ORYZA OIL & FAT CHEMICAL CO., LTD.

CE Maturation agent with a Circadian rhythm adjusting Effect

cosmeHerbest™ PASSIFLORA

Passiflora Incarnata Extract

ORYZA OIL & FAT CHEMICAL CO., LTD.

1 aza Numata Kitagata Kitagata-cho,

Ichinomiya-city Aichi-pref., 493-8001 JAPAN

TEL: +81 (0) 586 86-5141 / FAX: +81 (0) 586 86-6191

URL https://www.oryza.co.jp/

E-mail: info@oryza.co.jp

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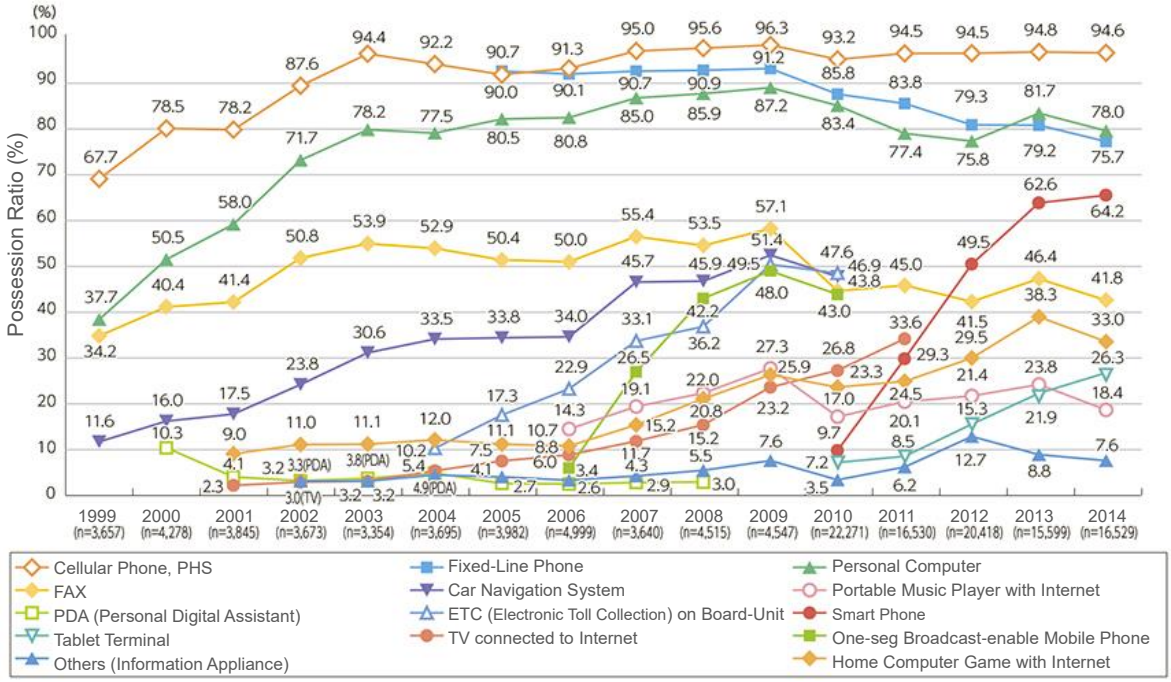
1. Introduction

The fact that we become sleepy after a fixed time passes after we wake up in the morning and naturally wake up after a fixed time passes after we sleep can be said to be due to the fact that our bodies beat to a rhythm. This rhythm is called the circadian rhythm and in addition to waking and sleeping, can be said to cover fluctuations in blood pressure, body temperature and the secretion of hormones. In this way, living creatures have daily life activity cycles like a body clock. As this is not exactly one day (24 hours), our bodies reset this rhythm based on stimuli such as light, temperature and food, and adapt to the actual time environment (day/night).

However, if the stimuli (light, temperature, food, and stress) that previously were applied at the correct time, are now applied in the wrong time frame due to us bathing in the blue light of a smartphone and PC during the sleeping hours, taking meals at night or being engaged in night work when being employed in shifts, the body rhythms are disturbed and we are faced with the phenomenon in which the clear distinction between night and day is decreased (Fig. 1). In actual fact, convenience stores, which are the typical 24 hour business providers, have increased to a surprising degree over the past 30 years (Fig. 2). As a result, we can eat any time we like, but we are exposed to light stimuli from the bright stores. According to this, the average time we spent sleeping is being reduced. This indicates that people's lifestyles are becoming more nocturnal and we are losing the clear distinction between day and night (Fig. 3).



(Quoted from "Communications Usage Trend Survey 2014" conducted by the Ministry of Internal Affairs and Communications)

Fig. 1: Changes ownership ratio of information-communication terminal

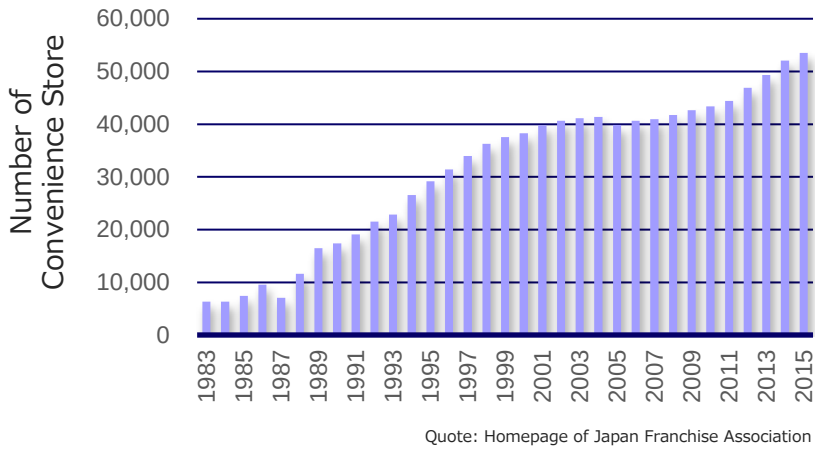
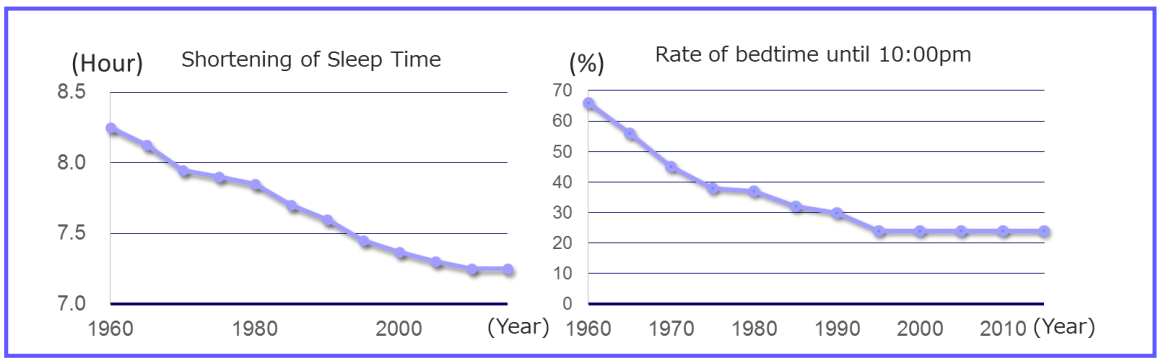


Fig. 2: Number of Convenience Store in Japan



Source: NHK Public Life Survey 2010

Fig. 3: Decrease in sleep time of Japanese people
and percentage that go to bed by 10:00 PM

2. Circadian Rhythms and Skin

Circadian rhythms are also found in the skin. As, during our daytime activities, we often go out and are easily exposed to external stress such as UV-rays and changes in air temperature, the skin can easily become damaged. And during our resting times at night, it is said that damage that we receive during daytime is repaired. In other words, if our sleep time is too short, the time for repairing our skin is also reduced. This means in turn that the damage received during the day cannot be reset and it is thought that this has negative effects on the skin, resulting in rough skin and cosmetics not applying well to the skin.

Development of this product started from the concept that if our disturbed body rhythms can be made to clearly distinguish between day and night, and encouraged to dedicate ourselves to repairing damage to our skin during the night and production of factors that will prepare us against damage, this will help improve the condition of our skin.

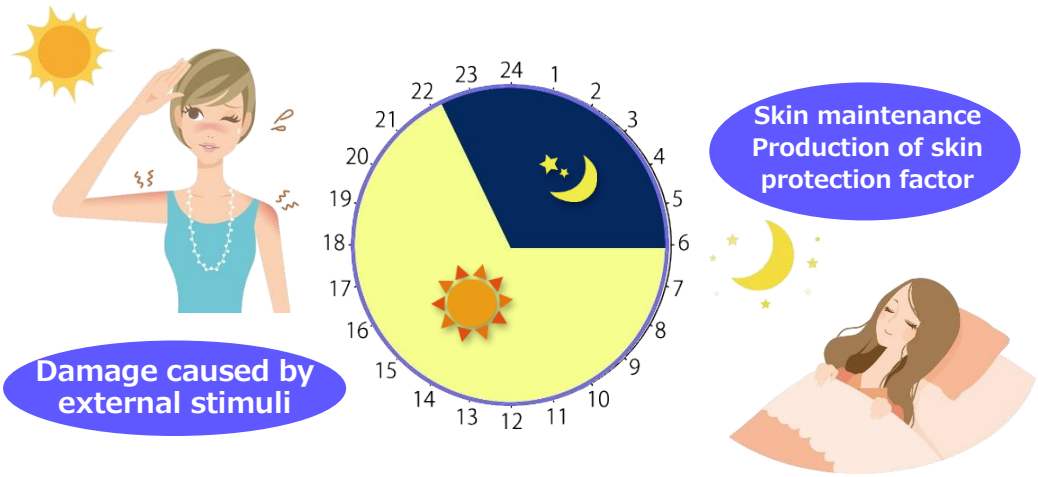


Fig. 4: Skin Role of Day and Night

3. Circadian Rhythm Adjusting Effect of Passionflower Extract

The Food Development Department of Oryza Oil & Fat Chemical Co., Ltd. conducted an experiment of the effects of Passionflower Extract on the circadian rhythms. The gene that controls the circadian rhythms is known as the clock gene, and is observed with the main indicators of period circadian clock2 (Per2) and cryptochrome circadian clock 1 (Cry1), the expression level of which increases during the daytime (activity period) and the Brain and Muscle Arnt-like 1 (Bmal1), the expression level of which increases during the night (rest period) (Fig.5).

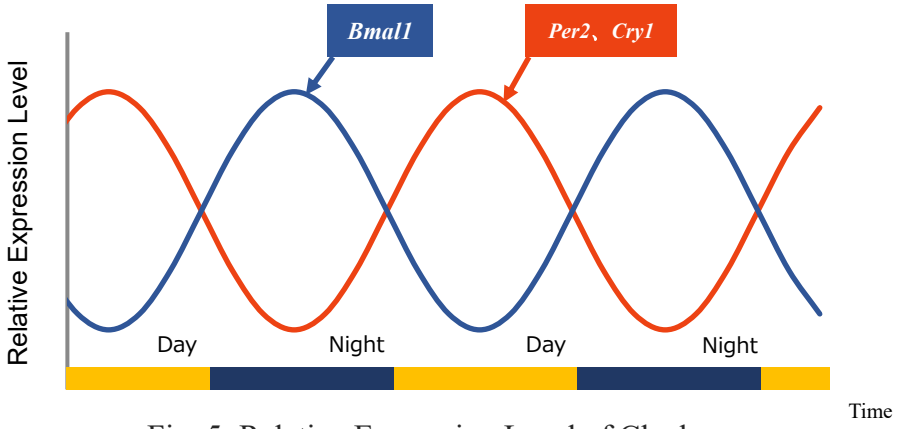


Fig. 5: Relative Expression Level of Clock gene

The experiment was carried out as follows.

Passionflower Extract (PFE) was added to mouse fibroblasts (NIH3T3) on which circadian rhythm has been tuned for a final concentration of 100 μg/mL, and mRNA expression level was measured 0, 4, 8, 12, 16, 20 and 24 hours after application. The respective data was corrected by the endogenous control (β -actin) expression level. As a result, the mRNA expression level reached its peak 20 hours after application for Per2 and 24 hours after application for Cry1. There was a significant increase for the PFE group in comparison with the control group.

The above result suggests that the expression level of Per2 and Cry1 that increases during daytime activity further increases with the application of passion flower extract and that passion flower extract works to provide a clear distinction between night and day. By providing this clear distinction between night and day, the disruption in the internal body rhythms can be corrected and the body will be able to resume its original levels of activity. For the skin as well, this promises to provide protection from the damage caused by UV-rays during the daytime and repair and full productivity during the night.

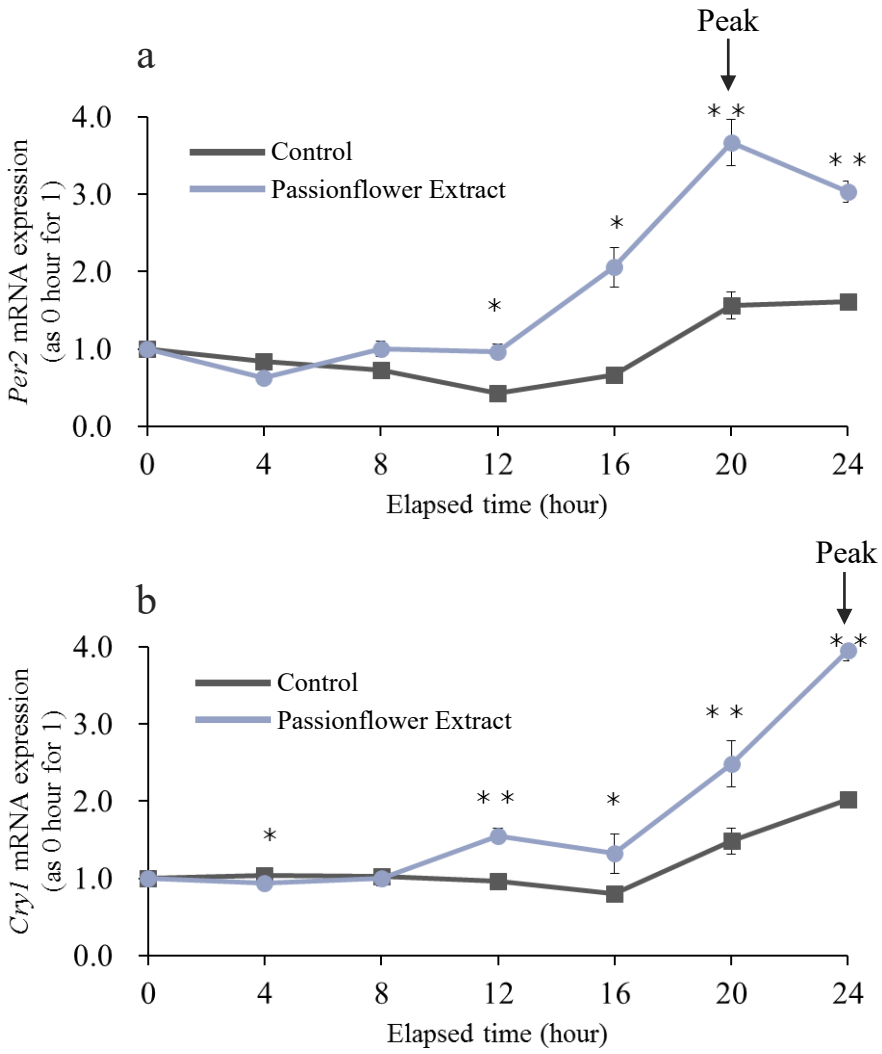


Fig. 6: mRNA Expression of *Per2*(a)、*Cry1*(b) on Passionflower Extract
The mean value±S.E. ($n = 4$) *: $p<0.05$, **: $p<0.01$ v.s. Control

Source: Catalogue of "Passionflower Extract (Food)" of Oryza Oil & Fat Chemical Co., Ltd.

