LUNAWHiTE

(Evening Primrose Seed Extract)

Moon Princess — *Evening Primrose which is a mysterious and pretty flower, bloom at the same time as the moon rises. It may be a present for contemporary women who demand whitening, from the Moon Goddess, Luna.*

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Summary of LUNAWHITE

What is "LUNAWHITE"?

"LUNAWHITE" produced from the raw material *Oenothera biennis* Linné seed is a new type of a starting material for cosmetics and quasi-drug products (additives).

Dr. Foo, New Zealand, made detailed examinations of polyphenols in the seed of a certain evening primrose species (*Oenothera biennis* Linné) of the genus *Oenothera*. The doctor made a confirmation that the seed contained a wider spectrum of polyphenols, compared with grape seed, pine bark, and green tea extracts.

Taking into account as much as possible the dermal safety profile of the polyphenols recovered from the seed of *Oenothera biennis* Linné, Ichimaru Pharcos Co., Ltd. examined the application of the polyphenols to cosmetics, based on the report of Dr. Foo's research and developed the Oenothera biennis Seed Extract as "LUNAWHITE".

It has already been verified that the *Oenothera biennis* Linné seed extract "LUNA WHITE" containing a wide spectrum of polyphenols has lots of actions, such as whitening effect and anti-aging effect, in addition to the anti-oxidation effect already reported. Ichimaru Pharcos Co., Ltd. is now under way of further examinations about the efficacy.

Ichimaru Pharcos Co., Ltd. proposes "LUNAWHITE" as a new type of a starting material with more effects and for whitening cosmetics and anti-aging cosmetics.

1. Introduction

1-1 What is *Oenothera biennis* Linné?

Oenothera biennis Linné is a biennial plant of the genus *Oenothera* of the family *Oenotheraceae*. There are 20 kinds of the group of *Oenothera* in Japan, and they are generally called "evening primrose", ambiguously. The genus *Oenothera* is derived from their blooming in the evening. In English, "Evening Primrose" means the primrose blooming in the evening. In German, *Oenothera tetraptera* Linné is called Nachtkerze with the meaning of "night candle".

The designation "Evening Primrose" is the genuine name of one *Oenothera biennis* Linné. It is said that *Oenothera biennis* Linné has its origin in North America. It grows one to two meters high. Yellow flowers bloom in the evening in early summer to early autumn but droop in the next morning. The evening primrose is a biennial plant of a height of about 60 cm.

1-2 γ-Linoleic acid contained in *Oenothera biennis* Linné

The seeds of the genus *Oenothera* contain high concentrations of γ-linoleic acid as a precursor of a hormone involved in human functions, namely prostaglandin. It has been elucidated that many of human diseases have a relation with the shortage of unsaturated fatty acids such as γ-linoleic acid. Accordingly, γ-linoleic acid is used for the therapeutic treatment of a wide range of diseases including alcoholic poisoning, allergy, cardiac diseases and atopic dermatitis. For the purpose of recovering γ-linoleic acid, *Oenothera biennis* Linné is cultivated mainly in England and Canada to promote its medical research works.

It is said that γ-Linoleic acid is contained in breast milk. A certain report tells that allergic symptoms can be observed in cases with no ingestion of γ-linoleic acid since childhood because of some reason or in cases with no good progress in γ-linoleic acid synthesis in biological organisms. Therefore, the therapeutic treatment of allergic eczema with γ-linoleic acid from *Oenothera biennis* Linné is recommended in many countries throughout the world. The extract containing unsaturated fatty acids as recovered from the seed of *Oenothera biennis* Linné is called evening primrose oil and is now applied to cosmetics.

1-3 Anti-oxidation effect of the seed of *Oenothera biennis* Linné

The seed of *Oenothera biennis* Linné contains high contents of γ-linoleic acid as well as other unsaturated fatty acids. The extract containing unsaturated fatty acids as recovered from the seed of *Oenothera biennis* Linné is called evening primrose oil and is now applied to cosmetics.

However, disadvantageously, the evening primrose oil readily decomposes in light, with heat and in air due to the presence of unsaturated fatty acids.

A certain farmer planted the seed of "evening primrose", which was harvested 6 years ago, and verified that the state of germination was great. On stimulation with the finding, Dr. Foo, a chemist, made detailed examinations about the stability of the seed of "evening primrose". Consequently, Dr. Foo confirmed that a wider spectrum of polyphenols was contained in the seed of the plant *Oenothera biennis* Linné, compared with grape seed, pine bark and green tea extracts. In other words, it is suggested that the *Oenothera biennis* Linné seed containing readily oxidizable unsaturated fatty acids, also contains the anti-oxidants to maintain the stability.

The polyphenols contained in the seed of *Oenothera biennis* Linné exist in the unsaturated fatty acids. Thus, it is suggested that the polyphenols are likely to be highly lipophilic. Based on the property, it is suggested that the polyphenols derived from the seed of *Oenothera biennis* Linné likely prevent the oxidation of fatty acids in cosmetics.

1-4 LUNAWHITE

"LUNAWHITE" produced from the raw material *Oenothera biennis* Linné seed serves as a starting material for cosmetics.

Two types of products with difference in solvent composition and one powder type product are available. These essentially contain the same ingredients. The solid is mainly composed of characteristic polyphenols and found very strong whitening effect, anti-oxidation effect and anti-aging effect under observation.

The designation "LUNAWHITE" is derived from "LUNA" meaning moon goddess because Evening Primrose is called in Japan as the name of “Tsukimisou” that it means the flower blooms at he same time as the moon rise, and is also derived from the very strong whitening effect ascribed to the actions of the characteristic polyphenols.

2. Main Components

2-1 Main Components

The composition of "LUNAWHITE" was determined using HPLC. "LUNAWHITE" was found to contain a broad spectrum of Fig.1 including gallic acid, catechin, OPCs, procyanidin B3, ellagic acid and pentagalloylglucose. This broad spectrum of polyphenolic compounds gives "LUNAWHITE" it's unique medicinal properties.

