Natural Cosmetic Ingredient

Clairju

(Hydrolyzed Prunus Domestica)

New Whitening agent
based on "theory of melanin diet".

ICHIMARU PHARCOS CO., LTD.

318-1 Asagi, Motosu-shi, Gifu 501-0475 JAPAN

Phone : (81) 58 320－1032

Fax : (81) 58 320－1039

http://www.ichimaru.co.jp

Published on June 28, 2005

Revised on December 12, 2005

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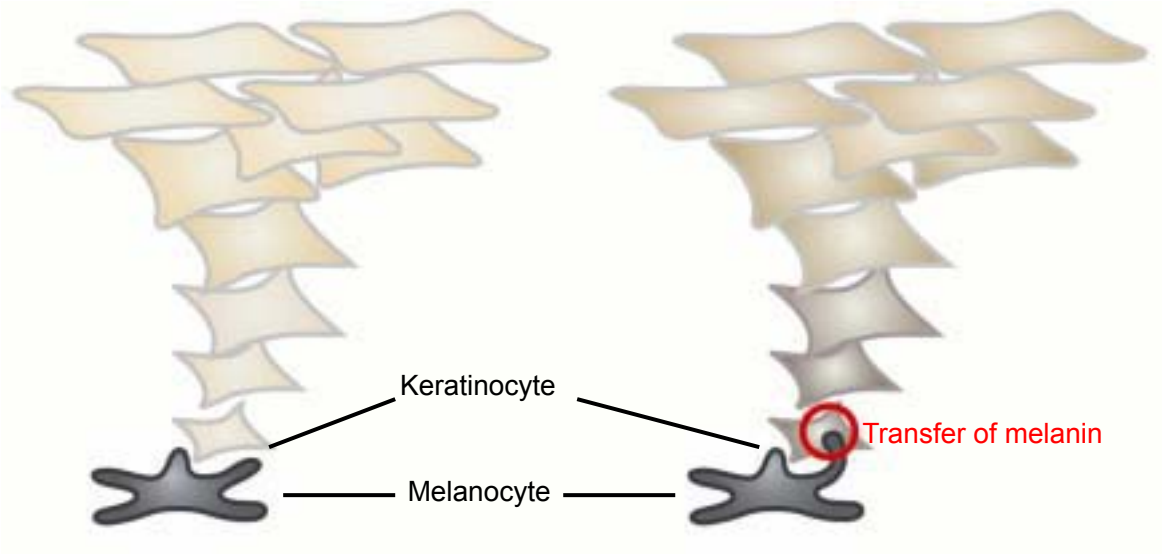
Novel Mechanism for Skin Whitening

What is the real cause for darkening of the skin?

Skin color is not the same among the races. What causes the difference in skin color? The most important factor for skin coloration is melanin pigment, though skin color is also influenced by blood circulation and the skin's surface condition. Melanin is synthesized by melanocyte in the skin. Melanin synthesized by the melanocytes are transferred to neighboring keratinocytes, and keratinocytes containing melanin determine skin color by moving to the skin surface in a 28-day turnover cycle. Skin color is changed mainly by transferring melanin synthesized by the melanocytes to the skin surface. In other words, the cause for the darkening of the skin is the transfer of melanin synthesized by the melanocyte to keratinocyte.



By recent research and technology development, various skin-whitening agents are marketed. A characteristic of most of them is to inhibit the synthesis of melanin.

By nature, however, melanin is synthesized to protect us from sunlight and the accumulation in the skin gives no adverse effect on the human body.

Thus --- Clairju was developed based on such a simple idea, as "Is it possible to make skin white by shielding melanin synthesized from the outside?"

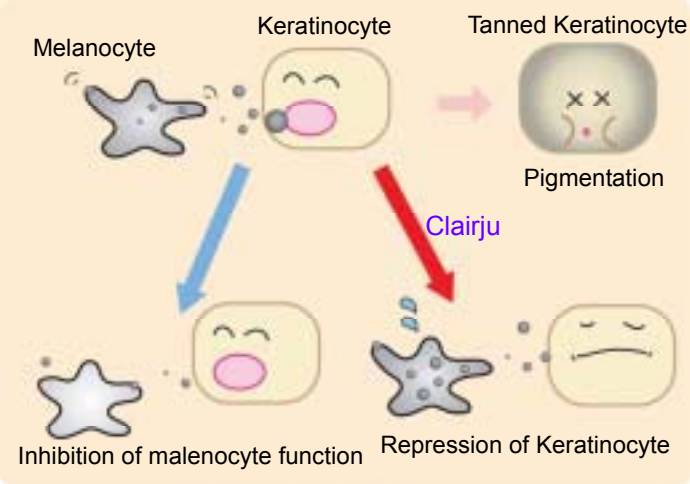
Novel skin-whitening theory, "Melanin diet theory"

Reports about the mechanisms for transferring melanin from the melanocytes to keratinocytes were published recently. As one of the mechanisms, the involvement of phagocytosis by keratinocytes was mentioned. Phagocytosis means that cells engulf foreign substances in the body, they can be said to be the appetite of the cells. Such reaction can be observed frequently in macrophages and leucocytes, which are involved in the defense reaction such as the immune reaction. Keratinocytes also have the phagocytotic action to eat melanin. Keratinocytes keep eating melanin synthesized by neighboring melanocyte. As the result, keratinocyte themselves turn to black. Blackening can be prevented by repressing the "appetite" of keratinocytes.

Clairju is a novel skin-whitening material developed for the "Repression of appetite" of keratinocyte.

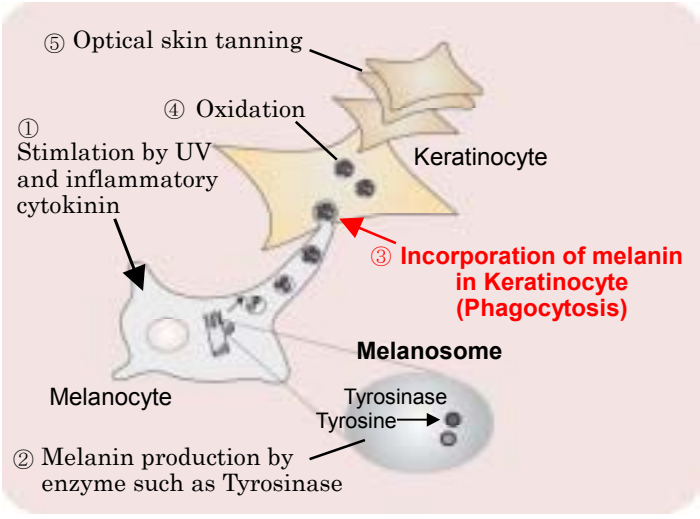
Traditional whitening agents allow skin to become white by "inhibition of melanin production".