4. Circadian Rhythm and Uneven Skin Color Tone

As stated above, circadian rhythms have an intimate relationship with the skin and when these rhythms are disturbed, the protective capacity of the skin as well as recover and production capacity drop, causing various skin-related concerns.

It is a law of nature that as we grow older, the cellular function of the skin and our metabolism gradually decrease. In addition, disturbing our circadian rhythms through irregular lifestyles from our twenties leads to multiple skin concerns such as dark spots, wrinkles, irregular pores, sagging, and uneven skin tone. These concerns are factors that affect our visual age. Among these, “uneven skin tone” is considered to be the greatest factor increasing our visual age. Although it is possible to hide uneven skin tone using base makeup products and concealers, we believe that it is an intrinsic wish of most women to be able to somehow reduce uneven skin tone without covering the skin.

The following photographs are to compare skin with severe uneven tone and skin with less uneven tone. The state of uneven skin tone was compared after editing the photographs to a brightness of +10. The photographs show that while unevenness is very prominent on skin with severe uneven tone even when the brightness is increased, raising the brightness of skin with less uneven tone makes the skin look clear (Fig. 7).

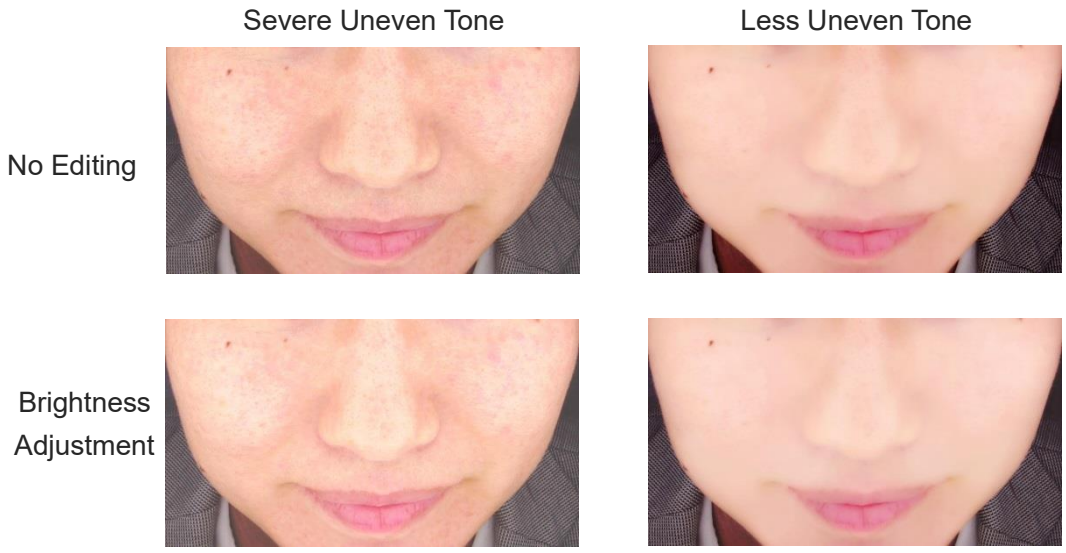


Fig. 7: Comparison of Uneven Skin Tone Areas and their Noticeability

The following elements are considered to cause uneven skin tone.

[Uneven Skin Tone due to Dryness in the Skin]
When the skin is dry, moisture is lost from the horny cells and skin's barrier function declines. In such condition, a protective function starts to work in the skin and horny cells are generated at rapidly in the basal layer of the skin. With the disturbance in turnover as new horny cells are formed at a rapid pace before old horny cells have exfoliated, the horny layer becomes thick (Hyperkeratosis). When multiple layers of horny cells that should have peed off pile up, it covers the color of the dermis and it skin reaches the "state in which only the dark colors of old horny tissues can be seen (uneven skin tone)."

[Uneven Skin Tone due to Rough Skin Texture]
There is a relationship between dryness in the skin and roughness in skin texture. Partial inconsistencies in the thickness of honey tissues due to disturbances in the skin turnover are a cause of rough skin texture. When skin texture becomes rough in places, the light is reflected in an uneven way and this leads to the "state in which skin color appears different depending on the areas (uneven skin tone)."

[Uneven Skin Tone due to Inflammation (Redness)]
Acne and inflammation caused by irritants lead to "redness (uneven skin tone)" in part of the skin or entire inflammation area. Inflammation makes skin rougher and chronic inflammation in a place causes excessive melanin generation, darkening the skin.

[Uneven Skin Tone due to Poor Blood Circulation]
When blood circulation becomes poor, oxygen and nutrition are not delivered to the skin efficiently and waste tends to be retained. As a result, some areas of skin become cloudy and dark. Skin tends to become rough in this condition because horny cells cannot grow healthily. As the skin itself is muddied with a dark color, the "skin color looks dull (uneven skin tone)."
In areas where the horny layer has become thin, redness is also seen due to the expansion of the capillaries.

[Uneven Skin Tone due to Pigmentation]
Pigmentation is caused by external stimuli such as UV-rays, hormone imbalances, and internal stimuli such as stress and lack of sleep. As we age, the areas with liver spots, small dark spots, and pigmentation increase, leading to "the skin appearing dark and dirty (uneven skin tone)."

Reduction of uneven skin tone is expected to lower our visual age and improve the appearance with makeup on. Dryness and rough skin texture, considered as the causes of uneven skin tone, are influenced by the state of the horny layer existing in the outermost layer of the skin. One of the factors causing dryness and roughness in the horny layer is cornified envelope (CE). When the cornified envelope is well formed, skin is protected against the entry of bacteria and dust into the inner part of skin. For this reason, cornified envelope contributes to the reduction in damage.

5. Stratum Corneum and Cornified Envelope (CE)

In the outermost layer of the skin, the stratum corneum forms a thin barrier of just 20 microns with the outside world. In addition to the barrier function to prevent the infiltration of foreign matters from the outside world, the stratum corneum plays a biologically important role of maintaining moisture within the body. As this structure is thought of in terms of blocks and mortar, it is constructed of corneocytes that are equivalent to the blocks and the intercellular lipids that act like the mortar. The corneocytes are filled with keratin fibers and they include natural moisturizing factors (NMF) mainly consisting of amino acids that play an important role in the previously described moisture retention function (Fig. 8).

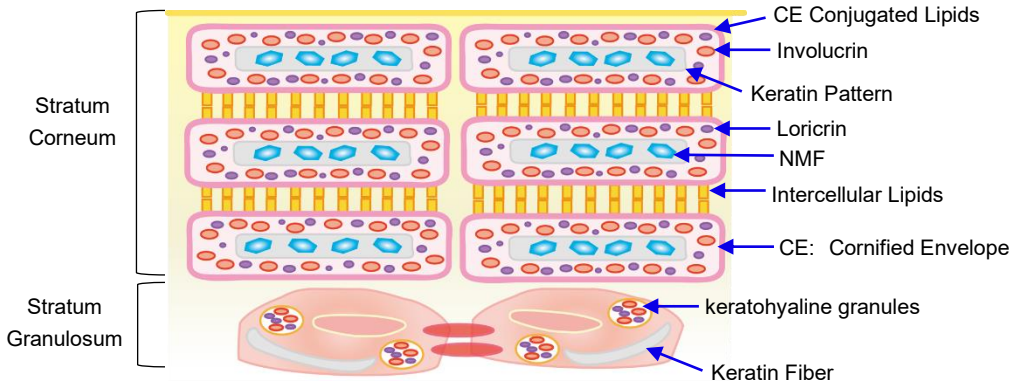


Fig. 8: The Components of Epidermis

On the other hand, the intercellular lipids making up the mortar section consist of ceramide, cholesterol, and free fatty acids, and these are organized into a repeated structure (lamellar structure) of oil layers and water layers. As the barrier function changes greatly based on quantitative and qualitative changes to these fats and disturbances in their orientation, these intracellular lipids are considered to play a vital role in the barrier function of the stratum corneum.

To form corneocytes, keratinocytes first divide in the basal membrane, they produce keratin, and they move toward the upper layer while differentiating and maturing. At this time, keratin 5 and keratin 14 form a pair in the vicinity of the stratum basal and keratin 1 and keratin 10 in the prickle cell layer and granular layer respectively. The keratin fibers in the granular layer, at the time of keratinization, are aggregated with filaggrin protein, causing dramatic shape changes in the keratin patterns. The keratohyaline granules in the granular cells contain large quantities of profilaggrin, which is the precursor to fillagrin, and filagrin is decomposed through the action of dephosphorylation at the time of keratinization. The isolated filaggrin aggregates keratin fibers within the cytoplasms of the corneocytes and then it is decomposed into amino acids and other substances in the upper epidermis.

Concerning the structure of corneocytes, there is a growing awareness about cornified envelope (CE) (also called cornified thick membrane, limbic body, and keratinocyte outer membrane). CE is formed when a variety of proteins such as involucrin and loricrin form bridges with each other and become insoluble. It forms a bag-shape structure wrapping corneocytes (Fig. 9).

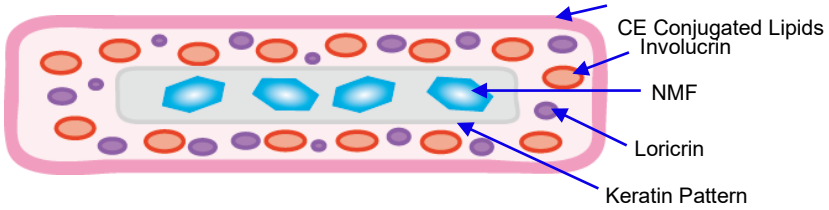


Fig. 9: The Components of Cornified Envelope (CE)

The precursor protein comprising CE is manifested from the prickle cell layer to the granular layer following the differentiation of the epidermal keratin sites. Involucrin created from prickle cells and lolicrin created from granular cells are the main components. Bag-shaped CE is formed when these proteins are sufficiently created and they are bridged by enzymes such as transglutaminase. Further, the wrapping in the CE of the keratin patterns and amino acids etc. existing within the CE can be considered as the maturing of the CE. As the CE matures, it forms a firm barrier in combination with the surrounding intracellular lipids. CE can also become immature and the barrier function declines in the case of sleep deficiency or skin receiving large volumes of UV-rays during the daytime, causing insufficient cell dispersion, differentiation, and synthesis of proteins such as keratin, involucrin and lolicrin.

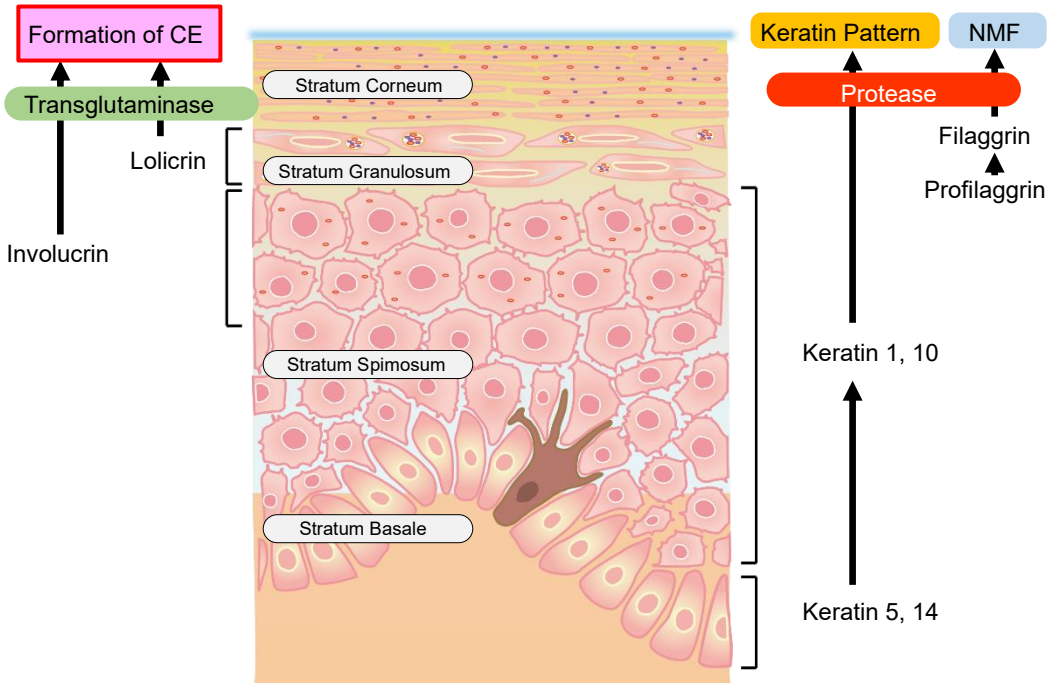


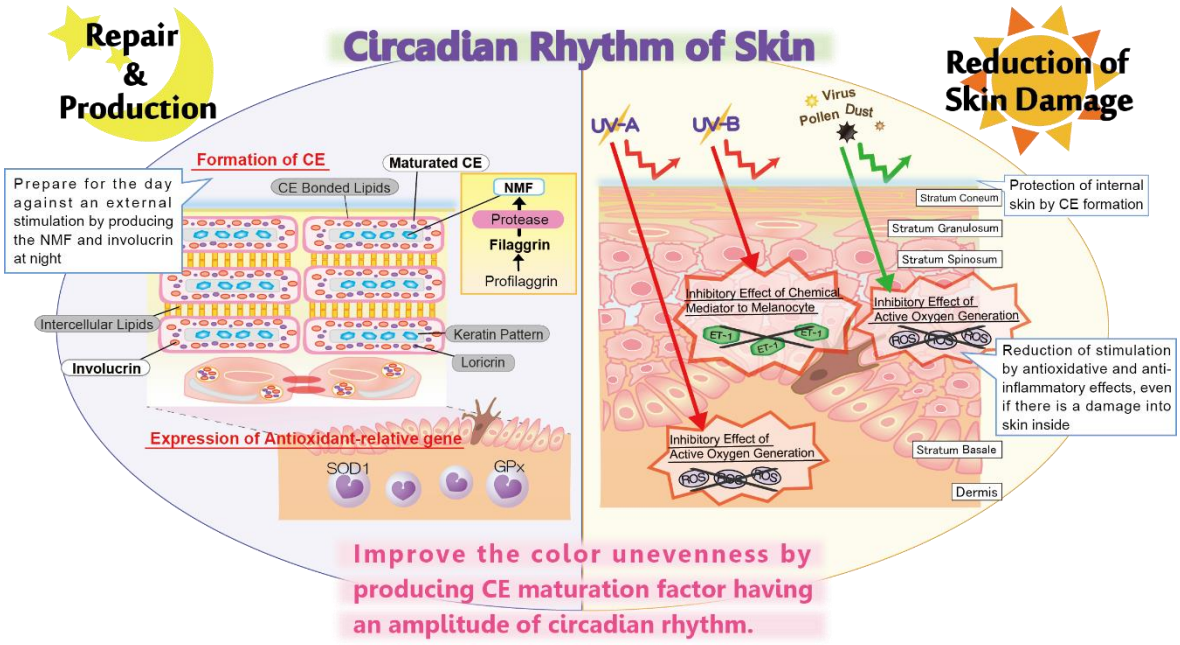
Fig. 10: Formation Process of Cornified Envelope

6. Circadian Rhythm and CE Maturation

Studies in circadian rhythms and corneocytes have been carried out recently. According to Gotsu et al. [1)], keratin patterns are formed by keratin fibers aggregating with fillagrin in the granular layer where keratinization of the horny cells takes place. The study group discovered that the expression of these fillagrin genes change in a 24 hour cycle rhythm. Based on this, they state that the production of fillagrin needs to be promoted in consideration of this rhythm in addition to increasing fillagrin production and suppressing the reduction in fillagrin production due to dryness in order to promote the production of fillagrin and maintain the healthy state of skin.