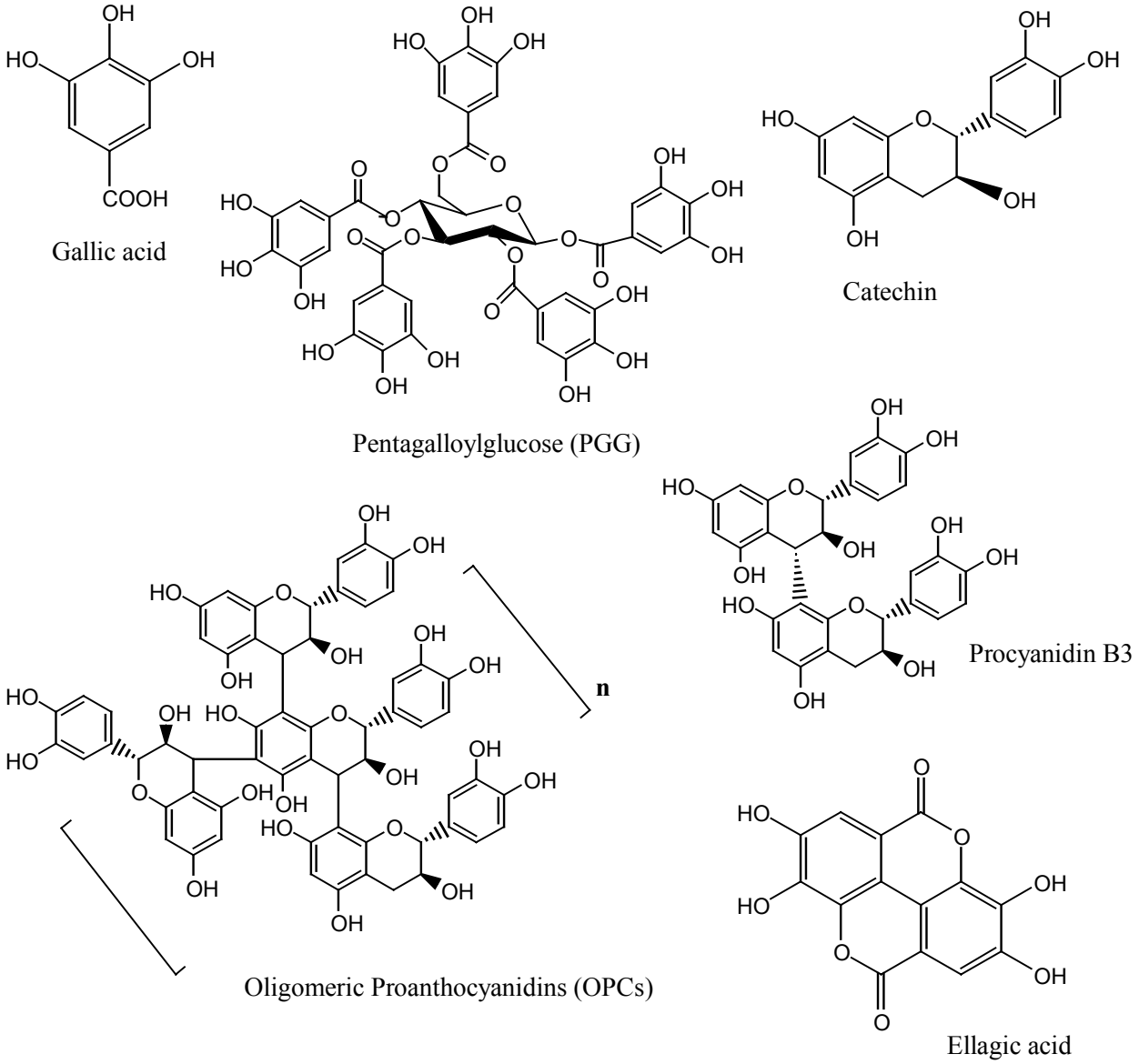

Fig.1 Polyphenolic Compounds

2-2 Polyphenols in LUNAWHITE and Others

Pine Bark, Grape Seed and Green Tea Extracts do not contain ellagic acid or pentagalloylglucose, which are very important free radical scavengers and have unique biological properties.

	LUNAWHITE	Pine Bark	Grape Seed	Green Tea
Gallic acid	○	○	○	○
Catechin	○	○	○	○
OPC	○	○	○	×
Procyanidin B3	○	○	×	×
Ellagic acid	○	×	×	×
Pentagalloylglucose	○	×	×	×

3. Efficacy

Whitening

- Tyrosinase Inhibition Effect
- B16 Melanoma cell Inhibition Effect
- Inhibition of Pigmentation Effect

Anti-aging

- Collagenase Activity Inhibition Effect
- Elastase Activity Inhibition Effect
- Hyaluronidase Activity Inhibition Effect

Anti-oxidation

- Lipid Peroxidation Inhibition Effect
- Super Oxide Dismutase (SOD) like Effect

3-1 Whitening Test

3-1-1 Tyrosinase Inhibition Effect

Tyrosine is changed to DOPA, and DOPA is changed to DOPA-quinone by Tyrosinase. So, it is thought important for whitening to inhibit Tyrosinase Activity. Kojic acid and Arbutin are known to inhibit Tyrosinase Activity.

The efficiency of inhibiting the formation of melanine reacting tyrosinase with tyrosine, is found by measuring the absorbance of the DOPA-chrome.

Test Method

To 1.0mL of L-tyrosine solution (0.3mg/mL) add 1.0ml of Mcllvain buffer at pH 6.5 and allow to stand in a water bath at 37℃ for 10minutes. After adding 1.0mL of each test samples or blank solution, add 0.1mL of tyrosinase solution (about 2, 500 unit/mL, derived from mushroom) in water bath at 37℃ for 20 minutes. After reacting, determine the absorbance of the producing amount of red colored DOPA-chrome on each test solution at 475 nm.

Results

According to Fig.2, inhibition of tyrosinase activity was observed. Therefore, LUNAWHITE has an inhibition effect of formation of melanin.

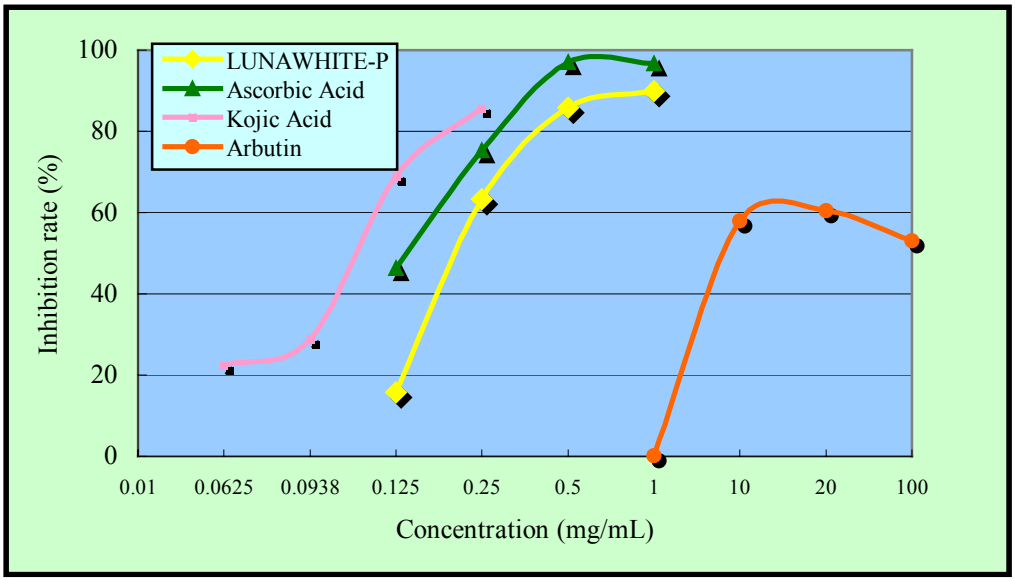

Fig.2 Tyrosinase Inhibition Effect

Test Sample	IC_{50} (mg/mL)
LUNAWHITE-P	0.21
Ascorbic acid	0.14
Kojic acid	0.11
Arbutin	8.7

3-1-2 B16 Melanoma cell Inhibition Effect

We investigated melanin production inhibition test on B16 melanoma cells of LUNAWHITE.

Test Method

a. Cells and the culture condition

B16 mouse melanoma cells were used and were cultured in an MEM culture medium including 10% Fetal Bovine Serum (made by GIBCO BRL). The culture conditions were 37 ° C, 5% CO_2.

b. Test Sample

After evaporating the solvent of LUNAWHITE-P, dissolve 50% ethanol, add to be 25 ppm concentration to culture medium. Separately, Arbutin (Sigma), Kojic acid (Kishida Chemical) and Magnesium ascorbyl phosphate (Kishida Chemical) were used as control samples.

