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Assessment of Stability and Safety of Maskne Cosmetic

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Abstract

Wearing a mask is still advised since COVID-19 continues to spread. However, masks may also irritate the skin and cause mask acne, often known as "maskne", which is a type of acne mechanica caused by friction between the skin and clothing. Therefore, there is a need to develop an effective maskne cosmetic. In this study, we made the maskne cosmetics containing *humulus lupulus* extract and copper tripeptide-1 and investigated its stability and safety. To measure stability, a centrifugation test and heat-cool cycling were done, and changes in viscosity and pH were measured for 8 weeks. The Cumulative Irritation Test (CIT, WKIRB-202111-HR-096) was performed and positive reactions were determined by the ICDRG criteria. The results indicated that the samples were stable after centrifugation, temperature cycling, viscosity, and pH tests. In addition, cosmetic safety test results revealed that maskne cosmetics containing *humulus lupulus* extract and copper tripeptide-1 did not cause any skin responses. These findings indicate that prepared maskne cosmetics' stability and safety were comparable to those of currently available commercial cosmetics.

This research is part of a master's thesis.

I. Introduction

Wearing a face mask is one of the key measures recommended by the Center for Disease Control and Prevention and WHO to mitigate the spread, of COVID-19. However, masks may cause irritation leading to a local acne outbreak in the atea covered by the mask, which popularly called "maskne or mask acne."(Kosasih, 2020).

Wearing a mask for long periods may irritate and degrade the skin barrier function(Damiani, Gironi, Grada, Kridin, Finelli, Buja, Bragazzi, & Pigatto, 2021). Furthermore, due to breathing and talking, a warm and humid environment is formed in the mask. This environment is conducive for bacteria multiplication and increased sebum secretion, which makes the skin sensitive and prone to skin conditions. The stratum comeum, which is the epidermis' outermost layer and serves as the skin's barrier, helps to prevent the loss of nutrients and moisture. When the skin barrier is damaged, the skin's lipid and water balance disintegrate, making the skin dry and more susceptible to external stimuli, and consequently prone to skin conditions. Therefore, there is a need for a maskne cosmetic that strengthens the skin barrier.

Cosmetic companies in the U.S. and Japan have recently introduced maskne cosmetics containing anti-inflammatory and skin barrier-strengthening ingredients. Similarly, maskne cosmetics are also being introduced in Korea.

Based on the need for maskne cosmetics containing active ingredients that are effective in protecting and improving skin barrier, and with anti-inflammatory effects, in this study, humulus lupulus extract and copper tripeptide-1 were chosen; they are easily absorbed into the skin and are projected to have anti-inflammatory and skin barrier-strengthening effects.

Humulus lupulus is a common tannin-containing plant in Korea, with significant quantities of polyphenols 6- and 8-prenylnaringenin. It possesses antibacterial, antioxidant, and anti-inflammatory effects (Stevens & Page, 2004; Liu, Hansen, Wang, Qiu, Dong, Yin, Qian, Yang, & Miao, 2015; Zanoli & Zavatti, 2008). Owing to its efficacy in soothing sensitive skin, allergies, inflammation, itching, and atopic skin, its efficacy against maskne is expected. One of its chemical components is a peptide, which has a low molecular weight and an amino acid bond. It has a similar structure to the skin and a high skin absorption rate, therefore it has a high utilitarian value (Moh, Jung, Kim, Cho, Seo, & Kim, 2011).

Copper tripeptide-1, a compound containing tripeptide—1 and copper, is a natural peptide generated from the human body and a representative material of carrier peptides. A huge body of scientific evidence supports the essential role of copper tripeptide-1 in the acceleration of wound healing (DeHaven, 2014). This compound is released during any tissue injury to signal repair processes to begin. It boosts anti-inflammatory proteins including superoxide dismutase and decorin while suppressing inflammatory cytokines like TGF-1, TNF-a, and IL-1 (Pickart, 2008; Wegrowski, Maquart, & Borel, 1992). In other words, copper tripeptide-1 is a functional material with high-efficiency, easy absorption, and anti-inflammatory properties and therefore a useful maskne cosmetics ingredient.

