



ORYZA OIL & FAT CHEMICAL CO., LTD.



Bio-defense Promotor
cosmeHerbest™ NADESHIKO
Dianthus Longicalyx Seed Extract



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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

Our skin is constantly exposed to stress. Stress is roughly classified into external stress and internal stress. Examples of external stress are UV-rays, dryness, polluted air, smoke, and irritation from metals and friction. Examples of internal stress are fatigue, irregular lifestyle such as unbalanced diet and lack of sleep, and mental stress caused by human relationships or other reasons. Since skin is constantly influenced by these external and internal stresses, it often looks dull or makeup does not go on smoothly because of them.

These stress factors are complexly combined and affect the skin. The factor most noted as a stressor to the skin is its relationship with reactive oxygen that is a cause of oxidative stress. Not all reactive oxygen is bad for us. It is necessary to maintain our body's immune power because it kills bacteria and viruses. However, when we are exposed to UV-rays, the amount of reactive oxygen increases in the body. This changes the amount of melanin production and timing and influences the synthesis of collagen that keeps skin elastic, inducing problems such as rough skin and inflammation. To protect the body from oxidative stress, people have often used a method to directly apply an antioxidant that can directly react with reactive oxygen species and eliminate them such as vitamin C, vitamin D, and polyphenols¹). We started a project to develop a phytochemical that can increase the expression of antioxidant enzyme (substance) in the body.

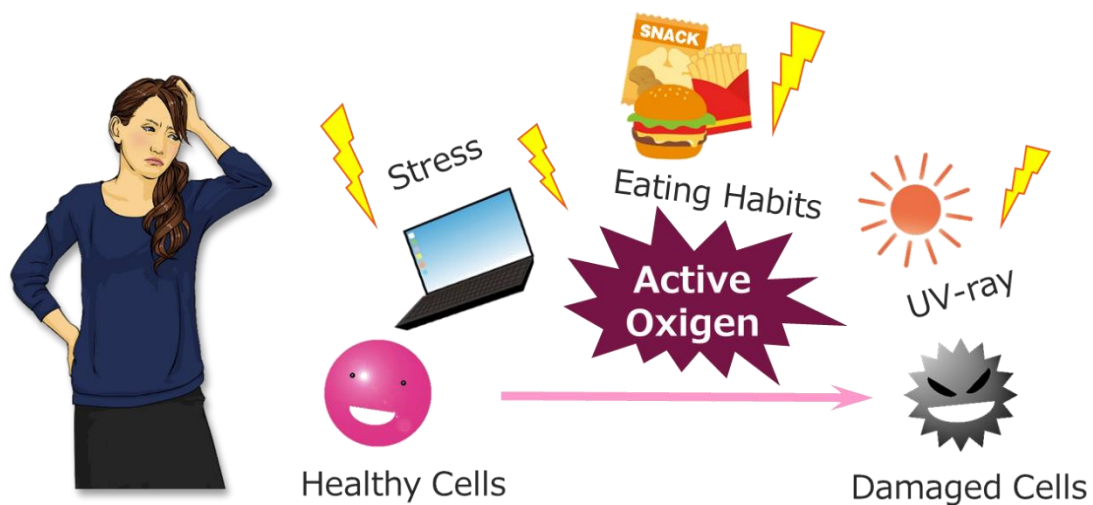


Fig. 1: Various Daily Stress

2. Redox (Reduction-Oxidation) Reaction in the Body

Reduction-oxidation is called “redox” in short. Aerobic microorganisms produce energy by energy metabolism using reduction-oxidation reaction in the body. Although reactive oxygen species are generated as a by-product of energy metabolism, living bodies have an anti-oxidative defense mechanism to combat them. Reactive oxygen species are generated by various factors. Physiological factors include breathing, white blood cells’ activities to process of foreign matter and bacteria, and metabolic process of drugs. Pathological factors include ischemia reperfusion, excessive exercise, mental/physical stress, infection, inflammation. External factors include smoking, UV-rays, radiation, polluted air, and heavy metals. It has been reported that reactive oxygen species that cannot be eliminated by the body’s anti-oxidative defense mechanism cause oxidative modification of biogenic substances such as proteins, lipids, carbohydrates, and nucleic acids. According to the reports, they also lower physiology, develop and accelerate disorders, and induce aging. Reactive oxygen species (ROS) is a collective term for highly-reactive oxygen species. Examples include superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\bullet OH$), and singlet oxygen (1O_2) (Fig. 2) and they act as an oxidant in the body. Superoxide and hydroxyl radical are also called “free radical” because they have an unpaired electron.

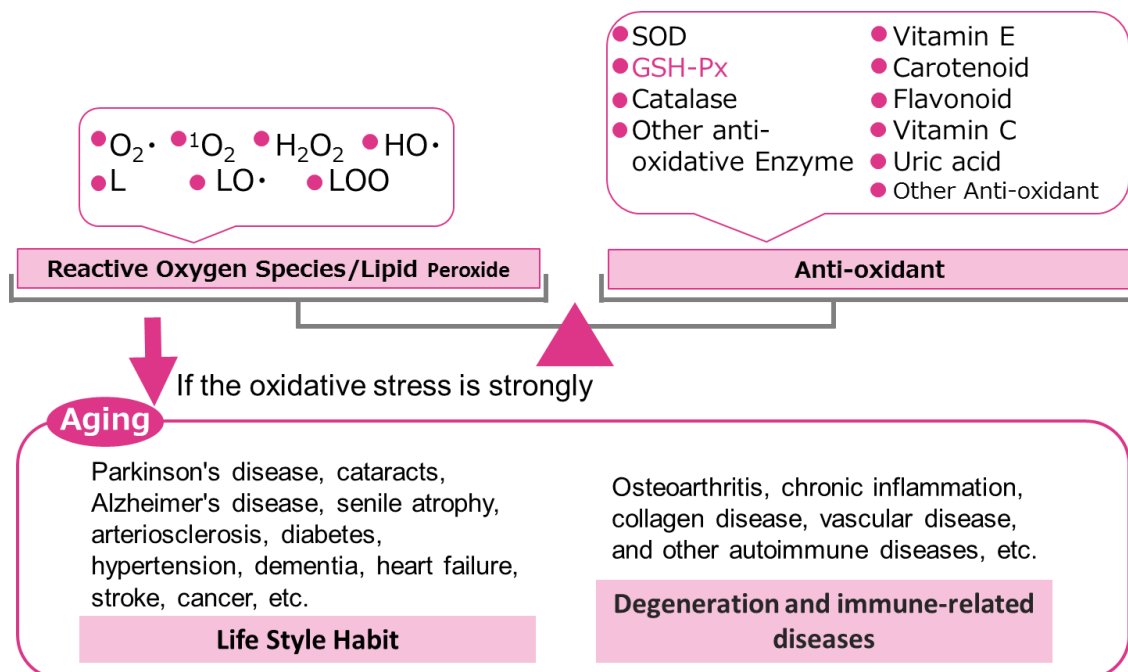


Fig. 2: Reactive Oxygen Species and Antioxidant

3. Antioxidant-Related Substances and Repair / Regenerants

Lifestyle diseases, cancers, and aging are considered as the end products of biological oxidative stress process. The body has a number of antioxidants such as prevention type antioxidants that suppress the generation of reactive oxidant and free radicals, antioxidants with a radical scavenging type mechanism of action, and substances with a repair and regeneration type mechanism of action. Combination of these substances creates a stronger self-defense mechanism.

GST: Glutathione-S-transferase

GSH: Glutathione, NAC: N-Acetyl Cysteine

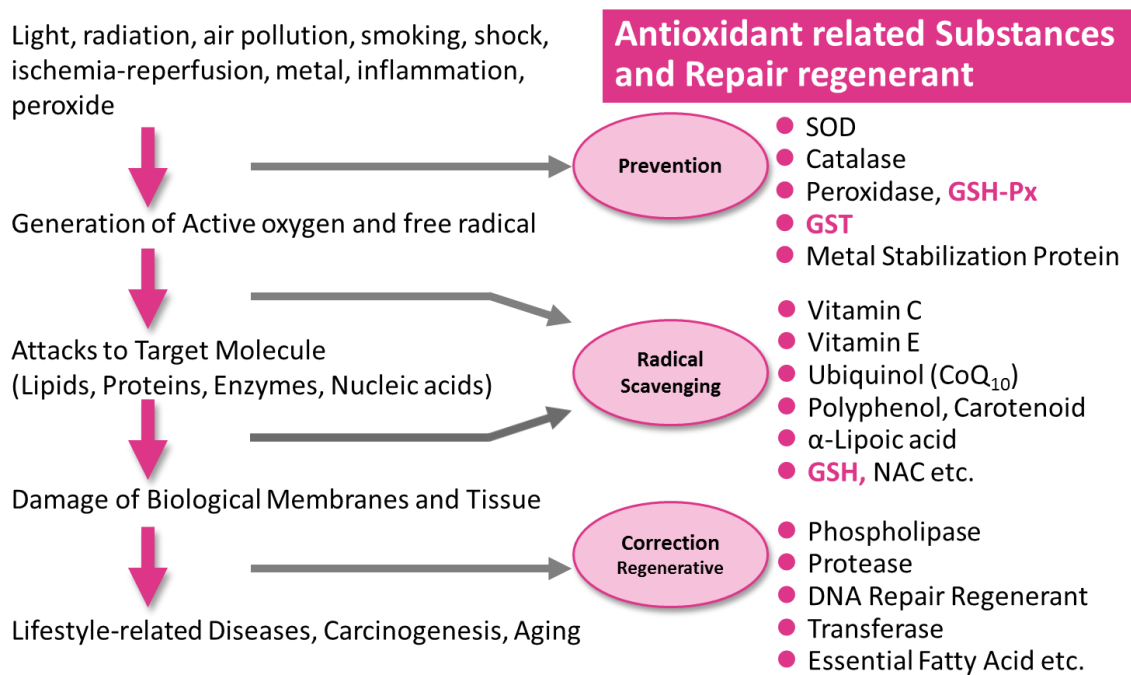
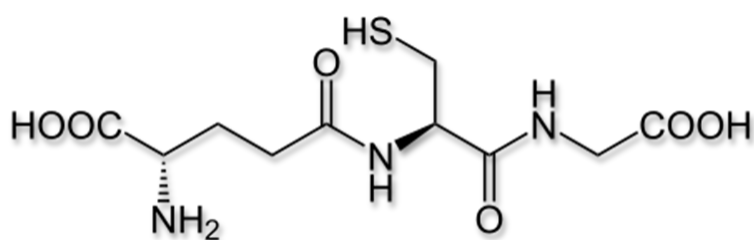


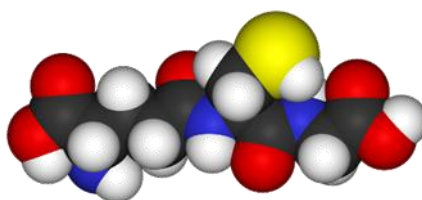
Fig. 3: Various Antioxidant (Repair Regenerant)

4. Glutathione

Glutathione is a tripeptide consisting of three types of amino acids: glutamic acid, cysteine, and glycine and it exists in the body as an antioxidant (Fig. 4). As an antioxidant, glutathione protects cells from oxidative stress caused by reactive oxygen produced by UV-rays in the body. It is an official drug listed in the Japanese pharmacopoeia and Japanese Standards of Quasi-drug Ingredients. Although glutathione is a water-soluble substance that demonstrates a higher anti-oxidative action than polyphenols, its amount reduces as you age according to a report^{2),3)} (Fig. 5). We studied various types of plant extracts' action to induce Nrf2 expression and searched for plant extracts that can increase the expression level of glutathione. Glutathione reduces reactive oxygen in the body and suppresses oxidative reaction of tyrosinase in melanocytes. It has also been confirmed that the synthesis of pheomelanin is induced under the existence of glutathione (Fig. 6). Glutathione is expected to assist the body's defensive response against oxidation, prevent sagging of the skin, and recover the skin's original functions instead of supplementing antioxidants externally.



Reduced Glutathione(GSH)



- IUPAC Name : (2S)-2-amino-5-[[[(2R)-1-(carboxymethylamino)-1-oxo-3-sulfanylpropane-2-yl]amino]-5oxopentanoic acid
- Synonym : γ -L-Glutamyl-L-Cysteinylglycine
- Chemical structure : $C_{10}H_{17}N_3O_6S$
- Molecular weight : 307.33
- Melting point : 195 °C, 468 K, 383 °F

Fig.4: Structure of Glutathione

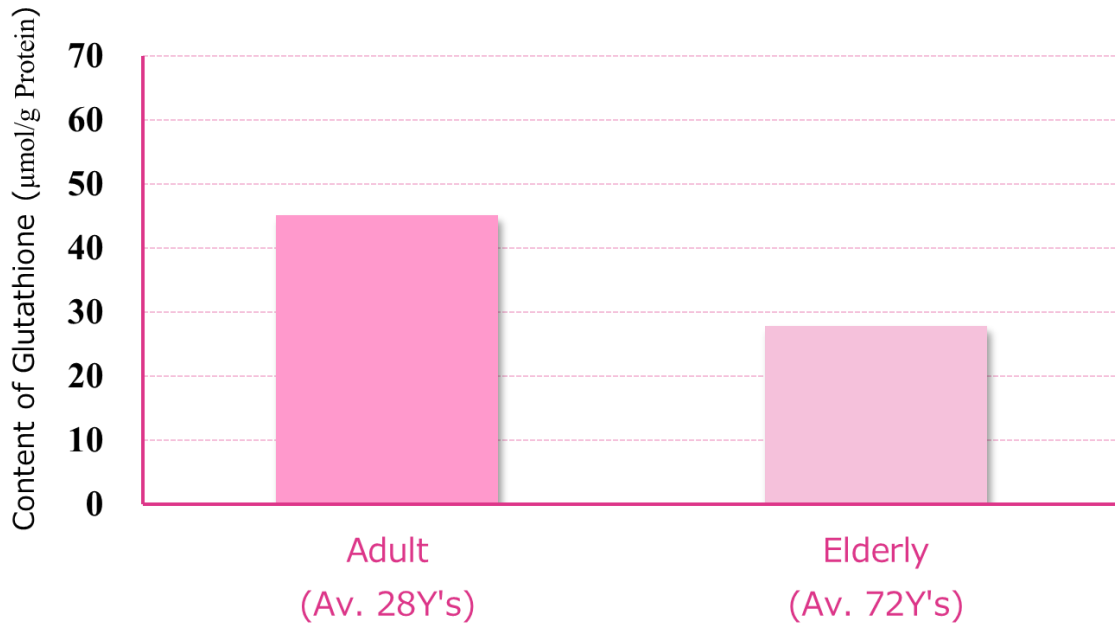


Fig.5: Aging and Change of Glutathione amount in the body

Source : Hernanz.A et al. Life Sci 67 (11), 1317-1324 (2000)