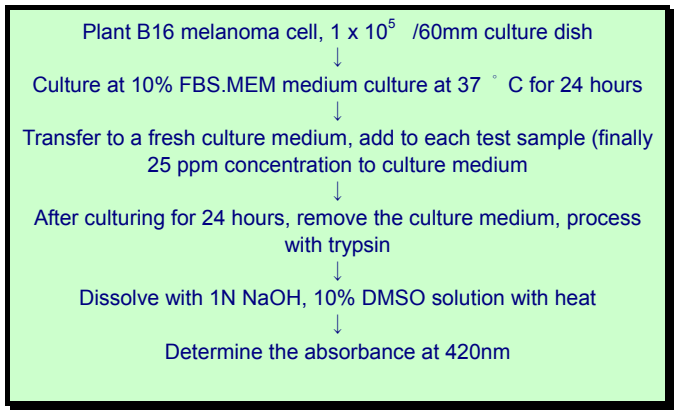

Fig.3 Melanin production inhibition test

c. Measurement

3 x 10^4 B16 melanoma cells were planted in a 60 mm plastic culture dish and were pre-cultured 24 hours. Then they were transferred to a fresh culture medium and test materials were added to be 25 ppm concentration to culture medium. The cells were collected by processing with trypsin after culturing for three days. After the cell were removed non-melanin matter by 5% TCA, Ether/Ethanol mixture and Ether Dehydrated, they were dissolved in 1N NaOH and 10% DMSO and then absorbance was measured at 420nm. At the same time the activity of the cells which were supplemented with various materials was measured by the MTT reduction method.

Results

Melanin production inhibition on each test samples was showed in Fig. 4. Whitening effect on LUNAWHITE is superior to other whitening products. Also cell toxicity on LUNAWHITE was not observed on MTT Test at same time.

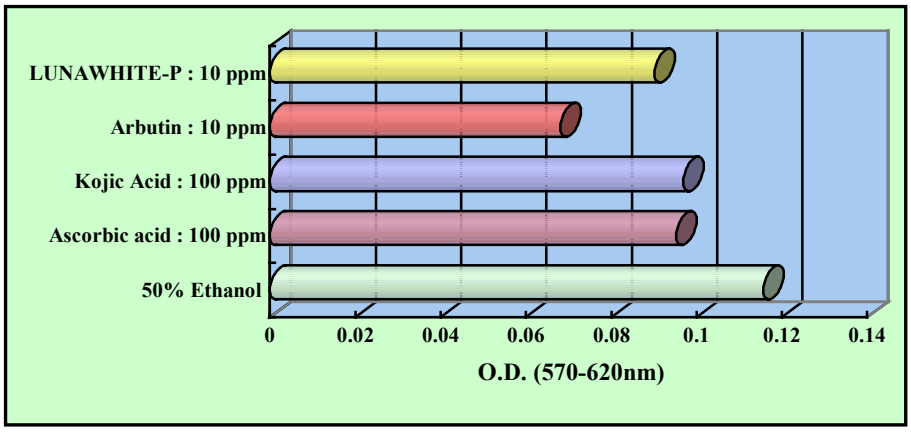

Fig.4 Test Result of Melanin Production Inhibition on LUNAWHITE

3-1-3 Pigmentation Inhibitory Test in W-M Guinea Pigs

We investigated pigmentation inhibitory test in W-M Guinea Pigs on LUNAWHITE.

Test Method

This test was performed according to the method of Ohbayashi et al. [1]. Going into the details, three brown guinea pigs (male W-M guinea pigs aged 13 weeks purchased from Japan Biomaterials Center Co., Ltd.) in each group, a total of six guinea pigs, were subjected to back hair removal with hair clippers to prepare 4 test sites (3 x 3 cm) on each animal's back. To each test site, 72μL of a 0.005 % (w/v) solution of 8-methoxysoralen in acetone was applied and then UVA (2 J/cm^2) was irradiated with FL-20BLB lamp (Toshiba). After irradiation, the test substance solution and the vehicle used to dissolve the test substance was applied to two test sites each once daily, 5 times a week for 3 weeks. On the day after the last application, the skin lightness of each site was measured with a color difference meter (Minolta CR-200) in triplicate. The difference in skin lightness value between before and after the test was regarded as a pigment deposition index. The two test substances used were LUNAWHITE-P and arbutin dissolved in the following milky lotion at a concentration of 1 % (w/w).

< Formulation of the milky lotion >

Stearic acid	2.5
Cetyl alcohol	1.5
Petrolatum	5.0
Mineral oil	10.0
NIKKOL TO-10 (Nikko Chemical)	2.0
Polyethylene glycol 1500	3.0
Triethanolamine	1.0
Carbomer	0.05
Arbutin	1.0
Sodium bisulfite	0.01
Ethylparaben	0.3
Purified water	73.64
Total	100.0

Results

The pigmentation index was calculated from the mean value of six skin lightness values of the two test sites to which the test substance was applied (each test site was measured in triplicate).

As a result, in the LUNAWHITE-P application group, the pigmentation index was 11.59 in Animal No. 1 (control site : 13.69), 12.94 in Animal No. 2 (control site : 11.85) and 12.73 in Animal No. 3 (control site : 13.58). Since one control site of Animal No. 2 showed no pigmentation, the skin lightness of the concerned site was not measured.

In the albutin application group, the pigmentation index was 10.50 in Animal No. 1 (control site : 13.46), 11.97 in Animal No. 2 (control site : 13.05) and 10.62 in Animal No. 3 (control site : 13.65). When the pigmentation indices of three animals were averaged and subjected to significance test (Student's t-test), a significant suppression (suppression rate : 17.57 %) was observed in the albutin application group with a significant level of 1 %, as shown in the Fig. 5.

For the LUNAWHITE-P application group, the average values are shown but no significance test was performed, since the experimental conditions were somewhat different as compared with the albutin application group.