Clairju does not allow skin to become black by the "**Repression of appetite**" of keratinocyte.



We recommend Clairju be used together with a traditional whitening agent

Several kinds of whitening agents have been developed. Main mechanisms are "inhibition of tyrosinase", "inhibition of melanin production", "anti-oxidation", "promotion of turn over effect" and "inhibition of mediator (inhibition of inducer of melanin synthesis).



Prune and Clairju [1) 2) 3)]

Clairju is the product, which is obtained by fibrinolysis of fruit pulp of *Prunus cerasifera* (= *Prunus domestica*) Lindel. (*Rosaceae*) harvested in California, U.S.A.

The Prune is a kind of European plum, a plant transplanted from Europe to West Asia. Although this was once called a damson plum, the name prune is now generally used through the recent health care boom. Another species different from "plum", probably a hybrid of *Prunus spinosa* and *Prunus cerasifera*, has also been named plum.

The birthplace of the prune is the Caucasus district internationally known as a longevity district, and it has been said that the prune was spread over Europe as a handy food of traveling a caravans. The history of the cultivation of the prune in Europe is long, and it can be understood that the prune is a very familiar fruit, judging from the fact that the botanical name of *domestica* was used for a poem in ancient Greece meaning "under cultivation."

Prune seedlings were transferred to California by a French gardener in 1856, and the production of the prune in California is now 99% of that in the U.S. and 70% of that in the world. In Japan, the prune was entered into "Honzozufu" and published in the later Edo period by the name of "Aokawasumomo (blue peel prune)," and it has been said that the prune for cultivation was introduced in the early Meiji period. The prune is a deciduous small tree with 7 to 8 m of height, and the leaves are smaller than those of a plum; thick and glossy. The flowers are white in color and 2 cm in diameter and bloom thickly like a bouquet. When the fruit is ripe, the outer cover becomes a beautiful purple and the flesh becomes amber.

The prune fruit contains compounds such as β-carotene, β-cryptoxanthin, lutein, neochlogenic acid as well as vitamins and minerals such as vitamin A, vitamin C, vitamin E, vitamin B group, folic acid, iron, potassium, magnesium, calcium, copper, manganese and zinc.

Clairju is named in the image of "Fruit (extract) makes skin clear" by using Clair and Jus that mean clearness and fruit in French, respectively.

Introduction

Property

Clairju is a product, which is obtained by fibrinolysis of the fruit pulp of *Prunus cerasifera* [*Prunus domestica* Lindel. (*Rosaceae*)]. Clairju is a mixture composed of Hydrolyzed Prunus Domestica, Butylene Glycol and Water.

Efficacy

● New Whitening Efficacy (Inhibition of Uptake of Melanosomes by Keratinocytes)

Clairju inhibited the incorporation of fluorescence-labeled beads into the keratinocytes of human normal epidermis (phagocytosis), suggesting that Clairju could inhibit the transfer of melanosomes containing melanin granules to keratinocytes. Thus, it can be expected that Clairju has a skin-whitening action with a novel mechanism (melanin diet).

● Whitening Efficacy on human skin

Clairju is observed to increase value of skin whiteness by human monitoring test. According to this result, Clairju has whitening effect for human beings.

● Moisturizing effect

Clairju is observed to inhibit TEWL by human monitoring test. According to this result, Clairju is expected to improve the skin barrier effect and increase the moisturizing effect.

● Improvement of skin texture

Clairju is observed to improve rough of Scale and Coarseness of skin surface by human monitoring test. According to this result, Clairju is expected to improve skin texture.

Recommendation

- ***Whitening cosmetic***
- ***Base cosmetic for middle-aged to elderly people***
- ***Improving agent of rough skin***

Inhibition of Uptake of Melanosomes

For the research on the transfer of melanosome to keratinocyte, an alternative method, in which phagocytosis is induced by using pseudomelanosome such as latex beads, has been reported [4].

With reference to these reports, the test was performed in the model system of phagocytosis in the epidermal cells.

1.Evaluation of inhibition of incorporation of fluorescence-labeled beads by fluorescence microscope observation

Test Sample

Clairju is applied 1% and 3% as final concentration in the test. 50% 1,3-Butylene Glycol is used as control.

Test Method

Keratinocyte of human normal epidermis (NHEK, Kurabo Industry) were seeded on chamber (177399, Nunc International K.K.) to make 1 x 10^3 cells/well, and cultivated in a epidermis growth medium (Eplife-KG2, Kurabo Industry) at 37°C under 5% CO_2 condition. Twenty-four hours later, the medium was changed to a fresh medium and added to each test sample, and fluorescence-labeled microbeads (Tetra Speck microsphere, 0.5 μ m, fluorescent, Molecular probes) were added at 2 $\times$ 10^7 cells/well.

After 48-hr cultivation, the cells were washed with PBS (-), and observed by fluorescence microscope observation.

Result

Fluorescence photograph and optical photograph; which samples are applied are shown in Fig. 1. fluorescence-labeled microbeads are observed to incorporate in epidermis an control. But, incorporation of fluorescence-labeled microbeads is actually decreased in epidermis with the application of Clairju.