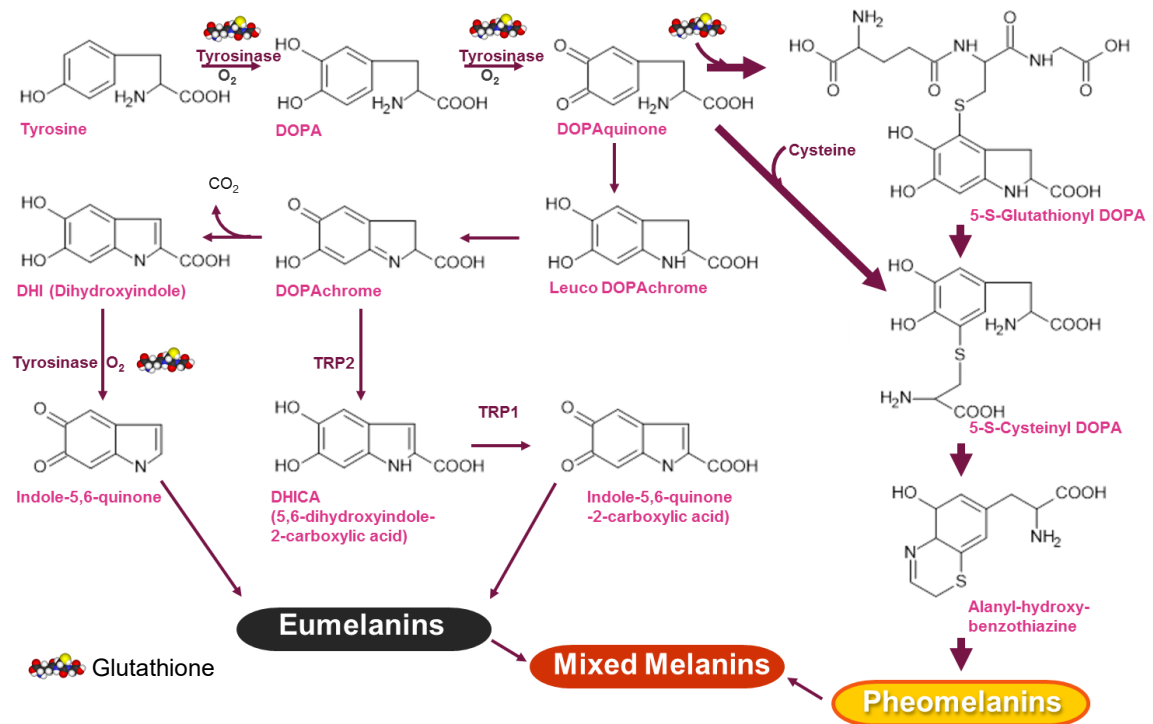


Fig.6: Melanin Synthetic Pathway and Glutathione

5. Action to Activate Transcription Factor Nrf2 Expression (= Action to Promote Glutathione Production)

NF-E2-related factor 2 (Nrf2) is a transcription regulator with a basic leucine zipper structure. It has the effect of enhancing the transcription of target genes through the activation of various reactive molecule species such as electrophiles, reactive oxygen, and active nitrogen species that attack biological polymers such as DNA and protein by oxidation.

The Nrf2-ARE pathway is known as a biological antioxidant system, which induces the expression of antioxidant enzymes (substances). Although Nrf2, a transcription factor, is normally bound with Keap1 and stored in cytoplasm, it is transferred into the nuclei of cells when the living body is exposed to oxidative stress. Then, by bonding with the ARE sequence that exists upstream of genes, Nrf2 uniformly regulates the expression of oxidative stress-responsive gene clusters such as glutamylcysteine synthetase, which synthesizes depleted glutathione through detoxification, including glutathione s-transferase (GST) that directly detoxifies electrophilic substances, heme oxygenase (Ho-1) and quinone oxidoreductase (NQO1); and glucose-6-phosphate dehydrogenase that synthesizes NADPH which has a major intracellular reduction ability (Fig. 7).

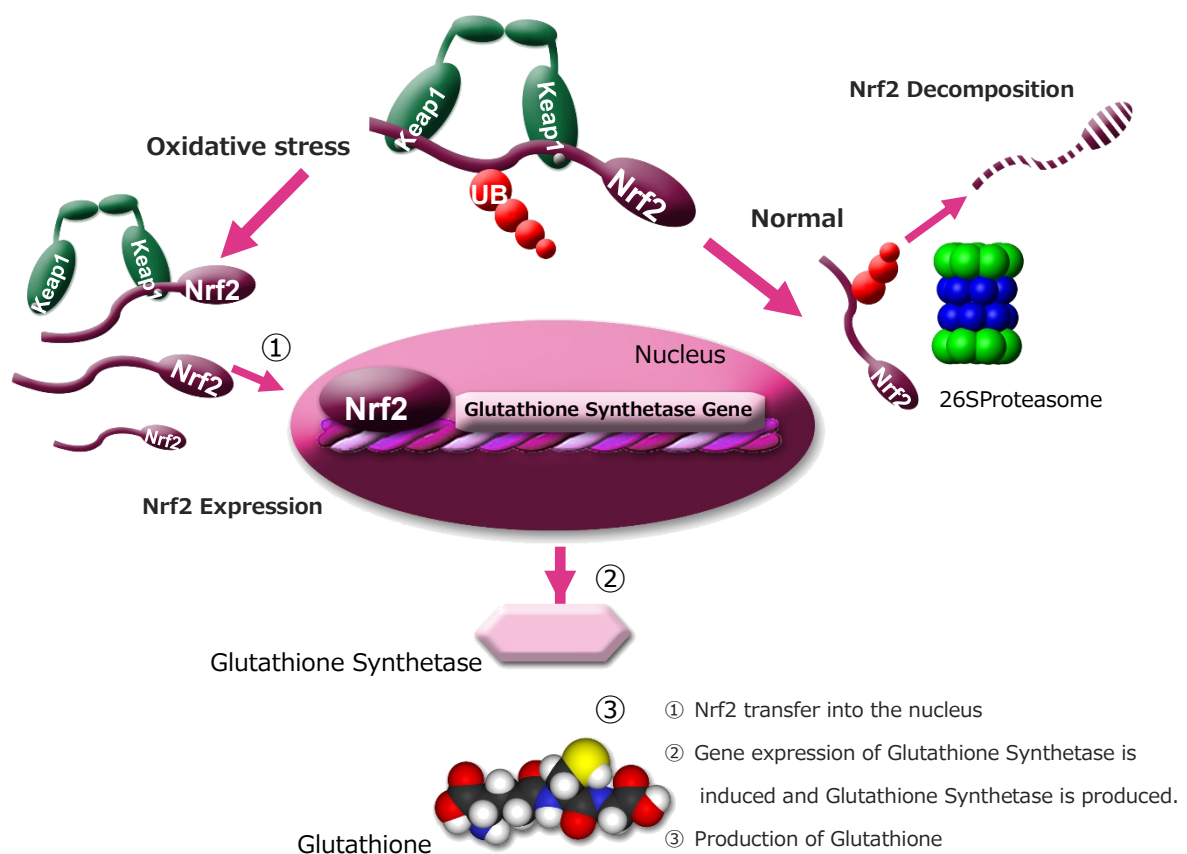


Fig.7: Glutathione Production Pathway due to Activation of Nrf2 Expression

6. Activate Expression of Nrf2 due to Plant Extracts

A joint research was undertaken on the expression of Ho-1 (Heme Oxygenase-1) caused by Nrf2 activation by various plant extracts at the Efficacy Analysis Laboratory, Gifu Pharmaceutical University.

Expression level of Ho-1 was examined using our existing products such as Coffee Bean Extract that is rich in chlorogenic acid, Purple Rice Extract that contains anthocyanidins, and Walnut Shellcoat Extract that is rich in polyphenols. As a result, an increased expression level of Ho-1 was observed with dianthus seed extract. Based on this result, we undertook various types of effectiveness evaluations on dianthus seed extract (Fig. 8).

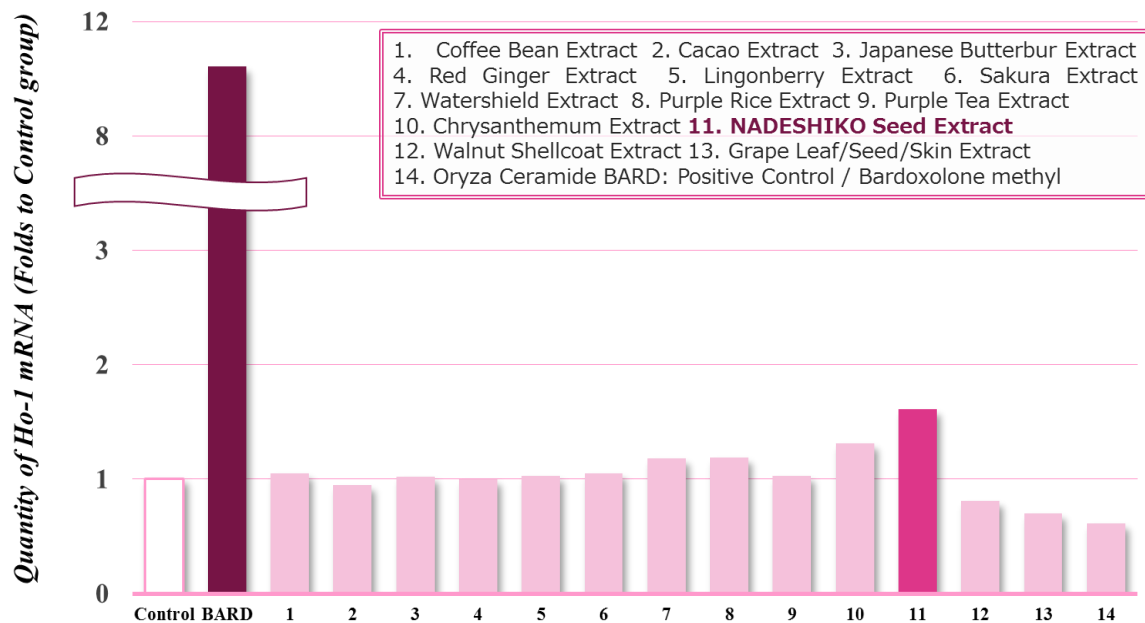


Fig.8: Ho-1 Expression of Various Plant Extracts

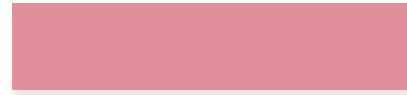
7. Nadeshiko (Dianthus)

The ingredient plant, dianthus, is a perennial plant and has the binomial name *Dianthus superbis* L. var. *longicalycinus* (*Caryophyllaceae*), which is commonly known as fringed pink or large pink. In ancient times, the plant was called Tokonatsu in Japan. The plant is widely distributed in the western part of Honshu (the main island) and the Shikoku and Kyushu regions, and is also sparsely found in parts of the Okinawa Islands (Kume Island and Tonaki Island). In



several areas, the plant is designated as an endangered species by local governments, and areas where it grows are decreasing annually. Outside Japan, the plant is found in Korea, China, and Taiwan. The height of the plant is several tens of centimeters, its leaves are linear shaped, and the plant produces pink flowers with five deeply cut fringed petals from summer to fall (August to September). Its seeds are small, black, and flat.

It is said the English word “pink” originally meant dianthus, and the word was not yet used to indicate the color during Shakespeare’s age. Later, the word came to indicate the color of dianthus flowers, which is pink. The color of “Nadeshiko” (dianthus) exists even in the Japanese Industrial Standards (JIS), which is described as C:0 M:48 Y:16 K:4, R:225 G:143 B:155 [#E18F9B].



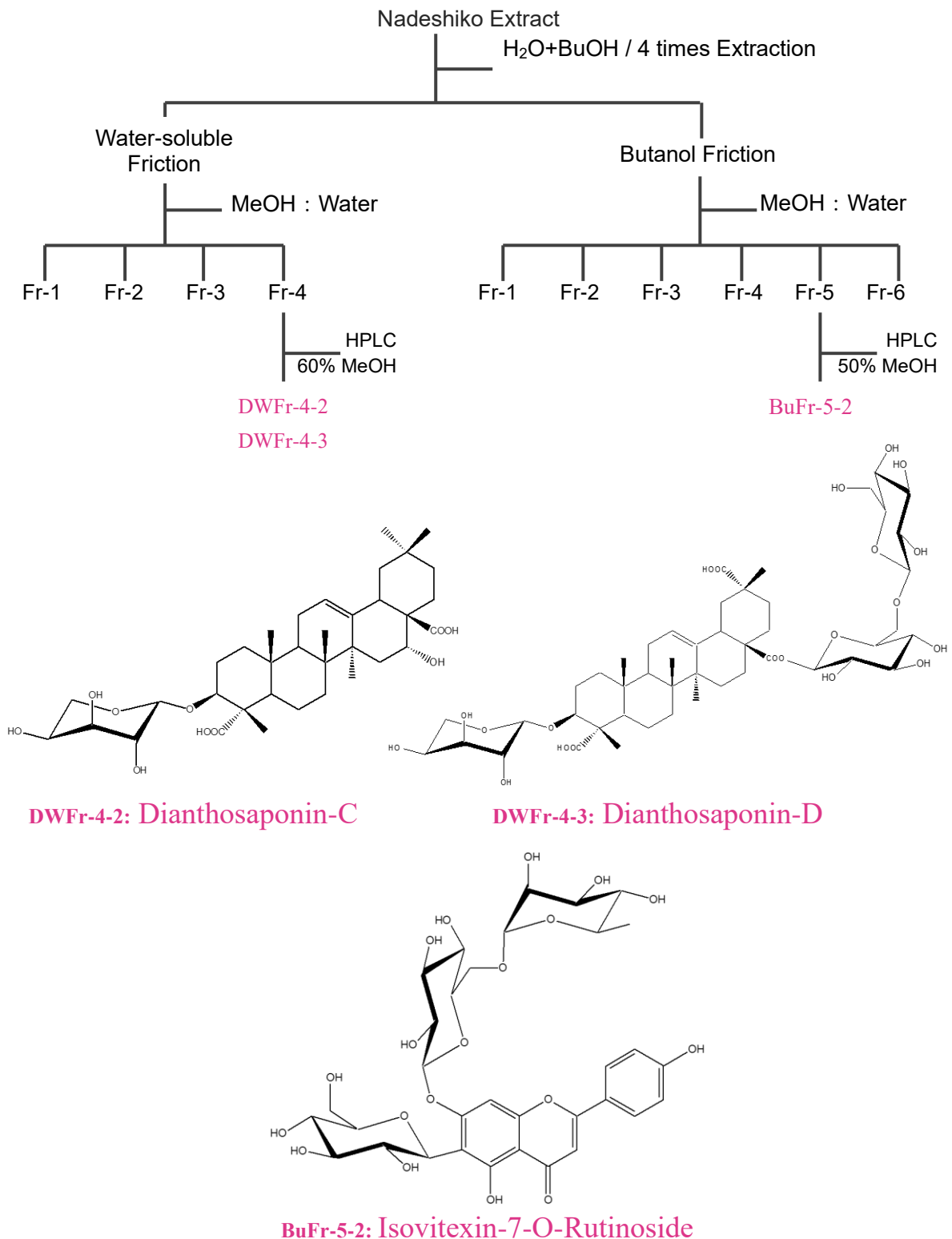
“Nadeshiko” color
(R : 225, G : 144, B : 155)

In herbal medicine, dianthus, as a whole plant during its blooming stage, is called Kubaku, and its seeds are called Kubakushi, and they are used for anti-inflammation and diuretic purposes as well as emmenagogues. Kubaku (the whole plant) is regulated as a pharmaceutical product by Japanese law, and cannot be used for cosmetic purposes. However, Kubakushi (the seed of fringed pink) is not regarded as a pharmaceutical product.