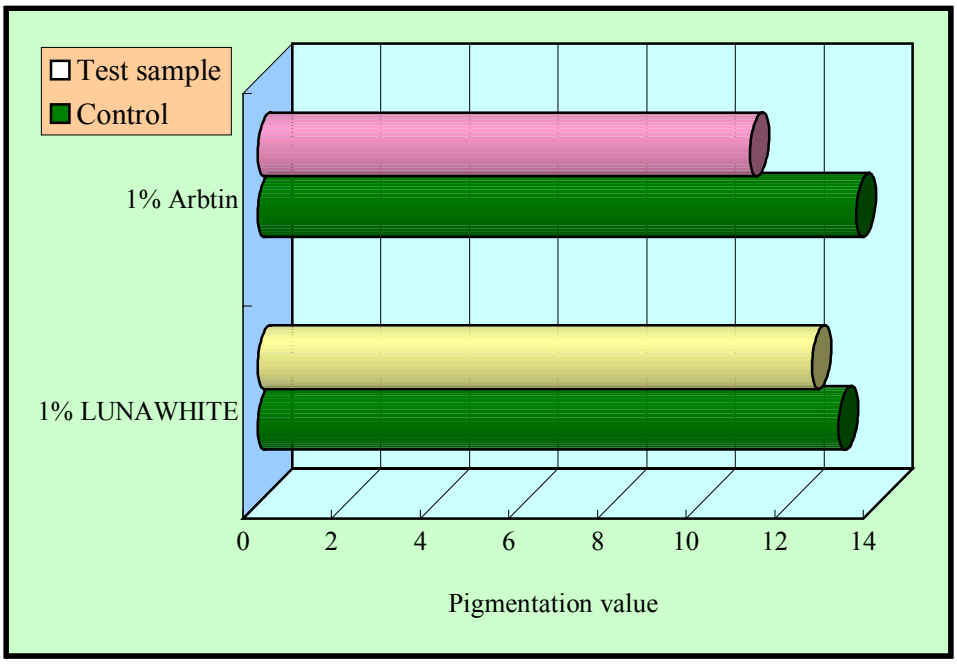

Fig.5 Mean pigment deposition indices of 3 animals in each group

Discussion

LUNAWHITE-P and albutin were subjected to a pigmentation inhibitory test in guinea pigs. As a result, albutin was considered to inhibit pigmentation, but in case of LUNAWHITE-P, there were two problems in the experiment, i.e., 1) that LUNAWHITE-P was not completely soluble in the vehicle used this time and 2) one control site did not show pigmentation due to some trouble in experimental procedures, so further investigation was considered to be necessary.

References

1) Ohbayashi et al. Journal of Society Cosmetic Chemists Japan Vol.31, No. 4,447 (1997)

3-2 Anti-aging Test

3-2-1 Collagenase Activity Inhibition Effect

We observed the collagenase activity inhibition effect of LUNAWHITE.

Test Method

This test method is an evaluating method using actinomycete-derived collagenase and synthetic substrate PZ-peptide. As the buffer solution, 0.1-M tris (hydrochloric acid) buffer (pH 7.1, containing 20-mM $CaCl_2$) was used. With this buffer, the enzyme and the substrate solutions were prepared to have a concentration of 0.1 mg/mL and 0.5 mg/mL, respectively. First, 50 μL of the test sample, 50 μL of the enzyme solution and 400 μL of the substrate solution were mixed together and the resulting solution was kept at 37°C for 30 minutes to promote its reaction. Then, its reaction was stopped by adding 1 mL of 25-mM citric acid solution to the solution. Next, 5 mL of ethyl acetate were added to the solution and the resulting solution was stirred to transfer the reaction product to the ethyl acetate layer. After the centrifugation of the stirred solution (at 3000 rpm for 5 minutes), the absorbance of the extractant containing the reaction product was measured at 320 nm of wavelength. The inhibition rate was calculated by the following formula. Here, the extractant for the test sample was used as the reference solution. Setting up a blind test in which the substrate was added to the solution whose reaction had been stopped, the inhibition rate was calculated from the difference in absorbance between the actual test and the blind test according to the following formula.

Inhibition Rate (%) =

$$100 - \frac{\text{Absorption Rate of Actual Test of Sample} - \text{Absorption Rate of Blind Test of Sample}}{\text{Absorption Rate of Actual Test of Control} - \text{Absorption Rate of Blind Test of Control}} \times 100$$

Results

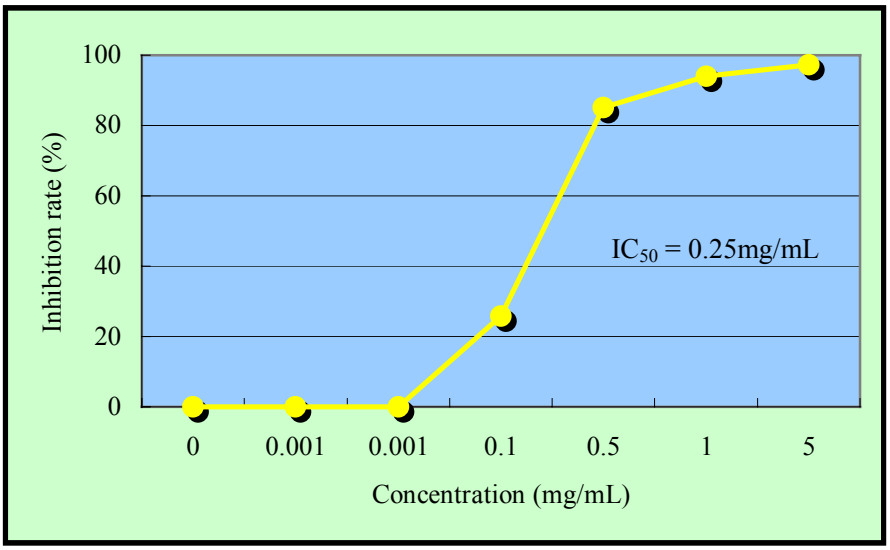

Fig.6 Collagenase activity inhibition effect

As for the typical change which is seen to aging of the skin which is, wrinkle and slack and so on are raised, but for the forming, it thinks that they are one because of elasticity's decline of the skin and the factor includes an influence by the aging, the sun light (the ultraviolet rays), the dryness, the oxidation and so on.

As the phenomenon having to do with learning of the concrete organization at the skin, the change of the matrix ingredient of the collagen, elastin, the hyaluronic acid in the corium and so on out of the cell is given and it finds the thing that the change of the collagen which is a corium main composition ingredient specifically and so on is important.

In other words, it thinks that collagenase concerns that Three-Dimension structure to act on the resolution of the collagen is denatured about the decrease of the skin elasticity which occurs in the wrinkle and slack process of the forming.

Therefore, it thinks that to obstruct to do the collagenase activated and to prevent from the oddity of the collagen prevent the forming of wrinkle and slack and that obstruct and prevent connect with aging prevention by the skin.

According to this test, as LUNAWHITE was observed inhibition effect of collagenase activity, as it was expected to inhibit wrinkle and slack.

3-2-2 Elastase Activity Inhibition Effect

We observed the elastase activity inhibition effect of LUNAWHITE.

Test Method

This test method is an evaluating method using pig's pancreas-derived elastase and synthetic substrate N-succinyl-Ala-Ala-Ala-*p*-nitroanilide. As the buffer solution, 0.05 M tris (hydrochloric acid) buffer (pH 8.8) was used. With this buffer, the enzyme and the substrate solutions were prepared to have a concentration of 0.05 unit/mL and 0.1 M by dimethyl sulfoxide, respectively. First, 50 μL of the test sample, 50 μL of the enzyme solution and 100 μL of the substrate solution were mixed together and the resulting solution was kept at 37°C for 30 minutes to promote its reaction. It was measured at 405 nm of wavelength. The inhibition rate was calculated by the following formula. Here, the extractant for the test sample was used as the reference solution. Setting up a blind test in which the substrate was added to the solution whose reaction had been stopped, the inhibition rate was calculated from the difference in absorbance between the actual test and the blind test according to the following formula.

$$\text{Inhibition Rate (\%)} = 100 - \frac{\text{Absorption Rate of Actual Test of Sample} - \text{Absorption Rate of Blind Test of Sample}}{\text{Absorption Rate of Actual Test of Control} - \text{Absorption Rate of Blind Test of Control}} \times 100$$