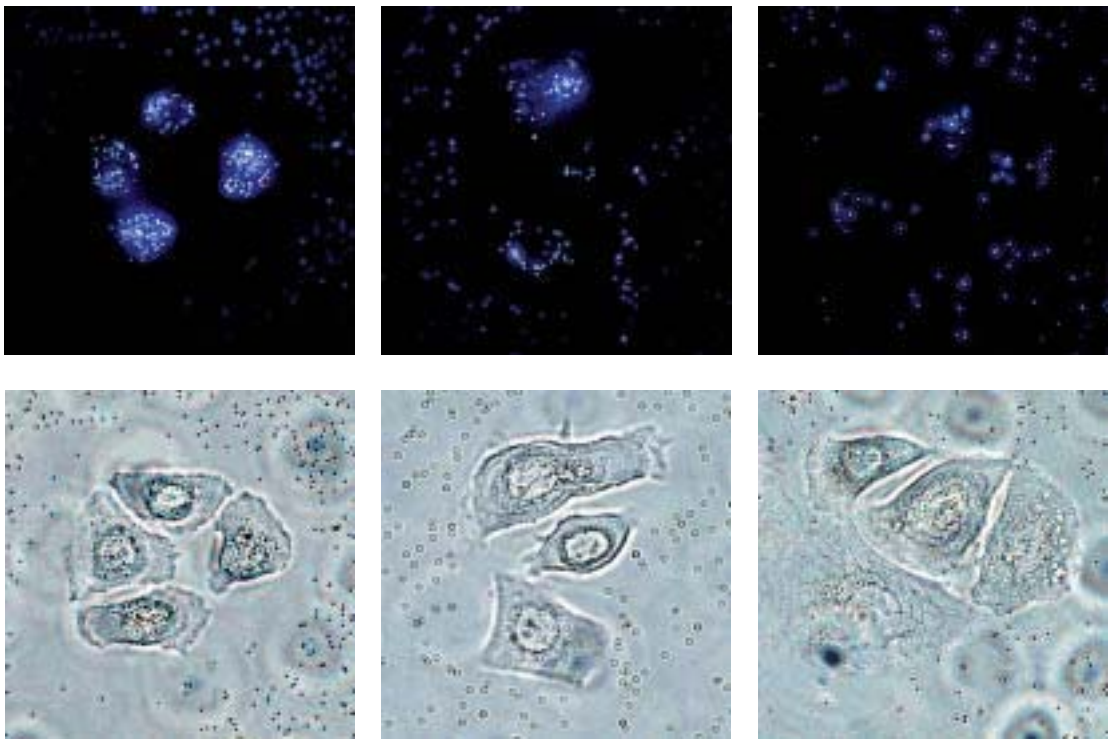


Fig.1: Inhibition of Incorporation of Melanin

Upper photograph is indicated location of fluorescence-labeled microbeads and Lower photograph is indicated location of keratinocyte. According to gathering fluorescence-labeled microbeads around keratinocyte, beads are incorporated in keratinocyte.

2.Evaluation of inhibition of incorporation of fluorescence-labeled beads by measurement of fluorescence intensity.

Test Sample

Clairju is applied 1% and 3% as final concentration in the test. 50% 1,3-Butylene Glycol is used as control.

Test Method

Keratinocyte of human normal epidermis (NHEK, Kurabo Industry) were seeded on a 96-well plate to make 1 × 10^4 cells/well, and cultivated in a keratinocyte growth medium (EpiLite-KG2, Kurabo Industry) at 37°C under 5% CO_2 condition. Twenty-four hours later, the medium was changed to a fresh medium added each test sample, and fluorescence-labeled microbeads (Tetra Speck microspheres, 0.5 μ m, fluorescent, Molecular probes) were added at 5 × 10^6 cells/well.

After 72-hr cultivation, the cells were washed with PBS (–), and the fluorescent intensity was measured at 360 nm of excitation wavelength and 450 nm of fluorescence wavelength by a fluorescent plate reader. At the same time, the number of cells was counted by a WST method to calculate the amount of fluorescence-labeled beads transferred into keratinocyte by the following equation.

Amount of incorporation of fluorescence-labeled beads =

Each fluorescence intensity / number of cells (WST value)

Result and Discussion

Amount of incorporation of fluorescence-labeled microbeads in keratinocyte of each sample are shown in Fig.2. Clairju inhibit the ability to incorporate fluorescence-labeled microbeads in keratinocyte.

According to this result, Clairju has an inhibition of phagocytosis of keratinocyte, and is expected to inhibit the ability to incorporate melanosome and prevent skin pigmentation.

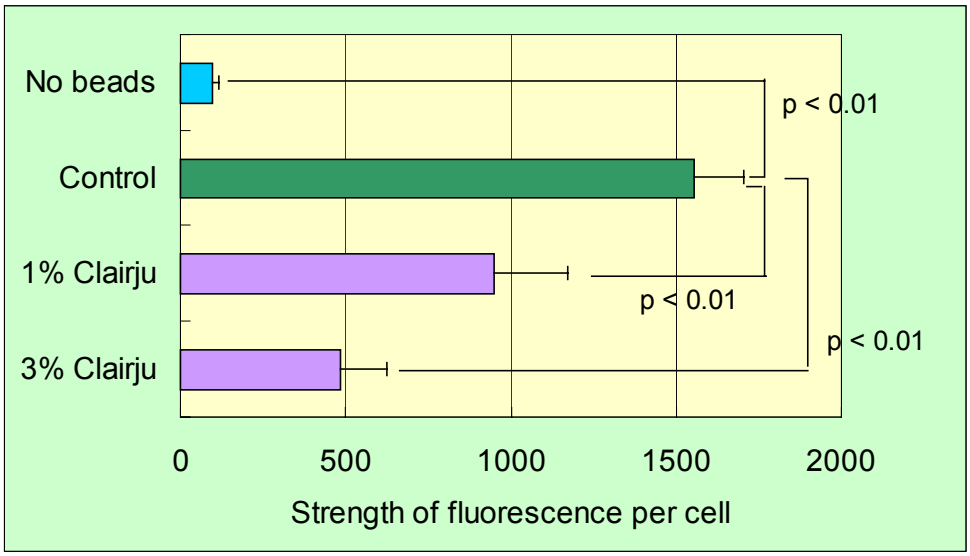


Fig.2: Inhibition of incorporation of fluorescence-labeled beads by measurement

3.Evaluation of the inhibition of uptake of melanosomes in co-cultured melanocytes and keratinocytes

Test Sample

Clair is applied 1% as final concentration in the test. 50% 1,3-Butylene Glycol is used as control.

Test Method

Melanocytes of human normal epidermis (HEM, TOYOBO) were labeled against cell membrane to become red by fluorescence-labeled kit (PKH26 Red Fluorescent Cell Linker Kit, Sigma). And seeded on chamber (177399, Nunc International K.K.) to make 2.5 × 10^4 cells/well, and cultivated in a melanocyte growth medium (Melanocyte Growth Medium, TOYOBO).