While dianthus is commonly known as a symbol of the beauty and modesty of Japanese women, it is also familiarly known as the nickname of the women’s national soccer team. The pink color of dianthus has been adopted as one of the team’s colors and is used as an accent color on its uniforms.

8. Components of cosmeHerbest™ NADESHIKO

The main component of cosmeHerbest™ NADESHIKO is saponin which has a high foamability and cleansing ability. As a result of analysis of components of the product, the following components were contained in high concentrations.



9. Effectiveness Evaluation

Dianthus seed contains saponin called “dianthosaponin.” Therefore, cosmeHerbest™ NADESHIKO is expected to have a cleansing action as well as an action to assist foaming due to the physical properties of saponin in addition to its chemical properties to produce glutathione by activating Nrf2 and the action demonstrated in the melanin formation suppression test on human melanocytes. This means that the product is expected to have an action to reduce visible pores. In order to confirm the actions above, we evaluated the cosmetic functionality of cosmeHerbest™ NADESHIKO.

9-1 Chemical Property

9-1-1 Nrf2 and GCLM Activating Action

Test Sample

cosmeHerbest™ NADESHIKO was subjected to the test, after adjusting to be in the 0.01 to 0.1% plant extracts as a final concentration, it was subjected to the test.

Test Method

Human epidermal keratinocytes were cultivated in a 24 well plate, cosmeHerbest™ NADESHIKO containing dianthus seed extract was added so that the concentration becomes 0.01 to 0.10%, and the samples were cultivated for 24 hours. Then, cells were collected, RNAs were extracted, and mRNA expression level of Nrf2 and glutamate cysteine ligase modifier (GCLM, glutamate cysteine ligase regulation sub unit) in the obtained RNAs was quantified using quantitative RT-PCR method. In the measurement, GAPDH was used as the endogenous control.

Results and consideration

When cosmeHerbest™ NADESHIKO was added, the expression level of both GCLM and Nrf2 increased as compared to the control (Figs. 9, 10). It is expected that adding cosmeHerbest™ NADESHIKO accelerates the synthesis of glutathione by the increase of GCLM expression (Figs. 9, 10).

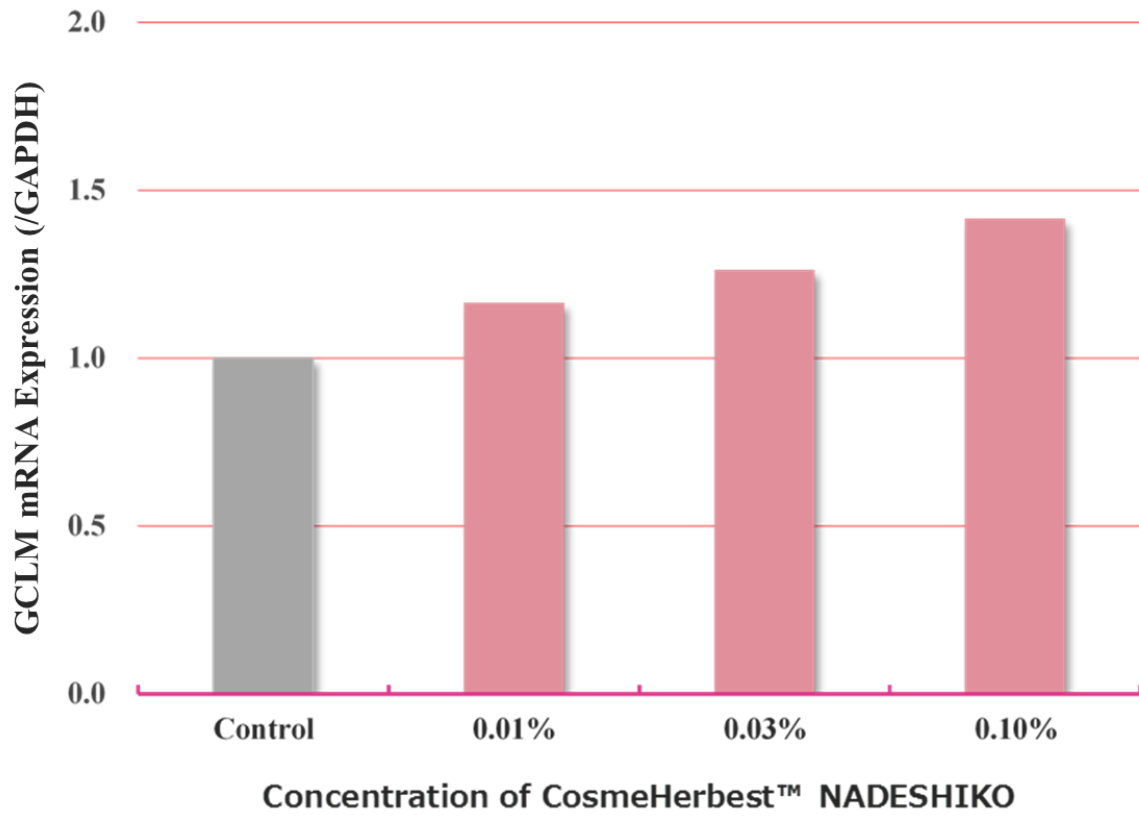


Fig.9: Expression of GCLM

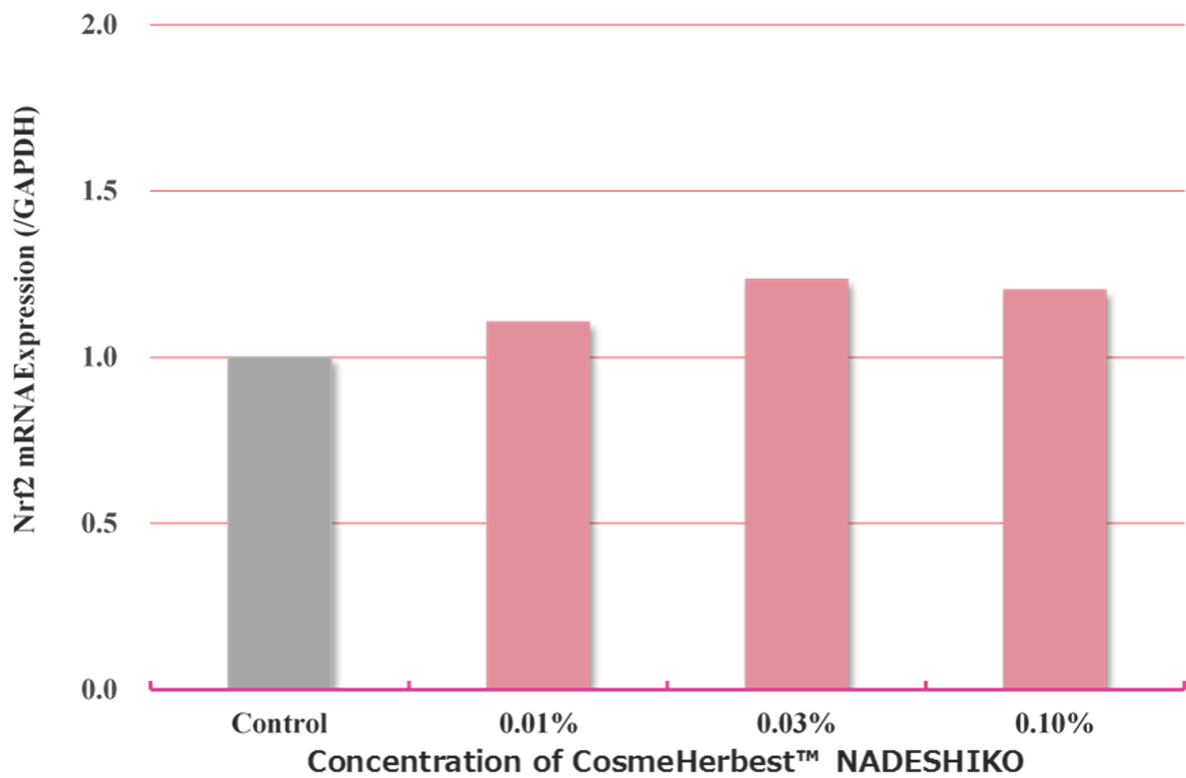


Fig.10: Expression of Nrf2

9-1-2 Inhibitory Effect of Melanin Formation on Human Melanocyte

Test Sample

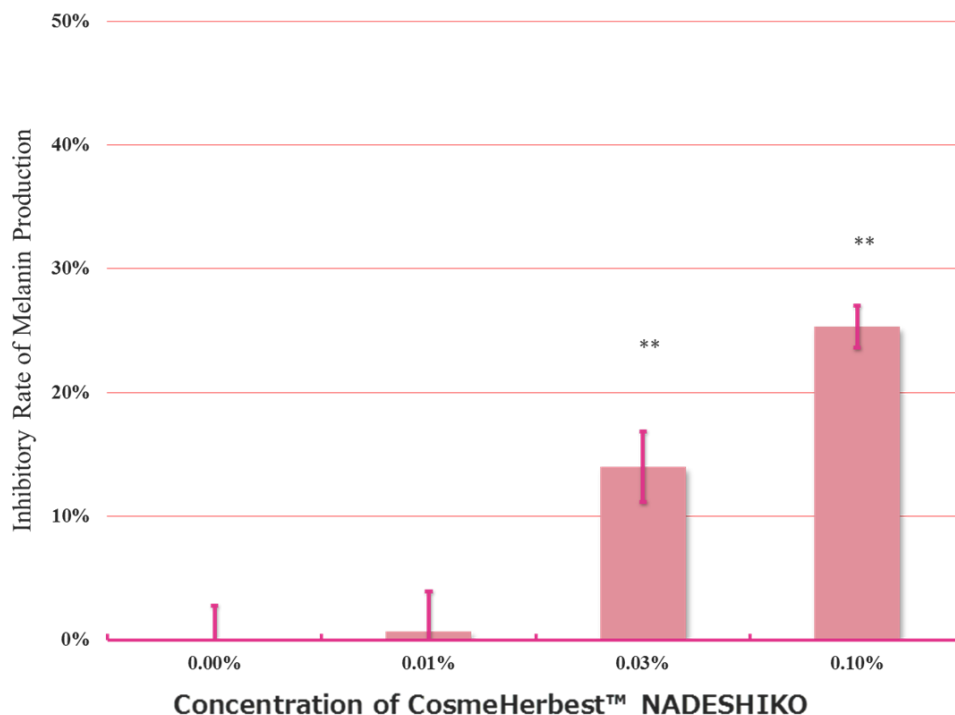
cosmeHerbest™ NADESHIKO was subjected to the test, after adjusting to be in the 0.01 to 0.1% plant extracts as a final concentration, it was subjected to the test.

Test Method

Endothelin-1, a type of melanin formation stimulating factor, was added to human epidermal melanocytes, cells were exposed to external irritation, and they were cultivated. Then, cosmeHerbest™ NADESHIKO was added so that the concentration becomes 0.01 to 0.10 % and its influence to melanin formation was observed. After two weeks of cultivation, the amount of melanin formation was compared against the control group by its absorbance.

Results and consideration

As shown in Fig. 11, melanin formation was significantly inhibited in the groups 0.03 % and 0.10 % of the material was added concentration-dependently (Fig. 11). Since cosmeHerbest™ NADESHIKO has been confirmed to produce glutathione synthetase which is a biological antioxidant, the result above is assumed to be provided by glutathione performing its action to suppress melanin formation in melanocytes.



** p<0.05

Fig.11: Inhibitory effect of Melanin Formation on Human Melanocyte

9-2 Physical Properties

cosmeHerbest™ NADESHIKO contains dianthosaponin-C and dianthosaponin-D that are saponins unique to dianthus. Therefore, it is expected that the material has foaming, foam retention, and cleansing abilities.

9-2-1 Foaming / Foam Durability Test

A foaming and foam retention ability test was conducted by a test by shaking and an improved version of the foaming test by the JIS K3362 Ross-Miles method. A fixed amount of sample solution was dripped from a fixed height and the height of foam was measured over time.

(1) Test by Shaking

Test Sample

cosmeHerbest™ NADESHIKO was prepared so that the final product concentration becomes 3 % by adding purified water, Amino Soap® AR-12 (Ajinomoto Co., Inc.) amino acid soap, and 30 % propanediol solution to be used in the test.

Test Sample	Water	Amino Soap® AR-12	30% Propanediol	cosmeHerbest™ NADESHIKO
①	100.00	—	—	—
②	99.95	0.05	—	—
③	96.95	0.05	3.00	—
④	96.95	0.05	—	3.00

Test Method

Sample solutions ① to ④ were prepared as shown in the table and were put in 50 mL glass bottles by 25 g. They were vigorously shaken up and down 20 times and photos were taken 30 seconds later to observe foam conditions.

Form Condition after 30 seconds by shaking



Results and consideration

Sample ① did not foam and foam of samples ② and ③ disappeared immediately after shaking. Foam of sample ④ containing cosmeHerbest™ NADESHIKO remained seven minutes after shaking. The test result confirmed that cosmeHerbest™ NADESHIKO performs an action to assist foaming of soap and help to retain foam.

(2) Foaming ability test by an improved version of JIS K3362 Ross-Miles method

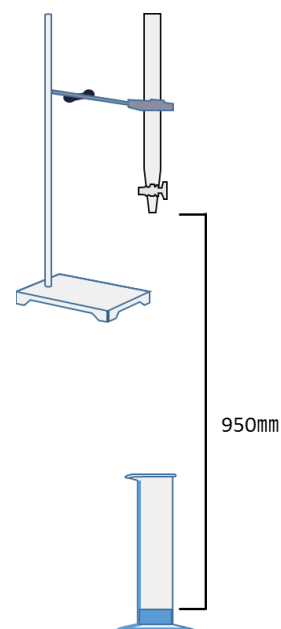
Test Sample

cosmeHerbest™ NADESHIKO was prepared so that the final product concentration becomes 3 % by adding purified water, Amino Soap® AR-12 (Ajinomoto Co., Inc.) amino acid soap, and 30 % propanediol solution to be used in the test.