In this study, we made a lotion formulation containing humulus lupulus extract and copper tripeptide-1 as active ingredients. The stability and safety of the formulation were examined to confirm the commercialization potential of the created maskne cosmetic

II. Material and Method

1. Material

Humulus lupulus extract (Senseryn™) was purchased from Changhyup trading Co., Ltd.,(Seoul, Korea). Copper tripeptide-1(BIO-CTP 500) was bought from Natural solution(Osan, Korea). Figure 1 shows the chemical formula of copper tripeptide-1.

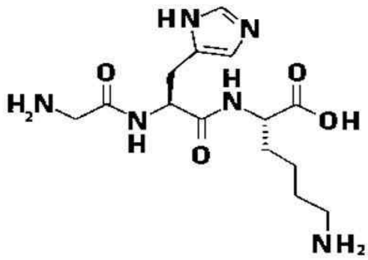

Figure 1. *Chemical formula of copper tripeptide-1*

Table 1. *Formulation of the vehicle and maskne cosmetics* (unit: g)

Phrase	Ingredients	Vehicle	Sample A	Sample B	Sample C
A	distilled water	64.2	61.2	57.2	58.2
	carbomer	0.20	0.20	0.20	0.20
	butylene glycol	5.00	5.00	5.00	5.00
	betaine	0.30	0.30	0.30	0.30
	erythritol	0.20	0.20	0.20	0.20
	glycereth-26	3.00	3.00	3.00	3.00
	glycerin	5.00	5.00	5.00	5.00
B	isopropyl myristate	8.00	8.00	8.00	8.00
	stearic acid	1.50	1.50	1.50	1.50
	glyceryl stearate	2.00	2.00	2.00	2.00
	polysorbate 60	3.00	3.00	3.00	3.00
	cetearyl alcohol	2.00	2.00	2.00	2.00
	phenoxyethano	0.40	0.40	0.40	0.40
C	distilled water	5.00	5.00	5.00	5.00
	triethanolamine	0.20	0.20	0.20	0.20
D	*humulus lupulus* extract	-	3.00		3.00
	copper tripeptide-1	-		3.00	3.00
	Total	100	100	96	100

Vehicle : Base cream
Sample A : Vehicle containing 3% *hunulus lupulus* extract
Sample B : Vehicle containing 3% copper tripeptide-1
Sample C : Vehicle containing 3% *hunulus lupulus* extract and 3% copper tripeptide-1

2. Preparation of the Vehicle and Maskne Cosmetics

The maskne cosmetics were prepared using *humulus lupulus* extract and copper tripeptide-1 as the active ingredients (Table 1). The right uantities of distilled water and thickener were measured and then mixed, while complete dispersion was confirmed with a high-speed stirrer (Hei-TORQUE Expert 100, Heidolph North America, IL, U.S.A.). Each ingredient required in phase A was weighed and mixed sequentially under conditions of 50 ℃, 800 rpm, 10 min. Phase B ingredients include solvents, antioxidants, and preservatives. The mixture was completely dissolved under conditions of 50° C, 800 rpm, and 10 min. using a high-speed stirrer.

Phases A and B were emulsified and mixed using a homo-mixer (T.K. Homo Mixer Mark Model 2.5, MJ Research Co., Ltd, Incheon, Korea) at 75 ℃, respectively. After cooling the mixed A+B phase to 45℃, phase C ingredients were added and mixed with a homomixer (neutralization reaction). Phases A, B, and C of the manufacturing process a cream formulation (vehicle) was prepared. Then, 3g copper tripeptide-1 and 3g *humulus lupulus* extract were added to the vehicle to prepare Sample A and Sample B, respectively. Sample C was prepared using the vehicle, 3 g copper tripeptide-1 and 3 g *humulus lupulus* extract. The manufacturing process is shown in Figure 2.