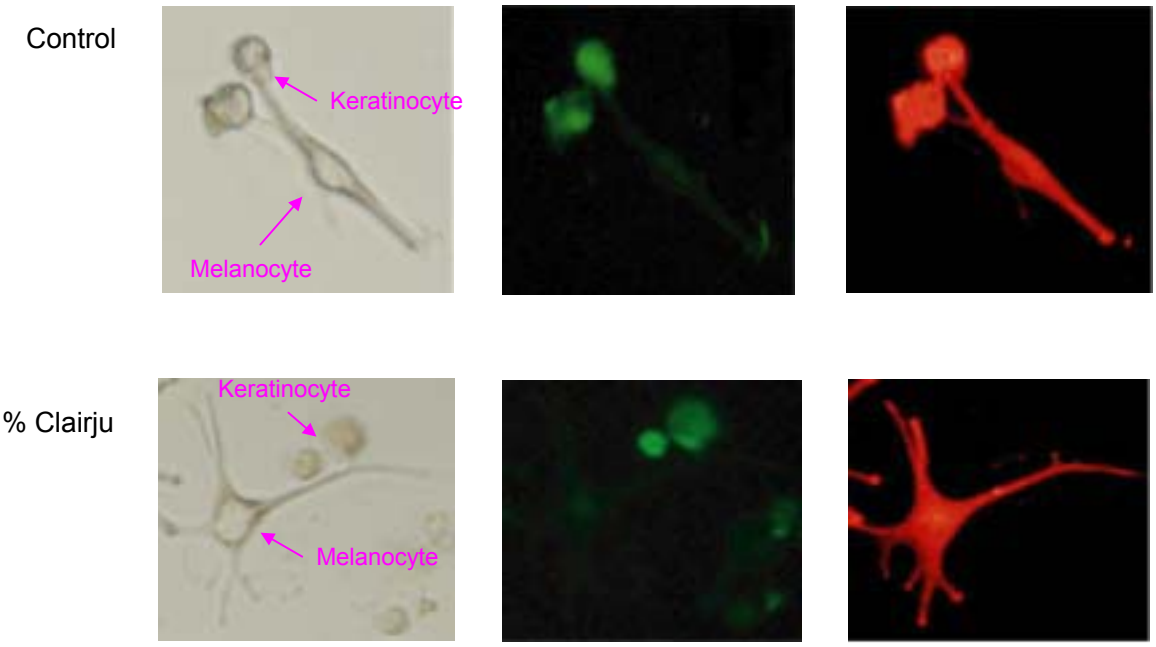
6 hours later, Keratinocytes of human normal epidermis (NHEK, Kurabo Industry) were labeled against cell membrane to become green by fluorescence-labeled kit (PKH67 Green Fluorescent Cell Linker Kit, Sigma) in the same way. And seeded on chamber (177399, Nunc International K.K.) to make 2.5 × 10^4 cells/well, and cultivated in an epidermis growth medium (Eplife-KG2, Kurabo Industry). (Cells and medium adjusted keratinocytes : melanocytes = 1 : 1.)

After 48-hr cultivation, the cells were washed with PBS (-), and observed by fluorescence microscope.

Result

It is known that phagocytosis by keratinocytes is the main process of incorporation of melanosomes. One part of cell membrane of melanocytes is incorporated to melanosome.

According to this result, red fluorescence-labeled was transferred to keratinocytes. Clairju inhibit this transfer.



Keratinocyte: Green Color, Melanocyte: Red Color

Whitening Efficacy on Human Skin

We investigate the whitening effect of Clairju on human skin.

Test Sample

Clairju is diluted 20 times by 50% 1,3-Butylene Glycol, adjusted to 5% solution and use as test sample. 50% 1,3-Butylene Glycol is used as control.

Test Method

The test detail is explained to volunteers in advance, and after confirming consent, they cooperated in these tests. 5% Clairju solution and control was applied on both cheeks twice a day for 3 months. Black (dark) value was measured before application, after application and one to three months later by MEXAMETER MXI8 (Courage + Khazka electronic GmbH, Germany).

Result and Discussion

Transition of melanin index of 5 volunteers is shown in Fig. 3. Melanin index is down on skin applied with Clairju and found that white value of skin goes up from a large point of view.

According to this result, Clairju has a whitening effect on human.

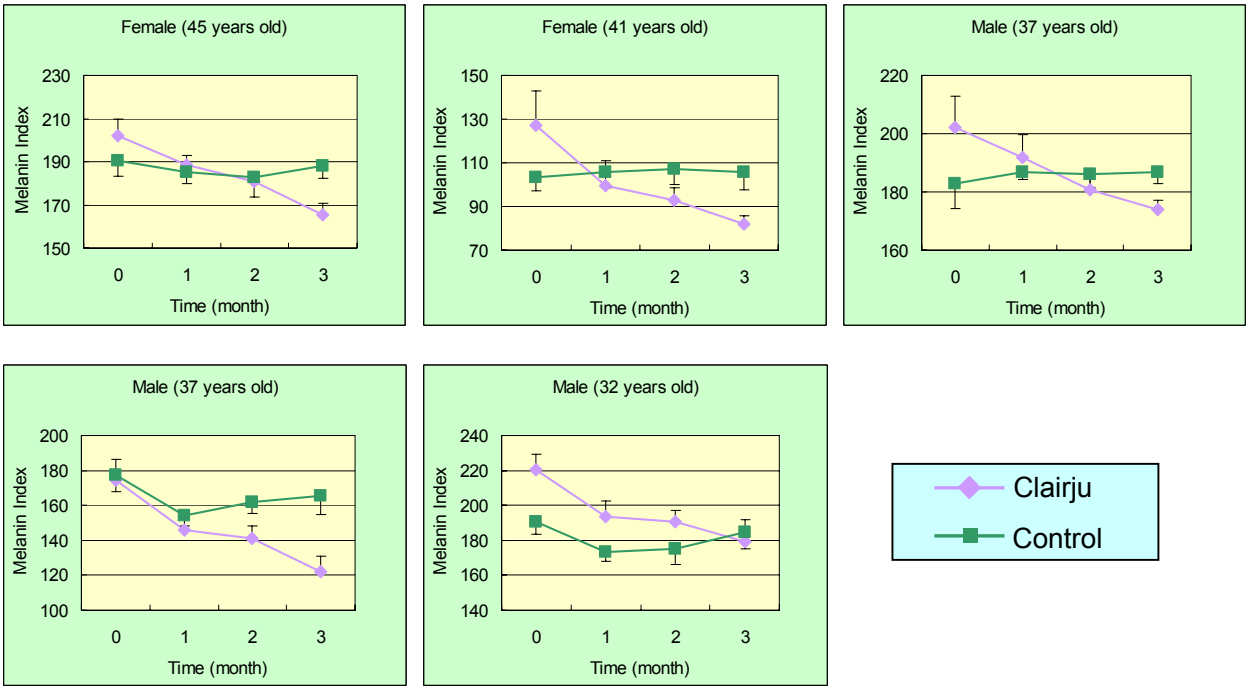


Fig.3: Whitening effect

Moisturizing Effect

It is important to protect from dryness and keep water content in order to protect skin from aging. Water content in the horny layer and trans-epidermal water content loss are measured.

