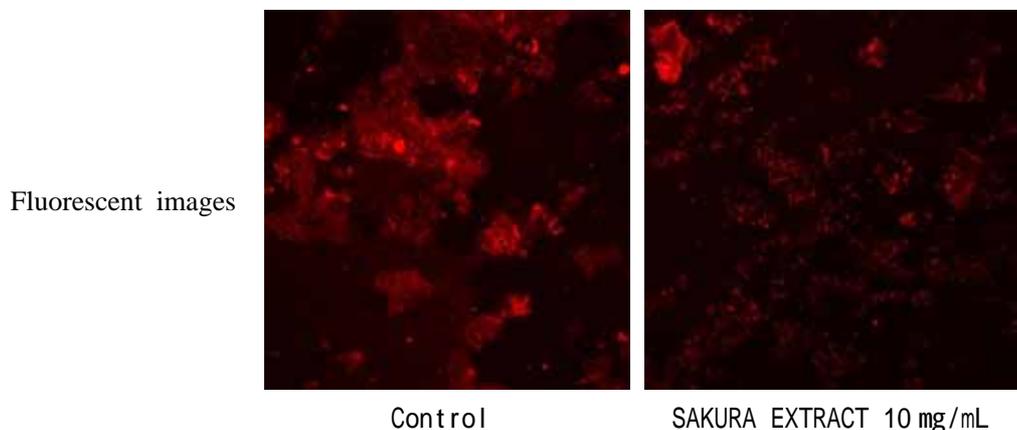


Fig. 33. Effect of SAKURA EXTRACT on epidermal AGEs

We collected corneum from female subject aged 30 years old and evaluated the effect of SAKURA EXTRACT on AGEs production. The corneum was treated with glyoxal and SAKURA EXTRACT for 3 days (37°C). AS a result, increased in AGEs by glyoxal was suppressed by the treatment of SAKURA EXTRACT (Fig. 34). Therefore, SAKURA EXTRACT is thought to suppress epidermal AGEs production enhance AGEs reduction.

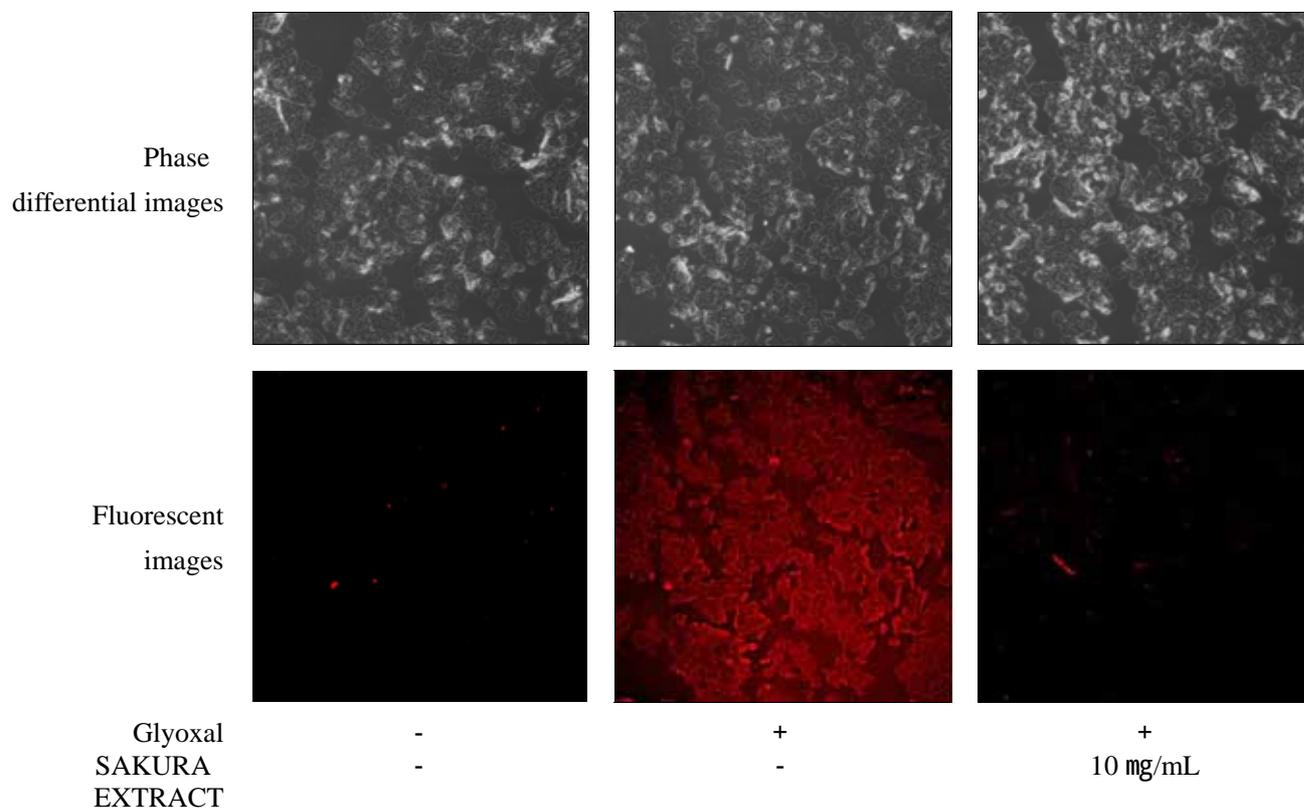


Fig. 34. Effect of SAKURA EXTRACT on epidermal AGEs formation

4. Product 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. 35, the bioactive components remained stable upon heating at 120°C for 1 hour. SAKURA EXTRACT is highly stable upon heating in food processing.