3. Assessment of Stability

Physical characteristics, including the composition stability, heat stability, pH, and viscosity of the prepared maskne cosmetics were determined. The composition stability was measured using a centrifugation method (HA-1000-3 benchtop centrifuge; Hanil Science Medical, Daejeon, Korea). Test tubes were filled with 10 mL samples in each and centrifuged at 30 rpm with spindle LV S63, for 2 min.

The stability of the prepared maskne cosmetics was determined by hot-cool cycling from 5℃ to 50℃, with a 24h storage time at each temperature using a multi-room temperature humidity incubator (DS-114; Dawon Science, Seoul, Korea). Five cycles were performed, and the appearance of the maskne cosmetics was observed at the end of each cycle. Stability was determined based on the appearance, color, and whether the maskne cosmetics preparation separated.

For 8 weeks, samples' viscosity and pH were tested at 5° C, 25° C, and 50° C, respectively. The viscosity of the samples was determined at 30 rpm using a Brookfield viscometer (DV-E Viscometer, Brookfield, MA, U.S.A) with the LV spindle. The pH of samples was monitored using a pH meter (sensitivity: ± 0.255) (3110 WTW, pH meter, Oberbayern, Germany).

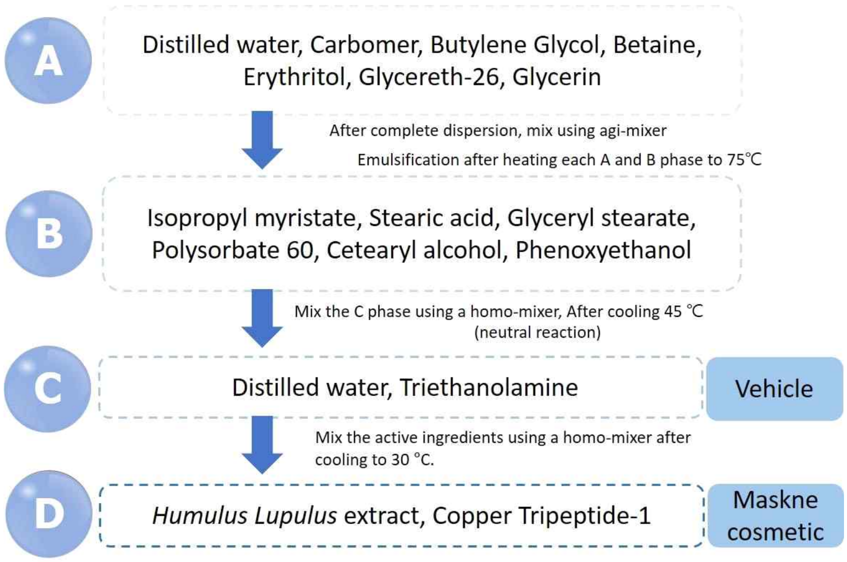

Figure 2. *Diagram of maskne cosmetic manufacturing process*

4. Cumulative Irritation Test (CIT)

Informed consent was obtained from all participants after providing them with written and oral information about the study. The study protocol was approved by the ethics committee of the Wonkwang University (Registration No. WKIRB-202111-HR-096).

The maskne cosmetics were evaluated by the Cumulative Irritation Test (CIT). Healthy, non-pregnant Korean women (aged 20~50 years) were screened and 30 were enrolled. The patch test was performed on skin with no scars, moles, freckles, and other skin problems to evaluate safety. 25 μL of samples (IQ chamber) were applied on the skin under scapula for 3 consecutive weeks using an occlusive patch, which was changed once a week, and followed by a challenge phase after a minimum 1 week rest period. The patch test results were interpreted according to the International Contact Dermatitis Research Group (ICDRG) guidelines, Table 2.

5. Data Analysis

All statistical produres were conducted using the SPSS statistical package (version 24.0 program). Data were analyzed as the descriptive statistics, mean, standard deviation. The results were subjected to one-way ANOVA. Significant differences were determined at $p < 0.05$.