Test Sample

Clairju is diluted 20 times by 50% 1,3-Butylene Glycol, adjusted to 5% solution and used as test sample. 50% 1,3-Butylene Glycol is used as control.

Test Method

The test detail is explained to volunteers in advance, and after confirming by consent, they cooperated in these tests. 5% Clairju solution and control was applied on both cheeks twice a day for 3 months. The Trans Epidermal Water loss (TEWL) value was measured before application, after application and three months later by TEWAMETER TM210 (COURAGE + KHAZAKA Electronic GmbH, Germany). For further detail, the test was done in a constant environmental room at 25 ° C, 50%RH after acclimation for 20 minutes.

Result and Discussion

The change of TEWL value is shown in Fig.4. When Clairju is applied, it is observed that water evaporation from the skin surface is inhibited.

According to this result, Clairju is expected to improve the skin barrier effect and have a superior moisturizing effect.

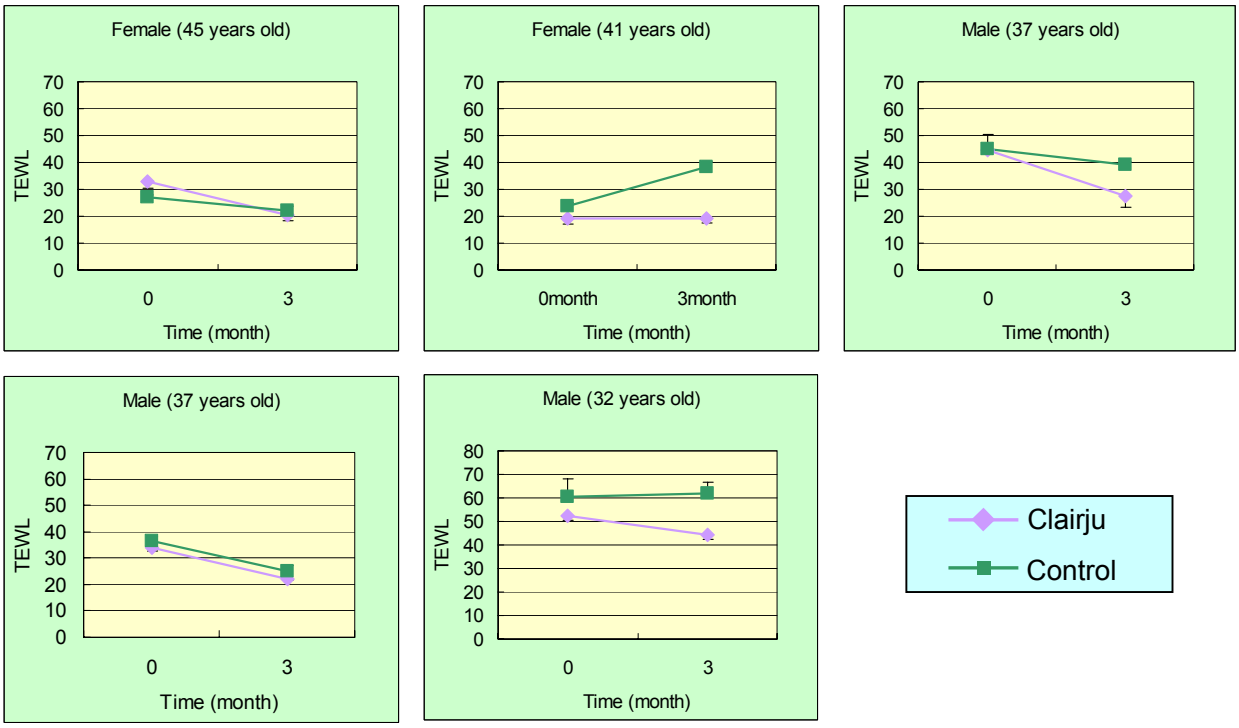


Fig.4: Improvement of barrier effect

Improvement of Skin Texture

We investigate improvement of skin texture by observation of skin surface using VISIOSCAN (COURAGE + KHAZAKA Electronic GmbH, Germany)

Test Sample

Clairju is diluted 20 times by 50% 1,3-Butylene Glycol, adjusted to 5% solution and used as test sample. 50% 1,3-Butylene Glycol is used as control.

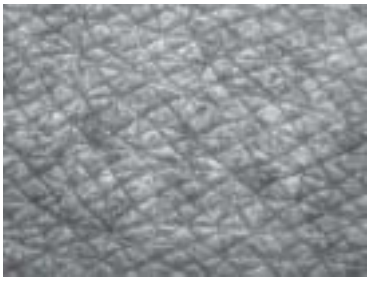
Test Method

The test detail is explained to volunteers in advance, and after confirming consent, they cooperated in these tests. 5% Clairju solution and control was applied on both checks twice a day for 3 months. Sesc. (scale) and Ser (Coarseness) are analyzed before application, after application and at the end of each month by Two-dimensional skin surface analyzer / VISIOSCAN (COURAGE + KHAZAKA Electronic GmbH, Germany).

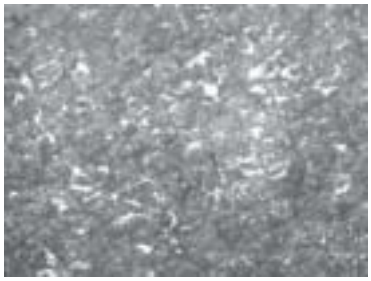
a. Scale

The rate of a clear portion of the scanning spots is calculated. Since the area where the horny layer in the skin comes up due to dryness is brighter than the neighboring one, the dry condition of the skin can be evaluated by the rate of the clear portion.

It can be said that the moisture retaining ability of the skin increases as this value decreases.



Small scale (Small Sesc value)



Large scale (Large Sesc value)

b. Coarseness

This value is obtained by calculating the rate of a dark portion of the scanning spots that is dependent on the number and width of wrinkles. Since the area of wrinkles and skin furrow is darker than the neighboring one, it can be evaluated for coarseness of the skin and the condition of wrinkles by the rate of the darkness.

The area of wrinkles is smaller as this value decreases, and it can be said that the skin surface is in a good condition.

Small wrinkle (Small Ser value)

Large wrinkle (Large Ser value)

Result and Discussion

Skin scale value before application is set up 1, and change of scale for three months is shown in Fig5. Skin scale value before application is set up 1, and change of coarseness for three months is shown in Fig6. 5% Clairju solution let scale and coarseness improve compared with control from a large point of view.