Results

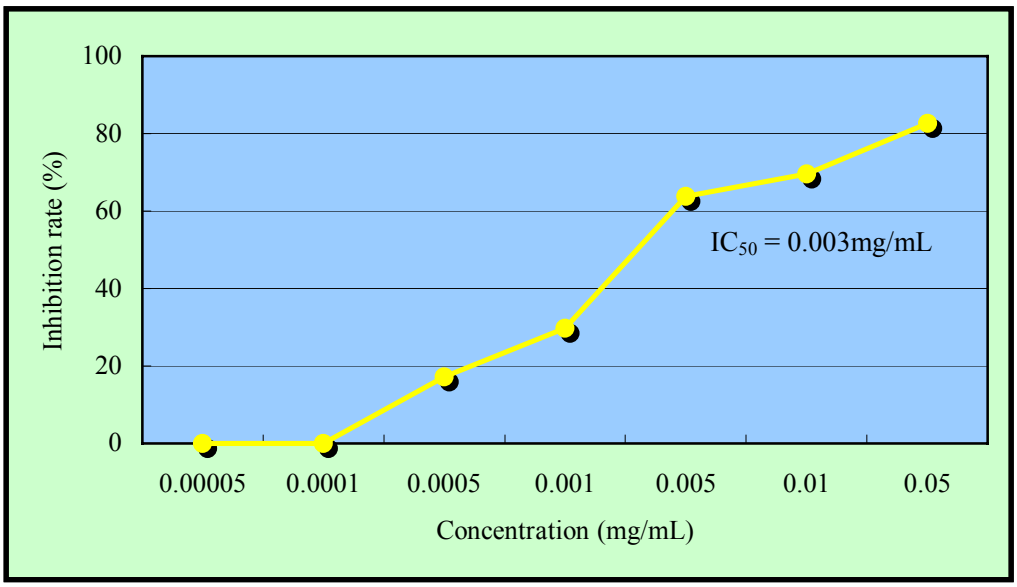

Fig.7 Elastase activity inhibition effect

According to this test, as LUNAWHITE was observed inhibition effect of elastase activity, as it was expected to inhibit wrinkle and slack.

3-2-3 Hyaluronidase Activity Inhibition Effect

We observed the hyaluronidase activity inhibition effect of LUNAWHITE.

Test Method

To 0.1 mL of the sample, 0.05 mL of the hyaluronidase solution was added, and after standing at 37 ° C for 20 minutes, 0.1 mL of the Compound 48/80 solution was added followed by another standing at 37 ° C for 20 minutes. After adding 0.24 mL of the hyaluronic acid solution, it was incubated at 37 ° C for 40 minutes. The reaction was suspended by adding 0.1 mL of 0.4N sodium hydroxide, and 0.1 mL of the potassium borate solution was added and heated for 3 minutes in boiling water. After cooling to room temperature, 3 mL of the *p*-dimethyl aminobenz aldehyde test solution was added. It was allowed to stand at 37 ° C for 20 minutes, and the absorbance was determined at 585 nm. Meanwhile, using that with purified water instead of the sample as the control, blanks without enzyme were prepared referring to the sample and control respectively.

Results

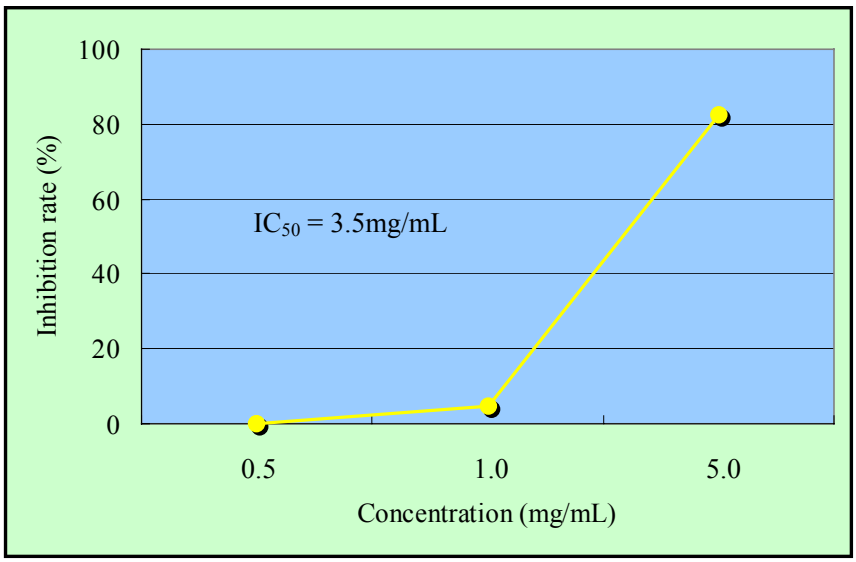

Fig.8 Hyaluronidase activity inhibition effect

According to this test, as LUNAWHITE was observed inhibition effect of hyaluronidase activity, as it was expected to inhibit wrinkle and slack.

3-3 Anti-oxidation Test

3-3-1 Lipid Peroxidation Inhibition Effect

Test Method

a. Test Sample

After evaporating the solvent of LUNAWHITE, dissolved in 50% ethanol, adjusted to 10mg/mL as solid content and diluted to 0.01mg/mL. DL-alpha-tocopherol as positive control dissolved in 0.8% sodium lauryl sulphate and adjust to 0.01mg/mL.

b. Measurement

Peroxidized lipid was determined by measuring the amount of a peroxidized compound by TBA method. 0.1% of linoleic acid was dissolved in 0.8% sodium lauryl sulphate and 4.9mL of this solution was used. To this solution, 0.1mL of the specimen were added and exposed to ultraviolet rays (irradiation by FL-20SE and FL-20BLB lamps of Toshiba standing in a row) for one hour. To 1mL of this solution, 0.02ml of 4.5% BHT (butylated hydroxytoluene) solution 1.5mL of 0.67% thiobarbiturate solution and 1.0 mL of 15% acetic acid were added and heated at 95 ° C for one hour. After cooling, 4mL of n-butanol solution including 15% methanol mixture were added and shaken well and centrifuged. The amount of lipid peroxide was determined by measuring absorbency (O.D.) of this butanol layer at 532 nm.

As a control, the amount of peroxidized fat without UV irradiation was determined and the difference from the specimen with the irradiation was defined as the amount of peroxidized lipid formation. The amount of peroxidized lipid without the addition of a specimen was set at 100 and the inhibition rate was calculated by determining the respective ratios.

Inhibition rate (%)

= [1- (sample O.D. – blank O.D.) / (sample control O.D. – blank control O.D.)] X 100

Results

According to Fig.9, lipid peroxidation inhibition effect was observed. And, it confirmed that this efficacy was stronger than DL-alpha-tocopherol being used for the cosmetics and so on. Therefore, LUNAWHITE has a strong inhibitory effect on the peroxidized lipid formation.

