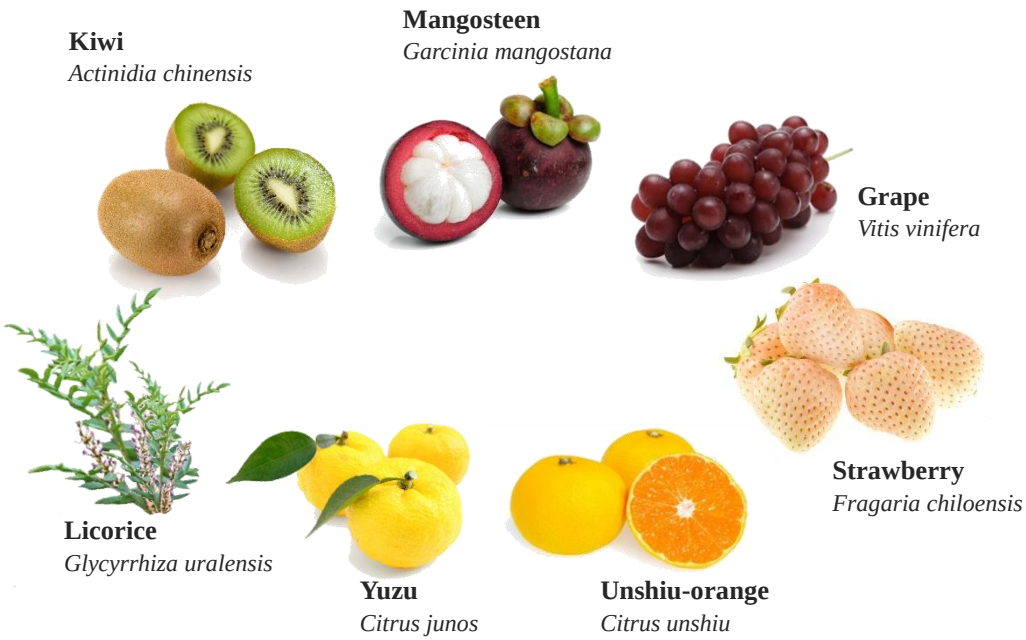
ORYZA OIL & FAT CHEMICAL CO.,LTD.

Anti-acne Fruits Complex

VeryBerry™ ACNEcare



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1. Introduction

Acne is a skin problem experienced by in people in a wide age range, from teenagers to adults. Although acne is a common skin disorder, it is very stressful for people in puberty who are conscious about their looks because eruption mainly occurs on their face. It is said that over 80% of men and women suffer from some type of acne problem during puberty and worry about it.

In general, acne is believed to naturally heal when you reach a certain age. However, there is a report indicating that more women in their twenties and over have acne and pimples because of a diversified lifestyle and increased mental stress. Because a high-stress society will continue to cause acne, demand for measures to treat adult acne as well as acne during puberty is estimated to continue to increase.

Many people with acne do not take any effect to treat it. When acne heals naturally, it leaves acne scars (cicatrix) that are often pointed out as cosmetic problems. Since cicatrix caused by acne is difficult to completely repair, starting acne-care treatment in an early stage is important.

For acne, many cosmetic products with a peeling effect and anti-oxidative effect are available. Peeling type products contain glycolic acid or other materials with a peeling effect. They can treat mild degrees of acne. Anti-oxidation type products contain antioxidants such as vitamins C and E as it is believed that reactive oxygen is deeply involved in the formation of acne.

However, acne cannot be treated simply by eliminating a single cause. You need to take a multi-varied approach.

People stressed by acne expect cosmetic products to perform effects to treat their acne. In order to satisfy their needs, we worked hard to develop an effective cosmetic ingredient that can be used safely.

2. About the Acne

2-1 How Acne Worsens

Acne is a skin disorder with chronic inflammation of hair follicles and sebaceous glands due to hormonal influence. One of the causes of acne is increased sebum production by sebaceous glands caused by changes in sexual hormones during puberty, especially effects of male hormone androgens (androgens are also produced in woman in their adrenal gland). Sebum is usually discharged through hair follicles. However, when abnormal keratinization increase around the opening of a hair follicle because of irritation from free fatty acids, the pore becomes clogged, sebum accumulates, and a first-stage eruption called comedo is developed. Then, *P. acnes* present around the opening of the hair follicle generates lipase and other substances that cause inflammation. In this condition, any irritation aggravates inflammation and worsens acne. Women's acne worsens before their period and by the use of cosmetics that clog pores such as foundation products. Lack of sleep, mental stress, eating snacks between meals, touching eruptions, and wearing your hair in a way it rubs against the skin are also factors that worsen acne for both men and women.

Many products are available to treat acne. However, we rarely see all-in-one products that can prevent acne formation, treat acne in each stage, and care the skin after acne is healed.

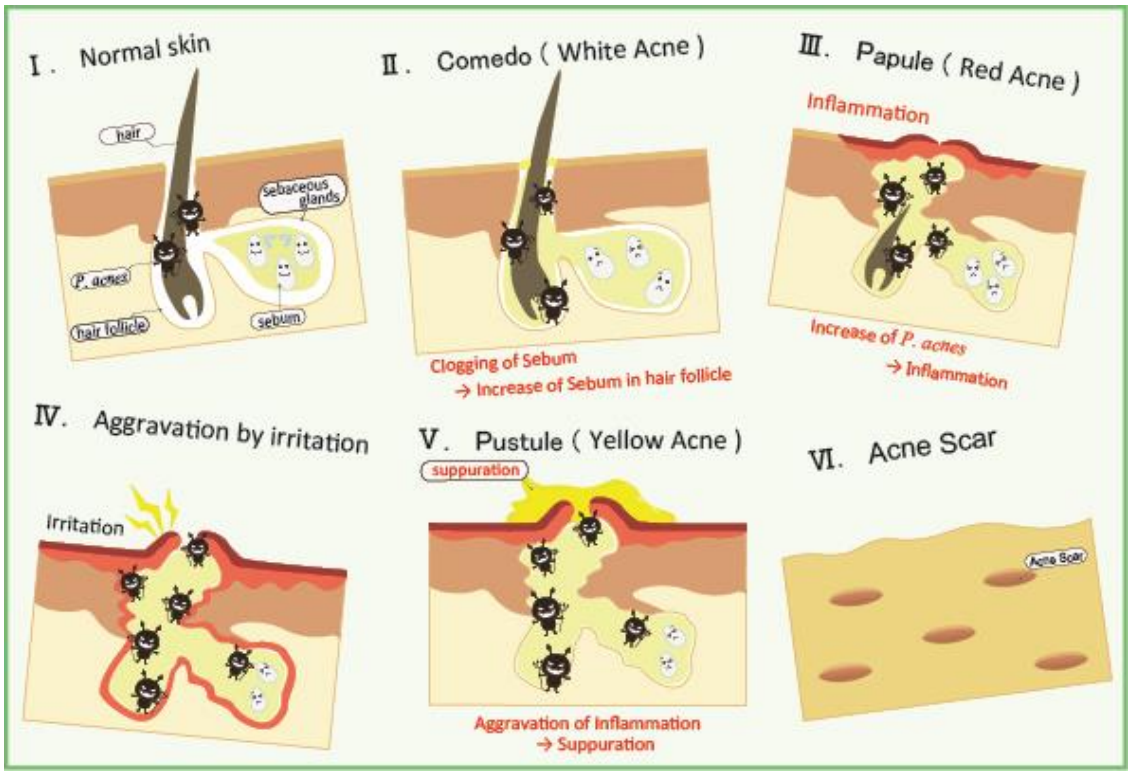


Fig.1 Mechanism of Acne Aggravation

2-2 Kind of the Acne

After formation, acne worsens as shown below and is named differently in each stage (Fig.2).

Stage Ⅱ (White Acne ; Comede)

The opening of a pore becomes thick and narrow due to declined turnover function. Sebum clogs the opening and acne is formed.

Stage Ⅲ (Red Acne ; Papule)

Sebum accumulates inside the pore, *P. acnes* and other bacteria consuming sebum grow rapidly, and inflammation occurs.

Stage Ⅴ (Yellow Acne ; Pustule)

Inflammation keeps occurring because of increased *P. acnes* and lipase generated by them. Various types of irritations worsen inflammation, generating suppuration.

White acnes, an early stage of acne, can be healed by correcting your basic skin care regimen. However, when they develop to red acnes or yellow acnes, they become difficult to treat. Caring for red acnes or yellow acnes incorrectly and popping them leave acne scars on the skin. It is very important to treat them correctly.

Stage Ⅱ White Acne | Stage Ⅲ Red Acne | Stage Ⅴ Yellow Acne

Fig.2 Kind of the Acne

3. Fruit Extracts and Active Component in VeryBerry™ ACNEcare

3-1 KIWI FRUIT（*Actinidia chinensis* Planch.）

Kiwi fruit (*Actinidia chinensis* Planch.) is originated from the central and southern regions of China. It was introduced to New Zealand in the early 20th century. New Zealand is now the main exporter of high quality Kiwi fruits of various species. The fruit was named “kiwi” after the national bird of New Zealand as the fruit resemble kiwi chick. In 1966, Kiwi fruit was introduced to Japan and cultivation started in late 1970’s. Japan is currently producing 40,000 tons of Kiwi fruits per annum. The physiological effects of Kiwi Seed Extract was researched and experimented. As the result, we found that Kiwi Seed Extract has an inhibitory effect of Dihydroteststerone production, which is cause of generation of acne, and an inhibitory effect of lipase originated from the *Propionobacterium acnes*.

3-2 STRAWBERRY (*Fragaria chiloensis* (L.) Mill.)

Strawberries are native to middle of Europe, and it has been brought to Japan by the Hollanders in the late Edo period. Now, strawberry is a popular fruit in Japan and its annual harvest amount is around 200,000 tons. Strawberry is rich in vitamin C, polyphenols including anthocyanin, flavonoid, phenylpropanoid and ellagitannin. Recently, the health functions of strawberry have been broadly investigated. The Strawberry Seed Extract powder contains tiliroside and kaempferol-3-O-glucoside, and we discovered superior moisturizing effect by those functional components.

3-3 YUZU (*Citrus junos* Siebold ex Tanaka)

Yuzu (*Citrus junos* Siebold ex Tanaka), or Japanese grapefruit, is originated in Sichuan and Yunnan, located in the upstream region of the Yangtze River in China. It was brought to Japan during the Heian period (about a thousand years ago). Yuzu is believed to be effective to recover from fatigue, release pain, and enhance beauty because of its high content of organic acids including citric acid and tartaric acid as well as vitamin C. In Japan, people bathe with yuzu floating in the water (yuzuyu) on the midwinter day (around December 22). This custom started in the Edo period (around 1600 to 1867). Yuzuyu is traditionally believed to treat chapped skin because of citric acid and vitamin C contained in yuzu peel and prevent the body from getting cold after taking a bath because its aromatic oil.

3-4 UNSHIU-ORANGE (*Citrus unshiu* Marc.)

Unshiu mikan (*Citrus Unshiu* Marc.) is commonly known as Satsuma, Satsuma Mandarin or Satsuma Orange in the Western society. Unshiu mikan is originated from Kagoshima (Satsuma). There are approximately 900 species of citrus in the world. Japanese oranges are unique to Japan and appeared 1,200 years ago. It was regarded as fruit for perpetual youth and longevity as described in Kojiki and Nihon Shoki. Japanese seedless orange was generated by mutation 400 years ago. The name Unshiu mikan originated from Wenzhou area in China where it is famous for production of oranges. There are various type of Citrus Unshiu, e.g. "Kumamoto mikan", "Ehime mikan", "Arita mikan" and "Sizuoka mikan". β-cryptoxanthin and citric acid, which is one of α-hydroxy acid are contained in its peel. Citric acid is often used for chemical peeling and is believed to be gentler and less irritating to the skin than other peeling materials.

3-5 LICORICE (*Glycyrrhiza glabra* L.)

Licorice is an herb also called glycyrrhiza. It is a perennial plant belonging to the *leguminosae* family and grows in China, Mongolia, and Siberia. It has been used as a drug throughout an extremely wide range of regions since ancient times. Its main effective component is glycyrrhizin with a detoxifying effect. It is effective for treating food poisoning, pufferfish poison, snake poison, and bacterial toxins. It is an important raw material for cosmetic products and quasi-drugs because it is believed to perform anti-allergic, anti-inflammatory, and skin-lightening effects.

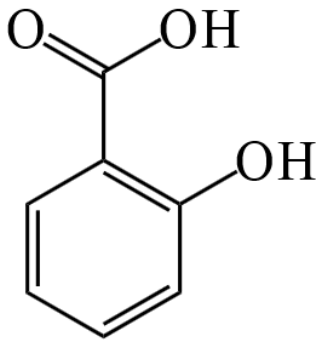
3-6 MANGOSTEEN (*Garcinia mangostana* L.)