According to this result, Clairju has an improvement effect on human skin texture.

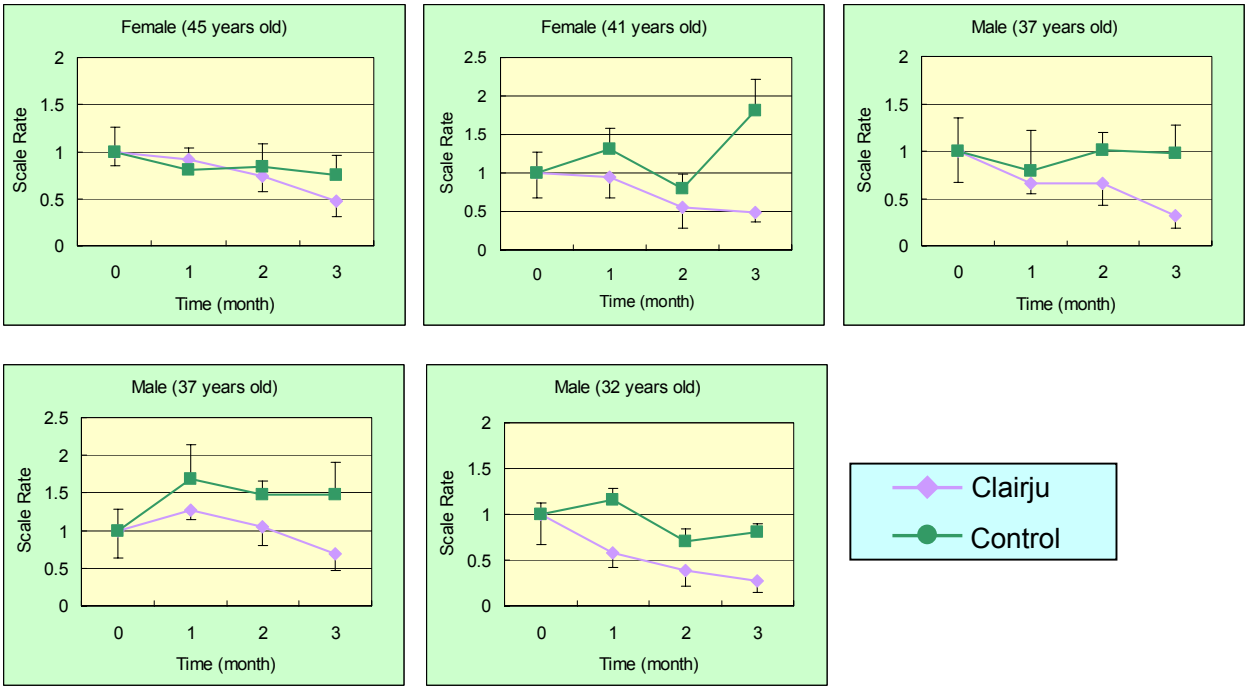


Fig.5: Improvement of skin texture (Scale)

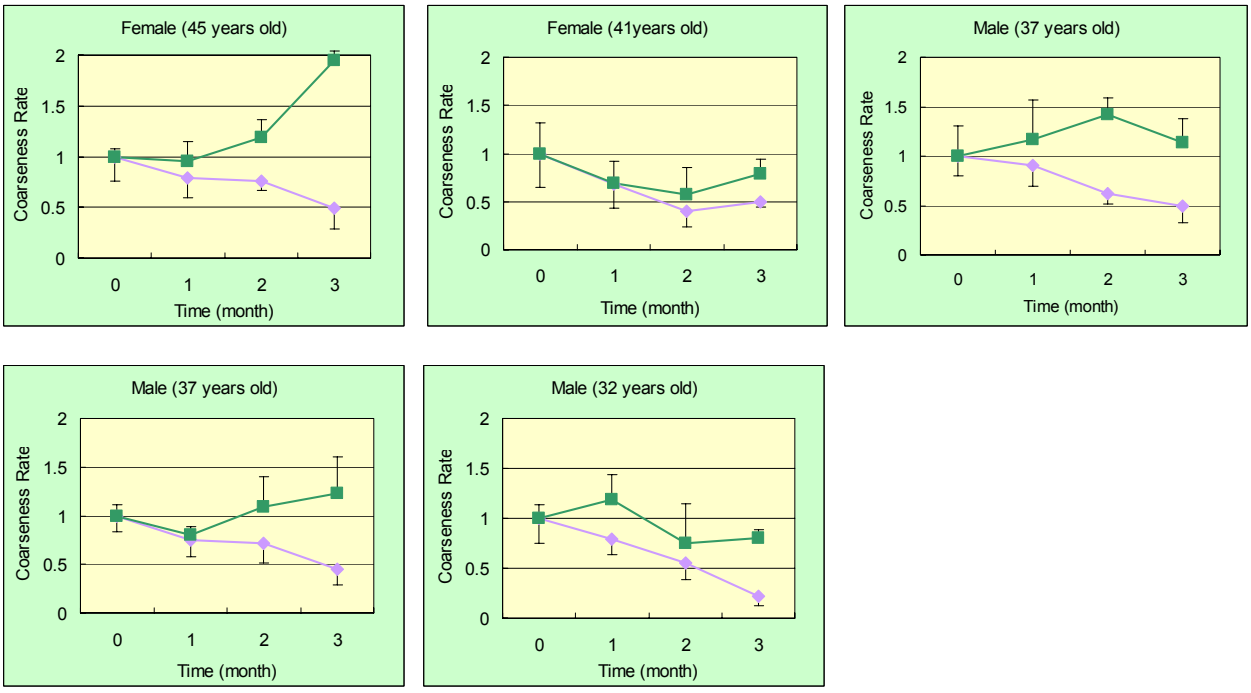


Fig.6: Improvement of skin texture (Coarseness)

Stability

1.Long Term Stability

Sample is placed at 4℃, room temperature, 50℃, and window side, and then . Absorbance value was measured at 470nm.

Result and Discussion

Change of Absorbance value is shown in Fig. 7. The tone color of Clairju is almost not changed at 4℃ and room temperature and appears stable.

At windows side, the tone color is observed temporarily to dilute, and it is observed to dilute more and more after 3 months. Also, the color tone tended to turn dark at 37℃ and 50℃ gradually.

According to this result, Clairju is stored in a place away from high temperature and window side (direct light).