Using Passionflower Extract that has the action of regulating circadian rhythms, we examined its effects on the skin in the following tests.

① mRNA Expression of PPAR in Keratinocytes
② mRNA Expression of Involucrin in Keratinocytes
③ mRNA Expression of Filaggrin in Keratinocytes
④ Acceleration Effect of CE Maturation
⑤ Inhibitory Effect of mRNA Expression of Endothelin
⑥ mRNA Expression of Antioxidant relative Gene



7. Passionflower

The raw material that we focused on in this test was a type of passionflower with the scientific name *Passiflora incarnata* L. and the whole of the plant was used. The product name is "cosmeHerbest™ PASSIFLORA" and we adopted the scientific name *Passiflora*. In English, it is called Purple Passion flower, and as this flower has a similar shape to a clock, it is given the name Chabotokeiso ("tokei" means clock) in Japan.
Its place of origin is said to be tropics and subtropical areas in North, Central and South America.

The passionflower has a long history, being used by the indigenous people as a folk medicine, and in 1569, the Spanish physician Monardez discovered the passionflower in Peru, which at that time was unknown in Europe[2]. By recording how the local indigenous people used the passionflower as a folk medicine and taking it back to Europe, he promoted the spread of this flower in Europe as herb tea with strong sedative effects.

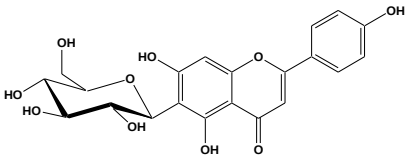
In addition, the Spaniards conquering Mexico and South America learned from the indigenous people of Mexico how to use the passion flower and this was spread to Europe where it was cultivated. The South American indigenous people of the 1800's used the root of the passion flower as a tonic and the leaves as a means of alleviating bruises and headaches, and its use as sedative spread in the United States. Since passionflower has an extremely strong sedative effect, it is called "botanical tranquilizer (sedative)", and it has long been used in Europe as a remedy for insomnia and other symptoms.

8. The Components in cosmeHerbest™ PASSIFLORA

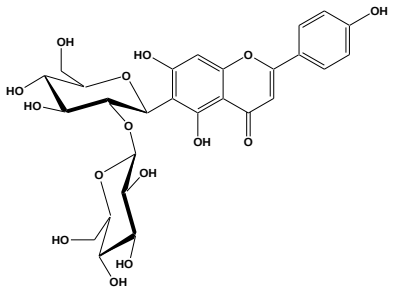
cosmeHerbest™ PASSIFLORA contains large amounts of flavone glycosides listed below, especially a large amount of isovitexin which is apigenin-6C-glucoside[3]. Isovitexin is also contained in rooibos originally from South Africa and is believed to have an action to help us sleep well.

Passionflower used in our cosmeHerbest™ PASSIFLORA is a type of herb listed in the European Pharmacopoeia. According to the Pharmacopoeia, it has an excellent mental stabilization action and soothing action to reduce tension and excitement. The test to study the expression of clock gene carried out in Oryza Oil & Fat Chemical confirmed that passionflower helps us to have physical states suitable to daytime and night time respectively. Flavone glycosides are believed to be its active center.

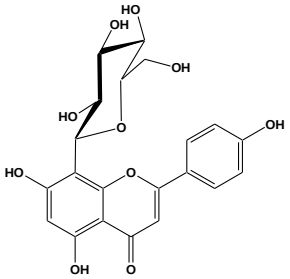
It has been reported that passion flower contains multiple flavonoid glycosides. Oryza separated its components and analyzed its structure with Kyoto Pharmaceutical University. As a result, the structure of the components was determined as shown in Fig. 11.



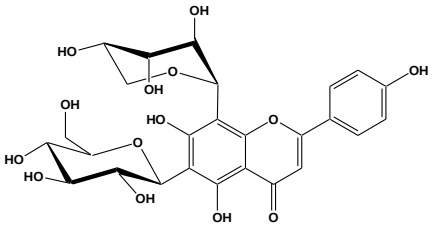
Isovitexin



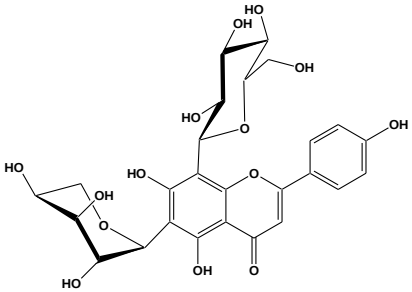
Isovitexin-*O*-gulcoside



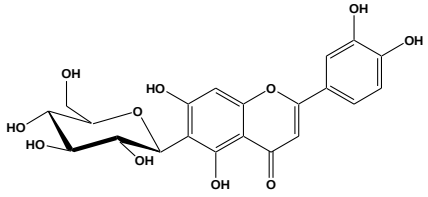
Vitexin



Schaftoside



Isoschaftoside



Homoorientin

Fig. 11: The Components in cosmeHerbest™ PASSIFLORA

9. Efficacy Evaluation

Passionflower (Purple Passionflower) contains isovitexin and other flavone glycosides. Oryza conducted a test to confirm its action to normalize the circadian rhythm. This test was conducted based on an idea that normalizing the circadian rhythm helps to recover from skin damage caused by UV-rays and other factors during daytime and correct the troubled horny layer at night. An in vitro verification was conducted for the action to promote the expression of involucrin and filaggrin that are CE maturation factors as well as the action to promote the expression of peroxisome proliferator-activated receptor (PPAR) that boosts skin's barrier function. Then, an in vivo test was carried out to check the effects of cosmeHerbest™ PASSIFLORA on skin and its functionality was evaluated from the viewpoint of cosmetology.

9-1 Screening Evaluation

9-1-1 mRNA Expression of PPAR in Keratinocytes

Test Sample

Passionflower Extract was prepared so that its final concentrations would be 100 and 300 μg/mL for the test. Concentrations 100 and 300 μg/mL of Passionflower Extract are equivalent to concentrations of 1.67% and 5% cosmeHerbest™ PASSIFLORA.

Test Method

Human epidermal keratinocytes (NHEK) were inseminated in a 12 well plate and cultured for 24 hours. Passionflower Extract (PFE) was added so that its final concentration would be 100 and 300 μg/mL and then the sample was cultured for another 24 hours. After cultivation, cells were collected and RNA was extracted. Then, cDNA was created from the obtained RNA and the mRNA expression level of PPAR α and PPAR γ was quantitatively determined by the quantitative PCR method. Data for them was corrected by the expression level of endogenous control (β-actin) and then a significance test was conducted by Student's *t*-test.

Test Result

It was confirmed that adding Passionflower Extract increases the mRNA expression level of PPAR α and PPAR γ (Fig. 12).

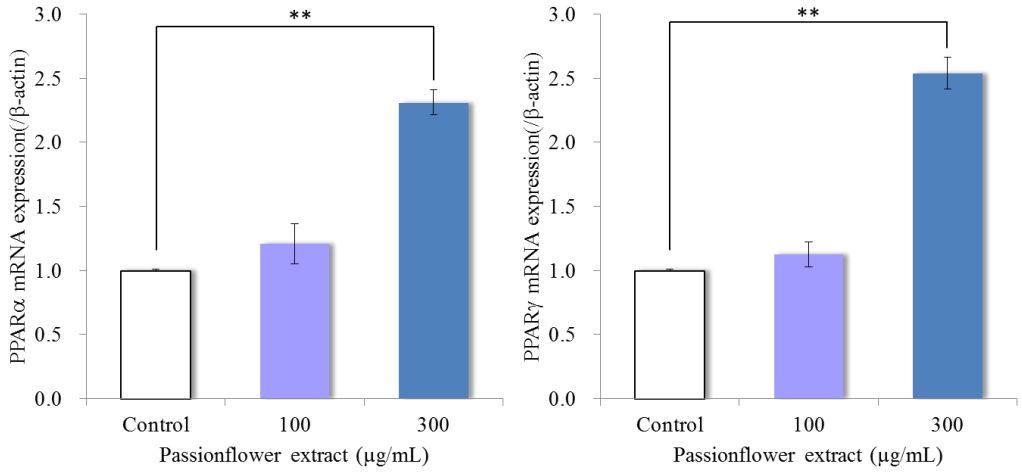


Fig. 12: mRNA Expression of PPARα (left) and PPARγ (right)
The mean value ± S.E. (n=4) *: p<0.05, **: p<0.01

9-1-2 mRNA Expression of Involucrin in Keratinocytes

Test Sample

Passionflower Extract was prepared so that its final concentrations would be 100 and 300 µg/mL for the test. Concentrations 100 and 300 µg/mL of Passionflower Extract are equivalent to concentrations of 1.67% and 5% cosmeHerbest™ PASSIFLORA.

Test Method

Human epidermal keratinocytes (NHEK) were inseminated in a 12 well plate and cultured for 24 hours. Passionflower Extract (PFE) was added so that its final concentration would be 100 and 300 µg/mL and then the sample was cultured for another 24 hours. After cultivation, cells were collected and RNA was extracted. Then, cDNA was created from the obtained RNA and the mRNA expression level of involucrin was quantitatively determined by the quantitative PCR method. Data for them was corrected by the expression level of endogenous control (β-actin) and then a significance test was conducted by Student's *t*-test.

Test Result

It was confirmed that adding Passionflower Extract increases the mRNA expression level of involucrin (Fig. 13).

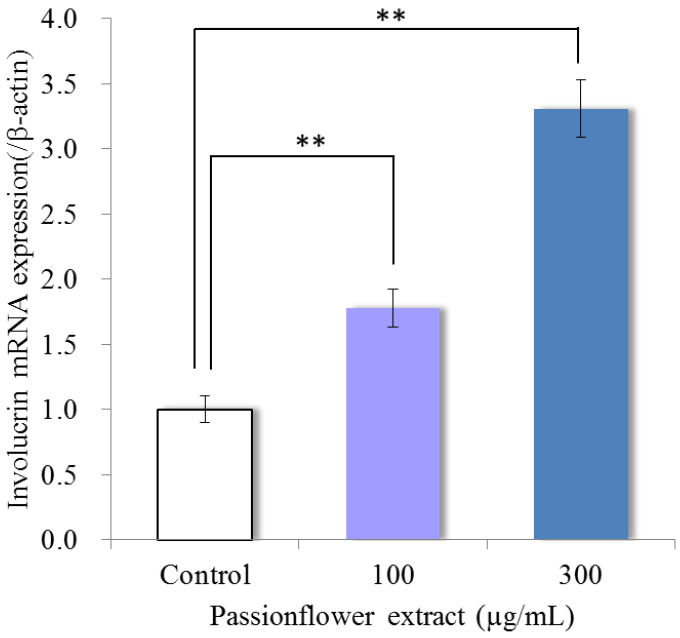


Fig. 13: mRNA Expression of Involucrin
The mean value ± S.E. (n=4) *: p<0.05, **: p<0.01

9-1-3 *m*RNA Expression of Filaggrin in Keratinocytes

Test Sample

Passionflower Extract was prepared so that its final concentrations would be 100 and 300 μg/mL for the test. Concentrations 100 and 300 μg/mL of Passionflower Extract are equivalent to concentrations of 1.67% and 5% cosmeHerbest™ PASSIFLORA.

Test Method

Human epidermal keratinocytes (NHEK) were inseminated in a 12 well plate and cultured for 24 hours. Passionflower Extract (PFE) was added so that its final concentration would be 100 and 300 μg/mL and then the sample was cultured for another 24 hours. After cultivation, cells were collected and RNA was extracted. Then, cDNA was created from the obtained RNA and the mRNA expression level of filaggrin was quantitatively determined by the quantitative PCR method. Data for them was corrected by the expression level of endogenous control (β-actin) and then a significance test was conducted by Student's *t*-test.

Test Result

It was confirmed that adding Passionflower Extract increases the mRNA expression level of filaggrin (Fig. 14).

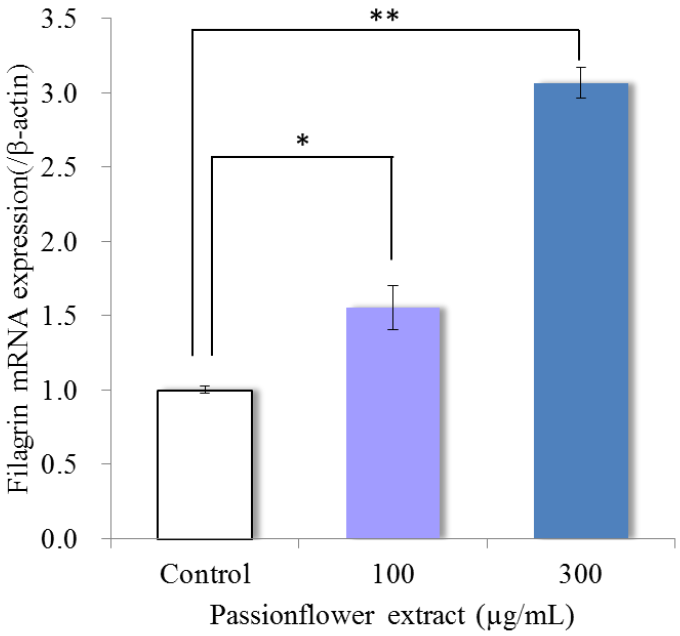


Fig.14: mRNA Expression of Filaggrin
The mean value ± S.E. (n=4) *: p<0.05, **: p<0.01

9-1-4 Acceleration Effect of CE Maturation

As described in the section above, the action of increasing gene expression of cornified envelope (CE) related factors involucrin and filaggrin has been confirmed. A monitoring test was carried out on human subjects to confirm that CE is formed well and matured.