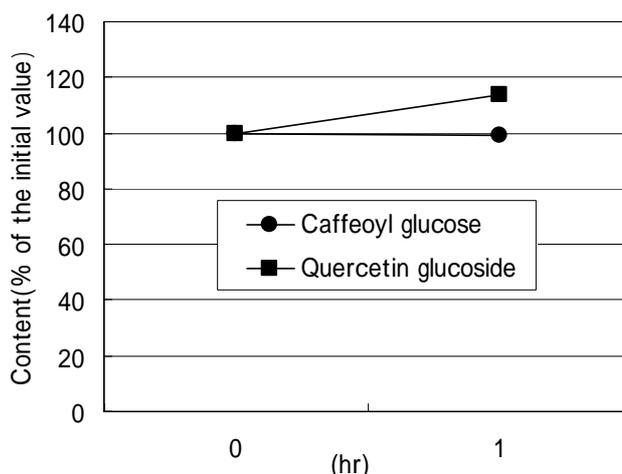


Fig 35. Heat stability of SAKURA EXTRACT-P

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 4. Water solubility of SAKURA EXTRACT-P at different concentrations.

Concentration of SAKURA EXTRACT-P	Neutral (pH6 - 7)		Acidic (pH3)	
	Room Temperature	5°C	Room Temperature	5°C
1%	Good	Good	Good	Good
2%	Good	Good	Good	Fair
5%	Good	Good	Fair	No Good
10%	Fair	No Good	No Good	No Good
20%	No Good	No Good	No Good	No Good

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. 36, bioactive component caffeoyl glucose was highly stable at acidic condition but began to disintegrate at alkaline condition.

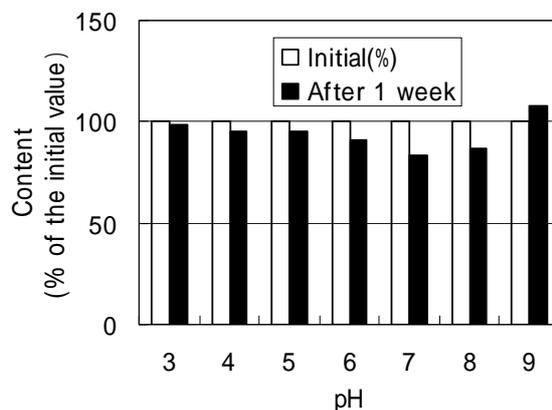


Fig 36. pH stability of caffeoyl glucose

4-4. Stability of aqueous solution

Aqueous solution of SAKURA EXTRACT-P of 0.5% at pH3.5 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.

	Stability of Aqueous Solution (0.5% aqueous solution, pH 3.5)			
	Room Temperature (light)	5°C	25°C	40°C
Precipitation	negative	negative	negative	negative
Turbidity	negative	negative	negative	negative

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. Acute toxicity (LD₅₀)

According to the Guidelines for Single-Dose Toxicity Tests of Pharmaceutical Products, SAKURA EXTRACT (in non-excipient form) was orally administered to male and female mice of the ddY strain (5 weeks old) at a dose of 2,000 mg/kg under a fasting condition, and then they were kept and observed for 14 days. Neither fatality, abnormalities in body weight gain compared to control group, nor macroscopic examinations of the organs in autopsy was observed. Thus, the LD₅₀ value (p.o.) of SAKURA EXTRACT was estimated to be over 2,000 mg/kg in both male and female mice.

5-3. 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.

5-4. 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-1 α and EVCAM was lower than the standard Risk Phase R38. SAKURA EXTRACT was considered as non-skin-irritating.

5-5. 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.

5-6. 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.

5-7. 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

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

- 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 + maltotetraitol), dietary fiber 2, sorbitol 3; maltitol 2; mannitol 2; maltotriitol 2; maltotetraitol 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

	Applications	Claims	Examples
Foods	Beauty Food Anti-glycation	1) Anti-glycation 2) Anti-ageing	Beverages (soft drinks etc), hard and soft capsules, tablets, candies, chewing gum, cookies, chocolate wafers, jelly, <i>etc.</i>
Cosmetics	Beauty Anti-glycation		Sunscreen, toner, lotion, body gel, shampoo, conditioner and bath salts, <i>etc.</i>

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

PRODUCT STANDARD

PRODUCT NAME

SAKURA SYRUP

FOOD

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. 1×10³ cfu/g (Analysis for Hygienic Chemists)

Moulds and YeastsMax. 1×10² cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

Composition

<u>Ingredients</u>	<u>Contents</u>
Glutinous starch syrup	99.4 %
Cherry blossom flower extract	0.2 %
Ascorbic acid	0.2 %
<u>Malic acid</u>	<u>0.2 %</u>
Total	100 %

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. 1×10² 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

<u>Ingredients</u>	<u>Contents</u>
Dextrin	69 %
Prunus lannesiana flower extract	25 %
Ascorbic acid	3 %
<u>Malic acid</u>	3 %
Total	100 %

PRODUCT STANDARD

PRODUCT NAME

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 As ₂ O ₃) | 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

Ingredients	Contents
Water	68.76 %
Butylene glycol	30.00 %
Prunus lannesiana flower extract	1.00 %
Ascorbic acid	0.12 %
Malic acid	0.12 %
Total	100.00 %

ORYZA OIL & FAT CHEMICAL CO., LTD. striving for the development of the new functional food materials to promote health and general well-being.

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