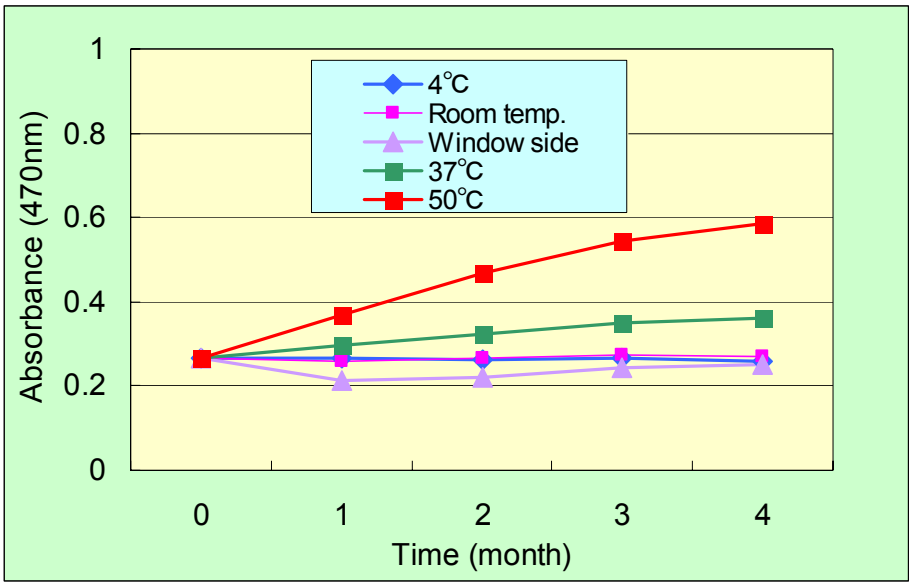


Fig.7: Long term stability

2.pH Stability

pH of Clairju is adjusted from 3 to 10 by HCl and NaOH, and . Absorbance value is measured at 470nm.

Result and Discussion

The visual change of appearance is shown in table 1. Absorbance value of Clairju is shown in Fig.8. Absorbance value tended to be up a little at more than pH 7, but turbid and precipitate were not observed at any pH range.

According to this result, it is safe when Clairju is used at alkali range.

Table 1: pH stability (Appearance)

pH	3	4	5	6	7	8	9	10
Appearance	○	○	○	○	○	○	○	○

○: Good, △: Slight Turbidity, ×: Precipitate

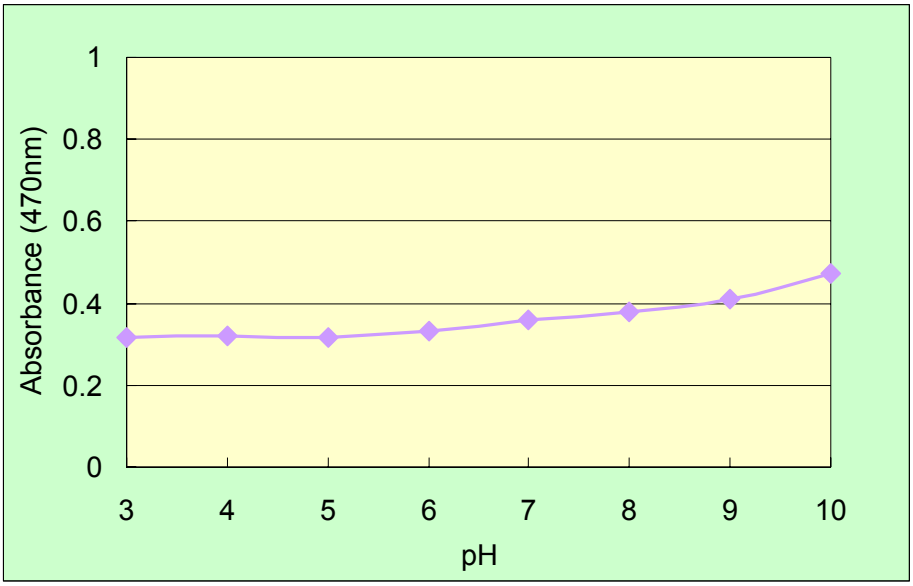


Fig. 8: pH stability

3. Thermal Stability

Store Clairju in water bath of 85℃. Visual observation and absorbance value at 470nm were determined.

Result and Discussion

Change of appearance by visual observation is shown in table 2, and the change of absorbance value is shown in Fig.9. Although the color tone turned dark by heating, turbid, precipitate and change of odor were not observed.

According to this result, it is safe when Clairju is heated for a long time.