Mangosteen is native to South East Asia and is believed to be a fruit tree that has been passing on the same genes since ancient times. It is called "manggis" in Malay and Indonesian and "mankut (มังคุด)" in Thai. Since the fruit is delicious, it is known as the "Queen of fruits." It is one of the most-used species in the *Clusiaceae* family. Its rind contains a high concentration of xanthones, a type of polyphenol. It has been proven that xanthones are very strong antioxidants with higher effects than other polyphenols and well-known antioxidants such as vitamin C and vitamin E. Because of its high effects, pathologists all over the world praise mangosteen as a "natural antioxidant." Mangosteen was used for medicinal purposes by native people in ancient times and is still widely used in folk remedies. It has been confirmed to have an effect to combat infectious diseases (malaria, dysentery, infection by parasites), treat skin disorders, and has effects to regulate the intestines, kill bacteria, reduce fever, relieve inflammation, and prevent oxidation.

3-7 GRAPE (*Vitis vinifera* L.)

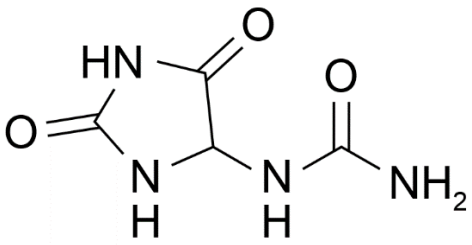
Grapes have been cultivated for a very long time. It is said that the cultivation of grapes began around BC 3000 in the Caucasus region and areas along the Caspian Sea. Grapes are the world's most produced fruit and it is said that there are over 10000 varieties. The purple color of grape skin is caused by anthocyanin polyphenol that is similar to a component in blueberries considered to be good for the eyes. Grapes also contain resveratrol which is currently attracting people's attention as a unique component of grapes. In addition to them, grape's fruit, skin, and seeds contain many types of polyphenols that are considered to be effective for a longer life and anti-aging.

3-8 SALICYLIC ACID



Salicylic acid is known as a substance often used to treat acne as well as for peeling and moisturizing purposes. Salicylic acid is used for acne care for roughly two purposes and effects. First, it softens corneum. Softening corneum helps to prevent acne formation because materials clogging pores and sebum plugs are easy to remove when the skin is soft. Effective ingredients of skin toner and treatment products can be absorbed by the skin better as well. Secondly, it prevents growth of germs. For its antiseptic effect, it prevents the growth of *P. acnes* on the skin. The skin can be kept in a condition where *P. acnes* cannot grow very well after it is washed and moisturized. Salicylic acid also has an anti-inflammatory effect to reduce inflammation by suppressing the activity of prostaglandin.

3-9 ALLANTOIN



It is said that allantoin was named after the amniote embryonic excretory organ (allantois) that oxidizes and concentrates uric acid produced by purine catabolism during development in most mammals, except humans and higher apes. Allantoin is extremely effective to treat allergies and rough skin. It prevents rough skin and promotes skin regeneration by treating wounds and increasing cells. It is used for anti-aging and acne care because it has effects to remove old skin tissues and hardened corneum smoothly. In addition to the acceleration of recovery from wounds and formation of new skin, allantoin makes scars less visible gradually and works on existing dark spots and wounds effectively. For these effects, allantoin is effective to treat red and swollen acne with inflammation and reduce acne scars as well.

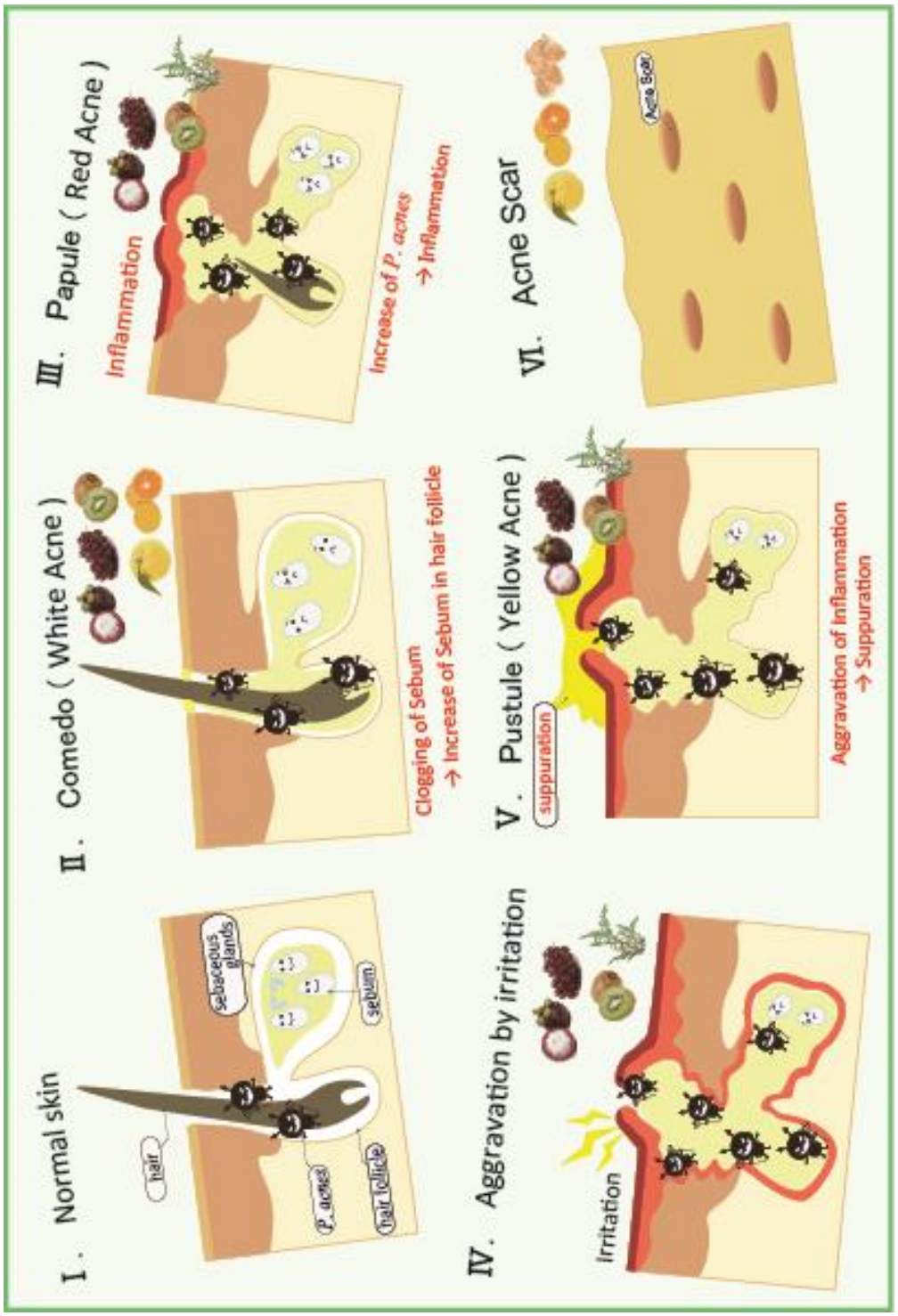


Fig.3 Anti-acne Effect of VeryBerry™ ACNEcare

4. Efficacy Evaluation of each Plant Extracts

4-1 Kiwi Seed Extract

4-1-1 Pathophysiology of Acne

① Sebaceous gland hypersecretion

5α-reductase is the enzyme responsible for the conversion of testosterone to dihydrotestosterone (DHT) which stimulate the enlargement of sebaceous gland resulting in sebaceous hypersecretion. Sebum produced act as an important nutritional source for anaerobic *P. acnes* which activates the inflammatory pathway underneath the skin. Thus acne develops due to the hyperproduction of DHT.

② Infectious *Propionibacterium Acnes* (*P. acnes*)

The growth of bacteria flora of the hair follicles, *P. acnes*, increases with sebum production. *P. acnes* produces bacterium lipase which breaksdown sebum triglyceride which travel as free fatty acids in the skin that activates the inflammatory cascade. Subsequently, inflammatory factors are released followed by leukocyte chemotaxis in the dermis leading to the formation of comedogenic acne on the epidermis（Fig.4）.

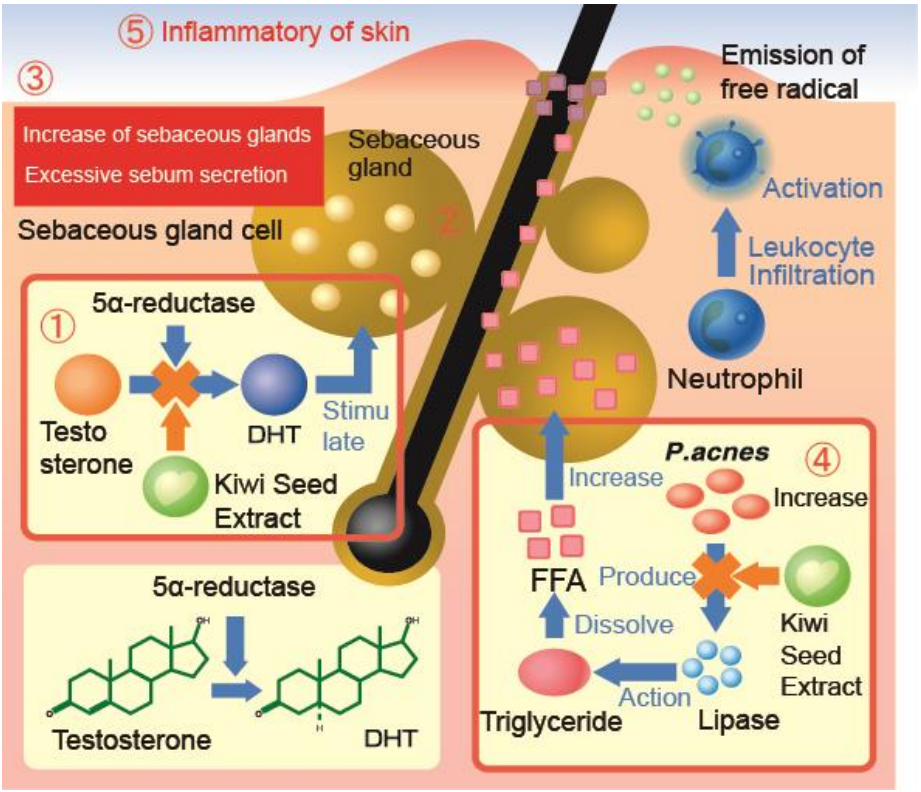


Fig.4 Mechanism of Acne Generation

4-1-2 Inhibitory Effect of 5α-reductase（*in vitro*）

Different concentration of Kiwi Seed Extract was added to testosterone containing enzyme 5α-reductase (S-9, Oriental Yeast) samples for reactions. The production of dihydrotestosterone (DHT) was analyzed using gas chromatography. Results confirmed that Kiwi Seed Extract prevents the production of DHT via its inhibition against 5α-reductase (Fig.5).

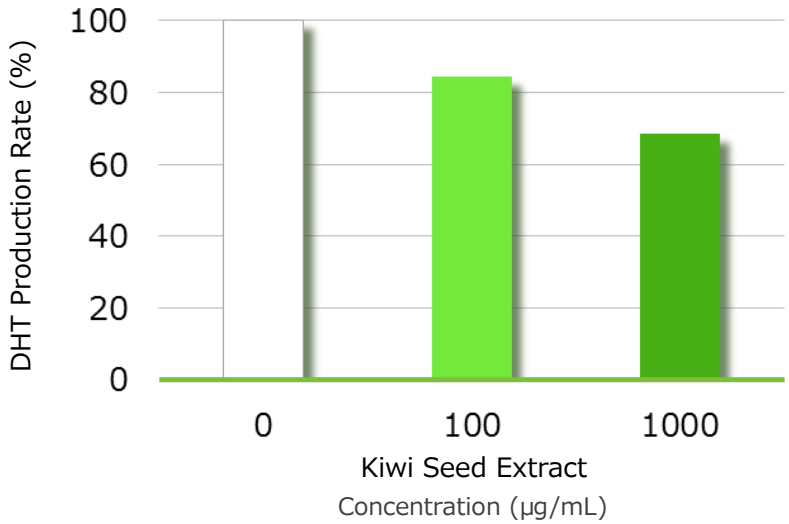


Fig.5 Inhibitory Effect of 5α-reductase

4-1-3 Inhibitory Effect of Lipase obtained from *P. acnes*（*in vitro*）

P. acnes was cultured in GAM medium to obtain the enzyme lipase. Kiwi Seed Extract of different concentration was added to evaluate its effect on lipase. Results demonstrated a dose-dependent inhibition against lipase of *P. acnes* (Fig.6).