Test Sample	Water	Amino Soap® AR-12	30% Propanediol	cosmeHerbest™ NADESHIKO
①	99.5	0.5	—	—
②	96.5	0.5	3.0	—
③	96.5	0.5	—	3.0

Test Method

As shown in the figure to the right, 10 mL of test solution was poured into a 38 mm diameter measuring cylinder to receive dropping solution and 40 mL of the same test solution was poured into an upper 24 mm diameter burette with an 8 mm diameter drip opening. The cock of the burette was opened, the test solution was dripped at a rate of 0.8 mL/sec., and the height of foam was measured when the dripped solution contacted the test solution in the lower measuring cylinder. The height of foam was measured immediately after the dripping finished (foaming ability) and also after 5 and 30 minutes after dripping finished (foam retention ability). The average of the highest position and lowest position was used as the foam height.



Test results and consideration

As shown in the graph in Fig. 12, sample ③ containing cosmeHerbest™ NADESHIKO showed the best results both in foam height and foam retention period as compared to samples ① and ②. This suggests that cosmeHerbest™ NADESHIKO has actions to assist and improve soap's foaming ability and foam retention ability.

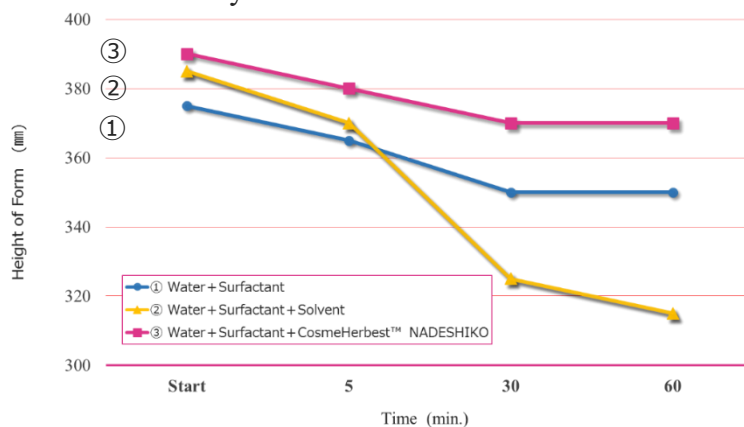


Fig. 12 Foaming ability test by an improved version of Ross-Miles method

9-2-2 Cleansing Assisting Property Test

Activated charcoal was applied to the skin evenly using a puff to mimic dirt. Then, it was cleansed using a solution containing a surfactant to observe how dirt was removed.

Test Sample

cosmeHerbest™ NADESHIKO was prepared so that the final product concentration becomes 3 % by adding purified water, Amino Soap® AR-12 (Ajinomoto Co., Inc.) amino acid soap, and 30 % propanediol solution to be used in the test.

Test Sample	Water	Amino Soap® AR-12	30% Propanediol	cosmeHerbest™ NADESHIKO
①	96.5	0.5	3.0	—
②	96.5	0.5	—	3.0

Test Method

The test areas on the back of the left and right hands were marked as shown in the photos and 0.2 g of activated charcoal (Hokuetsu ZN; Ajinomoto Fine Techno Co., Inc.) was applied evenly on the test areas using a puff. Sample (1) was applied on the right hand and sample (2) on the left hand. Each sample was dripped on the test areas by 5 mL and the areas were massaged in a circular stroke 20 times. Then, 20 mL of purified water was poured in a circular motion and moisture was lightly wiped off using KimWipes® paper. This process was repeated several times under observation using a microscope to test the cleansing effect of cosmeHerbest™ NADESHIKO.



Left : Sample②

Right : Sample①

Test results and consideration

It was observed that sample ② containing cosmeHerbest™ NADESHIKO removed fine grains of skin in a fewer number of times than the control sample ① (Fig. 13). Fine grains that could not be removed by the control were completely removed by cleansing using the solution containing cosmeHerbest™ NADESHIKO. According to the results, cosmeHerbest™ NADESHIKO is expected to improve soap's cleansing ability and also add an action to remove fine grains.

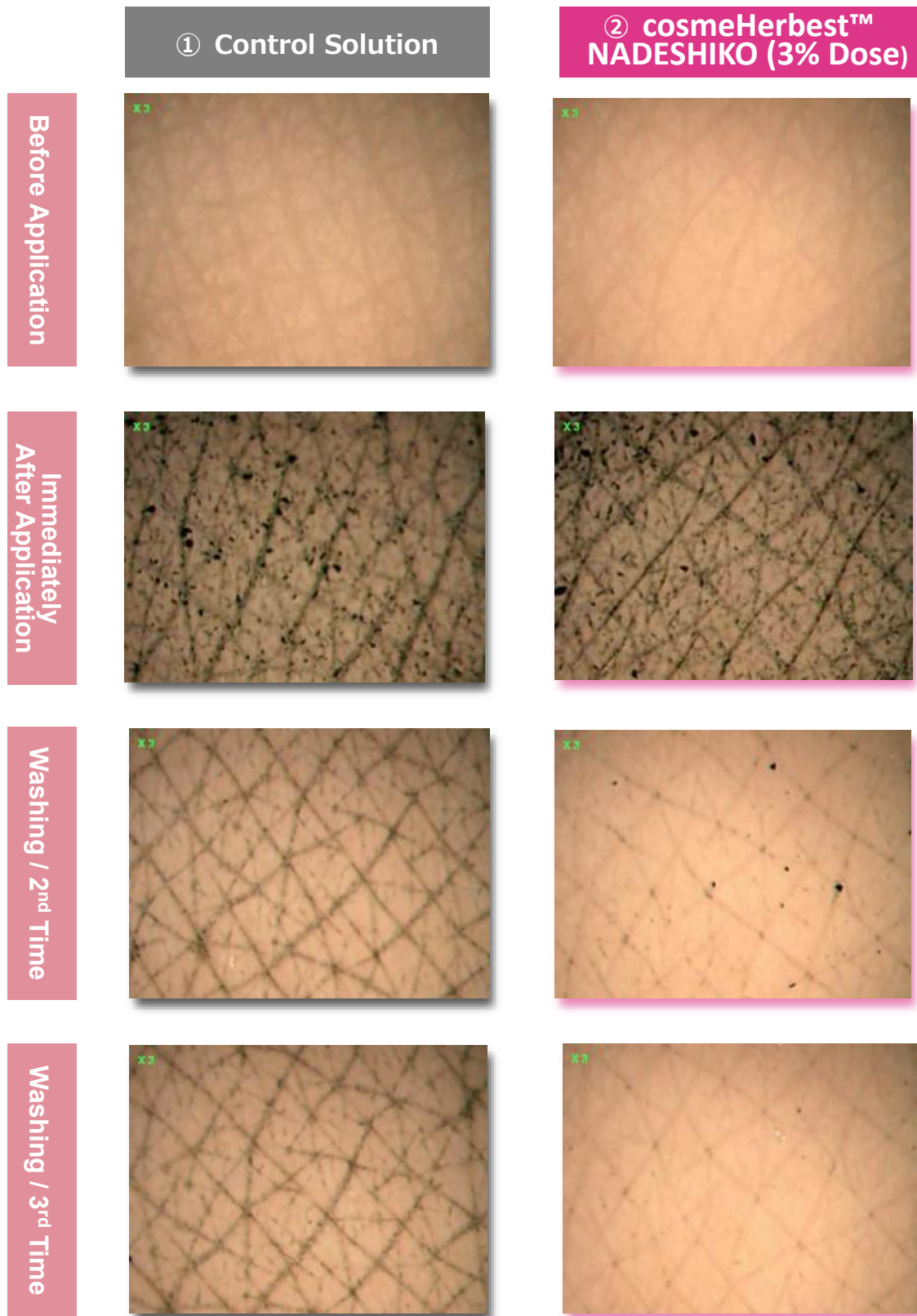


Fig.13: Washing Assisting Action

9-3 Action to Reduce Visible Pores (Human Monitor Test)

9-3-1 Comparison of Total Pore Areas by Race and Age

Visible facial pores are pointed out as a major skin problem by women in a wide range of age groups. It is known that pores stand out because excessive sebum remains in them when you are young. However, pores of people in their forties and fifties stand out because of a decrease of collagen around pores, in other words “sagging.” Sugata et al.⁴) studied visible pores on different races and observed that pores became larger as people age in all races although there was individual variation (Fig. 14). Among the races surveyed, American Caucasians had the largest pores and Thai people the second. Thai people include Chinese descent, Malay, and Thai people. The study confirmed that there was a correlation between pore size and skin tone (Fig. 15).

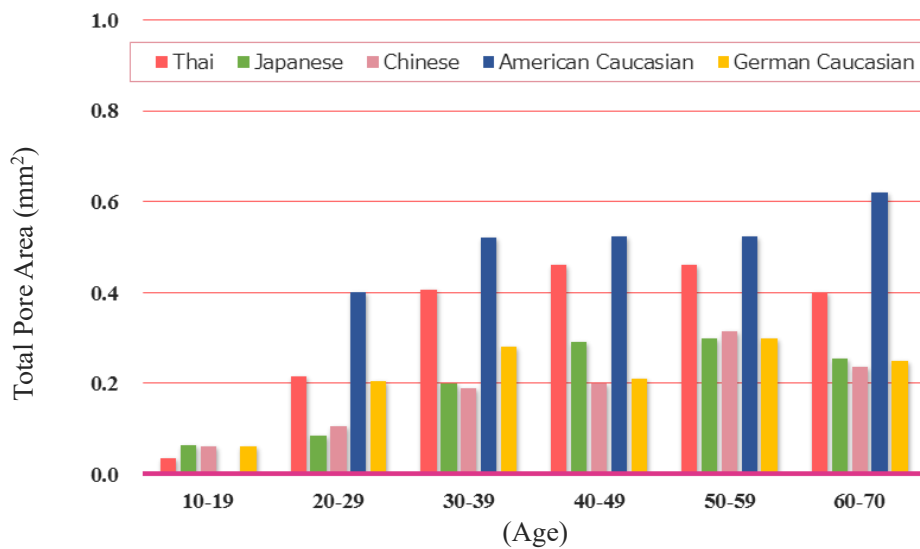


Fig.14: Comparison of Total Pore Area due to Various Race and Age

Source : Sugata et al.: Study of Regional and Ethnic Differences of Facial Pores. IFSCC Congress 2006 Osaka, Japan

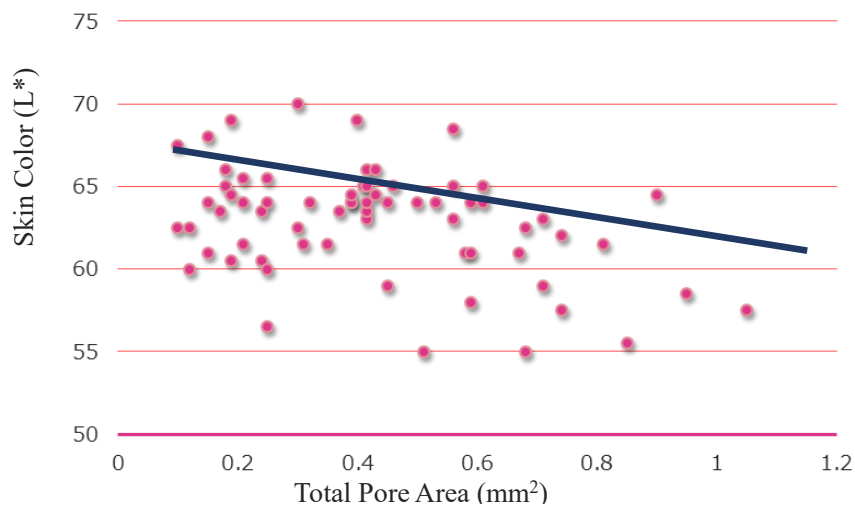


Fig.15: Skin Lightness (L* Value) and Total Pore Area

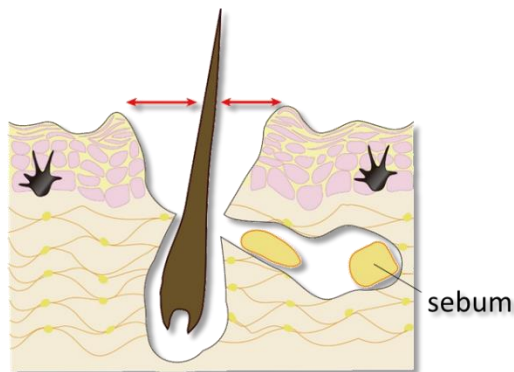
Source : Sugata et al.: Study of Regional and Ethnic Differences of Facial Pores. IFSCC Congress 2006 Osaka, Japan

9-3-2 Human Monitor Test

The in vitro tests described above confirmed that cosmeHerbest™ NADESHIKO has an action to produce glutathione which protects against oxidation in the body. The skin-lightening test confirmed that the product has an action to suppress melanin formation. An action to assist cleansing due to contained “Dianthosaponin” was also confirmed. This section describes effect of a lotion containing cosmeHerbest™ NADESHIKO to reduce visible pores when it is applied on human skin.

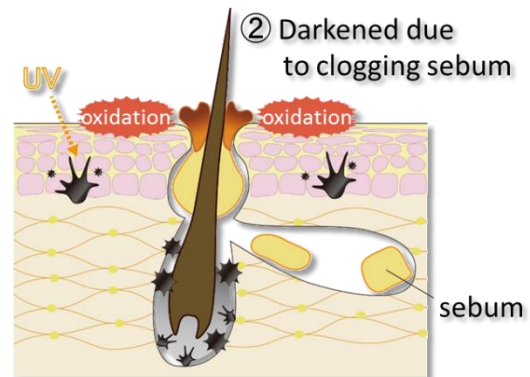
Type of Visible Pores

◆ Open Pores



Sagging skin due to the shortage of Bio-elements (collagen, elastin)
→ Open visible pores

◆ Darkened Pores



① Darkened by the melanin

Test Sample

Mix well 5mL of cosmeHerbest™ NADESHIKO and 95mL of 30% of propanediol solution adjusted in advance, and used this test solution as “NADESHIKO Lotion”.

Test Method

The test was carried on 14 subjects aged 23 to 61 who submitted a written agreement (9 men and 5 women). The subjects applied a lotion containing 1 mL of cosmeHerbest™ NADESHIKO on the entire face in the morning and at night every day. The number of facial pores was measured using Robo Skin Analyzer CS50 (Inforward Inc.) at start and eight weeks later. The following analysis indexes were used.



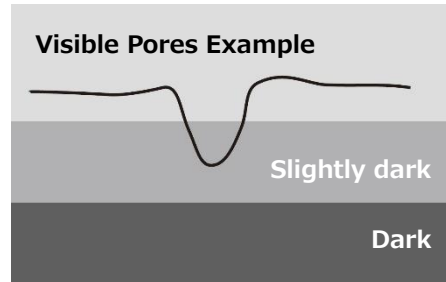
- ① Number of Visible Pores
- ② Number of Opened Visible Pores
- ③ Number of Darkened Visible Pores

Measurement was performed in a room where temperature was regulated to 21±3 °C and humidity 43±3 % after 15 minutes of conditioning. The test was started on March 6, 2015 and was carried out for eight weeks.

① Definition of visible Pores

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.