III. Results and Discussion

The physical stability of the vehicle and maskne cosmetic samples were evaluated using a centrifugation method. The centrifugation test results are shown in figure 3. Changes in the formulation and phase separation were noticed after centrifugation. All the samples maintained homogeneity.

All samples were stored in a multi-room temperature humidity incubator at temperatures ranging from 5 to 50℃, and then the stability was assessed based on the appearance, color, and phase separation of the samples. All samples remained homogeneous under these conditions and did not change as the temperature increased (Figure 4); therefore, the samples were considered to be stability.

The stability and application behavior of creams are important factors for subject acceptance. The viscosity of creams is influenced by the ingredients and production process. In this study, the viscosity was measured to assess the product's stability and application behavior on the skin (Saptarini & Hadisoebroto, 2020).

The viscosity of samples was measured at 5℃, 25℃, and 50℃ respectively for 8 weeks, as shown in Table 3. There was no significant difference in the viscosity

Table 2. *Grading criteria of the skin reactions by ICDRG guideline*

Positive/ Negative	Score	Criteria
-	0	No irritant reaction (Discrete patchy erythema without infiltration)
-/+	0.5	Doubtful reaction (Faint macular, No infiltration, Homogenous erythema)
+	1	Week Positive reaction (Erythema, Induration, Papules)
++	2	Strong Positive reaction (Erythema, Induration, Papules, Discrete vesicles)
+++	3	Extreme Positive reaction (coalescing vesicles, Bullous or ulcerative reaction)

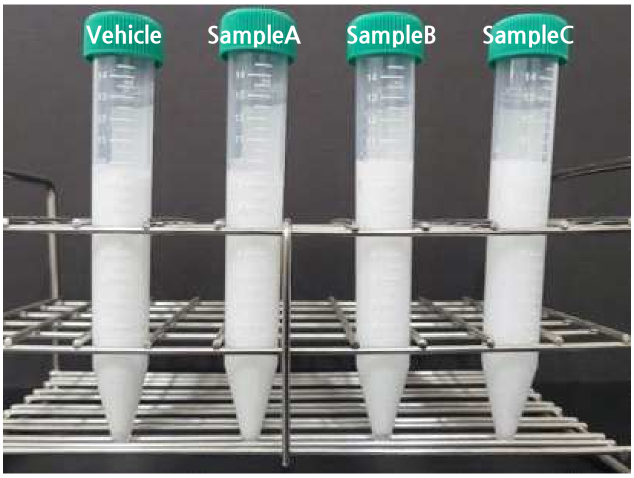

Figure 3. *Vehicle and maskne cosmetics after centrifugation test*
Vehicle : Base cream
Sample A : Vehicle containing 3% *hunulus lupulus* extract
Sample B : Vehicle containing 3% copper tripeptide-1
Sample C : Vehicle containing 3% *hunulus lupulus* extract and 3% copper tripeptide-1