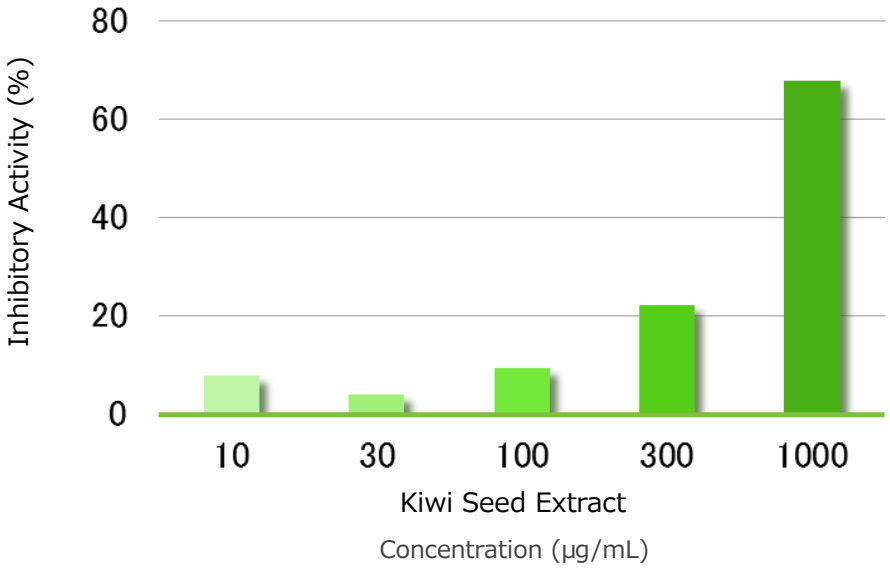


Fig.6 Inhibitory Effect of Lipase Activity

4-1-4 Inhibition of Prostaglandin E_2 (PGE2) Production (Anti- inflammatory Effect)

Further experiment was prompted to study the effect of Kiwi Seed Extract on prostaglandins (PG) production. Cultured macrophage cells (RAW264.7) were stimulated by lipopolysaccharide (LPS) to produce PG. Results showed that Kiwi Seed Extract significantly suppressed the production of PGE2 from cells RAW264.7 at concentration of 1-100 g/mL. Meanwhile, no cytotoxicity occurred at these concentrations (Fig.7).

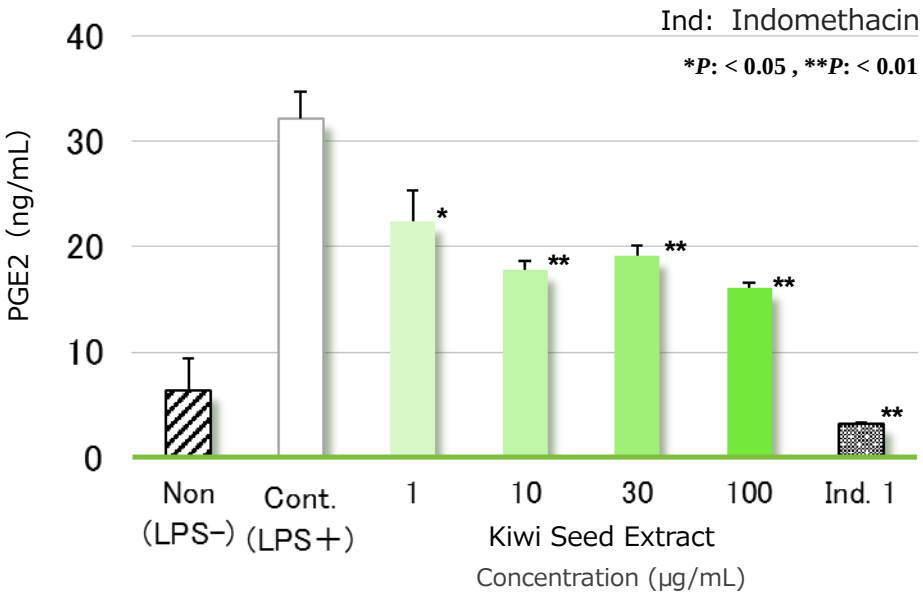


Fig.7 Inhibitory Effect of PGE2 production (*in vitro*)

4-1-5 Improvement Effect of Acne (Monitor Test)

The sebum production amount was studied before application and 2 weeks after application of Kiwi Seed Extract in the specific area (forehead). As shown in Fig. 8, sebum production significantly decreases while acne area subsided after 2-week application.

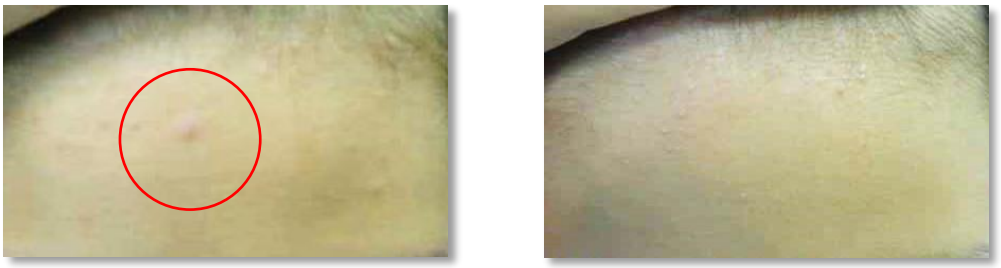


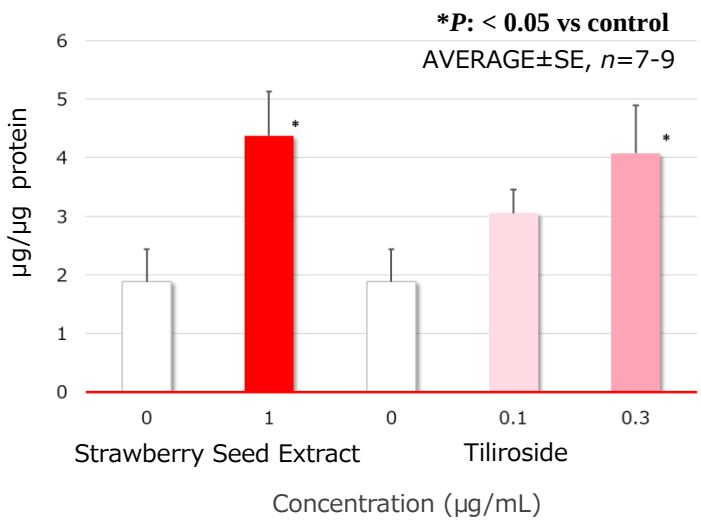
Fig.8 Anti-acne Effect of Kiwi Seed Extract-LC (*in vivo*)

4-2 Strawberry Fruit Extract

4-2-1 Promotion Effect of Ceramides Increase

Effects on epidermal tissues were examined using three-dimensional culture models of human epidermis. “Strawberry Seed Extract” or Tiliroside (substance containing Strawberry Seed Extract) was added to three-dimensional culture models of human epidermis, the models were cultured for five days, and ceramide content and ceramide synthesis were evaluated by TLC, immunostaining, and Western blotting methods. As a result, Strawberry Seed Extract (1 μg/mL) and Tiliroside (0.1, 0.3 μg/mL) increased ceramide II and ceramide V in the epidermal corneum (Figs. 9a, b). The increase of glucosylceramide was also confirmed (Fig. 10).

Ceramide Ⅱ



Ceramide Ⅴ

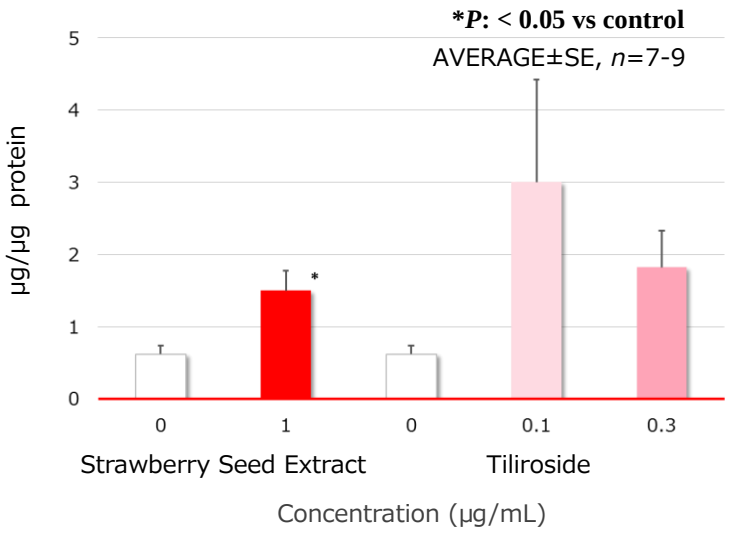


Fig.9a Promotion Effect of Ceramide Ⅱ & Ⅴ Increases

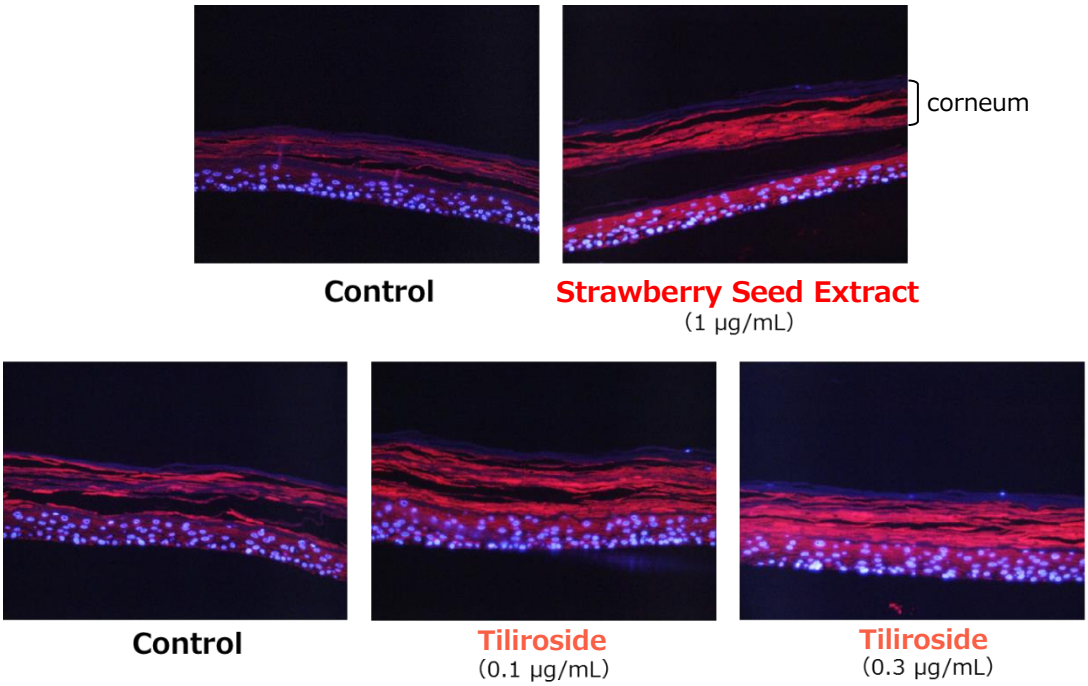


Fig.9b Promotion Effect of Ceramides Synthesis
(3D Skin Model / Red area; Ceramides)

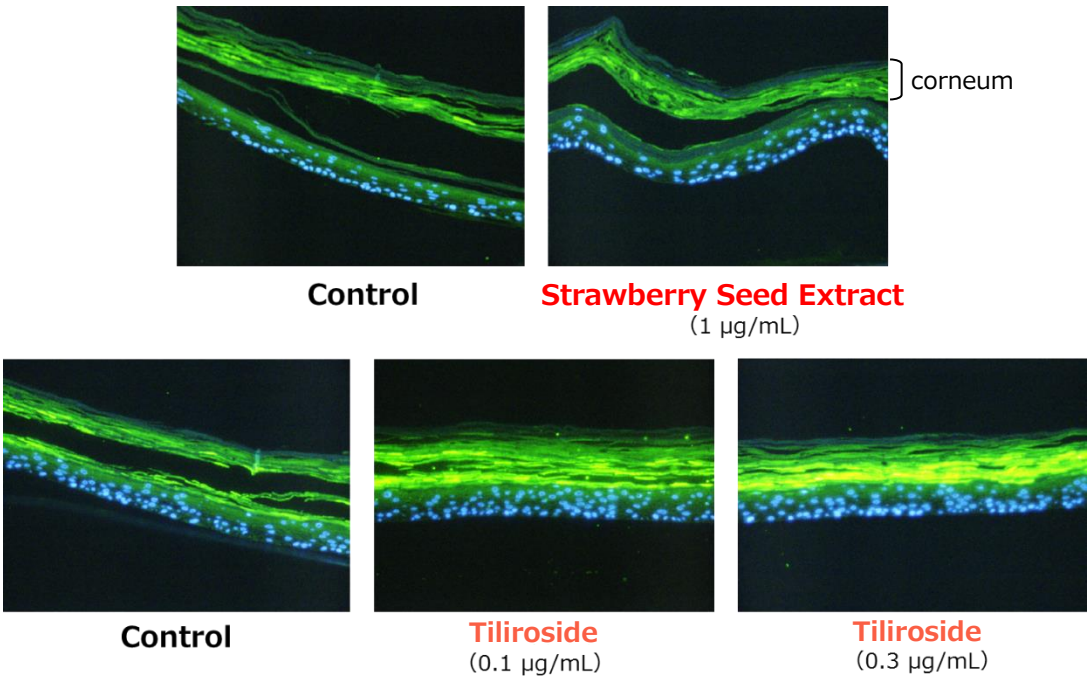


Fig.10 Promotion Effect of Glucosylceramides Synthesis
(3D Skin Model / Green area; Glucosylceramides)

4-2-2 Promotion Effect of Genes Expression Related to Skin Barrier Function (filaggrin, involucrin)

A test was carried out in an experimental system similar to that described in the previous page. As a result, Strawberry Seed Extract (1 μg/mL) and Tiliroside (0.1, 0.3 μg/mL) increased the expression of filaggrin and involucrin in the epidermal corneum as well (Figs. 11a, b, 12a, b).These results suggest that strawberry seed extract can boost skin's moisturizing function by increasing ceramides, enhancing skin barrier function through the increase of filaggrin, and normalizing cornification through the increase of involucrin.