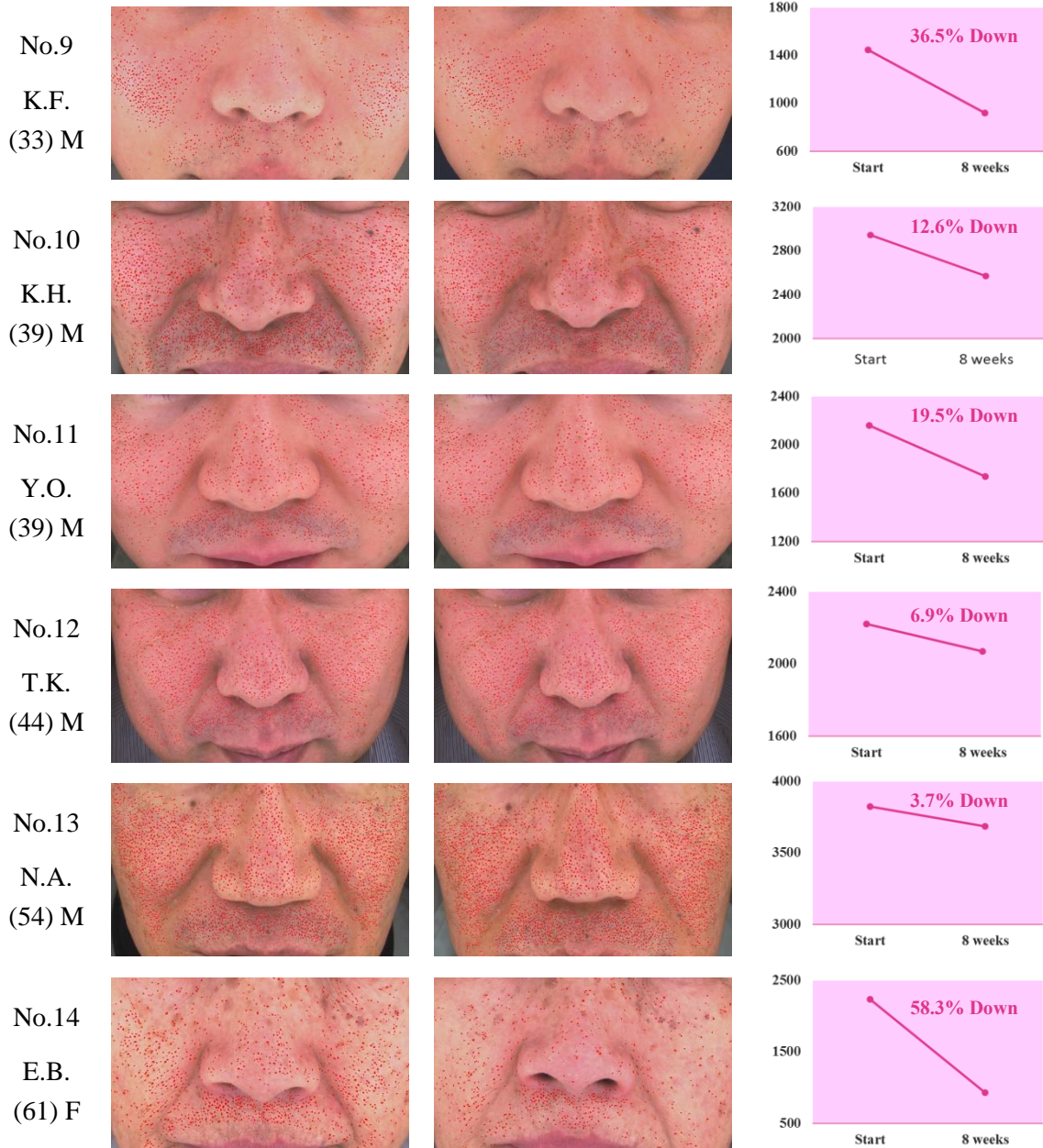
Continuous round areas with a size of 0.1 to 0.6 mm² among subject areas of which margin can be detected as “slightly dark areas” and “dark areas” as compared to surrounding areas in monochrome images are detected as “visible pores.”



Test Result

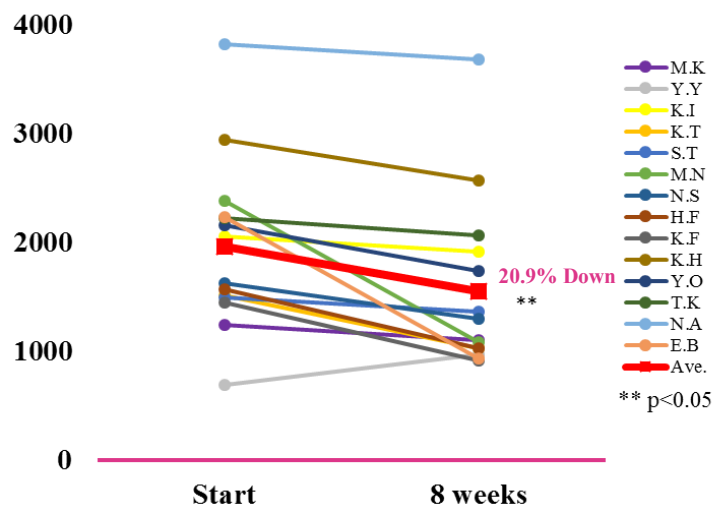
Subject	Age	Sex	Number of Visible Pores		Rate of Change (%)
			Start	8 weeks	Start as 100
M.K.	23	♀	1241	1096	88.32
Y.Y.	23	♀	690	967	140.14
K.I.	25	♂	2058	1916	93.10
K.T.	25	♂	1517	1027	67.70
S.T.	25	♂	1498	1359	90.72
M.N.	26	♀	2387	1084	45.41
N.S.	27	♂	1628	1296	79.61
H.F.	28	♀	1567	1029	65.67
K.F.	33	♂	1445	918	63.53
K.H.	39	♂	2942	2571	87.39
Y.O.	39	♂	2160	1738	80.46
T.K.	44	♂	2222	2068	93.07
N.A.	54	♂	3825	3684	96.31
E.B.	61	♀	2235	932	41.70
Ave.			1958	1549	79.10

	START	After 8 weeks	Change of Visible Pores
No.1 M.K. (23) F			 11.7% Down
No.2 Y.Y. (23) F			 40.19% Up
No.3 K.I. (25) M			 6.9% Down
No.4 K.T. (25) M			 32.3% Down
No.5 S.T. (25) M			 9.3% Down
No.6 M.N. (26) F			 54.6% Down
No.7 N.S. (27) M			 20.4% Down
No.8 H.F. (28) F			 34.3% Down



Consideration

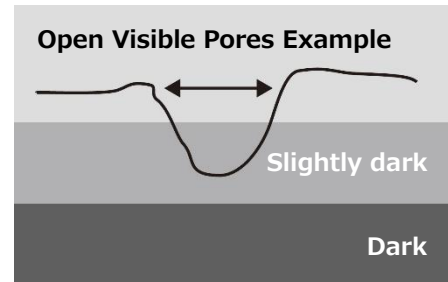
Visible pores of 13 test subjects out of 14 (93 %) reduced. The rate of decrease of visible pores was over 30 %. The test confirmed that cosmeHerbest™ NADEHSIKO is a natural ingredient with an extremely high effect to reduce visible pores.



② Definition of Open Visible Pores

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.

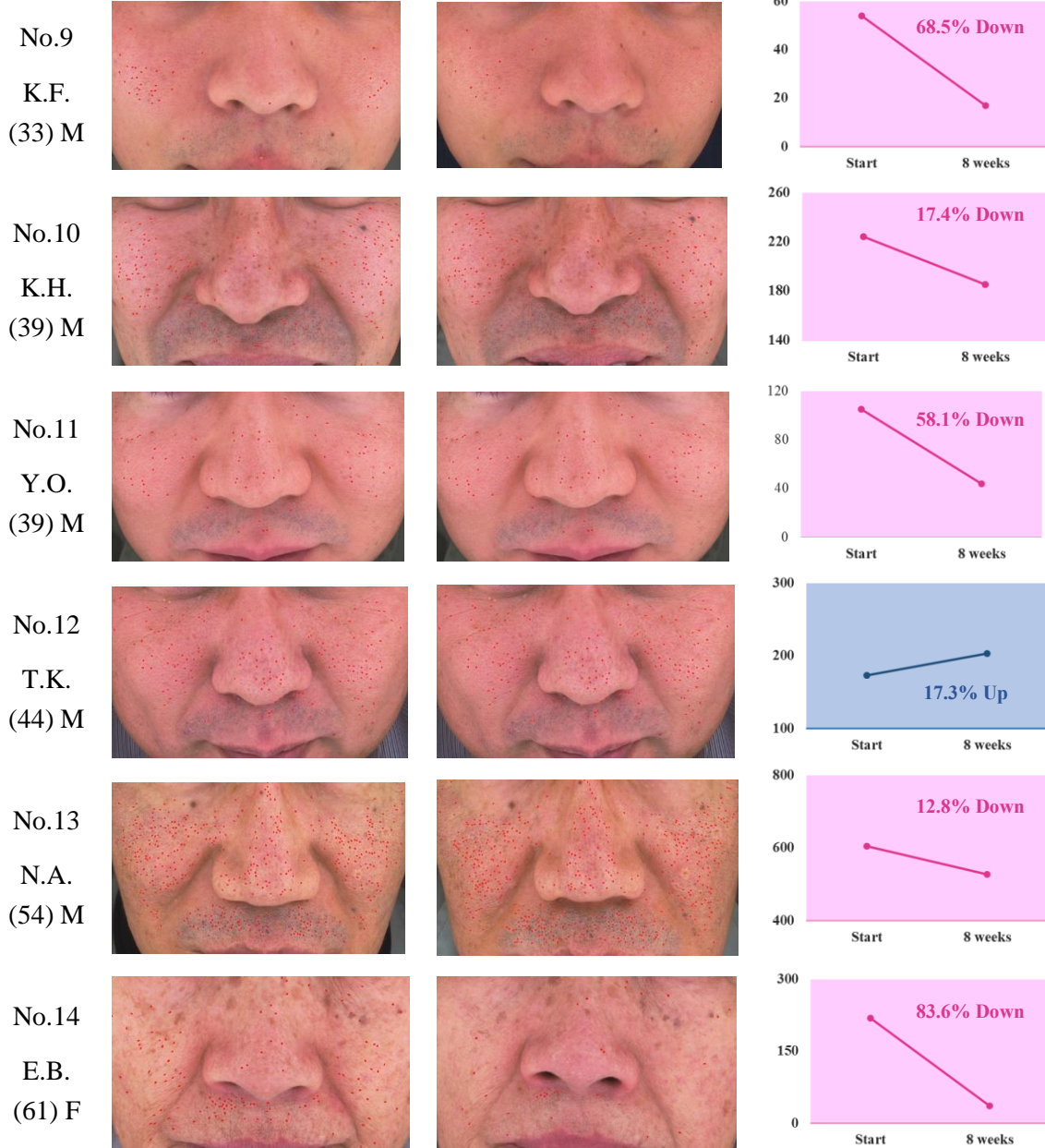
Round areas with a size of 0.3 to 0.6 mm² in the subject areas of which margin can be detected as “slightly dark areas” and “dark areas” as compared to surrounding areas in monochrome images are detected as “open pores” and shown in red points by image analysis.



Test Result

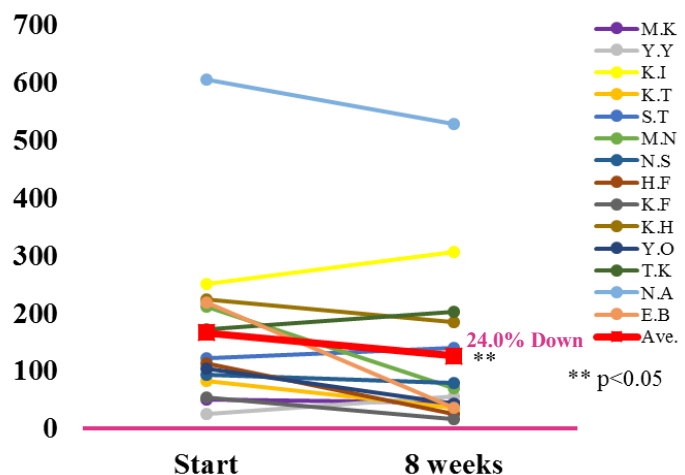
Subject	Age	Sex	Number of Visible Pores		Rate of Change (%)
			Start	8 weeks	Start as 100
M.K.	23	♀	50	46	92.00
Y.Y.	23	♀	26	56	215.38
K.I.	25	♂	251	306	121.91
K.T.	25	♂	82	36	43.90
S.T.	25	♂	123	140	113.82
M.N.	26	♀	212	70	33.02
N.S.	27	♂	94	79	84.04
H.F.	28	♀	113	25	22.12
K.F.	33	♂	54	17	31.48
K.H.	39	♂	224	185	82.59
Y.O.	39	♂	105	44	41.90
T.K.	44	♂	173	203	117.34
N.A.	54	♂	604	527	87.25
E.B.	61	♀	219	36	16.44
Ave.			166	126	75.97

	START	After 8 weeks	Change of Open Pores
No.1 M.K. (23) F			 8.0% Down
No.2 Y.Y. (23) F			 115.4% Up
No.3 K.I. (25) M			 21.9% Up
No.4 K.T. (25) M			 56.1% Down
No.5 S.T. (25) M			 13.8% Up
No.6 M.N. (26) F			 67.0% Down
No.7 N.S. (27) M			 16.0% Down
No.8 H.F. (28) F			 77.9% Down



Consideration

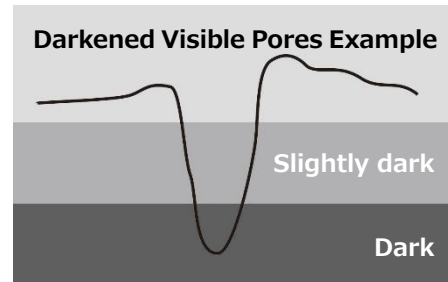
Open pores are often seen on middle-aged people and older. In the test, the rate of decrease of open pores was approximately 24 % in middle-aged subjects and older. Overall, open pores reduced on 10 subjects out of 14 (71 %).