Test Sample

Mix well 5% volume of cosmeHerbest™ PASSIFLORA and 95% volume of 30% of propanediol solution adjusted in advance, and used this test solution as "PASSIFLORA Lotion" twice a day in the morning and evening for three weeks to three subjects.

Test principle

Cornified envelopes (CE) are separated by removing soluble substances from the sample taken from skin and their maturity is evaluated based on the changes in the shape as CEs mature[4]. The disappearance of involucrin antigenicity accompanied with cross-linking and modification is evaluated by immunostaining and the acquisition of a hydrophobic property by lipid or protein binding is evaluated by Nile red staining. Through these evaluations, immature CEs and mature CEs can be distinguished[5].

Test method

Male and female persons aged from 24 to 55 (one male and two females) who submitted written consent participated in the test as the subjects. The subjects applied approximately 1 ml of lotion over their entire face and an area below their left knee in the morning and at night every day. The horny layer of each part was sampled by stripping it using cellophane tape before application and after three weeks of application.

The tape with the horny layer was shredded, soaked in 1 mL of dissociation buffer (2% SDS-20 mM dithiothreitol-5 mM EDTA-0.1 M Tris-HCl (pH8.5)), and then heated at 90 °C for 10 minutes. Only the dispersion liquid, without the tape base, was moved into a different tube and was centrifuged (4,000 g, 10 minutes). Then, the supernatant was removed. To the sediment (insoluble matter), 1 mL of new dissociation buffer was added and then the heating and centrifugation processes described above were repeated four times in total to thoroughly remove soluble substances. The obtained sediment was used as CE.

An appropriate amount of dissociation buffer was added to CE in dispersed form. The sample was dropped onto a slide glass, air-dried, and then fixed using cold acetone (-20 °C, 10 minutes). Then, the sample was hydrated with PBS and blocked by 3% BSA-PBS (room temperature, 1 hour). After blocking, the sample was left to rest in anti-involucrin antibody (Spring Bioscience, 1:100 in 3% PBS) at 4 °C overnight. Then, the sample was washed and stained with fluorescent labeled antirabbit antibody (Alexa 488, Life technologies, 1:100 in 3% PBS) at a room temperature for one hour sequentially. After washing, several drops of Nile red stain solution (3 µg/mL in 75% glycerol) were added, the sample was covered, and then it was observed under a fluorescence microscope.

In immature CEs, involucrin antigenicity is high and green fluorescence by immunostaining is enhanced. In mature CEs, fluorescence by immunostaining is less intense because involucrin antigenicity is lowered due to cross-linking of CE conjugated lipids and lipidation. Nile red is a pigment that produces fluorescence in a hydrophobic environment[6]. Mature CEs have obtained more-advanced hydrophobic properties due to CE conjugated lipids and other substances. For this reason, they produce intense red fluorescence by Nile red staining as compared to immature CEs.

Result and consideration

When comparing immunostaining photos before application and three weeks after continuous application, the photo taken three weeks after continuous application shows more tissues producing intense red fluorescence (Fig. 15). Intense red fluorescence suggests an increased ratio of mature CEs. For this reason, a lotion containing cosmeHerbest™ PASSIFLORA is expected to accelerate the maturation of CEs. The test results matched the results of the cell experiment in terms of the increase of gene expression of CE-related factors involucrin and filaggrin.

N.A (Male), 55's

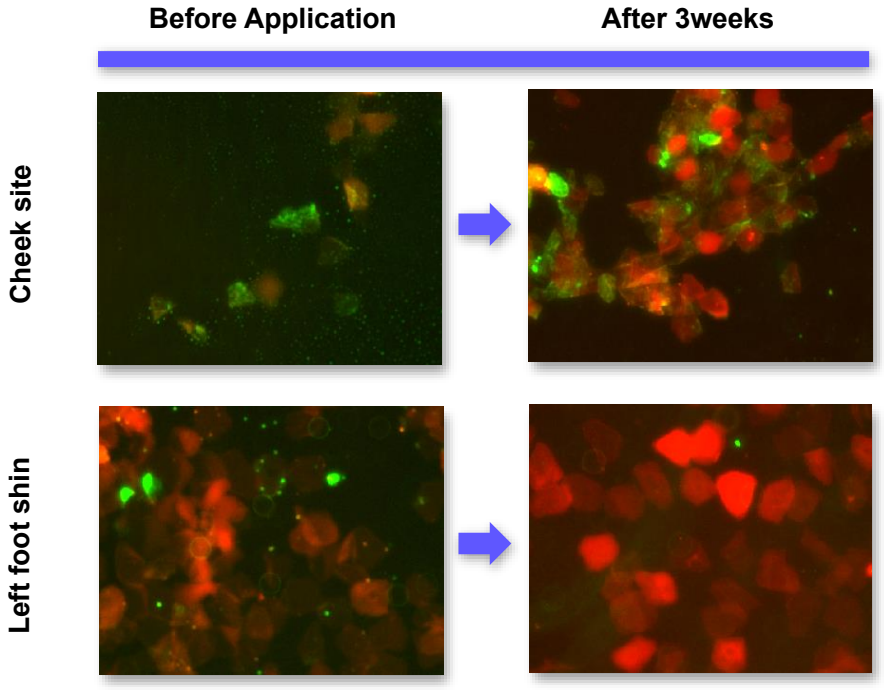


Fig. 15: Fluorescence analysis photo of the stratum corneum
(Green Color: Involucrin-antibody, Red Color: Nile red / Maturated CE)

9-1-5 Inhibitory Effect of *m*RNA Expression of Endothelin

Test Sample

Passionflower Extract was prepared so that its final concentrations would be 100 and 300 μg/mL for the test. Concentrations 100 and 300 μg/mL of Passionflower Extract are equivalent to concentrations of 1.67% and 5% cosmeHerbest™ PASSIFLORA.

Test Method

Human epidermal keratinocytes (NHEK) were inseminated in a 12 well plate and cultured for 24 hours. Passionflower Extract (PFE) was added so that its final concentration would be 100 and 300 μg/mL and then the sample was cultured for another 24 hours. After cultivation, the medium was replaced with PBS and 50 mJ/cm^2 of UVB was irradiated. After irradiation, the medium was replaced with PFE-supplemented medium prepared to achieve each concentration again and cultured for 24 hours. After cultivation, cells were collected and RNA was extracted. Then, cDNA was created from the obtained RNA and the mRNA expression level of endothelin-1 was quantitatively determined by the quantitative PCR method. Data for them was corrected by the expression level of endogenous control (β-actin) and then a significance test was conducted by Student's *t*-test.

Test Result and Consideration

Irradiation of UVB increased the mRNA expression level of endothelin 1 in keratinocytes. Endothelin is secreted from keratinocytes and used to transmit melanin production information to melanocytes. It was indicated that UVB irradiation stimulated keratinocytes to transmit melanin production information to melanocytes. However, when PFE was added by 300 μg/mL, the mRNA expression level of endothelin 1 reduced. This indicates that adding PFE may suppress the pigmentation caused by melanin.UVB (Fig. 16).

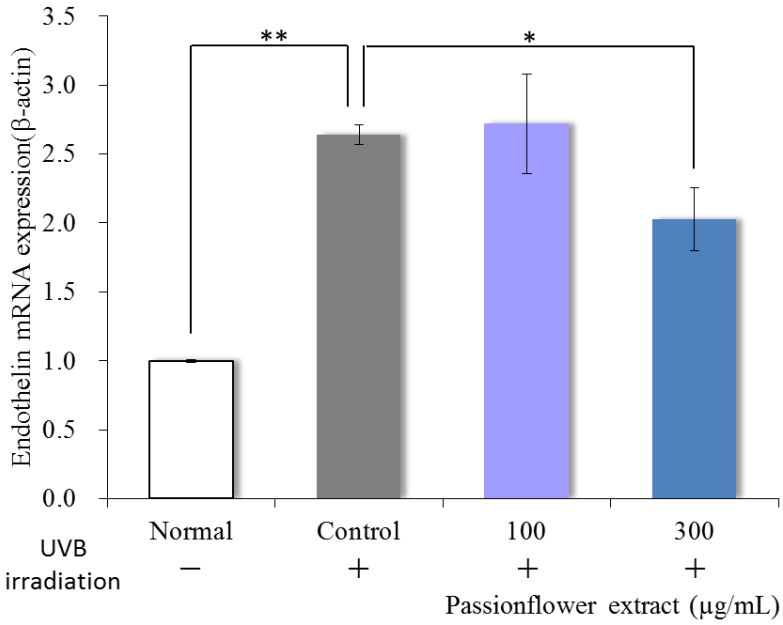


図 16 mRNA Expression of Endothelin-1
The mean value ± S.E. (n=4) ＊*: p<0.05, **: p<0.01

9-1-6 mRNA Expression of Antioxidant relative Gene

Reactive oxygen is generated in our body because of irritation such as UV-ray and stress. Reactive oxygen sends a command cornified cells to produce endothelin that causes pigmentation as described above. Reactive oxygen itself also damages inner areas of the skin. It causes inflammation inside the skin and destroys collagen and elastin that maintain skin supple. This may make skin dull and prone to wrinkles. Below is a report of a study conducted by the Food Development Department of Oryza Oil & Fat Chemical Co., Ltd.
Passionflower Extract was added to mouse fibroblasts (NIH3T3) with tuned circadian rhythm so that its final concentration would be 100 µg/mL and the sample was cultured for 16 hours in order to check the anti-oxidant action of passion flower extract. After cultivation, cells were collected and the gene expression level of anti-oxidation-related enzymes (GPx1, SOD1) was measured. As a result, the mRNA expression of both GPx1 and SOD1 significantly increased in the passion flower extract group as compared to the control group. According to the results, passion flower extract is expected to have an effect to enhance the action to protect skin from reactive oxygen generated due to UV-rays and stress (to prevent aging of skin).

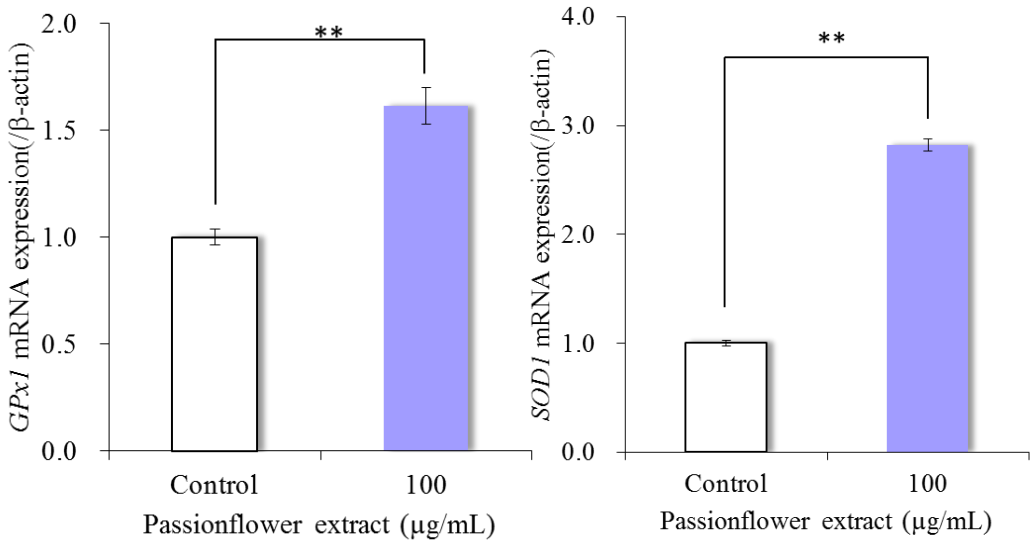


Fig. 17: mRNA Expression of GPx1 (left) and SOD1 (right)
The mean value ± S.E. (n=4) **: p<0.01

Source: Catalogue of "Passionflower Extract (Food)" of Oryza Oil & Fat Chemical Co., Ltd.

9-2 Clinical Study

The test was carried on 16 subjects aged 22 to 62 who submitted a written agreement (8 men and 8 women). A test was also conducted on 16 test subjects and they were separated in two groups considering their sex and age. Eight of the subjects used a placebo and the other eight subjects used the extract (single-blind test). Test subjects in the placebo group applied approximately 1 mL propanediol lotion and subjects in the extract group applied approximately 1 mL cosmeHerbest™ PASSIFLORA over their entire face in the morning and at night every day. Their skin condition was measured by the following items before application and after four weeks of application.

Measurement was performed in a room where temperature was regulated to 24±2 ℃ and humidity 58±2 % after 15 minutes of conditioning. The test was started on June 29, 2016 and was carried out for four weeks.

(1) Improvement of moisture and oil levels (skin quality)
(2) Improvement in skin texture
(3) Reduction of redness
(4) Reduction of pigmentation
(5) Improvement of skin tone clarity
(6) Reduction of uneven skin tone

Above-mentioned test number (3), (4), (5) and (6) were measured using Robo Skin Analyzer CS50 (Inforward Inc.).

Test Sample

Mix well 5% volume of cosmeHerbest™ PASSIFLORA and 95% volume of 30% of propanediol solution adjusted in advance, and used this test solution as “PASSIFLORA Lotion” to the Sample group for eight subjects. Use 30% of propanediol solution as Placebo Lotion to Placebo group for other eight subjects.

9-2-1 Improvement Effect of Moisture and Oil Balance

Test Method

The moisture level and oil level were measured using the WSK-P500U oil and moisture meter (manufactured by Wave Cyber Corporation) to measure the skin type improvement effect.