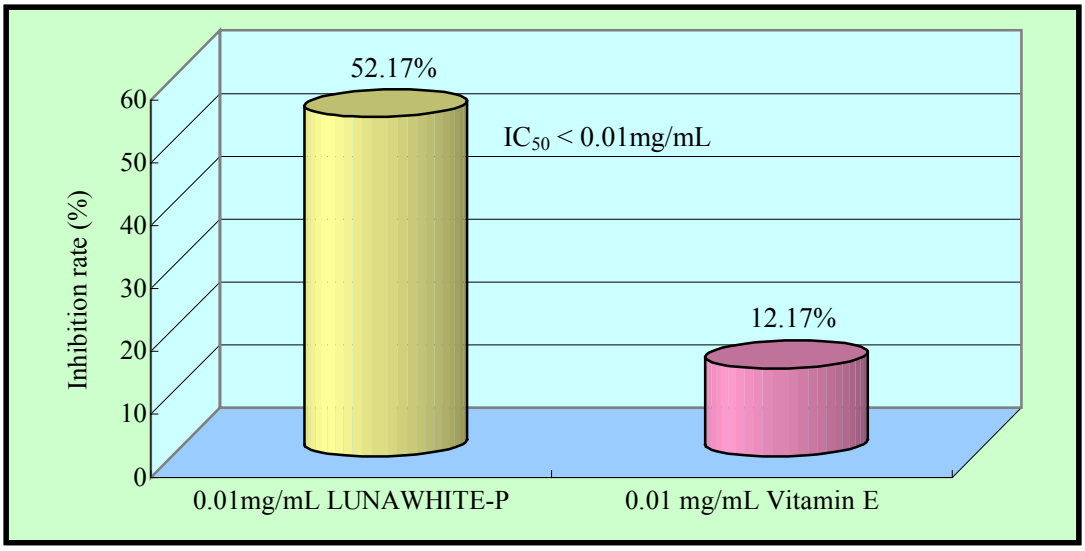

Fig.9 Lipid Peroxidation Inhibition Effect

3-3-2 Super Oxide Dismutase (SOD) like Effect

It knows that it is made a dismutation by super oxide dismutase (SOD) with super oxide which occurs in the living body. So, the influence of LUNAWHITE to super oxide which occurs in system of xanthine-xanthine oxidase was measured by the NBT method.

Test Method

a. Test Sample

After evaporating the solvent of LUNAWHITE, dissolved in 50% ethanol, adjust to 1% as solid content and diluted with the purified water if necessary. And, at the same time, as for BOTANPI Liquid E which was having anti-oxidation effect, it was diluted with the purified water in the same way.

b. Measurement

0.1mL of 0.75mM NBT solution, 0.1mL of 0.15mM BSA solution and 0.1mL of test sample were mixed to 0.02M sodium carbonate-hydrochloric acid buffer (pH 10.2), which is including 3.0mM of Xanthine and 3.0mM of EDTA, left for ten minutes with the room temperature. After that, it was added a xanthine oxidase, reacted for twenty minutes with the room temperature, added 0.1mL of 6.0mM cuprous chloride solution to stop this reaction and was determined by measuring absorbency (O.D.) at 560 nm.

As a control, it prepared for the sample control which a solvent was added to instead of the sample solution. Furthermore, before doing an enzyme reaction, it was prepared for the blank control that a reaction stop solution was added in advance. And, it showed the anti-oxidization effect of the 1% sample solution by setting the concentration which showed 50% of the deoxidise inhibition rate at a SOD 1 unit.

NBT deoxidise inhibition rate (%)

= [1- (sample O.D. – blank O.D.) / (sample control O.D. – blank control O.D.)] X 100

Results

According to Fig.10, SOD like effect was observed because 1% LUNAWHITE will be about twelve units convert to SOD. Therefore, LUNAWHITE has a strong anti-oxidation effect.

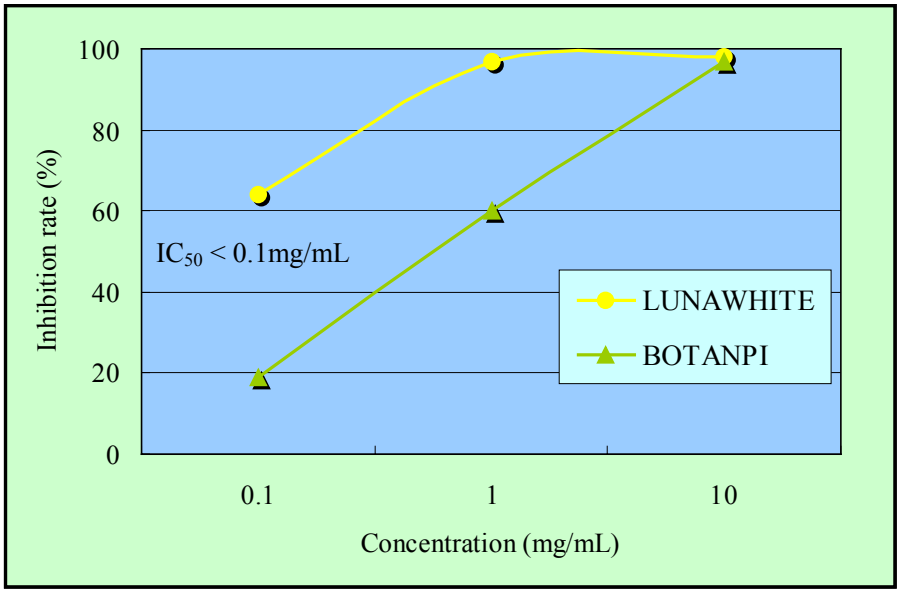

Fig.10 Super Oxide Dismutase (SOD) like Effect

3-4. Increase Stability of Ascorbic Acid

LUNAWHITE is a cosmetic ingredient; which contains polyphenol from *Oenothera biennis* Linné seed. Many kinds of unsaturated fatty acid such as γ-linoleic acid etc- which is easily oxidizable- are contained in *Oenothera biennis* Linné seed. Polyphenol is expected to be contained as an inhibition of oxidation of unsaturated fatty acid. In order to study well as far as anti-oxidation effect of LUNAWHITE, influence of the stability of ascorbic acid by LUNAWHITE is observed.

Influence of stability of ascorbic by LUNAWHITE

Ascorbic acid is very popular as an effective and safe anti-oxidation ingredient, and it is used in food etc. But stability is not good and reduction in water solution is observed with time. Regarding to change of ascorbic acid by LUNAWHITE with time, the stability effect is observed by measuring the survival rate of ascorbic acid.

Test Sample and method

Store 2 group at room temperature (25±5℃). One is 1mg/mL ascorbic acid acetic acid buffer solution and the other is ascorbic acid acetic acid buffer solution contained 0.1mg/mL LUNAWHITE-P (1% LUNAWHITE-B). pH of two of them is adjusted 5.5. Observe reduction of ascorbic acid by measuring absorption of 265nm; which is maximum absorption rate of ascorbic acid with time. At this time, absorption rate of LUNAWHITE is almost not affected by the result.

Result and Discussion

The result is shown in Figure 11. At room temperature, ascorbic acid is reduced with time. But, in the group; which added LUNAWHITE- reduction of ascorbic acid is memorably inhibited. Almost 12 days later, amount of ascorbic acid became less than 10 % when it is kept at room temperature. But, when LUNAWHITE is added, it takes almost 30 days until ascorbic acid became less than 10%. Also, regarding to comparison data of survival rate of ascorbic acid on the 9^{th} day, the group -which add LUNAWHITE- is 3 times more compared than with no additives.

According to these results, LUNAWHITE is confirmed to increase the stability of ascorbic acid.