③ Definition of Darkened Visible Pores



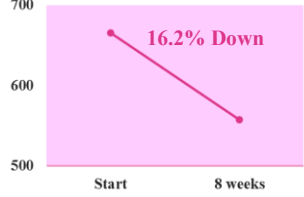


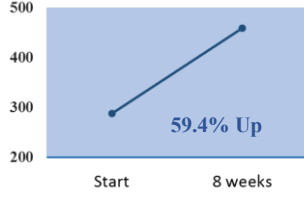


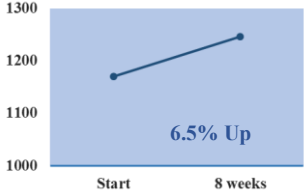


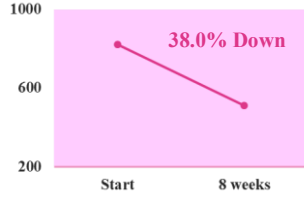


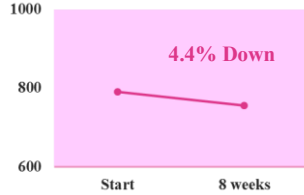


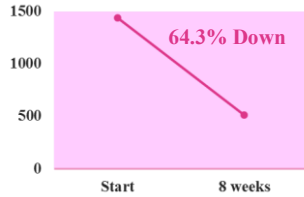


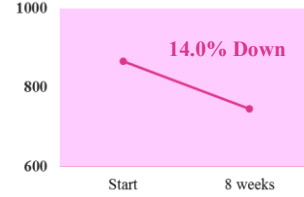


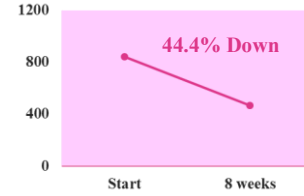
Among three color elements existing in color images (RGB), pores and pigmentation distribution is observed more in signal components of “BLUE (B).” Therefore, pores and pigmentation can be defined by the concentration and characteristics in shapes in monochrome images created by using BLUE signals.

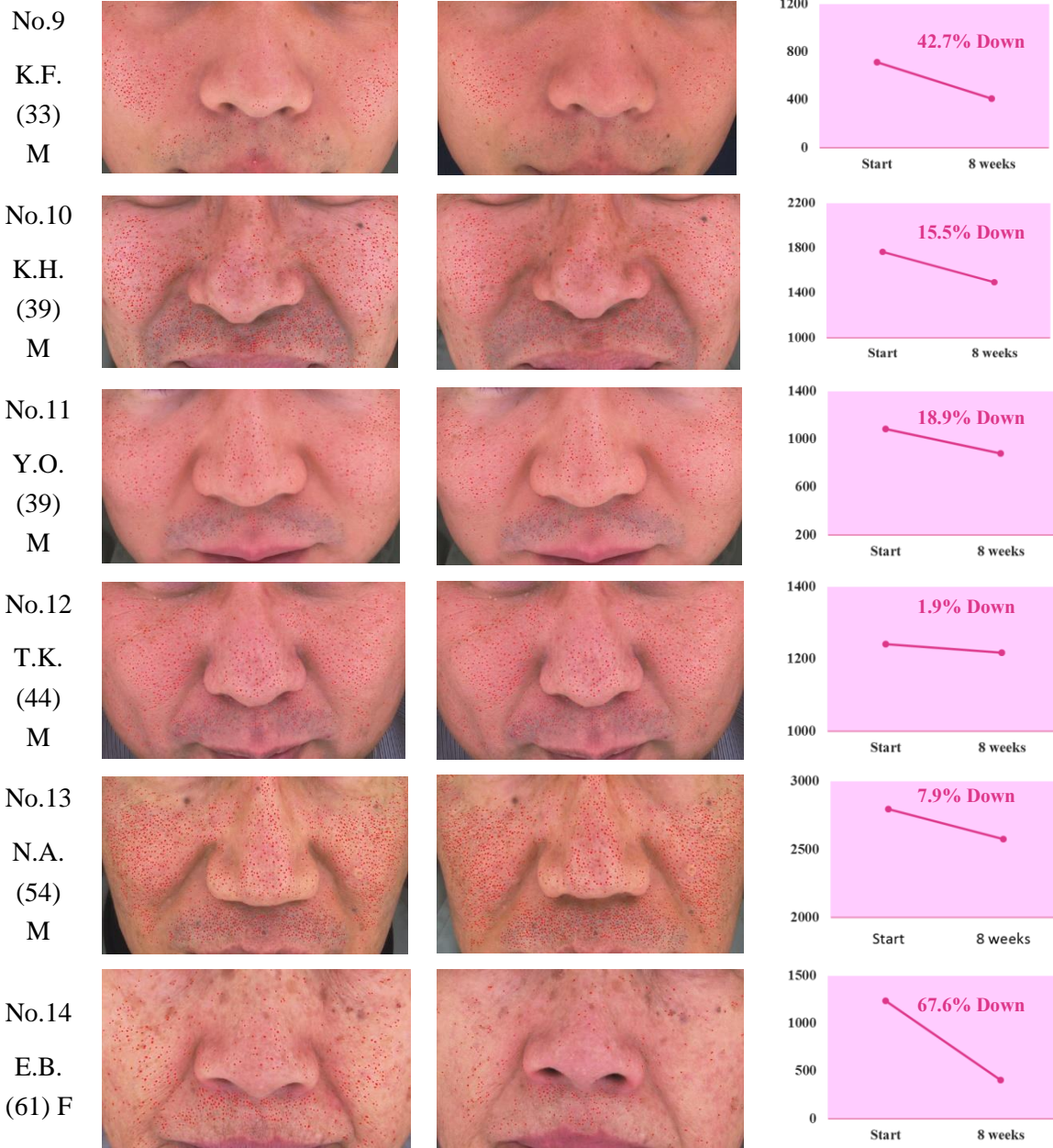
Continuous **round** areas with a size of **0.1 to 0.6 mm²** among subject areas of which margin can be detected as “**dark areas**” as compared to surrounding areas in monochrome images are detected as “**darkened pores.**”



Test Result

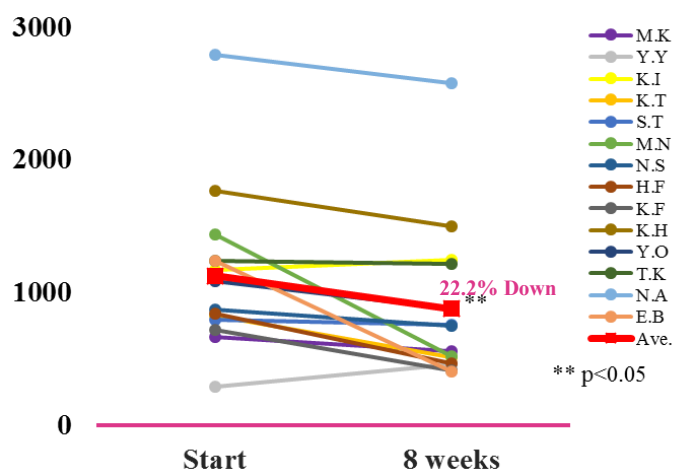
Subject	Age	Sex	Number of Visible Pores		Rate of Change (%)
			Start	8 weeks	Start as 100
M.K.	23	♀	666	558	83.78
Y.Y.	23	♀	288	459	159.38
K.I.	25	♂	1170	1246	106.50
K.T.	25	♂	822	510	62.04
S.T.	25	♂	790	755	95.57
M.N.	26	♀	1439	514	35.72
N.S.	27	♂	867	746	86.04
H.F.	28	♀	840	467	55.60
K.F.	33	♂	715	410	57.34
K.H.	39	♂	1768	1494	84.50
Y.O.	39	♂	1055	880	81.11
T.K.	44	♂	1241	1217	98.07
N.A.	54	♂	2795	2575	92.13
E.B.	61	♀	1239	402	32.45
Ave.			1123	874	77.79

	START	After 8 weeks	Change of Darkened Pores
No.1 M.K. (23) F			 Start: ~680, 8 weeks: ~570
No.2 Y.Y. (23) F			 Start: ~280, 8 weeks: ~450
No.3 K.I. (25) M			 Start: ~1150, 8 weeks: ~1250
No.4 K.T. (25) M			 Start: ~850, 8 weeks: ~530
No.5 S.T. (25) M			 Start: ~780, 8 weeks: ~750
No.6 M.N. (26) F			 Start: ~1450, 8 weeks: ~500
No.7 N.S. (27) M			 Start: ~880, 8 weeks: ~760
No.8 H.F. (28) F			 Start: ~850, 8 weeks: ~470



Consideration

It is said that darkened pores are common in younger generations with high lipid metabolism. It is assumed that saponin, a main component of cosmeHerbest™ NADESHIKO, removed lipids that darken pores. Darkened pores were reduced on 12 subjects out of 14 (86 %) in the test.



9-3-3 Pigmentation Reduction Test

The effect to reduce pigmentation areas was measured using the Robo Skin Analyzer just like the effect to reduce visible pores. As shown in the photos below, pigmentation level is not very clear in color photos. Continuous areas with a size of 1.2 mm² 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. Relatively light pigmentation is classified to level 1 and shown in red, dark pigmentation is classified to level 3 and shown in blue, and pigmentation in between the two is classified to level 2 and shown in yellow.

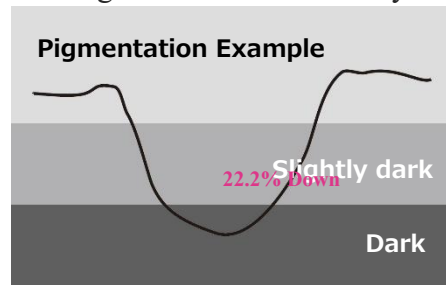




Image Example

Color photo (Nose)
Monochrome photo (Nose)

Pigmentation Tone Evaluation



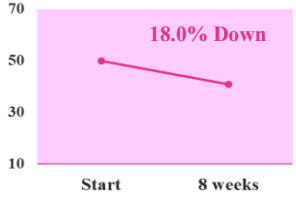


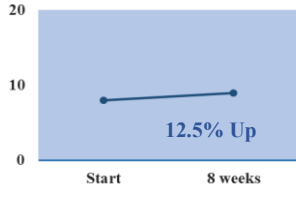
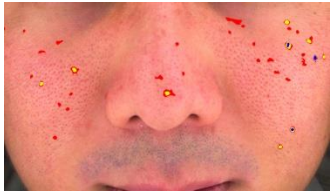

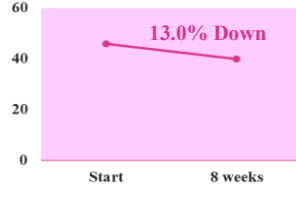


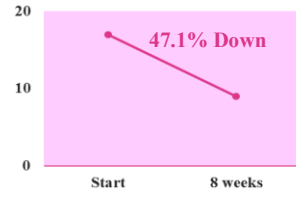


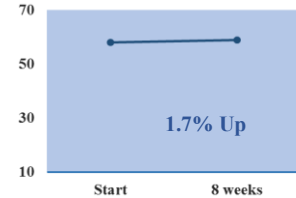
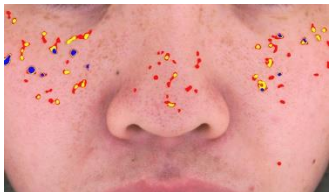

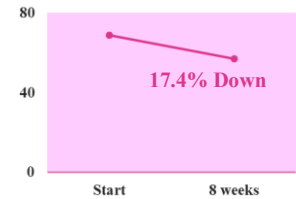


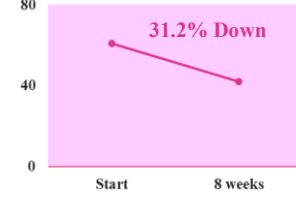


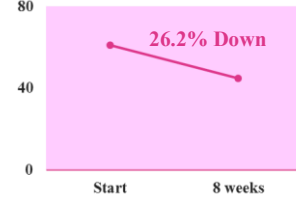
Level 1 ➔

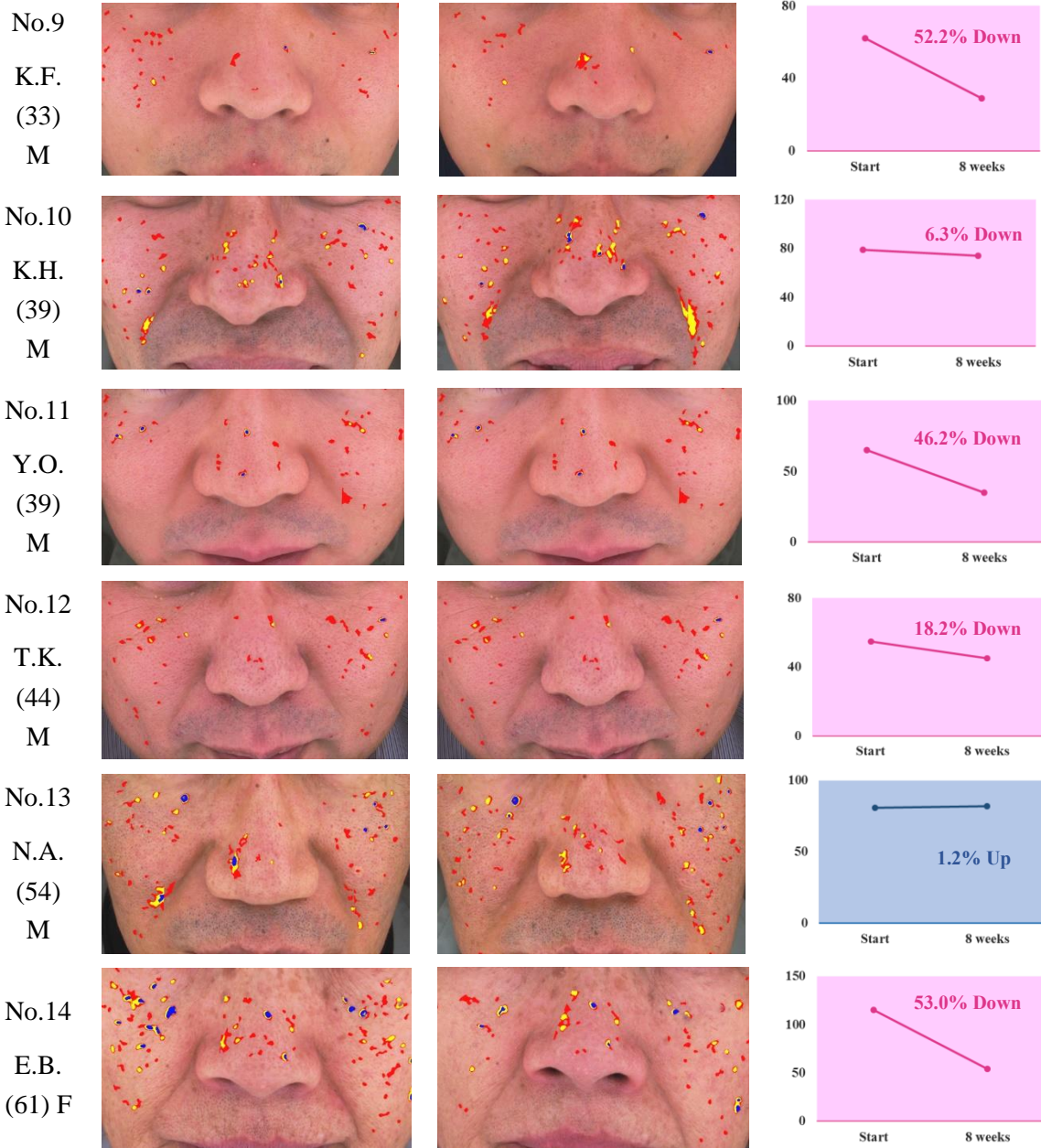
Level 2 ➔

Level 3 ➔

Test Result

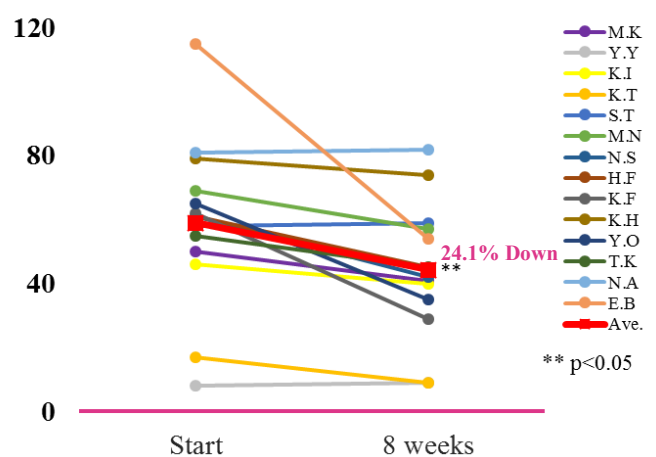
Subject	Age	Sex	Number of Visible Pores		Rate of Change (%)
			Start	8 weeks	Start as 100
M.K.	23	♀	50	41	82.00
Y.Y.	23	♀	8	9	112.50
K.I.	25	♂	46	40	86.96
K.T.	25	♂	17	9	52.94
S.T.	25	♂	58	59	101.72
M.N.	26	♀	69	57	82.61
N.S.	27	♂	61	42	68.85
H.F.	28	♀	61	45	73.77
K.F.	33	♂	62	29	46.77
K.H.	39	♂	79	74	93.67
Y.O.	39	♂	65	35	53.85
T.K.	44	♂	55	45	81.82
N.A.	54	♂	81	82	101.23
E.B.	61	♀	115	54	46.96
Ave.			59	44	75.09