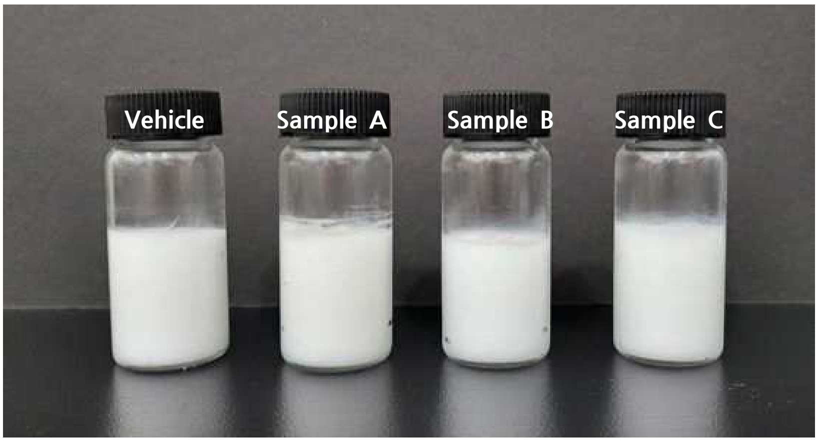

Figure 4. *Vehicle and maskne cosmetics after a cycling test*
Vehicle : Base cream
Sample A : Vehicle containing 3% *hunulus lupulus* extract
Sample B : Vehicle containing 3% copper tripeptide-1
Sample C : Vehicle containing 3% *hunulus lupulus* extract and 3% copper tripeptide-1

change between the vehicle and maskne cosmetics. The vehicle and maskne cosmetics had a high viscosity of approximately 162000 cps and assumed thickened cream state. The prepared maskne cosmetics were stable for use as a cosmetic.

Table 4 shows the pH change of vehicle and maskne cosmtics at 5° C, 25° C, and 45° C for 8 weeks. There was no significant pH alteration in the vehicle and

Table 3. *The viscosity changes from 1 week to 8 weeks* (Unit : cps)

Temp.	Sample	1 day	1 week	2 weeks	4 weeks	6 weeks	8 weeks	F	*p*
5℃	Vehicle	161533 ± 94.28	161867 ± 471.40	162267 ± 271.40	161533 ± 94.28	161600 ± 282.84	162000 ± 0.00	2.53	0.07
	SampleA	162100 ± 454.61	162033 ± 471.40	161500 ± 141.42	161700 ± 0.00	161467 ± 339.93	161933 ± 339.93	1.39	0.28
	SampleB	162133 ± 188.56	161900 ± 141.42	161700 ± 244.95	161733 ± 286.74	162100 ± 0.00	162233 ± 464.28	0.90	0.51
	SampleC	162100 ± 188.56	161900 ± 141.42	162133 ± 418.99	161567 ± 124.72	162100 ± 124.72	162233 ± 464.28	1.23	0.34
25℃	Vehicle	161800 ± 282.84	162267 ± 188.56	161800 ± 282.84	162333 ± 449.69	161600 ± 282.84	162300 ± 141.42	2.66	0.06
	Sample A	162000 ± 496.66	161900 ± 571.55	161500 ± 141.42	161700 ± 81.65	161733 ± 188.56	161467 ± 94.28	0.81	0.57
	Sample B	161600 ± 141.42	161933 ± 329.98	161800 ± 565.69	161467 ± 94.28	161800 ± 216.02	161833 ± 418.99	1.76	0.17
	Sample C	161700 ± 0.00	161933 ± 329.98	161400 ± 0.00	161533 ± 181.56	162000 ± 141.42	162267 ± 418.99	2.27	0.96
50℃	Vehicle	161367 ± 286.74	161000 ± 0.00	161333 ± 249.44	161667 ± 47,14	161867 ± 524.93	162067 ± 471.40	2.61	0.06
	Sample A	162500 ± 141.42	161700 ± 0.00	161767 ± 47.14	161,967 ± 518.54	161800 ± 163.30	161467 ± 329.98	0.91	0.51
	Sample B	161633 ± 169.97	161867 ± 524.93	161967 ± 449.69	161767 ± 169.97	161567 ± 418.99	161800 ± 141.42	0.31	0.91
	Sample C	161900 ± 509.90	162367 ± 205.48	161700 ± 244.95	162100 ± 454.61	161567 ± 418.99	161800 ± 141.42	0.98	0.47

Vehicle: Base cream
Sample A: Vehicle containing 3% *hunulus lupulus* extract
Sample B: Vehicle containing 3% copper tripeptide-1
Sample C: Vehicle containing 3% *hunulus lupulus* extract and 3% copper tripeptide-1
Data were measured done in triplicate.
Data represent means ± standard deviation of each triplicate data.
ANOVA analysis results between periods (1 day~8 week), such that there is no statistically significant difference.