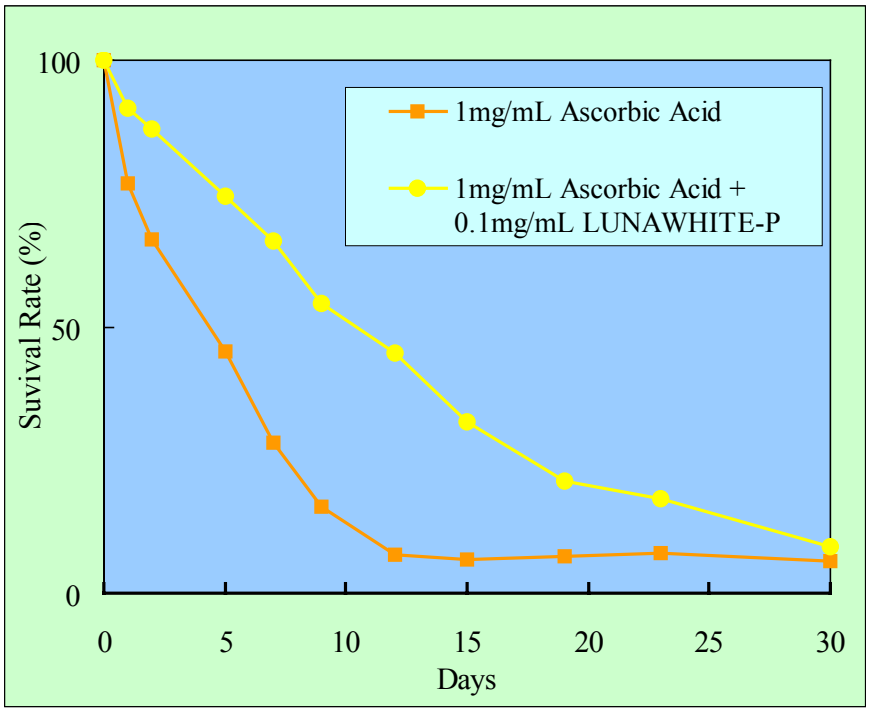

Figure 11, Stability effect of Ascorbic Acid

0.1mg/mL LUNAWHITE-P = 1% LUNAWHITE-B

4. Stability Test

4-1 Long term Stability

Test Method

Store 100g of LUNAWHITE B and LUNAWHITE E for 30 days under cool dark place, at room temperature, at a window side, at 40℃ and at 50℃. The absorbency of each condition was determined.

Result

Under 50℃ of conditions, the color of LUNAWHITE B and E was a little changed to dark. However, under the other condition, that color was not changed.

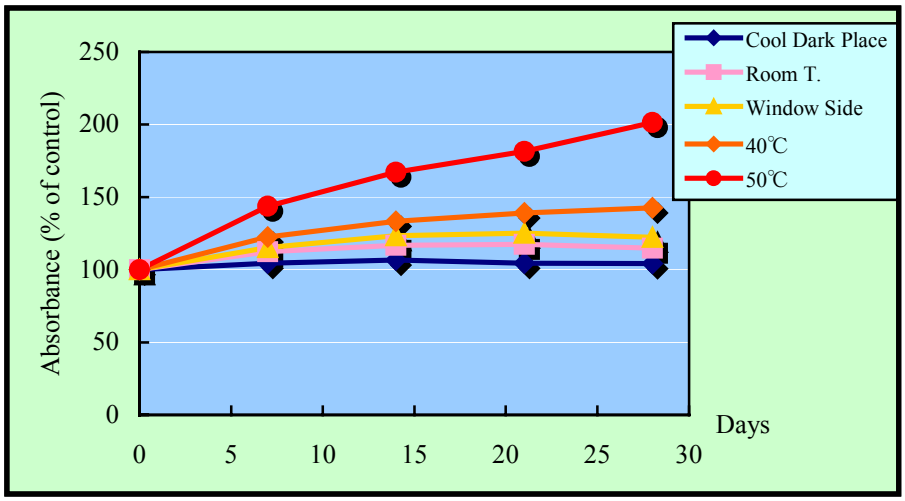

Fig.11 Long term Stability on LUNAWHITE B

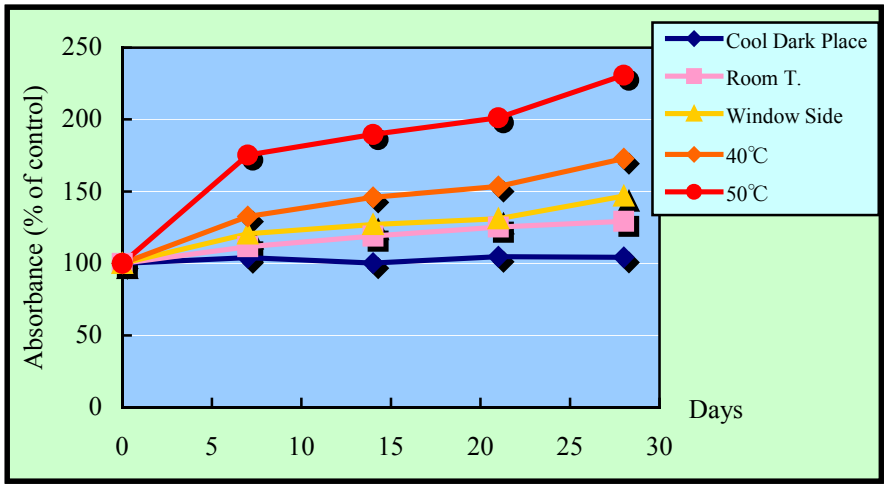

Fig.12 Long term Stability on LUNAWHITE E

4-2 pH Stability

Test Method

Adjust pH from 2 to 10 using hydrochloric acid or sodium hydroxide, color, precipitate and odor was determined.

Result

Color of LUNAWHITE B and E was a little bit changed on more than pH 7, but we observed each is stable in acid ranges. Odor of each was not changed on all of pH range. As a result, LUNAWHITE is stable from pH 2 to 7.

pH	2	3	4	5	6	7	8	9	10
LUNAWHITE B	○	○	○	○	○	○	○	○	○
LUNAWHITE E	○	○	○	○	○	○	○	○	○

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

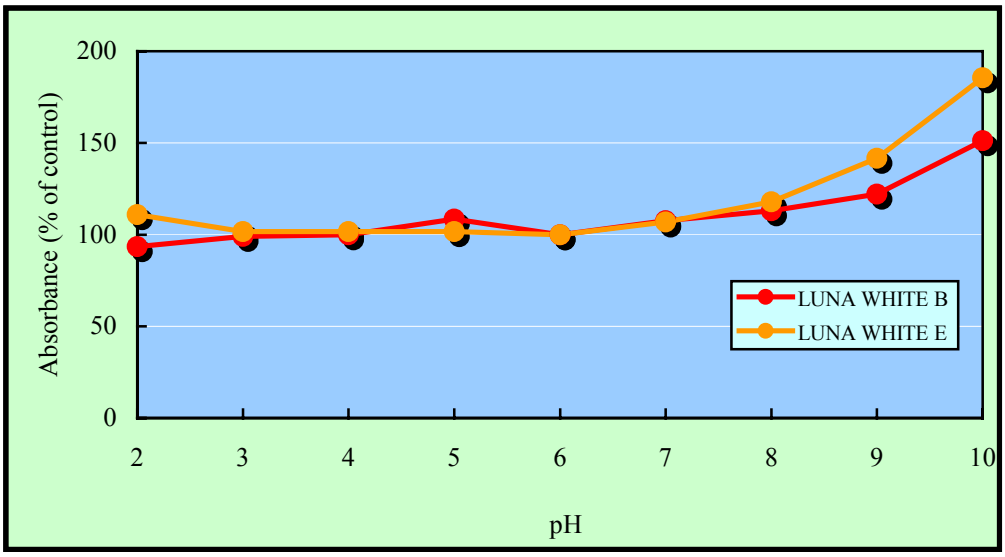

Fig.13 pH Stability on LUNAWHITE B and E

4-3 Thermal Stability

Test Method

Store 100mL of LUNAWHITE B and E for 8 hours in water bath of 85℃. Color and odor of each were determined.