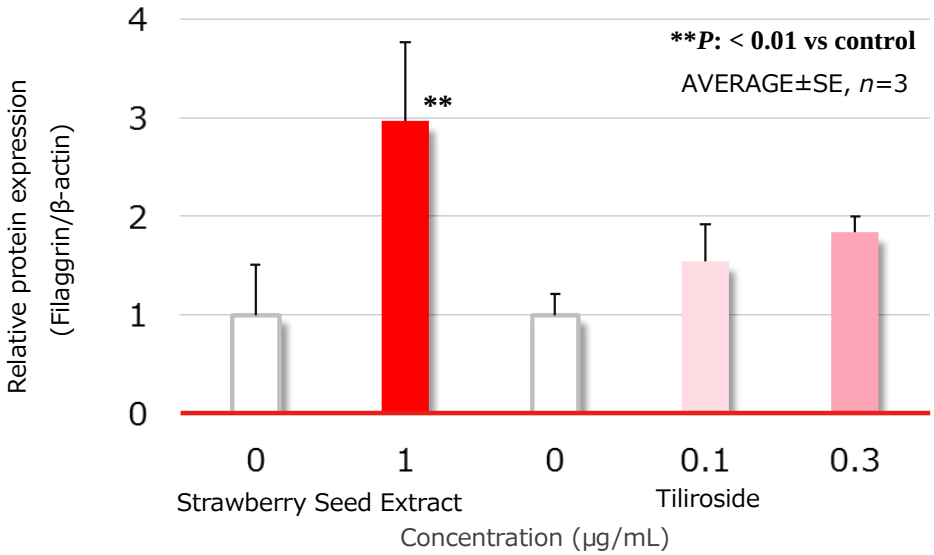


Fig.11a Promotion Effect of Filaggrin Expression

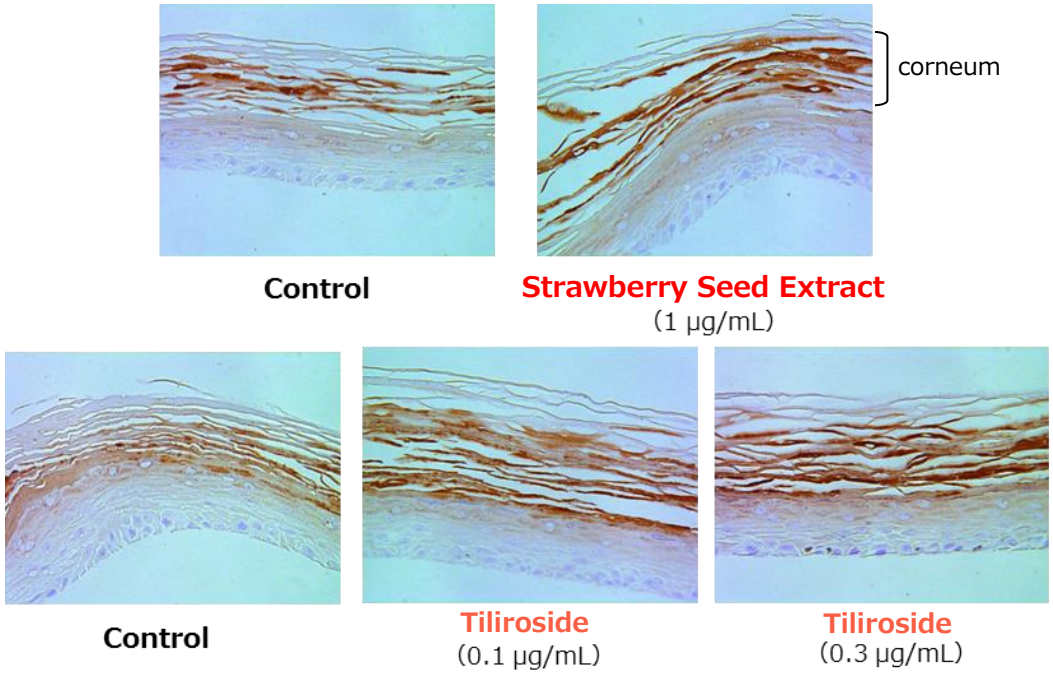


Fig.11b Promotion Effect of Filaggrin Production (3D Skin Model)

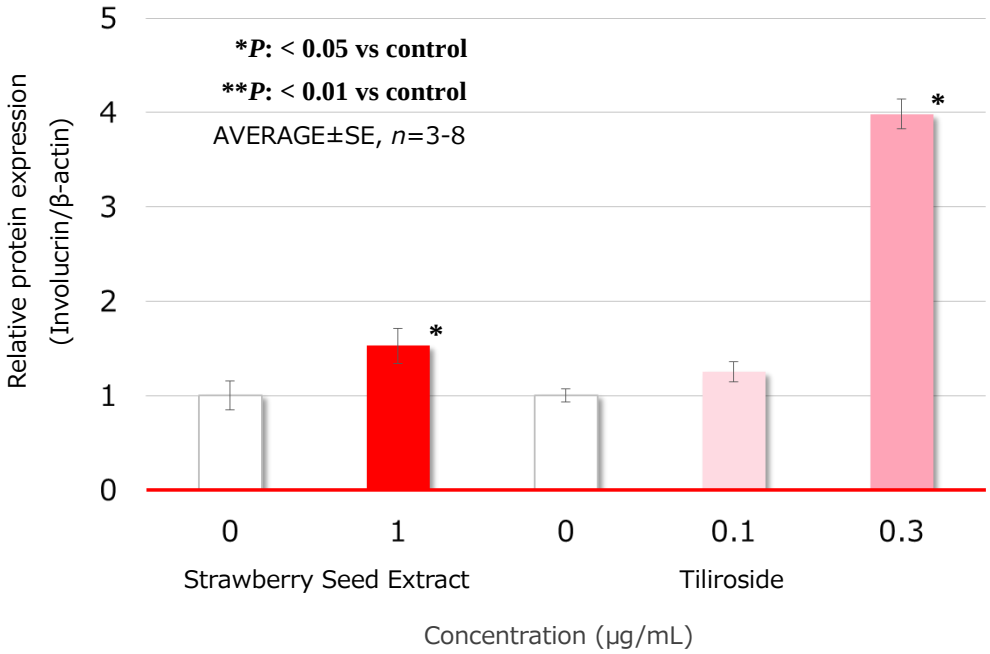


Fig.12a Promotion Effect of Involucrin Expression

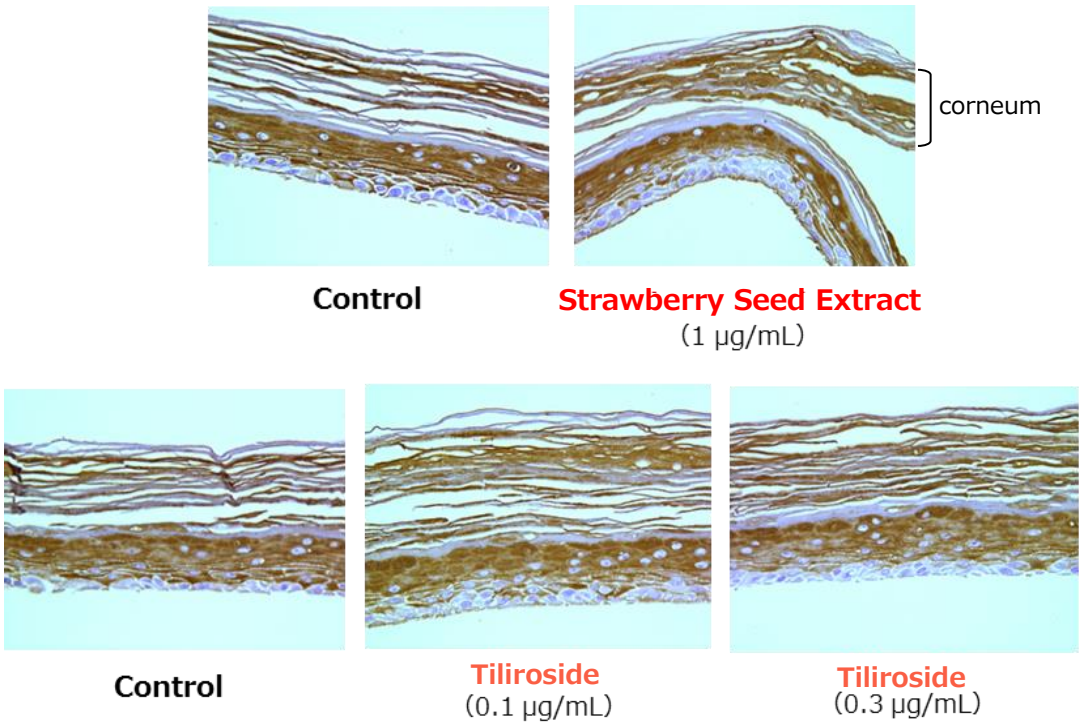


Fig.12b Promotion Effect of Involucrin Production (3D Skin Model)

4-3 Yuzu Seed Extract

Promotion Effect of Skin Turn-over

A reconstructed artificial human skin cell model was used to examine the effect of Yuzu Seed Extract on skin turn-over. The microscopic illustrations of skin dermal layer are shown in Fig 13. A pronounced thickening effect on skin dermal layers (epidermis and dermis) was observed in the models treated with Yuzu Seed Extract in a dose-dependent manner. It was hypothesized that Yuzu Seed Extract promoted collagen production and fibroblast growth, resulting in thickening of the skin dermal layer.

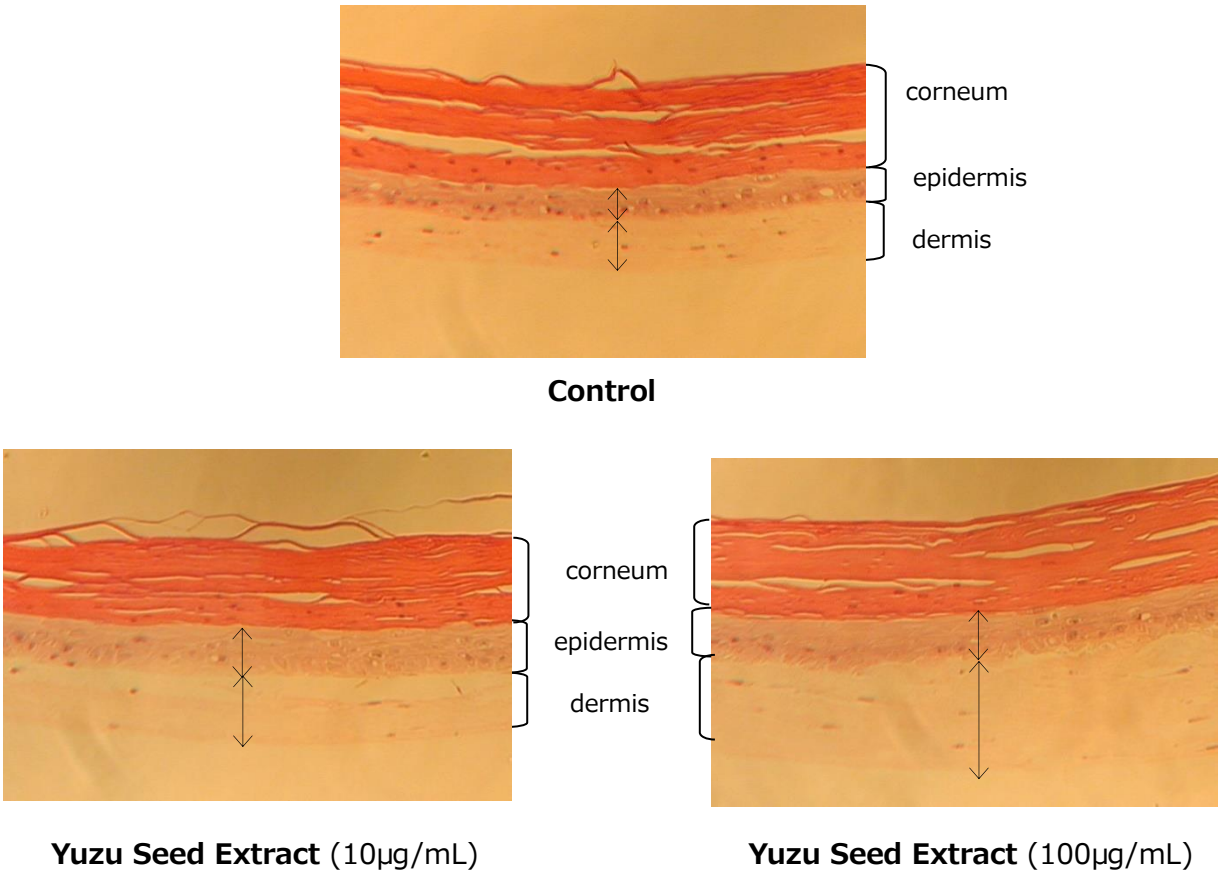


Fig.13 Microscopic Illustration of Reconstructed Skin Cell (artificial human skin cell model)

4-4 Citrus Unshiu Extract

4-4-1 Moisturizing Effect（Monitor Test）

The moisturizing effect of Citrus Unshiu Extract product [Citrus Unshiu Extract - LC, which contains 99% 1, 3 - butylene glycol (BG) and 1% Citrus Unshiu Extract; Lot No. Z-419] upon topical usage was evaluated and compared with vehicle (BG). Fig. 14 a and b revealed that the product Citrus Unshiu Extract - LC and its twice-diluted sample increased and maintained skin moisture up to 120 minutes while maintenance of 99% and 49.5% BG aqueous solutions lasted only for 80 minutes.