	START	After 8 weeks	Improvement of Pigmentation
No.1 M.K. (23) F			 Start: 50, 8 weeks: 41
No.2 Y.Y. (23) F			 Start: 8, 8 weeks: 9
No.3 K.I. (25) M			 Start: 45, 8 weeks: 39
No.4 K.T. (25) M			 Start: 18, 8 weeks: 9
No.5 S.T. (25) M			 Start: 58, 8 weeks: 59
No.6 M.N. (26) F			 Start: 70, 8 weeks: 58
No.7 N.S. (27) M			 Start: 60, 8 weeks: 41
No.8 H.F. (28) F			 Start: 60, 8 weeks: 44



Consideration

When cosmeHerbest™ NADESHIKO was applied for two months, pigmentation significantly reduced on 11 subjects out of 14. According to the result, cosmeHerbest™ NADESHIKO is expected to have an effect to reduce pigmentation as well as visible pores.



9-3-4 Skin Texture Improving Action

cosmeHerbest® NADESHIKO was applied for eight weeks and the skin condition was observed using Robo Analyzer to study the improvement in skin texture.

Definition of Skin Texture

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). To check the subjects' skin texture, skin condition in the left lower cheek was observed.



Test Result

Subject	Age	Sex	Number of Visible Pores		Improvement Ratio (%) ^{*1}
			Start	8 weeks	
M.K.	23	♀	19	63	231.58
Y.Y.	23	♀	16	64	300.00
K.I.	25	♂	45	51	13.33
K.T.	25	♂	68	63	-7.35
S.T.	25	♂	44	53	207.69
M.N.	26	♀	13	40	20.45
N.S.	27	♂	63	62	-1.59
H.F.	28	♀	14	66	371.43
K.F.	33	♂	32	36	12.50
K.H.	39	♂	59	55	-6.78
Y.O.	39	♂	52	53	1.92
T.K.	44	♂	40	54	35.00
N.A.	54	♂	52	45	-13.46
E.B.	61	♀	39	52	33.33
Ave.			39.6	54	35.99

*1 Improvement Ratio (%)

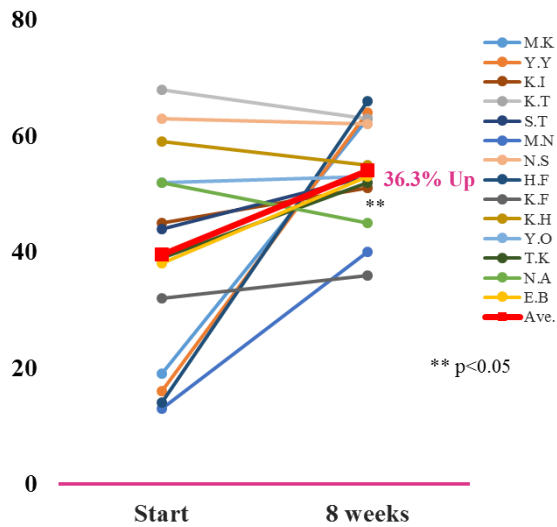
$$= \frac{\text{Score after 8 weeks from the application} - \text{Score before Application}}{\text{Score before Application}}$$

Beige Color area is equivalent to an improvement area of skin texture.

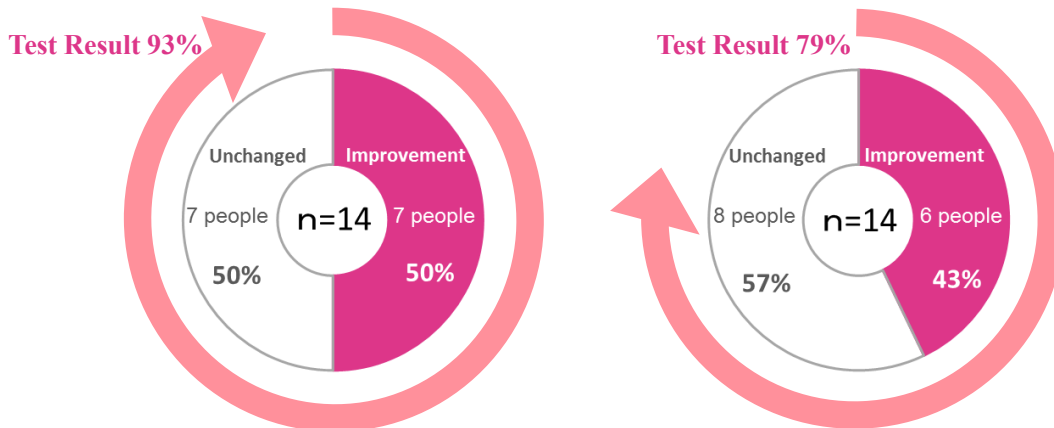
	Start	8 weeks		Start	8 weeks
No.1 M.K. (23) F			No.8 H.F. (28) F		
	Beige Area: 19 → 63			Beige Area: 14 → 66	
No.2 Y.Y. (23) F			No.9 K.F. (33) M		
	Beige Area: 16 → 64			Beige Area: 32 → 36	
No.3 K.I. (25) M			No.10 K.H. (39) M		
	Beige Area: 45 → 51			Beige Area: 59 → 55	
No.4 K.T. (25) M			No.11 Y.O. (39) M		
	Beige Area: 68 → 63			Beige Area: 52 → 53	
No.5 S.T. (25) M			No.12 T.K. (44) M		
	Beige Area: 44 → 53			Beige Area: 40 → 54	
No.6 M.N. (26) F			No.13 N.A. (54) M		
	Beige Area: 13 → 40			Beige Area: 52 → 45	
No.7 N.S. (27) M			No.14 E.B. (61) F		
	Beige Area: 63 → 62			Beige Area: 39 → 52	

Consideration

Skin texture was improved on 10 subjects out of 14 (71 %) in the test. It is assumed that dianthosaponin, a main component of cosmeHerbest™ NADESHIKO, removed excessive sebum and waste matter, contributing to the improvement of skin texture.



9-3-5 Summary of Monitor Tests [Questionnaire Results]



Consideration

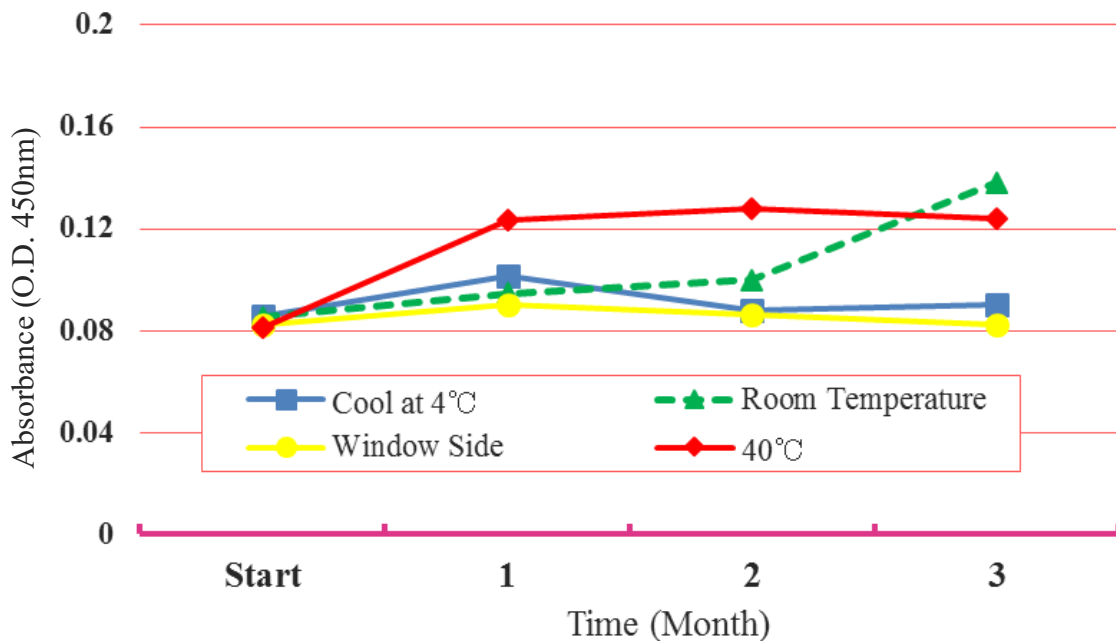
Visible pores were reduced on 13 subjects out of 14 (93 %), open pores were reduced on 10 subjects out of 14 (71 %), and darkened pores were reduced on 12 subjects out of 14 (86 %). In the pigmentation reduction test, pigmentation was reduced on 11 subjects out of 14 (79 %). The skin texture improvement test also produced good results (improvement on 10 subjects out of 14, 71 %). According to the results, cosmeHerbest™ NADESHIKO is expected to have an action to improve skin conditions as well.

It is assumed that these results were provided by the cleansing action of saponin which is a main component of dianthus as well as dianthus’s melanin formation suppressing action, Nrf2 expression promoting action, and glutathione production promoting action. In the questionnaire after the tests, over 50 % of the subjects said they experienced improvement in the condition of their facial pores and 40 % felt tendency of reduction in pigmentation. According to the results above, cosmeHerbest™ NADESHIKO is expected to have a skin lightening action. The best application of the material is to improve facial pore conditions. It is expected to provide the optimum effects when used in cleansing products such as soaps, shampoos, and makeup removers as well as hair conditioners and hair treatment products.

10. Stability Test

10-1 Long Term Stability Test

Store cosmeHerbest™ NADESHIKO as it was, in a cool dark place at 4°C, room temperature, window side and at 40°C and observed color change and determined the absorbance at 450nm for 3 months.



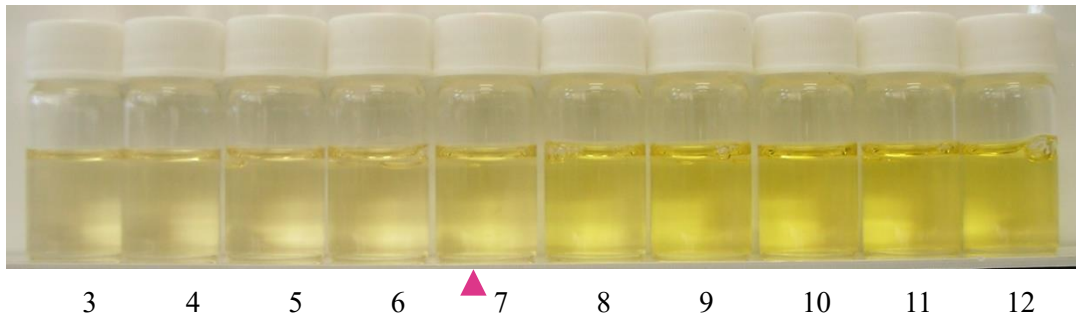
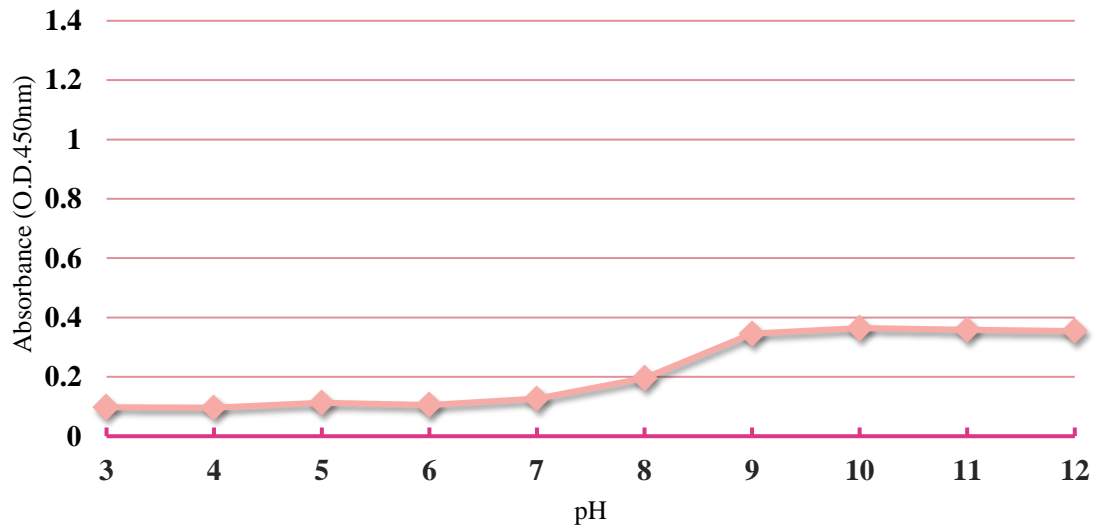
Test Result and Consideration

The test results have been rising absorbance at room temperature for 3 months after, but this rise will be colored trend of about hardly feel the difference with the naked eye. When incorporated into cosmetic formulations it can be inferred that have little effect on the cosmetic base of color.