Result

Color was a little bit changed by heating. But, odor and precipitate of each was not observed.

Heating time (85℃)	0	1	2	3	4	5	6	7	8
LUNAWHITE B	○	○	○	○	○	○	○	○	○
LUNAWHITE E	○	○	○	○	○	○	○	○	○

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

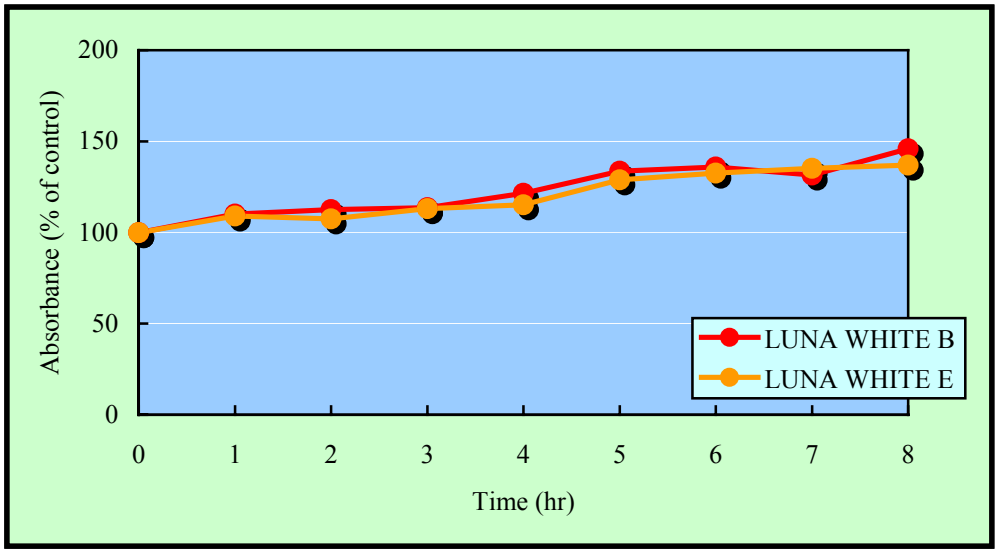

Fig.14 Thermal Stability on LUNAWHITE B and E

5. Compatibility

5-1 Compatibility of LUNAWHITE with Surfactant

Surfactants		B	E
Cation	10% Stearyl Trimethyl Ammonium Chloride	○	○
NONION	1% Polyethylene (50) Oleyl Ether	○	○
	1% Polyethyelene (5) Coconut Fatty acid Monoethanolamide	△	△
	1% Polyoxyethylene Sorbitan Monooleate (20 E.O.)	△	△
	1% Coconut Fatty Acid Diethanolamide	○	△
ANION	25% Triethanolamine Lauryl Sulfate	×	×
	25% Sodium Polyoxyethylene (4) Lauryl Ether Sulfate	×	△
	25% Triethanolamine Polyoxyethylene (4) Lauryl Ether Sulfate	○	○
	25% Sodium Polyoxyethylene (10) Lauryl Ether Phosphate	△	△
AMPHOLYTIC	10% Lauryl Dimethylaminoacetic acid Betaine	×	×
	10% 2-Alkyl-N-carboxymethyl-N-hydroxyethyl Imidazolium Betain	○	○
	10% 2- Coconut Alkyl-N-carboxyethyl-N-hydroxyethyl Imidazolium Betain	○	○

○: Good, △: Slight Turbidity ×: Precipitate

5-2 Compatibility of LUNAWHITE with high Molecular Compound and Solvent

High Molecular Composition	B	E
0.5% Carboxymethyl cellulose	△	○
1% Polyvinyl alcohol (aq.)	○	○
1% Cationic cellulose	△	△
1% Polyethylene glycol (400)	△	△
1% Polyethylene glycol (1500)	△	△
1% Polyethylene glycol (6000)	△	△
0.1% Carboxyvinyl polymer	○	○
0.5% Arginine	×	×
0.5% Xanthan Gum	○	○
50% Glycerin	○	○
50% 1,3-Butylene glycol	○	○
50% Propylene glycol	○	○
50% Ethanol	○	○
90% Purified water	○	○

○: Good, △: Slight Turbidity ×: Precipitate

5-3 Compatibility of LUNAWHITE with Our Cosmetic Ingredients

Cosmetic Ingredients	B	E
10% RYOKUCHA Liquid (Camellia Sinensis Leaf Extract)	○	×
10% CHITIN Liquid (Carboxymethyl Chitin)	○	○
10% Phyto COLLAGE (Natto Gum)	○	○
10% SILKGEN G Soluble (Hydrolyzed Silk)	○	○
10% LACTOSSACARIDE B (Yogurt Filtrate)	×	○
10% Biocellact ALOE VERA B (Aloe Barbadensis Leaf Extract)	×	×
10% Flavosterone SB (Soybean (Glycine Soja) Protein)	○	○
10% AQUACRUSTAR (Hydroxyethyl Chitosan)	△	○
10% LACTOFERRIN-S (Lactoferrin)	×	×
10% Bio antiage B (Mixed Plant Extract)	○	○
10% FM Extract LA-B (Lactobacillus / Whey Ferment)	○	○
10% ALPROTECTOR (Mixed Plant Extract)	△	△
10% MARINWORT IPC-14 SBW (Algae Extract)	○	○
10% TREHALOSE 30 (Trehalose Water Solution)	○	○
10% BOTANPI Liquid E (Paeonia Suffruticosa Root Extract)	○	○

○: Good, △: Slight Turbidity ×: Precipitate

6. Toxicological Safety Data

Test Commodity	Test Results *	Animal	Dose
Acute Oral Toxicity Test	LD_{50}: Not less than 10mL/kg	Mouse	5
Primary Skin Irritation Test	Not observe any irritancy response	Guinea-pig	3
Accumulated Skin Irritation Test	Not observe any irritancy response	Guinea-pig	22
Skin Sensitization Test	Not observe any sensitization response	Guinea-pig	5
Photo-Toxicity Test	Not observe any irritancy response	Guinea-pig	3
Primary Eye Irritation Test	Scarcely observe any irritancy response	Rabbit	3
Photosensitization Test	Not observe any sensitization response	Guinea-pig	10

* This safety data was observed on 1% LUNAWHITE-B.

7. Product Specification

	LUNAWHITE-B	LUNAWHITE-E	LUNAWHITE-P
Appearance			
Color	Reddish brown liquid		Reddish brown powder
Order	Slight specific order		
Identification			
Tannin	Positive		
PH (1→10)	4.0 – 7.0		----
Purity			
Heavy Metals	20 ppm max.		
Arsenic	2 ppm max.		
Residue on Evaporation	0.5 – 1.5 w/v%		----
Loss on Drying	----		10 % max.
Residue on Ignition	----		10 % max.
Assay as Polyphenol	0.3 – 0.7 w/v%		40 – 80 %