(Measurement condition：Temperature 26℃，Humidity 42％)

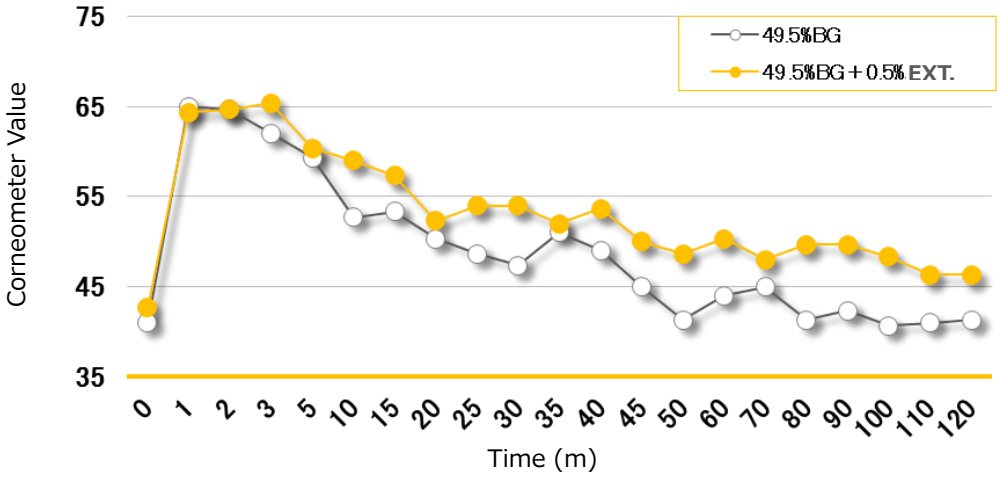


Fig.14a Moisturizing Effect (Control : 49.5% BG)

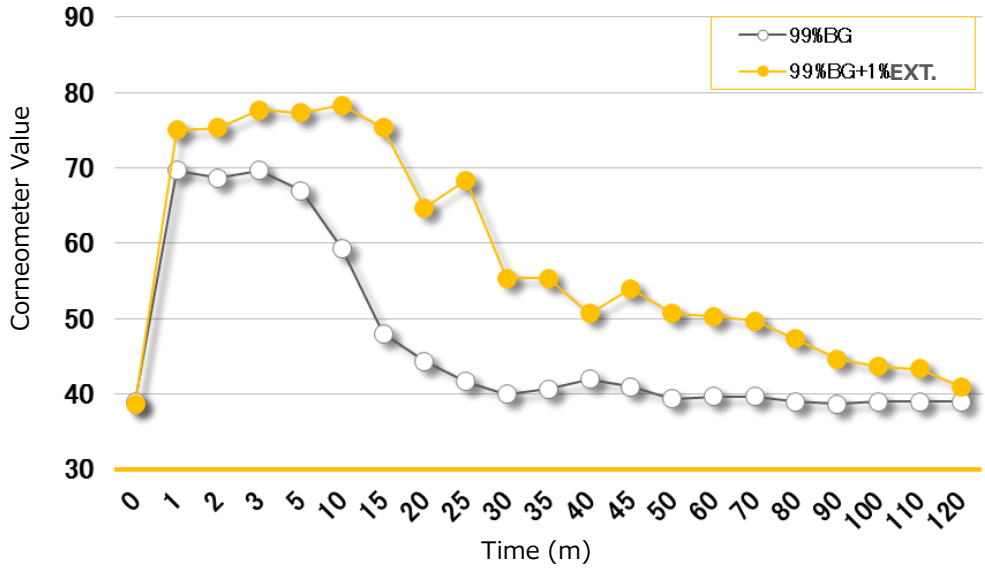


Fig.14b Moisturizing Effect (Control : 99% BG)

4-4-2 Promotion Effect of Skin Turn-over （Monitor Test）

Promotion effect of skin turn-over was investigated using CosmeHerbest™ ORANGE as it is. The tanning agent were applied on the left and right forearm inside by patch test plaster for three hours. The skin color changed to brown color after 24hours and color photo was taken (Fig15; Start). Then, the control lotion was applied on right forearm twice a day, and the sample lotion was applied on the left forearm twice a day for five days (Fig15; 5 days). Determination was performed by the naked eye by comparing the left and right.

- Tanning agent / 10% DHA solution
  (10% Dihydroxyacetone, mixture of ethanol: BG: water = 20: 30: 50)
- Control Lotion (30% Propanediol water solution)
- Sample Lotion (undiluted CosmeHerbest™ ORANGE, Lot. M-524)

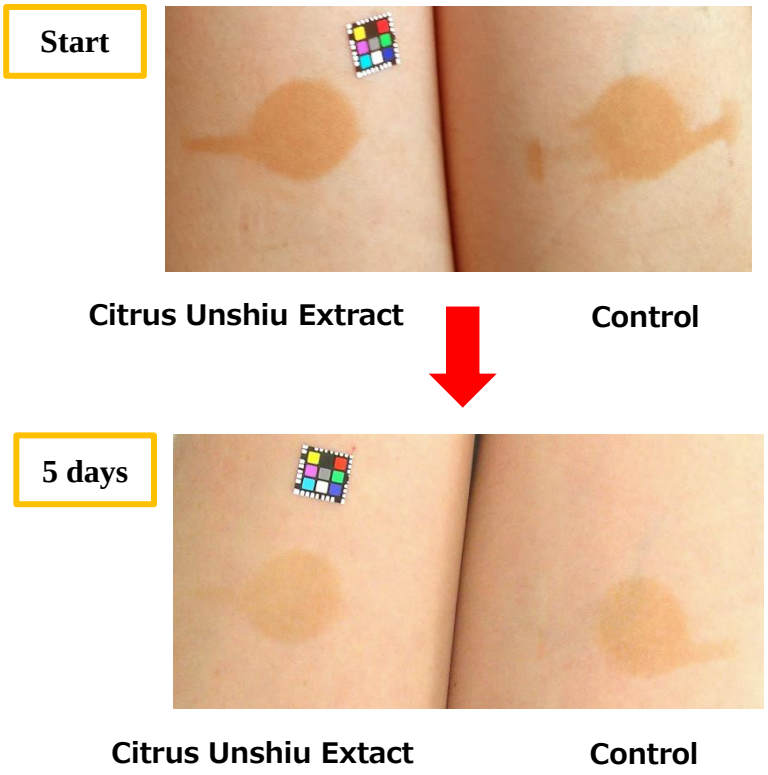


Fig.15 Promotion Effect of Turn-over

4-5 Mangosteen Peel Extract

Inhibitory Effect of *P. acnes*

There is a study report concluding that alpha-mangostin (AM) contained in peel of mangosteen has an effect to suppress the growth of *P. acnes*. According to the report, a test was carried out using dimethyl sulfoxide (DMSO) and brain-heart infusion broth as negative controls and amoxicillin as the positive control. In the test, the growth of *P. acnes* was suppressed when the concentration of AM was 1.0 μg/mL or higher [1] (Table 1.)

According to a different report, the growth of *P. acnes* was suppressed in the capsular solution side containing AM (area a) [1] in an inhibition ring test to see the effect to suppress the growth of *P. acnes* (Fig. 16).

Table.1 Determination of Minimum Inhibitory Concentration of AM for *P. acnes*

Test Solutions	*P. acnes* growth
0.5 μg/mL AM	+
1.0 μg/mL AM	−
2.0 μg/mL AM	−
4.0 μg/mL AM	−
8.0 μg/mL AM	−
16.0 μg/mL AM	−
32.0 μg/mL AM	−
DMSO (negative control)	+
Brain-heart infusion broth (negative control)	+
Amoxicillin (positive control)	−

+ growth, - no growth

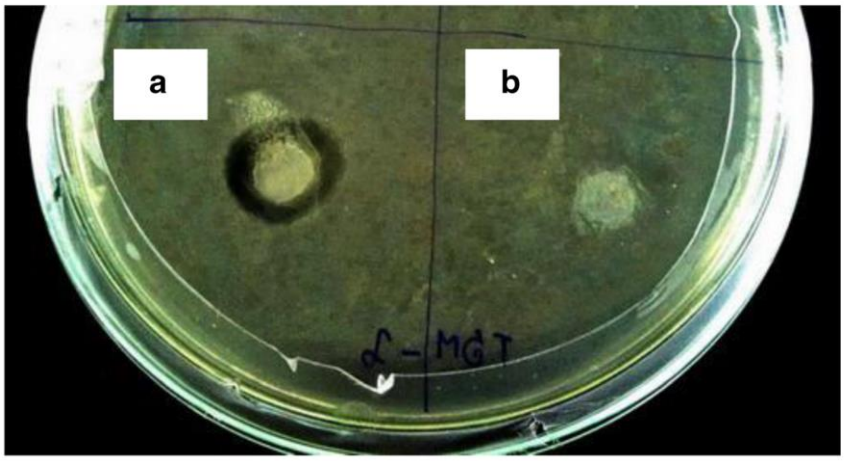


Fig.16 Inhibition zone (a : AM dose , b : control)

Source of reference :

1) Rathapon Asasutjarit et al., Physicochemical properties and anti-propionibacterium acnes activity of film-forming solutions containing alpha-mangostin-rich extract. *AAPS PharmScitech*, Vol.15, No.2, 306-316 (2014).

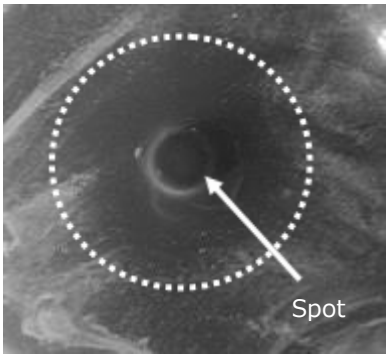
4-6 Grape Extract

Inhibitory Effect of *P. acnes*

Minimum inhibitory concentration of resveratrol was measured with the purpose of inhibition of *P. acnes* growth. This test, where resveratrol was added to an agar medium, proved that it controls the multiplication of *P. acnes* (antibacterial activity) at the concentration of 300 g/ml or higher (Fig.17a, b).

In addition, a study of the strong anti-inflammatory effect by the resveratrol is reported [2)] and can expect that inhibit the pathogenesis or aggravation of the acne in conjunction with antibacterial effect.

a)



b)

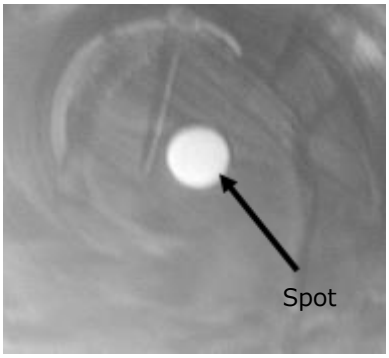
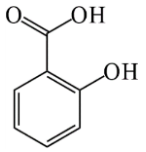


Fig.17 Inhibition zone (a : Resveratrol dose, b : control)

Source of reference :

2) Das S, Das DK. Anti-inflammatory responses of resveratrol. *Inflammation & Allergy-Drug Targets*, 6, 168-173 (2007).

4-7 Salicylic Acid



Palliation Effect of Inflammation

Purposes and effects of using salicylic acid for acne care are described in page 6 of this booklet. It has been reported that salicylic acid also has an effect to suppress aggregation of neutrophils (Fig. 18).

Sebum is decomposed by lipase, an enzyme generated by *P. acnes*, and becomes free fatty acid which is a strong factor to invite neutrophils. Neutrophils (white blood cells) have a strong weapon to attack bacteria, reactive oxygen. However, reactive oxygen is troublesome because it sometimes ends up destroying skin tissues. Tissues destroyed by reactive oxygen generated by neutrophils appear reddish acne to our eyes. When neutrophils aggregate in the dermis, yellow pimple is formed. Reactive oxygen is often pointed out as a cause of aging and illnesses. Reactive oxygen generated by neutrophil is also one of the causes of acne. In other words, suppressing the aggregation of neutrophils can ease inflammation and prevent worsening of inflammation of acne.