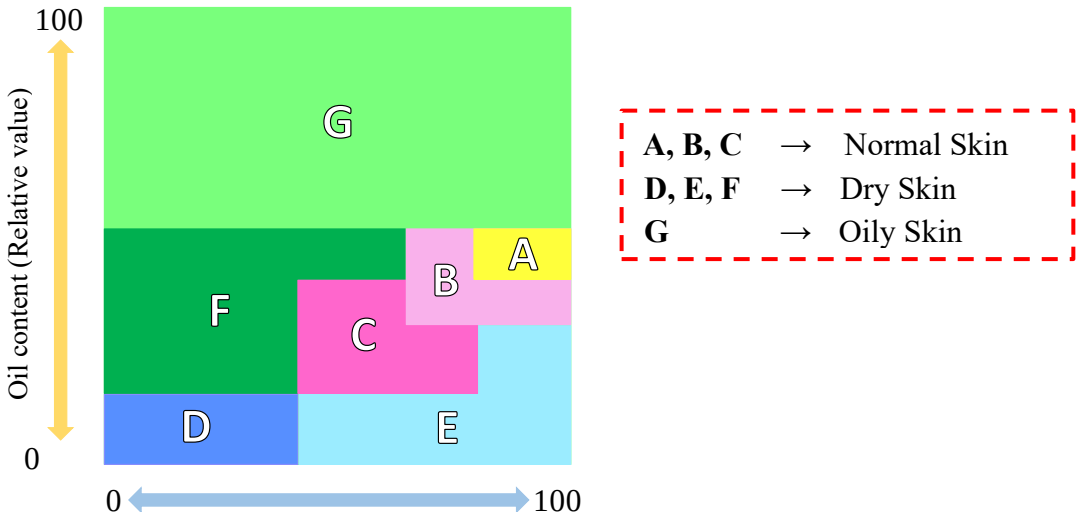
The moisture level was indicated by a value between 0 and 100. Electrostatic capacity of skin was determined by pressing the sensing part of the measuring equipment against skin and the result was used as a moisture value. The state where saline solution was detected is the "saturated state (100)" and the state with no moisture is the "no moisture state (0)."

The oil level is indicated by a value between 0 and 100 just like the moisture level. The sensing part of the measuring equipment was pressed against skin and the area of the spread out oil was determined by the refractive index. The state where oil covered the entire area is "oil-saturated state (100)" and the state with no oil is the "no oil state (0)."

Skin type was evaluated by the balance between the measured moisture and oil levels and evaluation ranks were categorized in three skin types (A-C: Normal skin, D-F: Dry skin, G: Oily skin). Skin type was also compared before the test and four weeks later by grading the skin type evaluation ranks.

Table 1: Evaluation Table of Skin Type and its Distribution

Rank	Moisture	Oil	Explanation	Score
A	81-99	41-50	Ideal state	7
B	66-99	31-50	Approximately good state	6
C	41-80	16-40	Moisture and oil are scant, but balance is good.	5
D	0-40	0-15	Lack of secretion	4
E	41-99	0-30	Balance of moisture and oil is bad. (Oil is scant.)	3
F	0-65	16-50	Balance of moisture and oil is bad. (Moisture is scant.)	2
G	0-99	51-99	Excessive serection	1



Test Result ar

Type of skin of the 16 test subjects was evaluated by the moisture and oil level measurement results and then the results before the test and four weeks later were compared. It was confirmed that oily and dry skin improved to the same level as normal skin in the group that used the sample containing cosmeHerbest™ PASSIFLORA (Fig. 18).

The evaluation ranks were graded and the improvement rates and the average improvement rate in each group were calculated. When the average value of the placebo group was 1, the average value of the extract group was 13. According to the results, cosmeHerbest™ PASSIFLORA is expected to regulate moisture and oil balance and improve skin type.

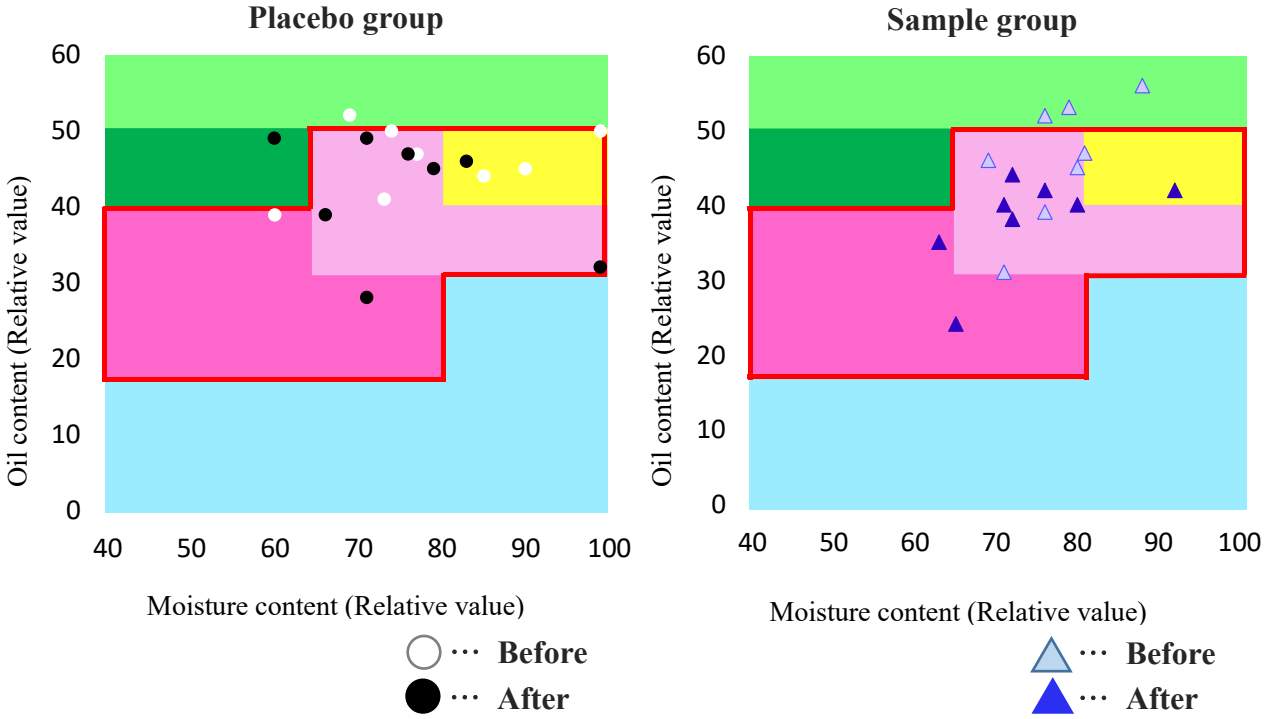


Fig. 18: Distribution of moisture level and oil level

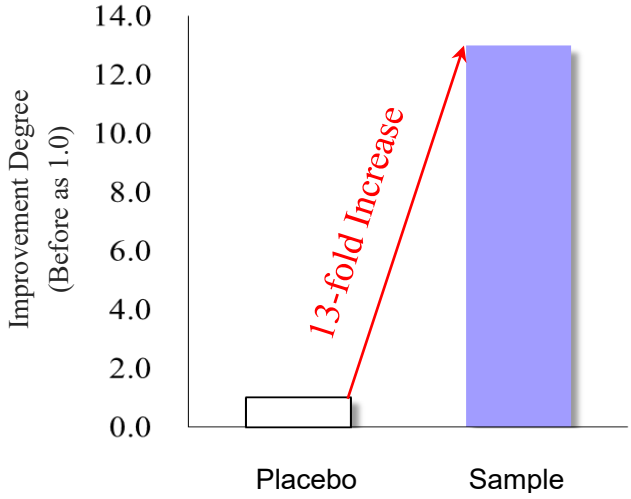


Fig. 19: Improvement Effect of Skin Type

9-2-2 Improvement Effect of Skin Texture

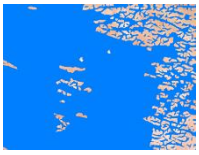
Test Method

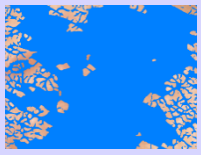
Dark and bright areas in monochrome images were enhanced on a computer to determine them as skin grooves and crista cutis respectively. The closer the binarized results are to the ideal skin texture model with 0.4 mm-equilateral triangles, the higher the point was (full score: 100).

Test Result and Consideration

Skin texture values measured on the 16 test subjects were compared before the test and four weeks later and the improvement rate was calculated respectively. When the improvement rate of the two groups was compared, the action to improve skin texture was not confirmed in the placebo group. However, skin texture of subjects in the group that used the extract containing cosmeHerbest™ PASSIFLORA significantly improved. According to the results, cosmeHerbest™ PASSIFLORA is believed to have an action to improve skin texture.

Table 2: Improvement of Skin Texture
(Image Comparison)

Placebo	Before	After
K.I. (27) M		

Sample	Before	After
Y.T. (33) M		

Areas with fine texture on skin are recognized and shown in beige color. The larger the beige areas is, finer the skin texture.

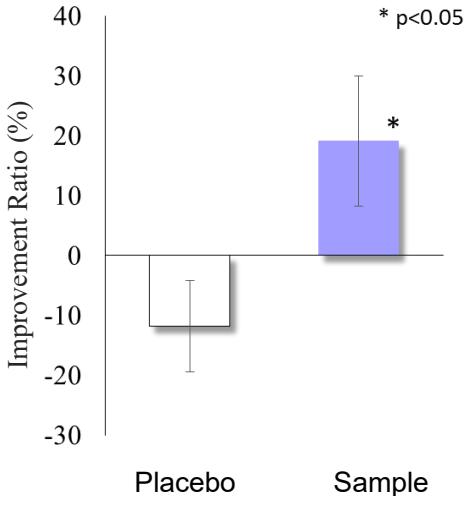


Fig. 20: Improvement Ratio of Skin Texture

9-2-3 Improvement of Skin Redness

Test Method

The effect to reduce redness was measured using a Robo Skin Analyzer. Two groups of areas, “group of skin areas with redness” and “group of skin areas without any redness or pigmentation” were defined in three-dimensional areas of color images (RGB). Color information of the images was processed into monochromatic-processed using the vector connecting these groups as the standard. Then, redness was detected based on the contrast in the monochrome images. Areas where redness was detected were considered as areas with inflammation or acne.

Image Example

Test Result and Consideration

When the improvement rate in the groups was compared, improvement was confirmed on only three test subjects among eight in the placebo group. However, redness was reduced on seven test subjects among eight in the group that used an extract containing cosmeHerbest™ PASSIFLORA, indicating a significant effect to reduce redness (Table 3, Fig. 21 and Fig. 22). According to the results, cosmeHerbest™ PASSIFLORA is expected to have an action to suppress inflammation and reduce redness of skin.

Table 3 Comparison of Improvement Effect of Skin Redness

Placebo group (8 subjects)

Subjects	Age	Sex	Area of Redness		Improvement Ratio (%)
			Before	After	
Y.Y.	24	F	687	**964**	-40.32
K.I.	26	M	2416	**2615**	-8.24
T.H.	26	M	1562	**1721**	-10.18
H.F.	29	F	864	**994**	-15.04
Y.F.	29	M	1583	**1222**	22.80
A.Y.	30	F	957	**842**	12.02
E.N.	43	F	759	**861**	-13.44
S.I.	58	M	1920	**1512**	21.25
Ave.			**1343.5**	**1341.38**	**-3.89**

Sample group (8 subjects)

Subjects	Age	Sex	Area of Redness		Improvement Ratio (%)
			Before	After	
M.K.	24	F	2049	**1942**	5.22
Y.M	25	F	1244	**817**	34.32
S.T.	26	M	1953	**1533**	21.50
E.W.	28	F	1000	**1098**	-9.80
N.S.	28	M	1248	**1225**	1.84
Y.T.	33	M	449	**418**	6.90
T.K.	45	M	2021	**1596**	21.03
T.S.	52	F	906	**558**	35.10
Ave.			**1358.75**	**1152.13**	**14.52**

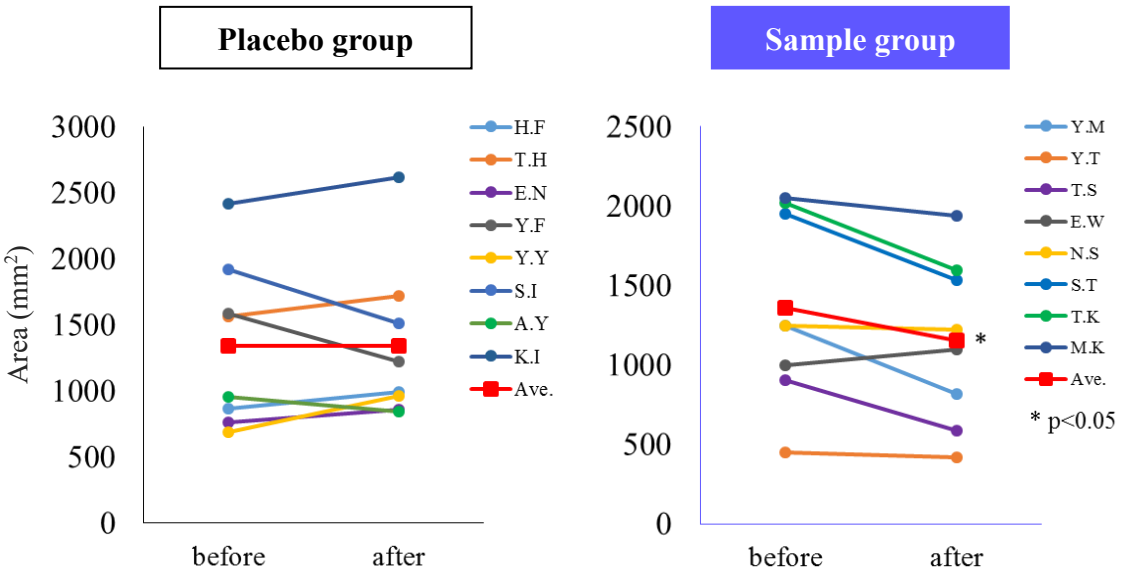


Fig. 21: Improvement Effect of Skin Redness

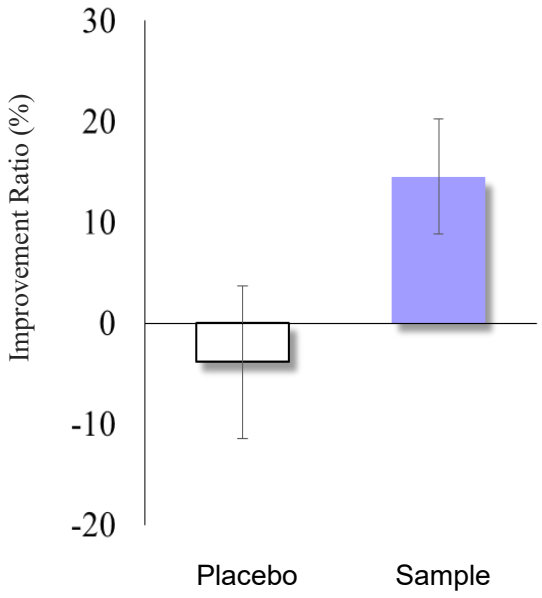


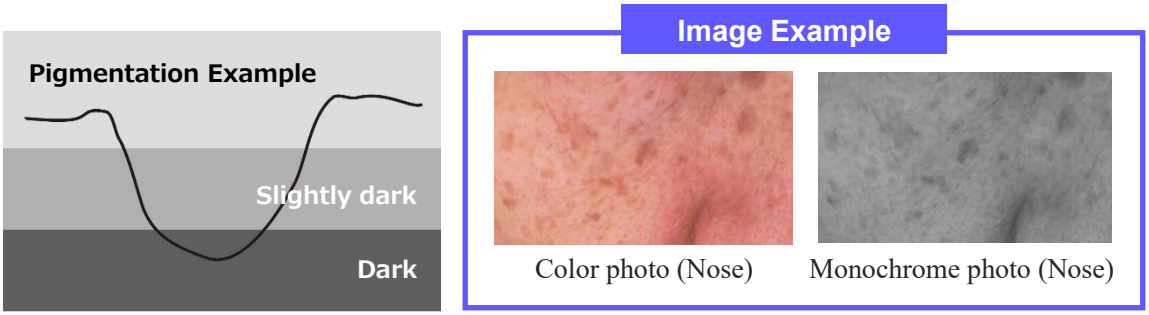
Fig. 22: Improvement Ratio of Skin Redness

9-2-4 Improvement Effect of Pigmentation

Test Method

Among three color elements existing in color images (RGB), pores and pigmentation distribution is observed more in signal components of "BLUE (B)." Therefore, pores and pigmentation can be defined by the concentration and characteristics in shapes in monochrome images created by using BLUE signals.