10-2 pH Stability Test

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

Test Result



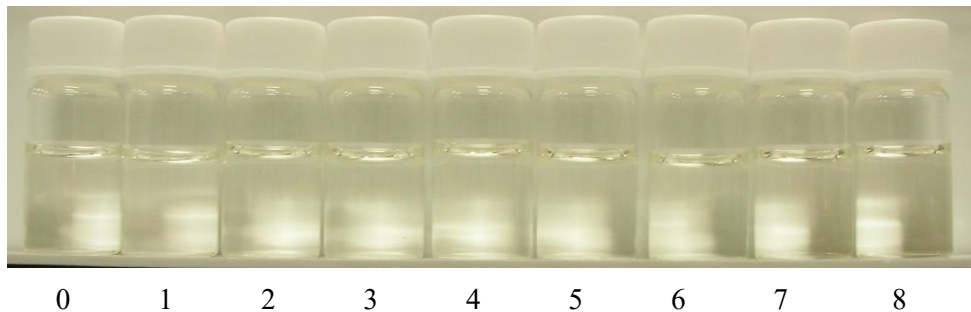
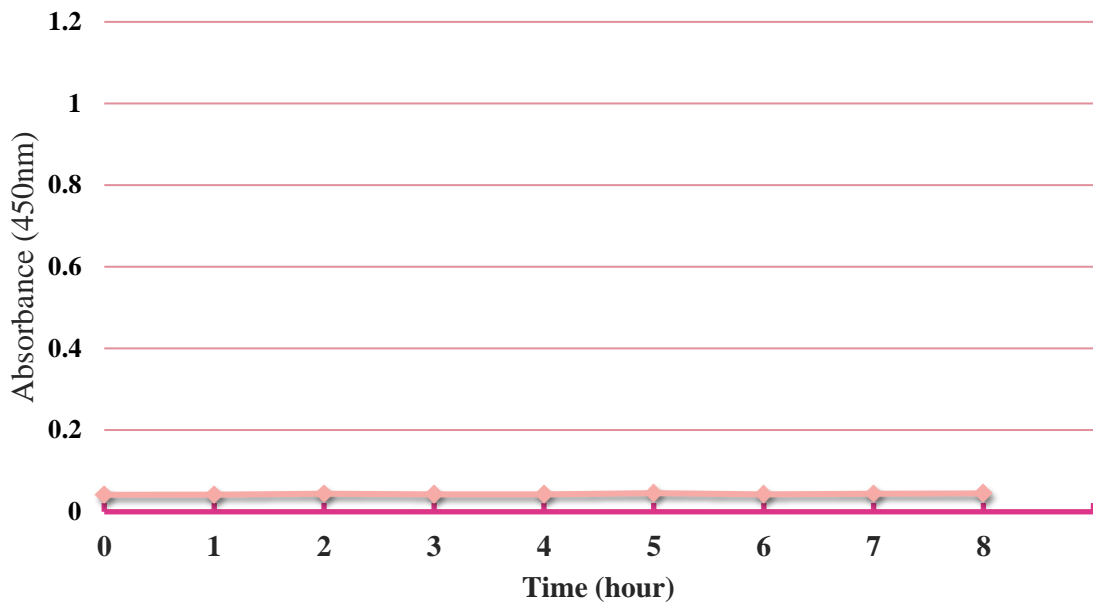
Consideration

From acidic, weakly acidic, and until the neutral zone is a stable and pale yellow color was stable and unchanged the color tone, but the color tone rapidly yellowish in the alkaline side. Please use in the neutral zone from the acidic side.

10-3 Thermal Stability Test

Adjust 10% concentration of cosmeHerbest™ NADESHIKO with purified water and heat at 90°C for 8 hours and observed the color change and determined the absorbance at 450nm.

Test Result



Consideration

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

11. Compatibility Test

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

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

12. Toxicological Safety Test

Trade Name	cosmeHerbest™ NADESHIKO	
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	No Irritation	RIPT method (50 people)
Sensitization Test	No Irritation	RIPT method (50 people)
Photo Toxicity Test	Not Performed	
Photo Sensitization Test	Not Performed	
Eye Irritation Test	No Irritation	SkinEthic™ HCE method
Mutagenicity Test	Negative	Ames Test (TA98, TA100)
Human Patch Test	Judgement for 24hours + (6), ± (1)	Closed-patch Test

13. Recommended Planning and Guide Formulation

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

- | | | |
|---|--|---|
| <ul style="list-style-type: none"> ● Cleansing Form ● Face-wash Cream ● Face-wash Soap | <ul style="list-style-type: none"> ● Serum ● Lotion ● Cream | <ul style="list-style-type: none"> ● Scalp Shampoo ● Scalp Rinse ● Scalp Treatment |
|---|--|---|

13-1 Formulation 1 Scalp Shampoo / RFAS-06

No.	Trade Name	Manufacturer	%	INCI Name
1	EMALEX EG-diS	Nihon Emulsion Co., Ltd.	0.50	PEG-2 Distearate
2	AMISOL PLME	Kawaken Fine Chemical	0.50	Lauramide MIPA
3	COMPERLAN 100	BASF	0.50	PPG-5Laureth-5
4	EMALEX PEIS-12EX	Nihon Emulsion Co., Ltd.	3.60	PEG-12 Isostearate
5	EMALEX 710	Nihon Emulsion Co., Ltd.	0.70	Laureth-10
6	SOFTAZOLINE CPB-R	Kawaken Fine Chemical	12.00	Cocamidepropylbetaine / Water
7	BEAULIGHT NA-25S	Sanyo Chemical Industries	32.00	Sodium Laureth Sulfate
8	SOFTAZOLINE CH	Kawaken Fine Chemical	3.00	Sodium Cocoamphoacetate / Water
9	D-PANTHENOL	Kawaken Fine Chemical	1.00	Panthenol
10	CITRIC ACID		0.20	Citric acid
11	LIPOFLOW MN	Lion Corporation	1.00	Poluquaternium-7 / Water / Methylparaben / Propylparaben
12	PURIFIED WATER		40.00	Water
13	PROPYLPARABEN		0.10	Propylparaben
14	METHYLPARABEN		0.20	Methylparaben
15	DL-CAMPHOR		0.05	Camphor
16	ℓ-MENTHOL		0.60	Menthol
17	cosmeHerbest™ NADESHIKO	Oryza Oil & Fat Chemical	2.50	Water / Propanediol / Dianthus Longicalyx Seed Extract
18	MENTHOL		0.50	Mentha Arvensis Leaf oil
19	FRAGRANCE		0.50	Fragrance
20	Blue 1 (0.1% Sol.)		0.20	Water / Blue 1
21	Yellow 5 (0.1% sol.)		0.35	Water / Yellow 5
			100.00	

Manufacturing Procedure

- 1) Stir and dissolve Ingredients No.1 to 14 at 80°C.
- 2) After dissolving 1), add and dissolve Ingredients No.15 and 16, and cool.
- 3) Then, add Ingredients No.17 to 21 at 60°C stir, furthermore cool, and pack to proper container at 30°C.

13-2 Formulation 2 Balancing Lotion / IBL-15A

No.	Trade Name	Manufacturer	%	INCI Name
1	ELDEW PS-203	Ajinomoto Co., Inc.	0.15	Phytosteryl/Octyldodecyl Lauroyl Glutamate
2	FZ-209	Dow Corning Corporation	1.22	Phenyl Trimethicone
3	EMALEX MTS-15L	Nihon Emulsion Co., Ltd.	0.98	PEG-5 Glyceryl Isostearate / PEG-8 Glyceryl Isostearate / PEG-20 Glyceryl Isostearate / PEG-40 Hydrogenated Castor Oil
4	EMALEX HC-20	Nihon Emulsion Co., Ltd.	0.05	PEG-20 Hydrogenated Castor Oil
5	EMALEX SLP	Nihon Emulsion Co., Ltd.	0.05	Hydrogenated Lecithin
6	SEABERRY FRUIT OIL	Oryza Oil & Fat Chemical	0.05	Hippophae Rhamnoides Fruit Oil
7	TOCOPHERYL ACETATE		0.05	Tocopheryl Acetate
8	PROPYLPARABEN		0.05	Propylparaben
9	METHYLPARABEN		0.10	Methylparaben
10	1,3-BUTYLENE GLYCOL		5.00	Butylene Glycol
11	AMILITE ACT-12 (30%)	Ajinomoto Co., Inc.	0.10	TEA-Cocoyl Alanine / Water
12	SODIUM CHONDROITIN SULFATE		0.05	Sodium Chondroitin Sulfate
13	TREHALOSE	Hayashibara Biochemical	1.00	Trehalose
14	GLYCERIN Conc.	NOF Corporation	2.00	Glycerin
15	cosmeHerbest™ NADESHIKO	Oryza Oil & Fat Chemical	1.00	Water / Propanediol / Dianthus Longicalyx seed Extract
16	cosmeHerbest™ SAKURA	Oryza Oil & Fat Chemical	1.00	Water / Butylene Glycol / Prunus Lannensiana Flower Extract
17	PURIFIED WATER		81.05	Water
18	FRAGRANCE		0.05	Fragrance
19	EMALEX HC-20	Nihon Emulsion Co., Ltd.	0.05	PEG-20Hydrogenated Castor Oil
20	ETHANOL		4.00	Alcohol
21	PURIFIED WATER		2.00	Water
			100.00	

Manufacturing Procedure

- 1) Heat and dissolve Ingredients No.1 to 8 at 80°C .
- 2) Heat and dissolve Ingredients No.9 to 17 at 75°C .
- 3) Dissolve Ingredients No.18 to 21 at room temperature.
- 4) While stir 2) by homogenizer and make emulsion adding 1) for 3 minute. (3000rpm)
- 5) Cool during stirring at 27°C and add 3).
- 6) Then, filter by 1.0 μ m membrane filter as the product.

13-3 Formulation 3 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	cosmeHerbest™ NADESHIKO	Oryza Oil & Fat Chemical	3.50	Water / Propanediol / Dianthus Longicalyx seed Extract
28	PURIFIED WATER		30.00	Water
			100.00	

Manufacturing Procedure

- 1) Mix and dissolve Ingredients No.1 to 16 at 85 °C . (Phase A)
- 2) Mix and dissolve Ingredients No. 17 to 28 at 85 °C . (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 °C and cool as the product.

13-4 Formulation 4 Body Lotion / INI-058

No.	Trade Name	Manufacturer	%	INCI Name
1	EMALEX SO-20BD	Nihon Emulsion Co., Ltd.	5.00	Cyclopentasiloxane / Dimethicone / Trimethylsiloxysilicate
2	PEMULEN TR-1	Nikko Chemicals Co., Ltd.	0.10	Acrylates/C10-30 Alkyl Acrylate Crosspolymer
3	EMALEX SO-20BD	Nihon Emulsion Co., Ltd.	4.00	Cyclopentasiloxane / Dimethicone / Trimethylsiloxysilicate
4	ISOSTEARIC ACID EX	Kokyu Alcohol Kogyo	1.00	Isostearic Acid
5	TOCOPHERYL ACETATE		0.10	Tocopheryl Acetate
6	ℓ-MENTHOL		0.05	Menthol
7	FRESCOLAT ML	Symrise	0.20	Menthyl Lactate
8	BUTYLPARABEN		0.05	Butylparaben
9	METHYLPARABEN		0.10	Methylparaben
10	CAFFEINE		0.10	Caffeine
11	CELLULOSE ACETATE	JNC Corporation	0.10	Cellulose Acetate
12	BEATAINE		1.00	Betaine
13	DEHYTON AB-30	BASF	0.50	Lauryl Betaine
14	EMALEX GWIS-180	Nihon Emulsion Co., Ltd.	0.20	PEG-60Glyceryl Isostearate
15	PEG#20000	NOF Corporation	0.10	PEG-400
16	1,3-BUTYLENE GLYCOL		5.00	BG
17	GLYCERINE Conc.	NOF Corporation	1.00	Glycerin
18	PURIFIED WATER		46.10	Water
19	POTASSIUM HYDROXIDE (10% aq.)		0.60	Potassium Hydroxide / Water
20	PURIFIED WATER		2.00	Water
21	KELTROL CG-T	Sansho Co., Ltd.	10.00	Xanthan Gum / Water
22	NATROSOL 250HHR (1% aq.)	Ashland Specialty	5.00	Hydroxyethyl Cellulose / Water
23	CARBOPOL 940 (1% aq.)	Lubrizol	10.00	Carbomer / Water
24	cosmeHerbest™ NADESHIKO	Oryza Oil & Fat Chemical	1.00	Water / Propanediol / Dianthus Longicalyx Seed Extract
25	cosmeHerbest™ LITCHI	Oryza Oil & Fat Chemical	1.00	Water / Propanediol / Litchi Chinensis Seed Extract
26	WHITE JERRY FUNGUS Extract-PC	Oryza Oil & Fat Chemical	0.50	Tremella Fuciformis Polysaccharide
27	FRAGRANCE		0.20	Fragrance
28	ETHANOL		5.00	Alcohol
			100.00	

Manufacturing Procedure

- 1) Mix well Ingredients No.1 and 2. (Phase A)
- 2) Dissolve Ingredients No.3 to 8 at 75 °C. (Phase B)
- 3) Heat Ingredients No.9 to 18 at 70 °C. (Phase C)
- 4) Mix well Ingredients No.19 and 20. (Phase D)
- 5) Weigh Ingredients No.21 to 23. (Phase E)
- 6) Dissolve Ingredients No.27 in 28. (Phase G)
- 7) While Stirring Phase C by homogenizer, add Phase A, furthermore add Phase B and make emulsion at 500rpm for 10 minutes.
- 8) Then, add Phase D into 7) at 1000rpm for 3 minutes, furthermore, add Phase E at 2000rpm for 3 minutes.
- 9) After making emulsion, cool at 40 °C, then, add Ingredients No.24 to 26 and Phase G, and cool at 30 °C as the product.

14. Product Specification

Commodity	:	Specification	Remarks
Trade Name		cosmeHerbest™ NADESHIKO	
Appearance			
· Color	:	Slightly yellow to slightly yellowish brown	
· Odor	:	liquid Slightly characteristic odor	
Identification			
· Saponin	:	Positive	
Purity			
1) Heavy Metals	:	20 ppm max.	
2) Arsenic	:	2 ppm max.	
Microbial Test			
1) Bacterial Count	:	1×10^2 /g max.	Hygiene Test
2) Fungi, Yeast	:	1×10^2 /g max.	Hygiene Test
3) Coli Form	:	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.

15. Labelling Name

- 15-1 **JP Labelling Name** : 水
プロパンジオール
カワラナデシコ種子エキス
- 15-2 **JP Quasi-drug Name** : None
- 15-3 **INCI Name** : Water
Propanediol
Dianthus Longicalyx Seed Extract
- 15-4 **已使用化粧品原料名称目录** : None
(IECIC2021)

16. Others

- 16-1 **Packaging**
1kg PE Bottle, 5kg PE Cubic container / Outer: Carton box
- 16-2 **Shelf Life and Storage Condition**
Store in a cool, dry, ventilated location. Keep away from high temperature and sunlight, store in the closed containers.

17. References

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- 3) Jung H. Suh et al., PNAS, **101**(10), 3381-3386 (2004)
- 4) Sugata et al.: Study of Regional and Ethic Differences of Facial Pores, IFSCC Congress 2006

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

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



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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