FMLP : *formylmethionyl*-leucyl-phenylalanine

AA : Arachidonic acid

PMA : Phorbol myristate acetate

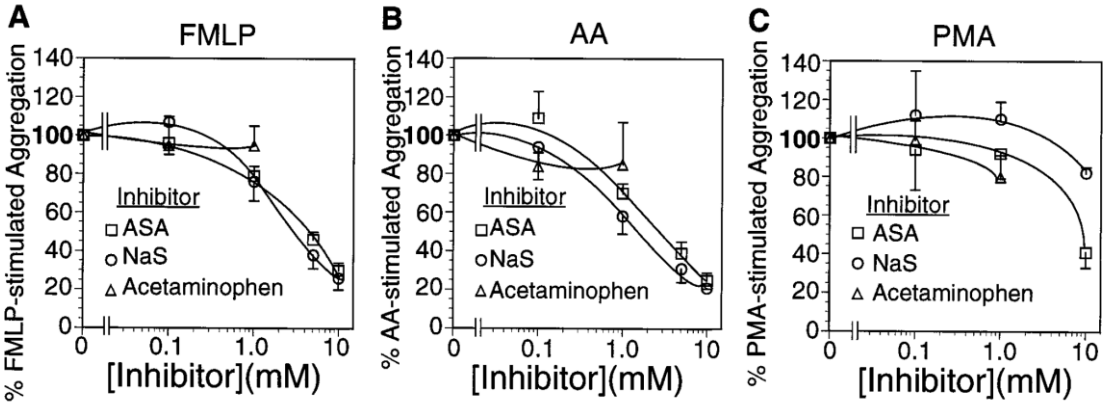


Fig.18 Effects of aspirin (ASA), sodium salicylate (NaS), and acetaminophen on aggregation

Neutrophils were incubated for 10 min in the absence or presence of ASA (□), NaS (○), or acetaminophen (△), followed by stimulation with FMLP ;**A**, AA ;**B**, or PMA ;**C** for 2 min and measurement of aggregation. Results are expressed as percent of stimulated aggregation in the absence of inhibitor and are the means 6 SEM of three experiments.

Source of reference :

3) Michael H. et al.,Modes of effect of aspirin-like drugs: Salicylates inhibit Erk activation and integrin-dependent neutrophil adhesion. *Proc. Natl. Acad. Sci. USA*, Vol.95,14540-14545 (1998).

Cause of Inflammation

When pores are clogged and sebum accumulates inside, *P. acnes* grows inside them.

1. Inflammation caused by free fatty acids

   Bacterial lipase generated from *P. acnes* decomposes sebum and produce irritant free fatty acids, causing inflammation.

2. Inflammation caused by neutrophil migration

   Production of bacterial lipase induces neutrophil cells to gather around acne. Protease, hyaluronidase, and other enzymes produced by *P. acnes* cause inflammation, which also induces neutrophil cells to pool in inflammation areas.

   Neutrophil cells consume, disinfect, decompose, and digest foreign matter, causing redness and pus.

   When neutrophil cells consume *P. acnes* in acne areas, reactive oxygen is produced. Reactive oxygen damages surrounding tissues, causing inflammation.

3. Inflammation caused by lipid peroxide

   Reactive oxygen generated by neutrophil cells reacts with unsaturated free fatty acid and unsaturated fatty acid of cell membrane and becomes lipid peroxide. Different from reactive oxygen with a short life span, lipid peroxide keeps attacking cells in the body for a long time, causing long period of inflammation.

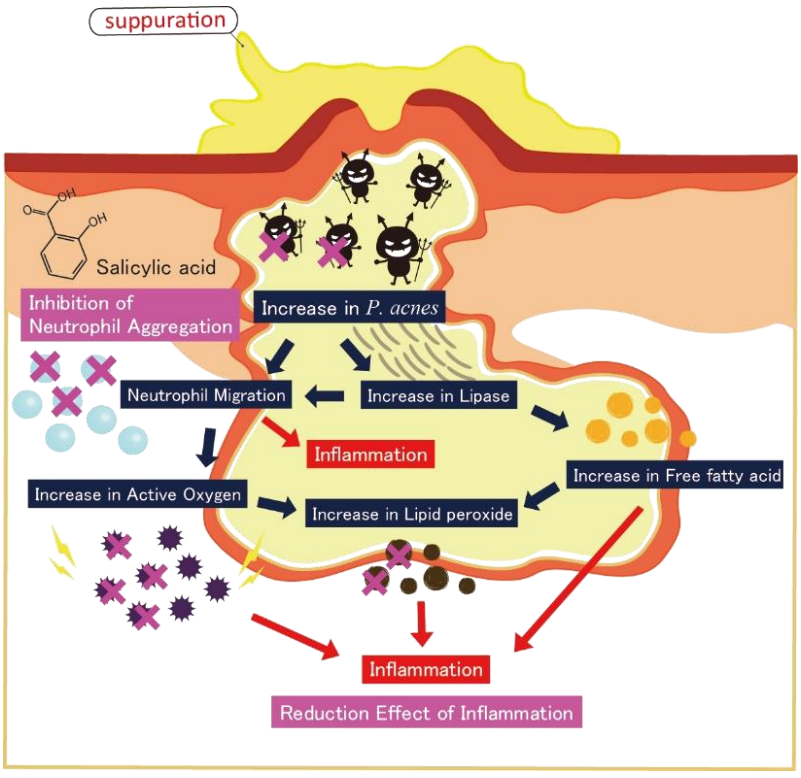


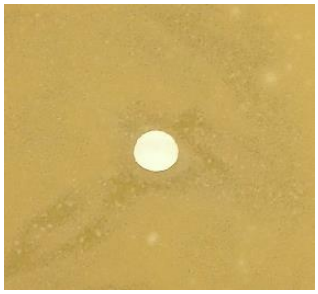
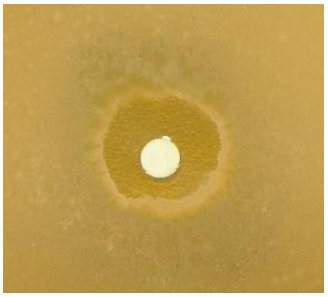
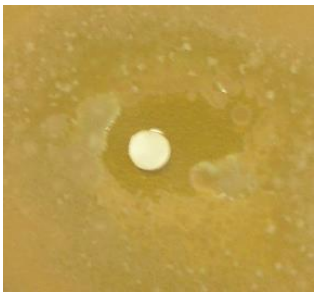
Fig.19 Cause of Inflammation

5. Anti-acne Effect-related Test on VeryBerry™ ACNEcare

5-1 Inhibition of *P. acnes* on VeryBerry™ ACNEcare (*in vitro* Test)

P. acnes was cultured in GAM liquid culture medium (NISSUI PHARMACEUTICAL CO., LTD.) beforehand. The liquid which cultured *P. acnes* was extended to GAM agar medium uniformly and a filter paper (diameter: 6 mm) was put in the center of this medium. 2 drops of each solutions (water, 50% resorcinol and VeryBerry™ ACNEcare) were subjected in the middle of paper disk. Two days later, inhibition circles were observed with naked eye and diameter of circles were measured (Table.2). As a result, it was confirmed that the growth of *P. acnes* was inhibited.

Table.2 Inhibitory Effect of *P. acnes*

	Water	50% Resorcinol	VeryBerry™ ACNEcare
Diameter of inhibition circle	0 cm	2.1 cm	1.7 cm
Photograph of inhibition circle			

5-2 Improvement of Acne on VeryBerry™ ACNEcare (*in vivo* Test)

A monitor test was conducted in order to check the ability of VeryBerry™ ACNEcare for the improvement effect against *P. acnes.* The test sample was prepared carbomer gel incorporated 3% of VeryBerry™ ACNEcare diluted with 30% Propanediol water solution.

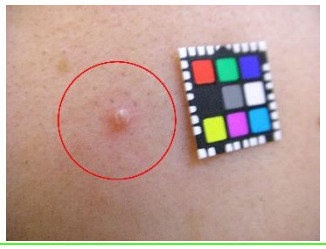
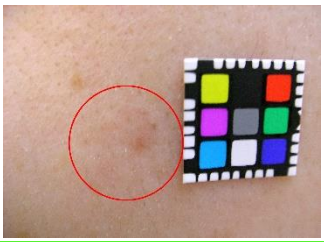
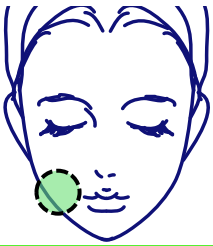
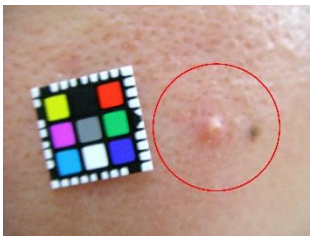
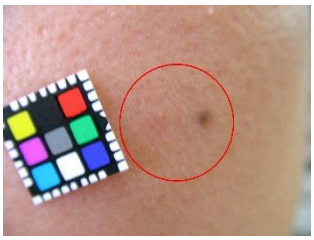
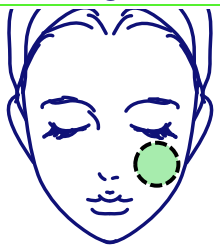
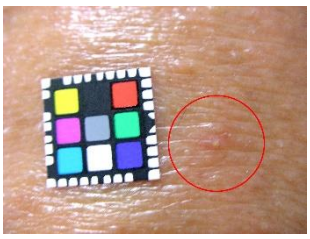
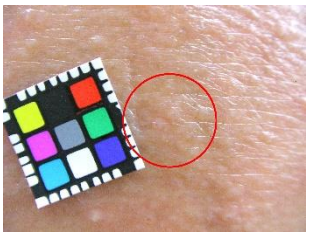
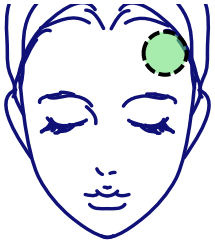
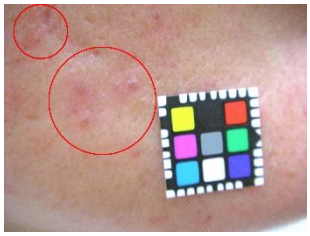
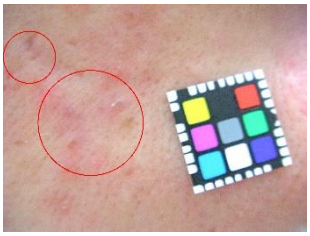
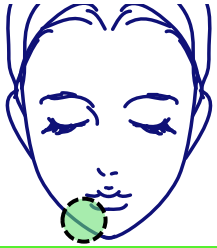
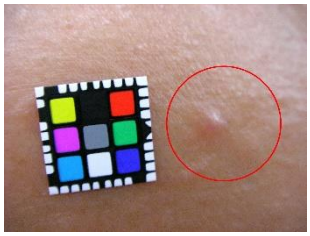
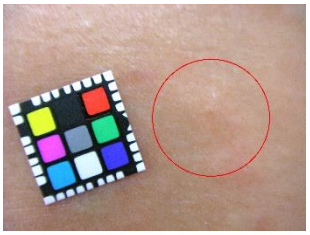
Formula	Percentage(%)
Water	67.73
1,3-Propnediol	28.83
VeryBerryTM ACNEcare	3.00
Carbopol™ 1382 Polymer	0.485
Triethylamine	0.437
TOTAL	100

Test method

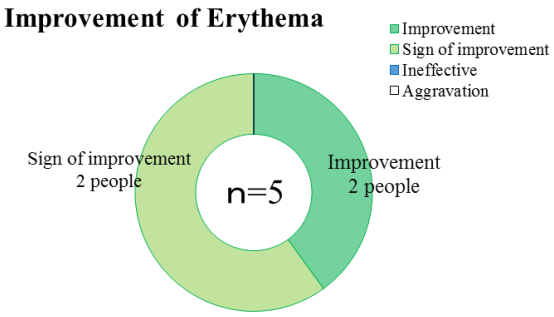
A gel containing VeryBerry™ ACNEcare was applied on acne areas of two male and three female subjects who submitted their written consent. Their acne conditions were visually checked two weeks after the acne formation. (When acne was completely healed within two weeks, the test was finished at that point.) After the test, the five test subjects answered a questionnaire about how their acne conditions improved during the test.