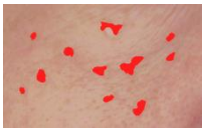
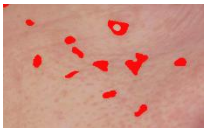
As shown in the photos below, pigmentation level is not very clear in color photos. Continuous areas with a **size of 1.2 mm^2 or larger** of which margin can be detected as "**slightly dark areas**" and "**dark areas**" as compared to surrounding areas in monochrome photos are detected as "**pigmentation areas**." Pigmentation was evaluated in three levels by their tone and contrasting intensity.

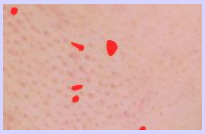
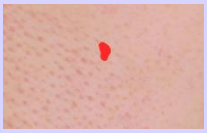


Test Result and Consideration

Pigmentation counts measured on the 16 test subjects were compared before the test and four weeks later and the improvement rates and average improvement rate were calculated respectively. When the average value of the placebo group was 1, the average value of the group that used the extract containing cosmeHerbest™ PASSIFLORA was 1.54 (Table 4, Fig. 22). According to the results, cosmeHerbest™ PASSIFLORA is expected to have an action to reduce pigmentation.

Table 4: Improvement of Pigmentation

Placebo	Before	After
S.I. (58) M		

Sample	Before	After
E.W. (28) F		

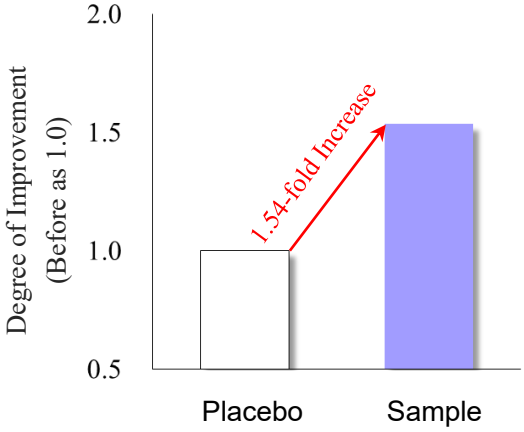
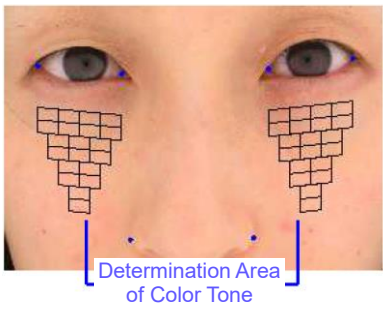


Fig. 23: Improvement Ratio of Pigmentation

9-2-5 Improvement Effect of Skin Brilliance

Test Method

As shown in the photo to the right, 40 measurement areas were located on areas under the right and left eyes. Three color tone items were measured in each measurement area. Then, measurement results were sorted from low values to high values and the average value of the middle 20 areas was used as the measurement result.



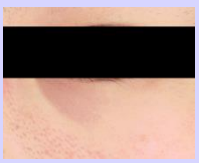
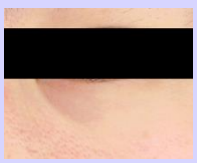
Brilliance indicates how brilliant colors are. This value can be used to evaluate blood circulation and cloudiness. Low values indicate that skin has darkened because the epidermis has become thicker and blood flow in the skin does not show clearly. High values indicate good blood flow in the skin and high clarity.

Test Result and Consideration

Pigmentation counts measured on the 16 test subjects were compared before the test and four weeks later and the improvement rates and average improvement rate were calculated respectively. When the average value of the placebo group was 1, the average value of the group that used the extract containing cosmeHerbest™ PASSIFLORA was 1.5 (Table 5, Fig. 24). According to the results, cosmeHerbest™ PASSIFLORA is expected to have an action to reduce pigmentation.

Table 5: Improvement Effect of Skin Brilliance

Placebo	Before	After
Y.Y. (24) F		

Sample	Before	After
E.W. (28) F		

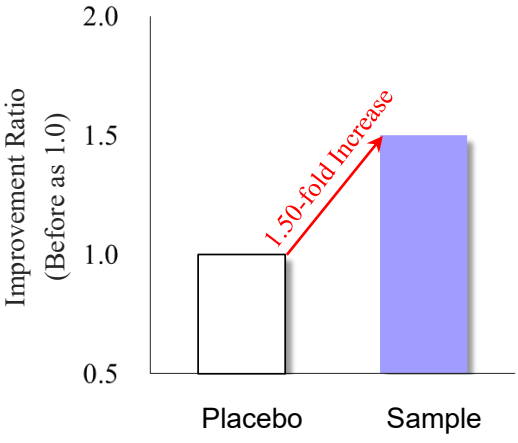


Fig. 24: Improvement Ratio of Skin Brilliance

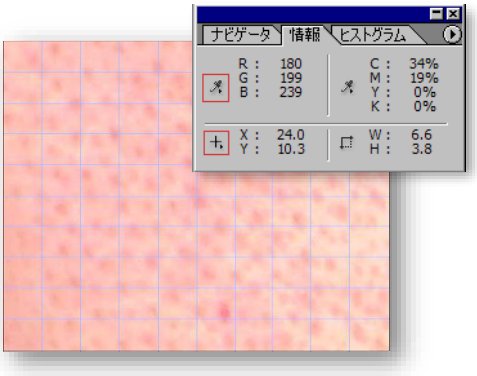
9-2-6 Improvement Effect of Uneven Skin Color Tone

According to the results of the monitor test on people described above, cosmeHerbest™ PASSIFLORA has effects to reduce causes of uneven skin tone including dryness, redness caused by inflammation, pigmentation, and poor blood circulation.

In order to confirm the reduction of uneven skin tone, the degree of uneven skin tone was analyzed using high-definition photos of test subjects taken by a Robo Skin Analyzer.

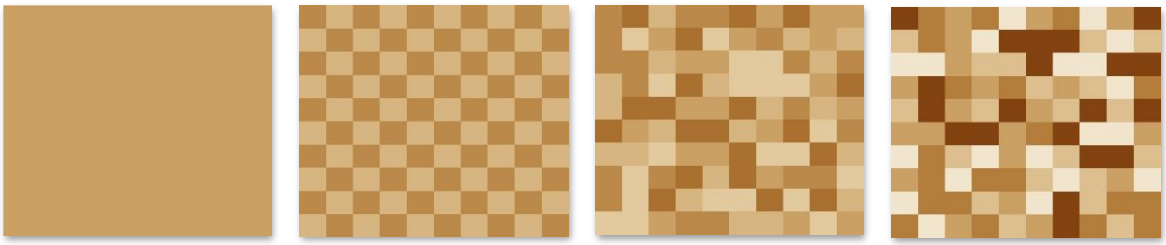
Test Method

Analysis method of Uneven Skin Color Tone[7)]



A 20 x 25 mm area on the right cheek was extracted from the high-definition photos taken by the Robo Skin Analyzer. The extracted area was divided into 100 cells by grid lines and the RGB values of the center of each cell were measured. Standard deviation of RGB values obtained for the 100 cells was calculated and then the "degree of uneven skin tone" for each of the 16 test subjects was calculated using the formula below. Respective standard deviation of RGB values was used to indicate variations of the R, G, and B values so that the "overall variation level" would be equal to the "degree of uneven skin tone.

Degree of Uneven Skin Tone = (SD of R value)×(SD of G value)×(SD of B value)



A B C D

Average of Brightness: A = B = C = D

SD / Standard Deviation: A < B < C < D

The average of the color of the 100 cells in A to D above was equivalent. However, the variation width of the overall color differs according to the color contrast and distribution (level of variation width: A<B<C<D). Color distribution causes uneven skin tone and influences skin's appearance.

Test Result

The degree of skin tone of the 16 test subjects was analyzed using photos. The difference between the results obtained before the test and ones obtained four weeks after was calculated and then the average value in each group was calculated. Improvement was observed on seven subjects that used the extract containing cosmeHerbest™ PASSIFLORA out of eight. The average improvement value was -38.2 points. In the placebo group in contrast, improvement was observed on only one of the eight subjects and the average improvement value was +57.5. The difference between the maximum and minimum R, G, and B values of each subject was calculated and the calculated values before the test and four weeks after were compared. As a result, the difference reduced in the extract group. As the degree of uneven skin tone increased in the placebo group, it significantly reduced in the extract group, indicating the sample reduced uneven skin tone.

Table 6: Improvement Effect of Uneven Skin Color Tone

Placebo group (8 subjects)

Subject	Age	Sex	Uneven Color Tone		Improvement Degree (After - Before)
			Before	After	
Y.Y.	24	F	201.52	242.72	+41.20
K.I.	26	M	162.94	219.16	+56.22
T.H.	26	M	219.98	281.41	+61.43
H.F.	29	F	180.33	279.48	+99.15
Y.F.	29	M	253.24	258.12	+4.88
A.Y.	30	F	67.36	138.47	+71.11
E.N.	43	F	84.03	53.41	-30.62
S.I.	58	M	242.76	399.07	+156.31
Ave.			**176.52**	**233.98**	**+57.46**

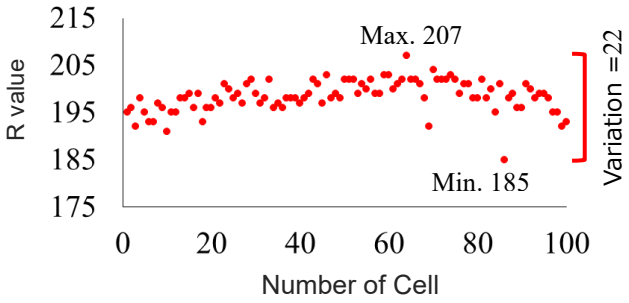
Sample group (8 subjects)

Subject	Age	Sex	Uneven Color Tone		Improvement Degree (After - Before)
			Before	After	
M.K.	24	F	163.60	152.41	-11.19
Y.M	25	F	349.89	311.10	-38.79
S.T.	26	M	157.80	85.79	-72.01
E.W.	28	F	161.55	125.89	-35.66
N.S.	28	M	290.35	228.06	-62.29
Y.T.	33	M	295.89	281.36	-14.53
T.K.	45	M	240.47	148.68	-91.79
T.S.	52	F	209.84	230.46	+20.62
Ave.			**233.67**	**195.47**	**-38.2**

Excerpt from the Sample group

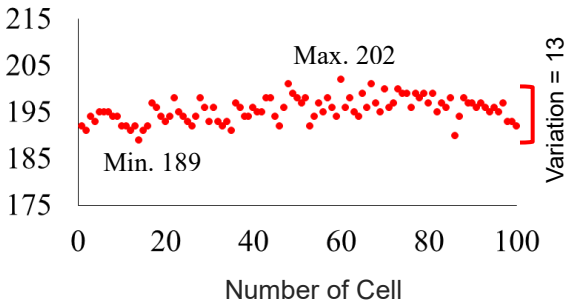
Subject	Age	Sex	Uneven Color Tone		Improvement Degree (After - Before)
			Before	After	
S.T.	26	M	157.80	85.79	-72.01

Before

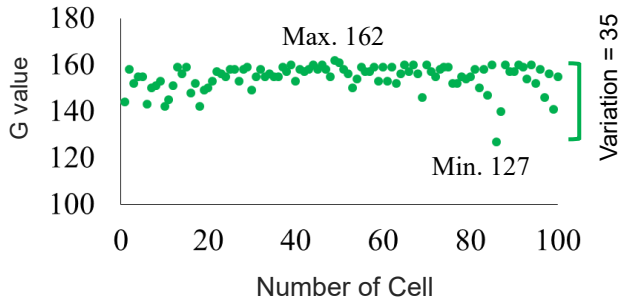


Max. 207 ⇔ Min. 185

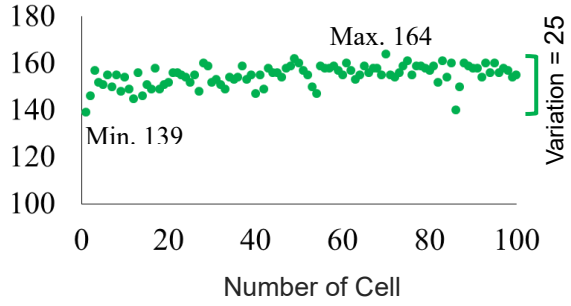
After



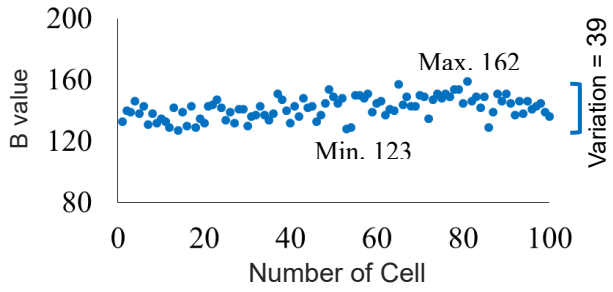
Max. 202 ⇔ Min. 189



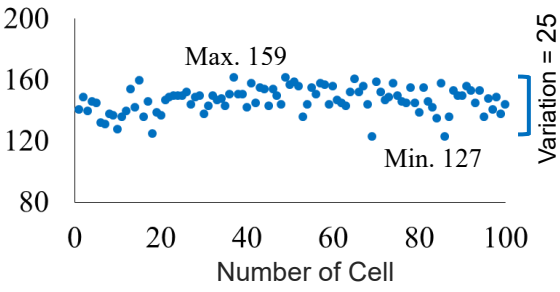
Max. 162 ⇔ Min. 127



Max. 164 ⇔ Min. 139



Max. 162 ⇔ Min. 123

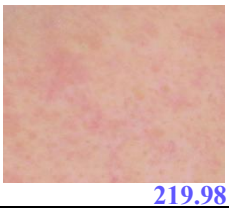
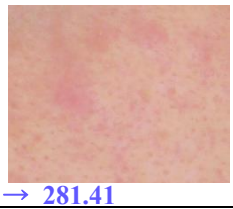
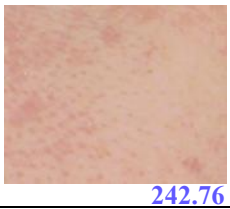
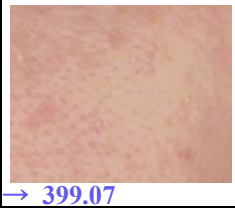