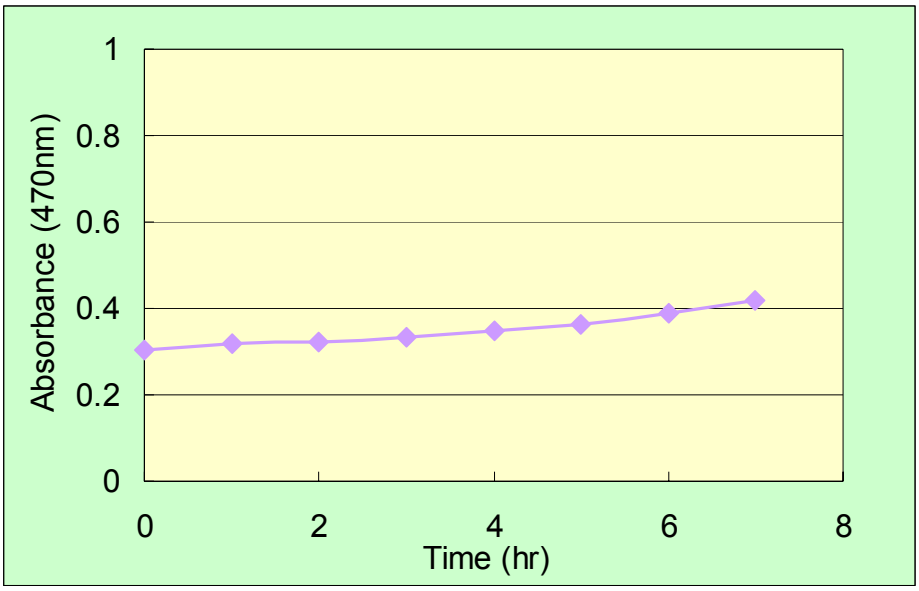


Fig. 9: Thermal stability

Compatibility

1.Compatibility of 10% of Clairju with Surfactant

	%	Ingredients	Result
Cation	2.8	Stearyl Trimethyl Ammonium Chloride	×
	3.0	Cetyltrimehylammonium Chloride	○
	2.7	Lauryltrimethylammonium Chloride	○
Anion	10.0	Triethanolamine Lauryl Sulfate	○
	25.0	Sodium Laureth Sulfate	○
	25.0	Triethanolamine Laureth Sulfate	○
	6.25	Laureth-6 Carboxylic Acid	○
	10.0	Potassium N-Cocoyl Glycinate	○
	7.5	Sodium Lauroyl Methylaminopropionate	○
	25.0	Sodium Tetradecenesulfonate	○
Nonion	10.0	Polyethylene Glycol (50) Oleyl Ether	○
	10.0	Coconut Tatty Acid Diethanolamide	○
	10.0	Sorbeth-60 Tetraoleate	○
	10.0	Polyoxyethylene Sorbitan Monooleate (20E.O.)	○
Silicone	10.0	Polyoxyethylene・Methylpolysiloxane Copolymer	○
Ampholytic	3.5	Lauryl Dimethylaminoacetic Acid Betaine	○
	4.0	Sodium N-Cocoyl-N-Carboxymethyl-N-Hydroxyethyl Ethylenediamide	○
	2.9	Lauroyl Amide Propylhydroxysulfobetaine	○

○: Good, △: Slight Turbidity, ×: Precipitate

2.Compatibility of 10% of Clairju with other ingredients

	%	Ingredients	Result
Solvent	50.0	Glycerin	○
	50.0	1,3-Butylene Glycol	○
	50.0	Propylene Glycol	○
	50.0	Isopropyl Alcohol	○
	50.0	Ethanol	○
Synthetic polymer	0.1	Carboxyvinyl polymer	○
	1.0	Polyvinylpyrrolidone	○
	1.0	Polyvinyl Alcohol	○
	1.0	Polyethylene glycol (6000)	○
Natural polymer	1.0	Sodium alginate	○
	1.0	Carboxymethyl cellulose	○
	1.0	Cationic cellulose	○
	1.0	Hydroxypropyl cellulose	○
Phospholipid	1.0	Lipidure-PMB	○
Vitamin-C derivative	2.0	Magnesium ascorbyl-2-phosphate	○
	2.0	Ascorbyl Glucoside	○

○: Good, △: Slight Turbidity, ×: Precipitate

3.Compatibility of 10% Clairju with other our products.

%	Product name	INCI Name	Result
10.0	FM Extract LA-B	Lactobacillus / Milk Ferment Filtrate	○
10.0	AQUACRUSTAR	Hydroxyethyl Chitosan	○
10.0	YEAST Liquid ZB	Yeast Extrcat	○
10.0	CHITIN Liquid (N)	Carboxymethyl Chitin	○
10.0	SUPER Hair Coat	Hydroxypropyl Chitosan	○
10.0	SAKURA Extract B	Prunus Yedoensis Leaf Extract	○
10.0	MARINWORT IPC-13 SBG	Algae Extract	○
10.0	MARINWORT IPC-14 SBW	Algae Extract	○
10.0	SILKGEN G Soluble	Hydrolyzed Silk	○
10.0	SILKGEN G Soluble-S	Hydrolyzed Silk	○
10.0	TREHALOSE 30	Trehalose	○
10.0	NEEM Leaf Liquid B	Melia Azadirachta Leaf Extract	○
10.0	Bio-PGA Solution HB	Polyglutamic Acid	○
10.0	Bio-PGA Solution LB	Polyglutamic Acid	○
10.0	Bio antiage B	Pueraria Lobata Root Extract Chlorella Vulgaris Extract Aloe Barbadensis Leaf Extract	○
0.1	Bio Sodium Hyaluronate	Sodium Hyaluronate	○
10.0	Biocellact ALOE VERA B	Aloe Barbadensis Leaf Extract	○
10.0	Fermentage Chardonnary B	Lactobacillus/Grape Juice Ferment (under applying)	○
10.0	Fermentage Pear B	Lactobacillus/Pyrus Communis (Pear) Fruit Juice Ferment (under applying)	○
10.0	Pharconix CTP-F (BG)	Hydrolyzed Collagen	○
10.0	JIOU Liquid	Rehmannia Chinensis Root Extract	○
10.0	SOUHAKUHI Liquid (BG)	Morus Alba Root Extract	○
10.0	CELLULE BLANC	Saxifraga Sarmentosa Extract Paeonia Suffruticosa Root Extract Pueraria Lobata Root Extract	○

%	Product name	INCI Name	Result
10.0	HIOUGI Liquid	Belamcanda Chinensis Root Extract	○
10.0	KOUBOKU Liquid B	Magnolia Obovata Bark Extract	
10.0	BOTANPI Liquid E	Paeonia Suffruticosa Root Extract	○
10.0	MARRONNIER Liquid B	Aesculus Hippocastanum (Horse Chestnut) Seed Extract	○
10.0	YUKINOSHITA Liquid MB	Saxifraga Sarmentosa Extract	
10.0	ROOIBOS Liquid B(N)	Aspalathus Linearis Extract	○
10.0	LEMONGRASS Liquid B	Cymbopogon Schoenanthus Extract	○
10.0	Phyto COLLAGE (N)	Natto Gum	○
10.0	ALPROTECTOR	Paeonia Suffruticosa Root Extract Tilia Cordata Flower Extract Althaea Officinalis Root Extract Arnica Montana Flower Extract	○
10.0	Phyto HYALURON B	Hibiscus Esculentus Fruit Extract	○
10.0	FLAVOSTERONE SB	Glycine Soja (Soybean) Protein	○
10.0	YUZU Ceramide B	Citrus Junos Fruit Extract	○
10.0	LACTOFERRIN-S	Lactoferrin	△
0.5	LEXSOD-P	Tannic Acid	○

○: Good, △: Slight Turbidity, ×: Precipitate

Specification

Subject	Specification
Appearance	Deep reddish brown to brown liquid, having a characteristic odor
Identification Sugar Phenol compounds	 Positive Positive
Purity Heavy metals Arsenic	 20 ppm max. 2 ppm max.
Residue on Evaporation	0.5 to 2.0 w/v%
INCI Name	Water Butylene Glycol Hydrolyzed Prunus Domestica
CAS Number	None
EINECS Number	None

Reference

1. Mitsuru HOTTA, *Useful Plants of the World , Heibonsya, Ltd.*, 854-867 (1989)

2. Tsunefumi YAMADA, *Weekly Asahidaihyakka plant of the world 5, Asahishinbunsya*, 92-93 (1977)

3.California Prune Board, http://www.prune.jp/index.html

4.Klaus Wolff, et al., *J. Ultrastructure research*, 39, 262-280 (1972)