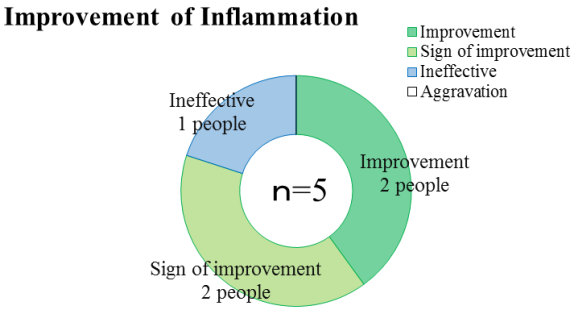
Result

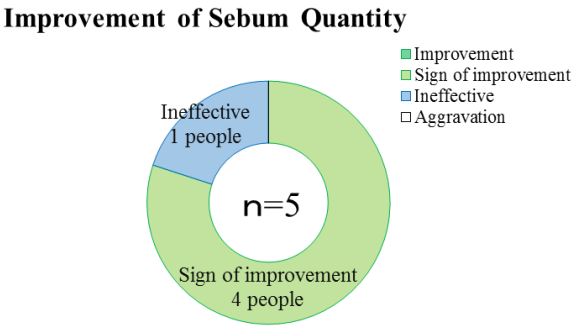
Table.3 Improvement of Acne on VeryBerry™ ACNEcare (*in vivo* Test)

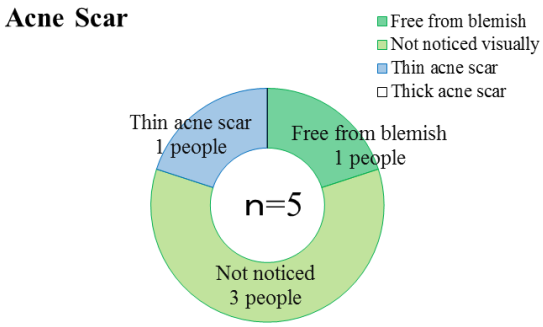
	START	After 2 weeks	Application part
No.1 M.M (23) F			
No.2 Y.Y (23) F			
No.3 Y.S. (25) M			
No.4 F.H (28) F			
No.5 M.O (29) M			

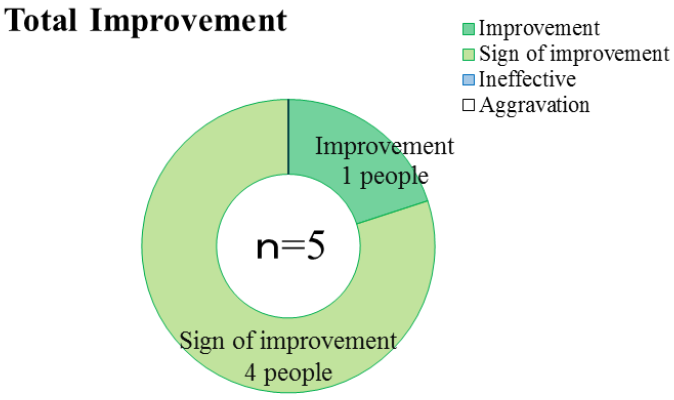
Results of Questionnaire











Consideration

As shown in Table 3, improvement in acne conditions was observed. Acne conditions were improved or there was a tendency for improvement on four test subjects among the five. In the questionnaire, 80% of the subjects answered "Improvement" or “Sign of improvement” for all items, indicating they felt effects of the sample. Judging from the results comprehensively, VeryBerry™ ACNEcare is expected to demonstrate an effect to improve acne conditions.

6. Stability Test

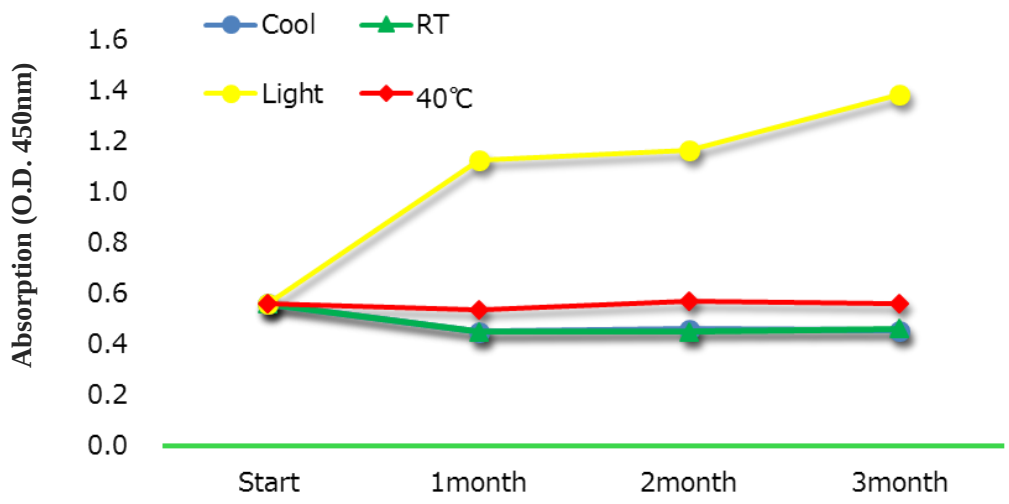
6-1 Long Term Stability

Store VeryBerry™ ACNEcare as it was, in a cool dark place at 4℃(Cool), room temperature (RT), window side (Light) and at 40℃ and observed color change and determined the absorbance at 450nm for 3 months.

Test Sample

VeryBerry™ ACNEcare (Undiluted; Lot No. S-524)

Test Result



Consideration

The test results have been rising absorbance at window side for 3 months after.
Please avoid high temperature and sun light, store the product in closed original container.

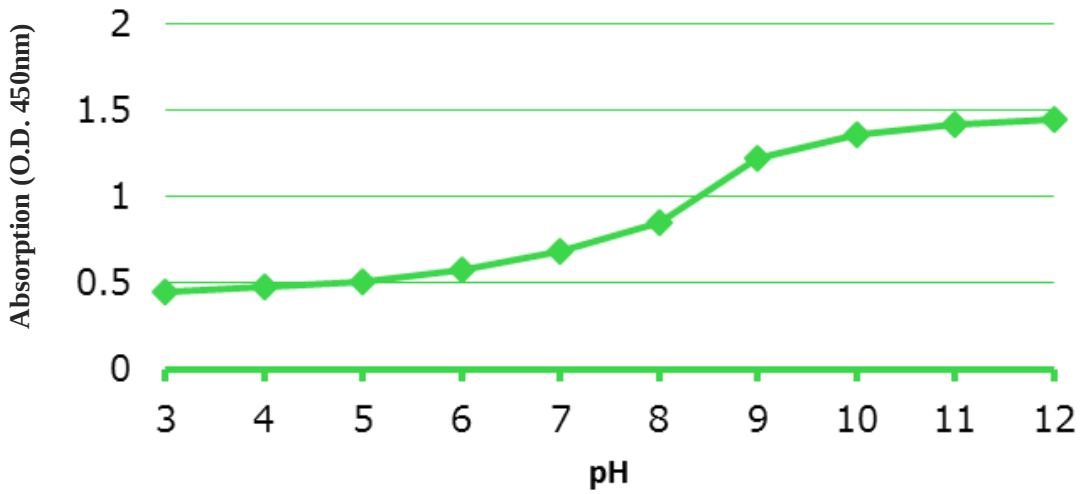
6-2 pH Stability Test

pH of VeryBerry™ ACNEcare was adjusted from 3 to 12 by hydrochloric acid and sodium hydroxide, observed the color change and determined the absorbance at 450nm.

Test Sample

VeryBerry™ ACNEcare (Undiluted; Lot No. B-503)

Test Result



Consideration

The color tone is pale reddish yellow from acidic to weak acidic zone, but the precipitate was observed and the color tone became dark brown in the neutral to alkaline zone more than pH 7. Thus, please use it from acidic to weak acidic zone in the cosmetic preparations.

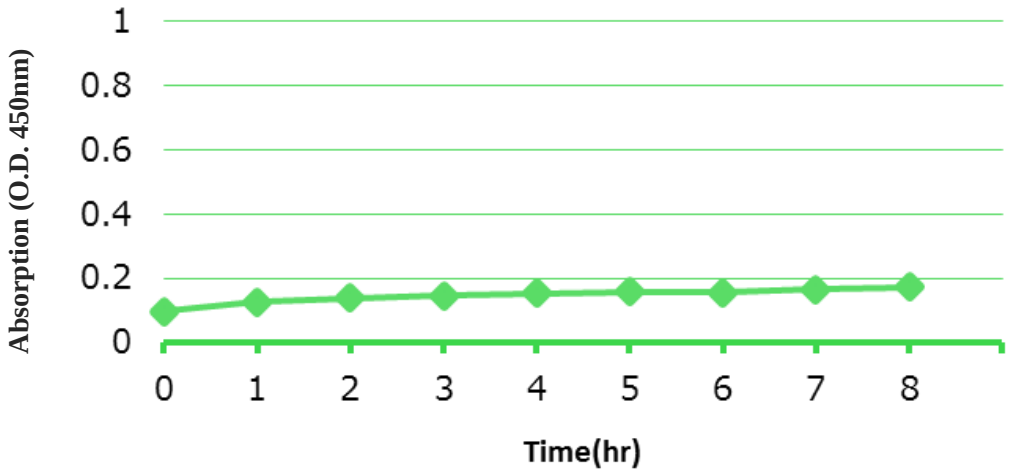
6-3 Thermal Stability Test

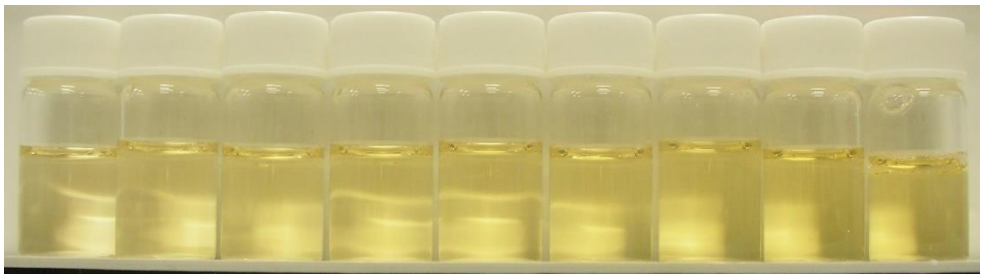
Adjust 10% concentration of VeryBerry™ ACNEcare with purified water and heat at 90℃ for 8 hours and observed the color change and determined the absorbance at 450nm.

Test Sample

VeryBerry™ ACNEcare (10% water solution; Lot No. B-503)

Test Result





Time (hr) 0 1 2 3 4 5 6 7 8

Consideration

10% aqueous solution of VeryBerry™ ACNEcare was heated for 8 hours at 90℃, as shown in the photo, the color tone was changed more yellow color little by little, but it is considered that thermal stability on VeryBerry™ ACNEcare relatively stable when heated for 8 hours.

7. 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	×	×

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

8. Toxicological Safety Test

Trade Name	VeryBerry™ ACNEcare	
Test Subject	**Result**	**Test Method**
Acute Oral Toxicity Test	Not Performed	
Primary Skin Irritation Test	Not recognize any stimulus	EpiSkin™ method
Accumulated Skin Irritancy Test	Not recognize any stimulus	RIPT method (50 people)
Sensitization Test	Not recognize any sensitization	RIPT method (50 people)
Photo Toxicity Test	Not Performed	
Photo Sensitization Test	Not Performed	
Eye Irritation Test	Not recognize any stimulus	SkinEthic™ HCE method
Mutagenicity Test	Negative	AmesTest (TA98, TA100, TA1535, TA1537, WP2uvrA)
Human Patch Test	Judgement for 24hours － (11)、 ± (1)	48hrs Closed Patch Test

9. Recommended Planning and Guide Formulation

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

- Cleansing Form
- Face Wash Cream
- Face Wash Soap
- Skin lotion
- Serum
- Milky lotion

9-1 Formulation 1 CLEANSING FOAM No.IFS-4478

No.	Trade Name	Manufacturer	%	INCI Name
1	ELDEW PS-306	Ajinomoto Co., Inc.	0.50	Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate
2	**EMALEX EG-di-SE**	**Nihon Emulsion Co., Ltd.**	4.00	Glycol Distearate
3	Lauric acid (NAA122)	NOF Corporation	1.00	Lauric acid
4	Myristic acid (NAA142)	NOF Corporation	3.00	Myristic acid
5	**EMALEX SEG-07**	**Nihon Emulsion Co., Ltd.**	4.00	Glyceryl Stearate, PEG-10 Stearate
6	**PYROTER CPI-40**	**Nihon Emulsion Co., Ltd.**	3.00	PEG-40 Hydrogenated Caster Oil, PCA Isostearate
7	Amisol LDE	Kawaken Fine Chemical	3.00	Lauramide DEA
8	**Oryza Tocotrienol-90**	**Oryza Oil & Fat Chemical**	qs	Tocotrienol, Tocopherol, Oryza Sativa (Rice) Bran Oil
9	Alanon ALE (30% AI)	Kawaken Fine Chemical	25.00	Sodium Lauroyl Methylaminopropionate, Water
10	Zemea Propanediol	DuPont	15.00	Propanediol
11	Purified Water		9.50	Water
12	**VeryBerry™ ACNEcare**	**Oryza Oil & Fat Chemical**	1.00	*1
13	**CosmeHerbest™ NADESHIKO**	**Oryza Oil & Fat Chemical**	1.00	Water, Propanediol, Dianthus Longicalyx Seed Extract
14	Amisoft LS-11F	Ajinomoto Co., Inc.	30.00	Sodium Lauroyl Glutamate
			100.00	

*1: Water, Propanediol, Salicylic Acid, Sodium Hydroxide, Dipotassium Glycyrrhizate, Citrus Unshiu Peel Extract, Citrus Junos Seed Extract, Allantoin, Actinidia Chinensis (Kiwi) Seed Extract, Fragaria Chiloensis Extract, Vitis Vinifera (Grape) Leaf Extract, Vitis Vinifera (Grape) Seed Extract, Vitis Vinifera (Grape) Skin Extract, Garcinia Mangostana Peel Extract

Manufacturing Method

1) Stir and dissolve Ingredients No.1 to 8 at 60℃.
2) After dissolving 1), add and dissolve Ingredients No.9 to 14 at 70℃.
3) Stir slowly by paddle at 70℃ for 1 hour and remove bubble.
4) Cool while stirring by paddle until at 40℃ as the product.