Max. 159 ⇔ Min. 127

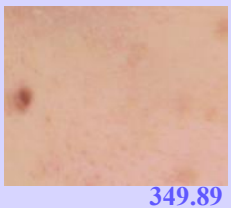
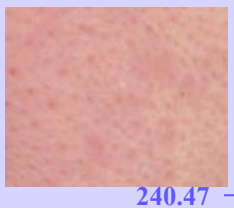
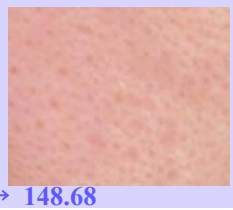
Fig. 25: Comparison before / after of Uneven Color Tone (Variation of RGB value)

Table 7: Improvement Effect of Uneven Skin Color Tone (Image Comparison)

Placebo group

	Before	After		Before	After
H.F. (29) F	 219.98	 → 281.41	S.I. (58) M	 242.76	 → 399.07

Sample group

	Before	After		Before	After
Y.M. (26) F	 349.89 → 311.10		T.K. (45) M	 240.47 → 148.68	

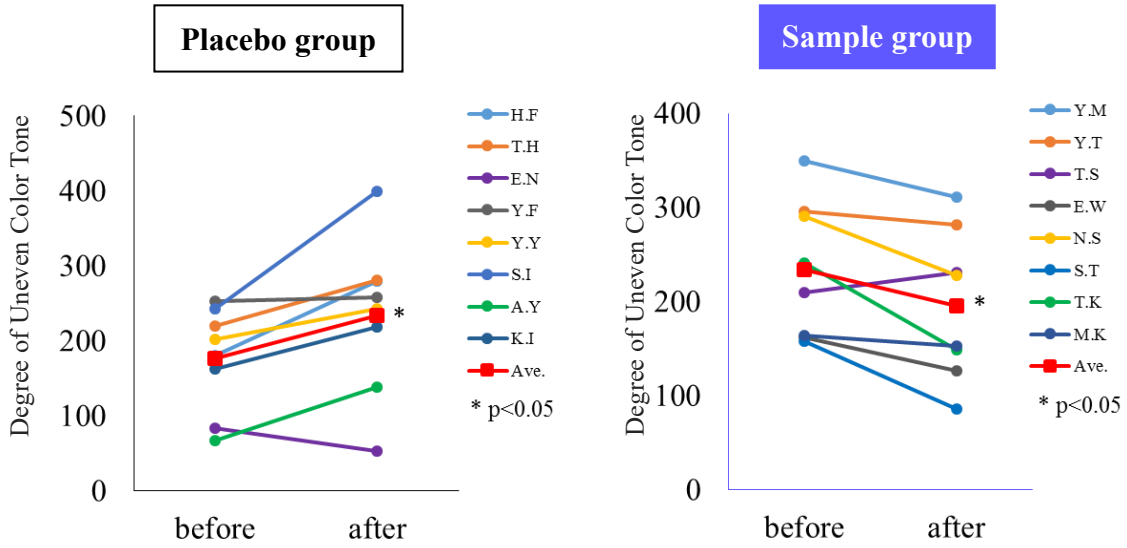


Fig. 26: Improvement Effect of Uneven Skin Color Tone

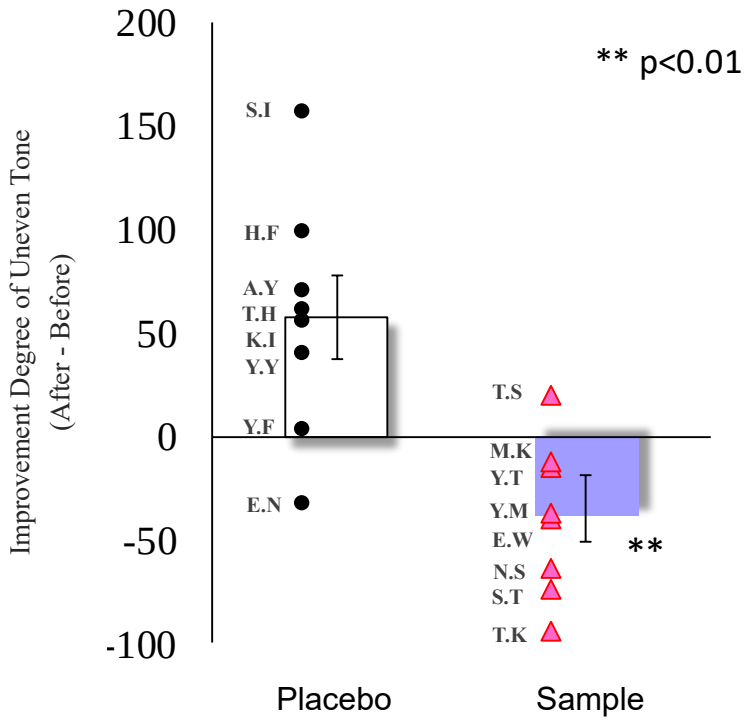


Fig. 27: Improvement Degree of Uneven Skin Color Tone

Conclusion

According to the results above, the following actions were confirmed in the monitor test on human.

1. Increase the gene expression of peroxisome proliferator-activated receptor (PPAR) that promotes skin's barrier function, adjust the moisture and oil level on skin by increasing gene expression of cornified envelope maturation, the cornified envelope formation factors involucrin and filaggrin, and improve skin texture
2. Reduce pigmentation by suppressing gene expression of endothelin produced by external irritation such as UV-ray
3. Reduce redness caused by inflammation and increase skin's brilliance by performing an anti-oxidant effect through the increase of gene expression of GPx and SOD activation to reduce uneven skin tone was also observed.

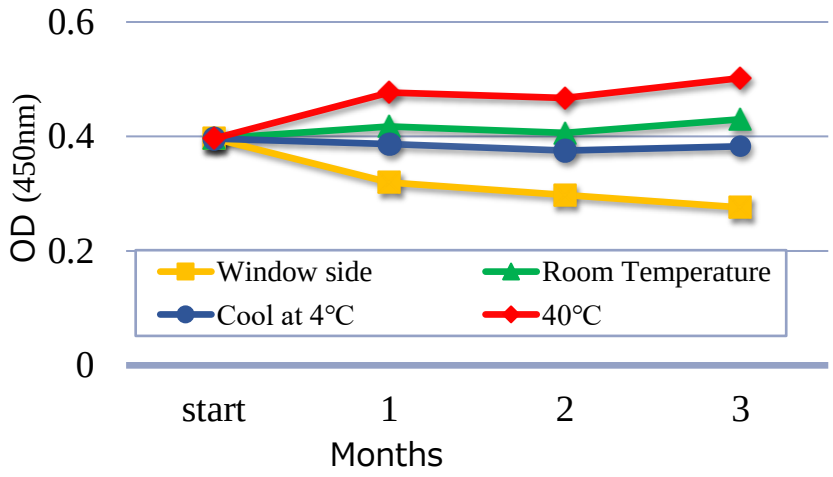
For the reasons above, cosmeHerbest™ PASSIFLORA is expected to have actions to produce cornified envelope formation factors and reduce uneven skin tone.

10. Stability Test

10-1 Long-term Stability Test

Store cosmeHerbest™ PASSIFLORA as it was, in a cool dark place at 4℃, room temperature, window side and at 40℃, observed for 3 months, and determined optical density at 450nm.

Test Result



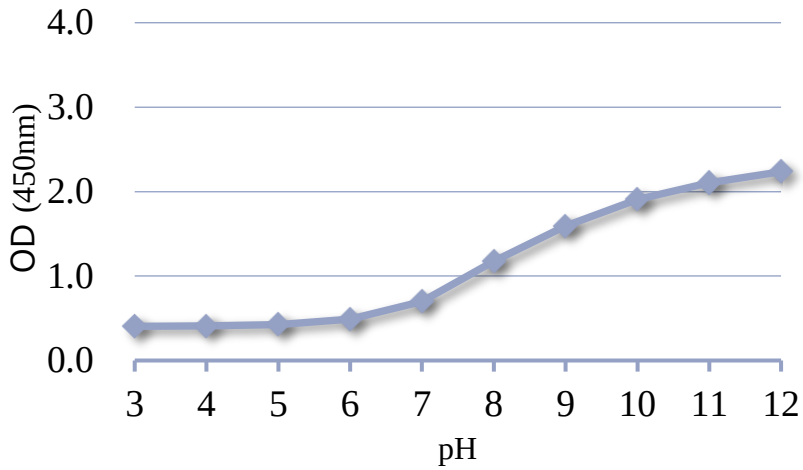
Consideration

The color changed when the sample was left at 40℃ and also when it was exposed to sunlight (by the window) throughout the three months. Because the color was stable when the sample was refrigerated (4℃), it is recommended to store cosmeHerbest™ PASSIFLORA in a refrigerator.

10-2 pH Stability Test

Adjust pH value of cosmeHerbest™ PASSIFLORA from 3 to 12 with hydrochloric acid and sodium hydroxide, observe the change of color tone and determine the optical density at 450nm.

Test Result



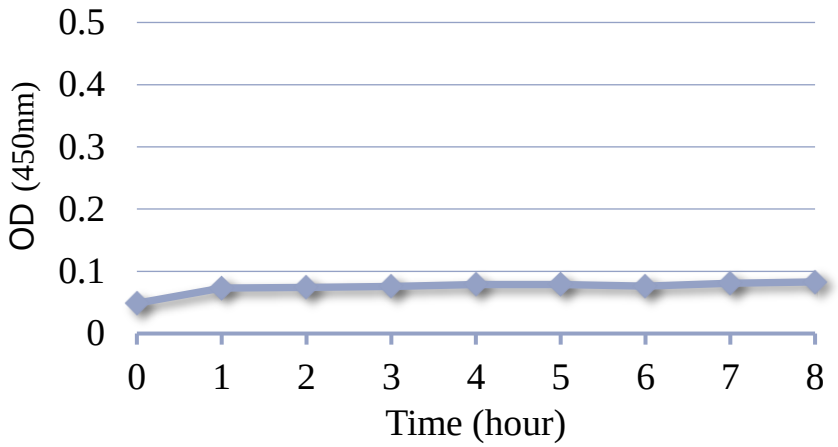
Consideration

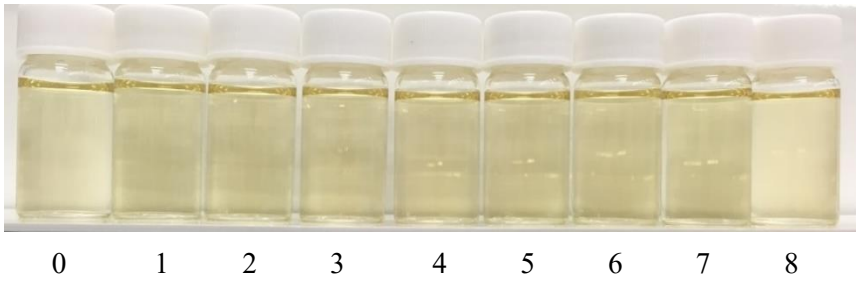
The sample is light brown in the acidic, weakly-acidic, and neutral regions. It turns yellow rapidly and showed a tendency to become reddish in the alkaline region. Use the product in acidic to neutral regions.

10-3 Thermal Stability Test

Heat 10% water solution of cosmeHerbest™ PASSIFLORA at 90℃ for 8hours and observe the change of color tone.

Test Result





Consideration

10% aqueous solution of cosmeHerbest™ PASSIFLORA was heated for 8 hours at 90 ℃, as shown in the photo, the color tone did not change, and it is considered that thermal stability on cosmeHerbest™ PASSIFLORA relatively stable when heated for 8 hours.

11. Compatibility Test

(○ : Clear, △ : Turbid, × : Precipitate)

	%	Trade Name / Manufacturer	INCI Name	Result 1hr	Result 24hr
Cation	3.0	QUARTAMIN 86W Kao Corporation	Steartrimonium Chloride / Water	○	○
Anion	10.0	SOYPON SLE Kawaken Fine Chemical Co., Ltd.	Sodium Lauroyl Sarcosinate	○	○
	10.0	EMAL 20C Kao Corporation	Sodium Laureth Sulfate / Water	○	○
	10.0	AMISOFT CT-12S Ajinomoto Co., Inc.	Water / TEA-Cocoyl Glutamate	○	○
Nonion	10.0	PYROTER GPI-25 Nihon Emulsion Co., Ltd.	Glycereth-25 PCA Isostearate	○	○
	10.0	SALACOS PG-218 Nisshin Oilio Group Co., Ltd.	Polyglyceryl-10 Dioleate / Tocopherol	×	×
	10.0	RHEODOL 460V Kao Corporation	Sorbeth-60 Tetraoleate	△	○
	10.0	RHEODOL TW-0120V Kao Corporation	Polysorbate 80	○	○
Amphoteric	5.0	AMPHITOL 20AB Kao Corporation	Lauramidopropyl Betaine	○	○
	10.0	SOFTAZOLINE LSB 29% aq. Kawaken Fine Chemical Co., Ltd.	Lauramidopropyl Hydroxysulfate	○	○
Silicone	10.0	KF-96A-10CS Shin-Etsu Chemical Co., Ltd.	Dimethicone	×	×
	10.0	KF-96A-300CS Shin-Etsu Chemical Co., Ltd.	Dimethicone	×	×
	10.0	KF-995 Shin-Etsu Chemical Co., Ltd.	Cyclopentasiloxane	×	×
	10.0	Silwet L-7604 Momentive Performance Materials	PEG-8 Dimethicone	○	○
	10.0	Silwet L-7622 Momentive Performance Materials	PEG-8 Dimethicone	×	×
Polymer	1.0	Bio-HA 1% Sol.（MP-PE）N Shiseido Co., Ltd.	Sodium Hyaluronate / Water	○	○

cosmeHerbest™ PASSIFLORA was adjusted to 10% concentration. Other products were adjusted to the concentration in the table with purified water, mixed cosmeHerbest™ PASSIFLORA and other ingredients, observe the compatibility at 1 hour and 24 hours after mixing.

12. Toxicological Safety Study

Trade Name	cosmeHerbest™ PASSIFLORA	
Safety Test Items	**Test Result**	**Test Method**
Acute Oral Toxicity Test	Not Performed	
Primary Skin Irritation Test	No Irritation	EpiSkin™ method
Accumulated skin Irritancy Test	Not recognize any stimulus	RIPTmethod (50 subjects)
Sensitization Test	Not recognize any stimulus	RIPTmethod (50 subjects)
Photo Toxicity Test	Not Performed	
Photo Sensitization Test	Not Performed	
Eye Irritation Test	No Irritation	EpiOcular™ method
Mutagenicity Test	Negative	Ames Test (TA98, TA100)
Human Patch Test	Not Recognize any stimulus	RIPTmethod (50 subjects)

13. Recommended Planning and Guide Formulation

(Formulation Provided by Nihon Emulsion Co., Ltd.)