9-2 Formulation 2 REVITAL CREAM No.MCC-476

No.	Trade Name	Manufacturer	%	INCI Name
1	Liquid Petrolatum (70")		8.00	Mineral Oil
2	**AMITER MA-HD**	**Nihon Emulsion Co., Ltd.**	4.00	Hexyldecyl Myristoyl Methylaminopropionate
3	KF-96A (10mm^2/s)	Shin-Etsu Chemical	4.00	Dimethicone
4	Behenyl Alcohol (BH-65)	Toho Chemical	2.00	Behenyl Alcohol
5	Kanette O	BASF	4.00	Cetearyl Alcohol
6	**EMALEX PG-M-S**	**Nihon Emulsion Co., Ltd.**	1.00	Propylene Glycol Stearate
7	**EMALEX SEG-07**	**Nihon Emulsion Co., Ltd.**	4.00	Glyceryl Stearate, PEG-10 Stearate
8	Butylparaben		0.10	Butylparaben
9	Amisoft HS-11P(F)	Ajinomoto Co., Inc.	0.30	Sodium Stearoyl Glutamate
10	Sorbitol Solution (70%AI)		5.00	Sorbitol, Water
11	Concentrated Glycerine		4.00	Glycerin
12	Methylparaben		0.30	Methylparaben
13	Keltrol T (1% soln.)	Sumitomo Dainippon Pharma	10.00	Xanthan Gum, Water
14	**VeryBerry™ ACNEcare**	**Oryza Oil & Fat Chemical**	1.00	*1
15	Purified Water		52.30	Water
			100.00	

*1: Water, Propanediol, Salicylic Acid, Sodium Hydroxide, Dipotassium Glycyrrhizate, Citrus Unshiu Peel Extract, Citrus Junos Seed Extract, Allantoin, Actinidia Chinensis (Kiwi) Seed Extract, Fragaria Chiloensis Extract, Vitis Vinifera (Grape) Leaf Extract, Vitis Vinifera (Grape) Seed Extract, Vitis Vinifera (Grape) Skin Extract, Garcinia Mangostana Peel Extract

Manufacturing Method

1) Heat and dissolve Ingredients No.1 to 8 at 70℃.
2) Heat and dissolve Ingredients No.9 to 15 at 75℃.
3) While stirring 1) by homogenizer and emulsify adding 2) at 3,000r.p.m. for 5 minutes.
4) Change to paddle and cool while stirring until at 40℃ as the product.

9-3 Formulation 3 SKIN LOTION No.3864F-8

No.	Trade Name	Manufacturer	%	INCI Name
1	EMALEX CC-168	**Nihon Emulsion Co., Ltd.**	1.90	Cetyl Ethylhexanoate
2	ELDEW PS-203	Ajinomoto Co., Inc.	0.10	Phytosteryl/Octyldodecyl Lauroyl Glutamate
3	AMITER LGOD-2(H)	**Nihon Emulsion Co., Ltd.**	0.30	Dioctyldodeceth-2 Lauroyl Glutamate
4	Pyroter CPI-30	**Nihon Emulsion Co., Ltd.**	0.50	PEG-30 Hyfrogenated Casttor Oil PCA Isostearate
5	**Oryza Tocotrienol-90**	**Oryza Oil & Fat Chemical**	0.05	Tocotrienol, Tocopherol, Oryza Sativa (Rice) Bran Oil
6	Propylparaben		0.05	Propylparaben
7	Methylparaben		0.05	Methylparaben
8	EDTA-3Na		0.01	Trisodium EDTA
9	Magnesium Chloride		0.01	Magnesium Chloride
10	Calcium Chloride		0.01	Calcium Chloride
11	EMALEX SLP	**Nihon Emulsion Co., Ltd.**	0.05	Hydrogenated Lecithin
12	Amisoft CS-11F	Ajinomoto Co., Inc.	0.05	Sodium Cocoyl Glutamate
13	Keltrol T (1% soln)	Sumitomo Dainippon Pharma	5.00	Xanthan Gum, water
14	Carbomer 940 (1% soln)		20.00	Carbomer, Water
15	1,3-Butylene Glycol		10.00	Butylene Glycol
16	Concentrated Glycerin		10.00	Glycerin
17	Glycine		0.10	Glycine
18	Purified water		48.52	Water
19	L-Arginine		0.30	Arginine
20	Purified Water		2.00	Water
21	**CosmeHerbest™ SAKURA**	**Oryza Oil & Fat Chemical**	0.50	Water, Butylene Glycol, Prunus Lannensianna Flower Extract
22	**VeryBerry™ ACNEcare**	**Oryza Oil & Fat Chemical**	0.50	*1
			100.00	

*1: Water, Propanediol, Salicylic Acid, Sodium Hydroxide, Dipotassium Glycyrrhizate, Citrus Unshiu Peel Extract, Citrus Junos Seed Extract, Allantoin, Actinidia Chinensis (Kiwi) Seed Extract, Fragaria Chiloensis Extract, Vitis Vinifera (Grape) Leaf Extract, Vitis Vinifera (Grape) Seed Extract, Vitis Vinifera (Grape) Skin Extract, Garcinia Mangostana Peel Extract

Manufacturing Method

1) Heat and stir Ingredients No. 1 to 6 at 75℃. (A phase)
2) Heat and stir Ingredients No. 7 to 18 at 75℃. (B phase)
3) Dissolve Ingredients No.19 and 20. (C phase)
4) Stir B phase by homogenizer, add A phase, then add C phase and emulsify at 3000rpm for 5 minutes.
5) Cool by water bath after emulsifying, add Ingredients No.21 and 22 at 45℃, furthermore, cool until at 30℃ as the product.

9-4 Formulation 4 NIGHT CREAM (2) / FSG-52

No.	Trade Name	Manufacturer	%	INCI Name
1	MULTIWAX W-445	Sonneborn <USA>	1.50	Microcrystalline Wax
2	WHITE VASELINE		3.50	Vaseline
3	PARLEAM 18	NOF Corporation	7.00	Hydrogenated Polyisobutene
4	SALACOS 5408	Nisshin Oilio Group	2.00	Pentaerythrityl Tetraethylheanoate
5	ELDEW APS-307	Ajinomoto Co., Inc.	2.00	Phytostearyl/Decyltetradecyl Myristoyl Methyl Beta-Alanine
6	NAA-422	NOF Corporation	2.00	Behenyl Alcohol
7	NAA-45	NOF Corporation	2.00	Stearyl Alcohol
8	EMALEX CC-18	**Nihon Emulsion Co., Ltd.**	1.50	Stearyl Stearate
9	EMALEX GMS-B	**Nihon Emulsion Co., Ltd.**	2.00	Glyceryl Stearate
10	EMALEX GM-5	**Nihon Emulsion Co., Ltd.**	1.00	PEG-5Glyceryl Stearate
11	AMITER MA-HD	**Nihon Emulsion Co., Ltd.**	2.00	Hexyldecyl Myristoryl Methylaminopropionate
12	KSG-016F	Shin-Etsu Chemical	3.00	Dimethicone,（Dimethicone/Vinyldimethicone）Crosspolymer
13	KF-96A-10cs	Shin-Etsu Chemical	2.00	Dimethicone
14	KF-995	Shin-Etsu Chemical	2.00	Cyclopentasiloxane
15	PHENOXYETHANOL		0.40	Phenoxyethanol
16	TOCOPHERYL ACETATE		0.10	Tocopheryl Acetate
17	METHYLPARABEN(2%), BG		5.00	Methylparaben / BG
18	GLYCERIN		11.00	Glycerin
19	PEG#1500	NOF Corporation	1.50	PEG-6 / PEG-32
20	EMALEX 8100		1.50	PEG-100 Stearate
21	HA 1% SOLUTION		2.00	SodiumHyaluronate / Water
22	SODIUM CITRATE		0.20	Sodium Citrate
23	CITRIC ACID		0.20	Citric Acid
24	EDTA-2Na		1.00	EDTA-2Na / Water
25	XANTHAN GUM (1% soln.)		10.00	Xanthan Gum / Water
26	MULTITOL (Powder)		0.10	Multitol
27	**VeryBerry™ ACNEcare**	**Oryza Oil & Fat Chemical**	3.50	*1
28	PURIFIED WATER		30.00	Water
			100.00	

*1： Water, Propanediol, Salicylic Acid, Sodium Hydroxide, Dipotassium Glycyrrhizate, Citrus Unshiu Peel Extract, Citrus Junos Seed Extract, Allantoin, Actinidia Chinensis (Kiwi) Seed Extract, Fragaria Chiloensis Extract, Vitis Vinifera (Grape) Leaf Extract, Vitis Vinifera (Grape) Seed Extract, Vitis Vinifera (Grape) Skin Extract, Garcinia Mangostana Peel Extract

Manufacturing Method

1) Mix and dissolve Ingredients No.1 to 16 at 85℃. (Phase A)
2) Mix and dissolve Ingredients No. 17 to 28 at 85℃. (Phase B)
3) While stirring Phase B by homogenizer, add Phase A and make emulsion at 3500rpm for 5 minutes.
4) Then, stir by paddle at 45℃ and cool as the product.

10. Product Specification

Commodity	:	Specification	Remarks
Trade Name		VeryBerry™ ACNEcare	
Appearance			
· Color	:	Reddish yellow to dark reddish brown	
· Odor	:	Slightly characteristic odor	
Identification			
· Anthocyanin	:	Positive	
· Flavonoid	:	Positive	
· Saponin	:	Positive	
· Polyphenol compounds	:	Positive	
· Sugar	:	Positive	
· Xanthone compounds	:	Positive	
· Salicylic acid	:	Positive	
· Allantoin	:	Positive	
· Dipotassium Glycyrrhizate	:	Positive	
pH (1→10)	:	4.0 - 6.0	
Purity Test			
1) Heavy Metals	:	20 ppm max.	
2) Arsenic	:	2 ppm max.	
Microbiological Examination			
1) Bacterial Count	:	1 × 10^2/g max.	Hygiene Test
2) Mold, Yeast	:	1 × 10^2/g max.	Hygiene Test
3) Coliform	:	Negative	Hygiene Test

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.

11. Labelling Name

INCI Name	化粧品国際中文標準名	CAS No.	EC No.
Water	水	7732-18-5	231-791-2
Propanediol	1,3-丙二醇	504-63-2	207-997-3
Salicylic Acid	水杨酸	69-72-7	200-712-3
Sodium Hydroxide	氢氧化钠	1310-73-2	215-185-5
Dipotassium Glycyrrhizate	甘草酸二钾	68797-35-3	272-296-1
Citrus Unshiu Peel Extract	温州蜜柑（Citrus Unshiu）果皮提取物	94266-47-4	304-454-3
Citrus Junos Seed Extract	香橙（Citrus Junos）籽提取物	94266-47-4	304-454-3
Allantoin	尿囊素	97-59-6	202-592-8
Actinidia Chinensis (Kiwi) Seed Extract	中华猕猴桃（Actinidia Chinensis）籽提取物	92456-63-8	310-127-6
Fragaria Chiloensis (Strawberry) Fruit Extract	草莓（Fragaria Chiloensis）果提取物	95193-64-9	305-877-6
Vitis Vinifera (Grape) Leaf Extract	葡萄（Vitis Vinifera）叶提取物	84929-27-1	284-511-6
Vitis Vinifera (Grape) Seed Extract	葡萄（Vitis Vinifera）籽提取物	84929-27-1	284-511-6
Vitis Vinifera (Grape) Skin Extract	葡萄（Vitis Vinifera）皮提取物	84929-27-1	284-511-6
Garcinia Mangostana Peel Extract	倒捻子（Garcinia Mangostana）果皮提取物	90045-25-3	289-884-9

12. Others

16-1 Packaging Style

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

16-2 Storage Condition

Store it in a cool, dry, ventilated area with desiccant.

Avoid high temperature and sun light, store the product in closed original container.

13. References

1) Rathapon Asasutjarit et al., Physicochemical properties and anti-propionibacterium acnes activity of film-forming solutions containing alpha-mangostin-rich extract. *AAPS PharmScitech*, Vol.15, No.2, 306-316 (2014).

2) Das S, Das DK. Anti-inflammatory responses of resveratrol. *Inflammation & Allergy-Drug Targets*, 6, 168-173 (2007).

3) Michael H. et al.,Modes of effect of aspirin-like drugs: Salicylates inhibit Erk activation and integrin-dependent neutrophil adhesion. *Proc. Natl. Acad. Sci. USA*, Vol.95,14540-14545 (1998).

From product planning to OEM

Please feel free contact if you need more additional information or our assistance :

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

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*Correction : Updated Tokyo office address

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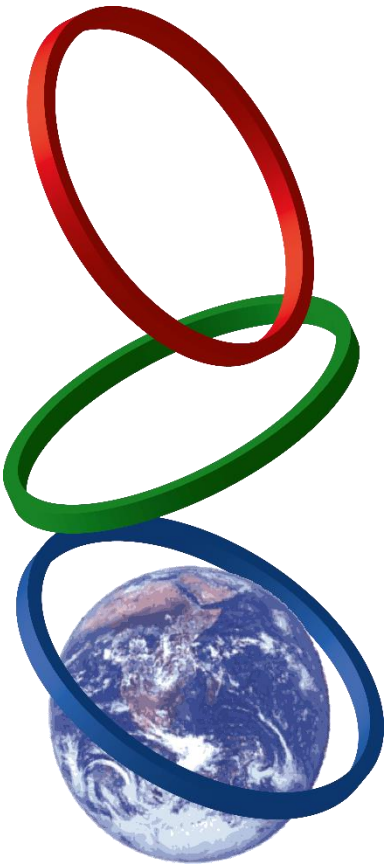

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