- Night Cream
- Day Cream
- Moisture Cream for Sensitized Skin
- Serum
- Lotion for Skin Roughness
- All-in-one-Gel

13-1 Guide Formulation 1: Night Cream / FMCC-476(M)

No.	Trade Name	Manufacturer	%	INCI Name
1	LIQUID PARAFFIN-70S	TOEI Chemical Co., Ltd.	8.00	Mineral Oil
2	**AMITER MA-HD**	**Nihon Emulsion Co., Ltd.**	4.00	Hexyldecyl Myristoryl Methylaminopropionate
3	KF-96A-10CS	Shin-Etsu Chemical	4.00	Dimethicone
4	CONOL 2265	New Japan Chemical	2.00	Behenyl Alcohol
5	CETANOL H	Kokyu Alcohol Kogyo	4.00	Cetearyl Alcohol
6	CERASYNT PA	ASHLAND	1.00	Propylene Glycol Stearate
7	**EMALEX GMS-B**	**Nihon Emulsion Co., Ltd.**	3.20	Glyceryl Stearate
8	**EMALEX 820**	**Nihon Emulsion Co., Ltd.**	0.80	PEG-20 Stearate
9	Butylparaben		0.10	Butylparaben
10	AMISOFT HS-11P	Ajinomoto Co., Inc.	0.30	Sodium Stearoyl Glutamate
11	SORBIT D-70	Mitsubishi Shoji Foodtech	5.00	Sorbitol / Water
12	Glycerin		4.00	Glycerin
13	Methylparaben		0.20	Methylparaben
14	Keltrol T	CP Kelco	10.00	Xanthan Gum / Water
15	**cosmeHerbest™ PASSIFLORA**	**Oryza Oil & Fat Chemical**	1.50	Water / Propanediol / Passiflora Incarnata Extract
16	**cosmeHerbest™ STRAWBERRY**	**Oryza Oil & Fat Chemical**	1.50	Water / Propanediol / Fragaria Ananassa Seed Extract
17	Purified water		50.40	Water
			100.00	

Manufacturing Procedure

1) Mix and dissolve Ingredients No.1 to 9 at 70℃. (Phase A)
2) Mix and dissolve Ingredients No. 10 to 17 at 75℃. (Phase B)
3) While stirring Phase B by homogenizer, add Phase A and make emulsion.
4) Then, stir by paddle at 40℃ and cool as the product.

13-2 Guide Formulation 2: Essence / FLG-04A(M)

No.	Trade Name	Manufacturer	%	INCI Name
1	Jojoba Oil		0.10	Simmondsia Chinensis (Jojoba) Seed Oil
2	LIQUID PARAFFIN-70S	TOEI Chemical Co., Ltd.	2.00	Mineral Oil
3	**AMITER MA-HD**	**Nihon Emulsion Co., Ltd.**	0.50	Hexyldecyl Myristoryl Methylaminopropionate
4	**EMALEX DSG-2**	**Nihon Emulsion Co., Ltd.**	0.50	Polyglyceryl-2 Distearate
5	CETANOL H	Kokyu Alcohol Kogyo	1.20	Cetearyl Alcohol
6	Pemulen TR-2 Polymeric Emulsifier	Lubrizol Corporation	0.07	Acrylates/C10-30 Alkyl Acrylate Crosspolymer
7	Carbopol 940	Lubrizol Corporation	0.06	Carbomer
8	**ORYZA Tocotrienol™-90**	**Oryza Oil & Fat Chemical**	0.10	Tocotrienol / Tocopherol / Oryza Sativa (rice) Bran Oil
9	Propylparaben		0.05	Propylparaben
10	Butylparaben		0.05	Butylparaben
11	**EMALEX GM-10**	**Nihon Emulsion Co., Ltd.**	1.00	PEG-10 Glyceryl Stearate
12	Glycerin		20.00	Glycerin
13	ACTIVONOL-3	ActivON Co., Ltd.	24.00	Propanediol
14	Methylparaben		0.10	Methylparaben
15	HA-Na 1%aq	Shiseido Co., Ltd.	4.00	Water / Sodium Hyaluronate / Methylparaben
16	PEG-75		0.20	PEG-75
17	Arginine		0.10	Arginine
18	**cosmeHerbest™ PASSIFLORA**	**Oryza Oil & Fat Chemical**	3.00	Water / Propanediol / Passiflora Incarnata Extract
19	Purified Water		42.42	Water
20	95% Ethanol		0.50	Alcohol
21	Fragrance		0.05	Fragrance
			100.00	

Manufacturing Procedure

1) Mix and dissolve Ingredients No.1 to 10 at 85℃. (Phase A)
2) Mix and dissolve Ingredients No. 11 to 19 at 80℃. (Phase B)
3) Dissolve Ingredients No.20 and 21 at room temperature. (Phase C)
4) While stirring Phase B by homogenizer, add Phase A and make emulsion.
   (2500rpm, 30min.)
5) Then, stir by paddle at 40℃, cool, and add Phase C as the product.

13-3 Guide Formulation 3: Moisture Cream / FEX-07(M)

No.	Trade Name	Manufacturer	%	INCI Name
1	**ORYZA Squalane™**	**Oryza Oil & Fat Chemical**	1.00	Squalane
2	Vaseline		2.00	Petrolatum
3	W-445	Shima Trading Company	0.50	Microcrystalline Wax
4	CONOL 2265	New Japan Chemical	2.50	Behenyl Alcohol
5	**ORYZA Tocotrienol™-90**	**Oryza Oil & Fat Chemical**	0.10	Tocotrienol / Tocopherol / Oryza Sativa (rice) Bran Oil
6	KALCOL 8688	KAO Corporation	3.00	Stearyl Alcohol
7	KF-96A-10CS	Shin-Etsu Chemical	2.00	Dimethicone
8	NAA-1850	NOF Corporation	0.20	Stearic Acid
9	**EMALEX MC-10**	**Nihon Emulsion Co., Ltd.**	10.00	Caprylic/Capric Triglyceride / Triethylhexanoin / PEG-30Glyceryl Isostearate / Glyceryl Diisostearate / PEG-10 Dimethicone / Dipotassium Glycyrrhizinate / Glycerin / Potassium Chloride / Water
10	Phenoxyethanol		Trace	Phenoxyethanol
11	PEG-20		4.00	PEG-20
12	AMISOFT HS-11P	Ajinomoto Co., Ltd.	0.10	Sodium Stearoyl Glutamate
13	Zemer Select Propanediol	DuPont	5.00	Propanediol
14	Arginine		0.05	Arginen
15	Keltrol CGT	CP Kelco	0.10	Xanthan Gum
16	Maltitol		2.00	Maltitol
17	Glycerin		7.00	Glycerin
18	**ORYZA Ceramide™-LC0.8**	**Oryza Oil & Fat Chemical**	1.00	Glycerin / Water/Polyglyceryl-10 Oleate / Oryza Sativa (Rice) Bran Oil/Glucosyl Ceramide
19	**cosmeHerbest™ PASSIFLORA**	**Oryza Oil & Fat Chemical**	1.00	Water / Propanediol / Passiflora Incarnata Extract
20	Purified Water		32.45	Water
21	Ultra Agar AX-100	Ina Food Industry Co., Ltd.	25.00	Water / Agar
22	Collagen 1%	DSM Nutrition Japan	1.00	Water / Soluble Collagen / Sodium Benzoate / Citric Acid
			100.00	

Manufacturing Procedure

1) Mix and dissolve Ingredients No.1 to 10 at 80℃. (Phase A)
2) Mix and dissolve Ingredients No. 11 to 21 at 80℃. (Phase B)
3) While stirring Phase A by homogenizer, add Phase B and make emulsion.
   (3000rpm, 5 minutes)
4) Then, stir by paddle at 40℃, cool, and add Ingredients 22 at 30℃ as the product.

13-4 Guide Formulation 4: All-in-one-Gel / AIG-1-10(M)

No.	Trade Name	Manufacturer	%	INCI Name
1	**AMITER MA-HD**	**Nihon Emulsion Co., Ltd.**	1.00	Hexyldecyl Myristoryl Methylaminopropionate
2	SH556 Fluid	Sow Coaning Corporation	1.00	Phenyl Trimethicone
3	ELDEW PS-203	Ajinomoto Co., Ltd.	0.50	Phytosteryl / Octyldodecyl Lauroyl Glutamate
4	Phenoxyethanol		0.10	Phenoxyethanol
5	Glycerin		10.00	Glycerin
6	HAI SUGARCANEBG	Kokyu Alcohol Kogyo	5.00	Butylene Glycol
7	**EMALEX ML-158**	**Nihon Emulsion Co., Ltd.**	1.00	PEG-50 Hydrogenated Castor Oil Triisostearate / PEG-60Hydrogenated Caster Oil / Ceteth-20
8	**EMALEX MCCG-10**	**Nihon Emulsion Co., Ltd.**	0.50	Polyglyceryl-10 Cocoate
9	Trehalose		0.20	Trehalose
10	Methylparaben		0.10	Methylparaben
11	**cosmeHerbest™ PASSIFLORA**	**Oryza Oil & Fat Chemical**	1.00	Water / Propanediol / Passiflora Incarnata Extract
12	**MAQUI BERRY Extract-LC**	**Oryza Oil & Fat Chemical**	0.05	Water / Butylene Glycol / Aristotelia Chilensis Fruit Extract
13	EDTA-2Na (1% soln.)		1.00	Water / Disodium EDTA
14	HA-Na1%aq	Shiseido Co., Ltd.	2.00	Water / Sodium Hyaluronate / Methylparaben
15	Carbopol 940 (1% soln.)	Lubrizol Corporation	50.00	Carbomer
16	Purified Water		23.55	Water
17	Potassium Hydroxide (10% soln.)		3.00	Water / Potassium Hydroxide
			100.00	

Manufacturing Procedure

1) Mix and dissolve Ingredients No.1 to 4 at 70℃. (Phase A)
2) Mix and dissolve Ingredients No. 5 to 16 at 70℃. (Phase B)
3) While stirring Phase B by homogenizer, add Phase A, furthermore make emulsion adding Ingredients No.17. (3000rpm, 5 minutes)
4) Then, stir by paddle and cool at 30℃ as the product.

14. Product Specification

Commodity	:	Specification	Remark
Product Name		**cosmeHerbest™ PASSIFLORA**	
Description			
· Color	:	Reddish brown to brown liquid	
· Odor	:	Characteristic odor	
Identification Test			
· Flavonoid	:	Positive	
· Sugar	:	Positive	
Purity Test			
1) Heavy Metals	:	20 ppm max.	JSQI Method 2
2) Arsenic	:	2 ppm max.	JSQI Method 3
Microbiological Examination			
1) Bacterial Count	:	1×10^2/g max.	Hygiene Test Method
2) Mold, Yeast	:	1×10^2/g max.	Hygiene Test Method
3) Coliform	:	Negative	Hygiene Test Method

These standards and test method are referred to General Notices and General Tests, Processes and Apparatus of The Japanese Standards of Quasi-drug Ingredients, unless otherwise specified.

15. Labelling Name

15-1 JP Labelling Name	:	水 プロパンジオール チャボトケイソウエキス
15-2 JP Quasi-drug Name	:	None
15-3 INCI Name	:	Water Propanediol Passiflora Incarnata Extract
15-4 已使用化汝品原料名称目录 (IECIC2021)	:	水 1,3-丙二醇 粉色西番莲(PASSIFLORA INCARNATA)提取物

16. Others

16-1 Packaging

1kg PE Bottle, 5kg PE Cubic container / Outer: Carton box

16-2 Shelf Life & Storage Condition

- Two (2) years from the manufacturing date which is indicated on Certificate of Analysis.
- Avoid high temperature and humidity, and store in cool place around 4 ℃ dry and dark place.

17. Reference

1) Gotsu et al., JP2014-55127 A
2) Miroddi M., et al. (2013). *Passiflora incarnata* L.: ethnopharmacology, clinical application, safety and evaluation of clinical trials. J. Ethnopharmacol. 150, 791-804.
3) Volker Fintelmann / Rudorf Fritz Weiss, Lehrbuch Phytotherapie (2012)
4) Hirao T et al., Identification of immature cornified envelopes in the barrier-impaired epidermis by characterization of their hydrophobicity and antigenicities of the components, Exp. Dermatol., 10, 35~44 (2001)
5) Hitoshi Masaki, “Evaluation and Experiment Manual of Moisturizing, Whitening, Anti-Wrinke, Anti-oxidant”, Fragrance Journal (2012)
6) Greenspan P et al., Nile red: A selective fluorescent stain for intercellular lipid droplets, J. Cell. Biol., 100, 965~973 (1985)
7) Kiyotaka Tanaka et al., The Pharmaceutical Society of Japan, Chapter 130 Annual Meeting Abstracts 3, P.222, 30P-pm136

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ORYZA OIL & FAT CHEMICAL CO., LTD.

striving for the development of the new functional cosmetic ingredients to promote health and general well-being.

Headquarters:
ORYZA OIL & FAT CHEMICAL CO., LTD.
1 aza Numata Kitagata Kitagata-cho,
Ichinomiya-city Aichi-pref., 493-8001 JAPAN
TEL : +81 586 86-5141
FAX : +81 586 86-6191
URL : https : //www.oryza.co.jp/
E-mail : info@oryza.co.jp

ISO 22716
BUREAU VERITAS
Certification

Factory in Ichinomiya

Tokyo Sales Office:
5F Diamant-building, Kandasuda-cho 1-5
Chiyoda-ku, Tokyo, 101-0041 JAPAN
TEL : +81 3 5209-9150
FAX : +81 3 5209-9151
E-mail : tokyo@oryza.co.jp

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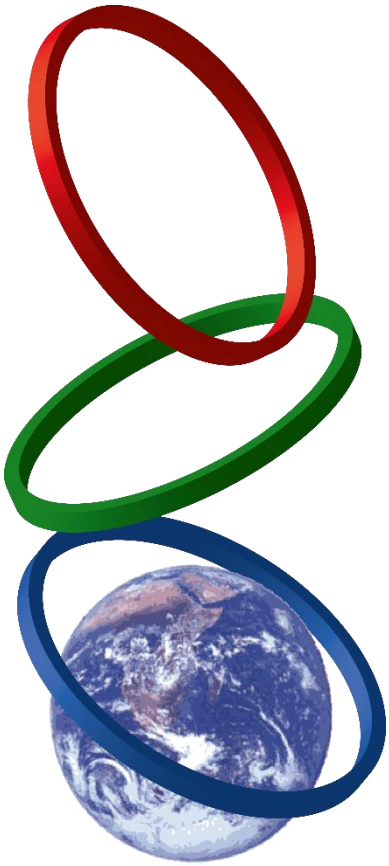

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