maskne cosmtics. The pH level of the vehicle ranged from 6.19 to 6.31 and that of the maskne cosmetics (sample A, B, and C) ranged from 5.85 to 6.06. The pH level of maskne cosmetics was lower than that of the vehicle, which could be attributed to *hunulus lupulus* extract and 3% copper tripeptide-1. Hop bitter acids consist of two related series, the α-acids (or humulones) and β-acids (or lupulones), which are both characterized as prenylated phloroglucinol derivatives. In addition, hop bitter acids are effective against inflammatory (Cleemputt, Cattor, Bosscher, Haegeman, Keukeleire, & Heyerick, 2009).

The pH levels of samples were typically around 6.0. The mean pH of the skin surface is 5.0-6.0 (Lambers, Piessens, Bloem, Pronk, & Finkel, 2006). Many studies have shown that the skin penetration of cosmetics is directly related to pH levels. Change in pH could lead to structural changes in keratin. It has been shown that corticosteroid cream has the ability to penetrate the skin effectively at the range of pH 6.2 ± 0.2. Therefore,

Table 4. *The changes in pH from 1 week to 8 weeks*

Temp.	Sample	1 day	1 week	2 weeks	4 weeks	6 weeks	8 weeks	F	*p*
5℃	Vehicle	6.31 ± 0.01	6.24 ± 0.04	6.25 ± 0.03	6.26 ± 0.02	6.19 ± 0.03	6.24 ± 0.00	3.84	0.18
	Sample A	5.95 ± 0.12	5.99 ± 0.08	5.95 ± 0.06	5.98 ± 0.08	5.97 ± 0.08	5.96 ± 0.07	0.98	0.99
	Sample B	6.01 ± 0.00	5.90 ± 0.02	5.98 ± 0.09	5.93 ± 0.09	5.93 ± 0.05	5.93 ± 0.08	1.63	0.23
	Sample C	6.01 ± 0.00	5.91 ± 0.01	5.98 ± 0.09	5.95 ± 0.02	5.92 ± 0.05	6.00 ± 0.05	1.72	0.18
25℃	Vehicle	6.22 ± 0.01	6.22 ± 0.01	6.25 ± 0.04	6.22 ± 0.01	6.23 ± 0.01	6.27 ± 0.04	1.55	0.23
	Sample A	5.88 ± 0.00	5.90 ± 0.10	5.87 ± 0.04	5.95 ± 0.05	5.95 ± 0.06	5.92 ± 0.05	0.66	0.68
	Sample B	5.88 ± 0.00	5.82 ± 0.05	5.91 ± 0.08	5.92 ± 0.04	5.90 ± 0.09	5.87 ± 0.01	0.57	0.74
	Sample C	5.88 ± 0.00	5.82 ± 0.05	5.91 ± 0.08	5.92 ± 0.04	5.90 ± 0.09	5.92 ± 0.07	0.53	0.77
50℃	Vehicle	6.23 ± 0.01	6.28 ± 0.03	6.25 ± 0.04	6.25 ± 0.03	6.22 ± 0.00	6.25 ± 0.03	1.00	0.46
	Sample A	5.92 ± 0.08	5.85 ± 0.05	5.97 ± 0.11	5.97 ± 0.03	5.95 ± 0.05	6.06 ± 0.03	1.71	0.19
	Sample B	5.97 ± 0.05	5.94 ± 0.07	5.96 ± 0.07	5.91 ± 0.04	5.96 ± 0.09	5.87 ± 0.01	0.61	0.71
	Sample C	5.93 ± 0.03	6.02 ± 0.02	6.02 ± 0.02	5.95 ± 0.06	5.98 ± 0.06	5.88 ± 0.01	2.68	0.06

Vehicle : Base cream
Sample A : Vehicle containing 3% *hunulus lupulus* extract
Sample B : Vehicle containing 3% copper tripeptide-1
Sample C : Vehicle containing 3% *hunulus lupulus* extract and 3% copper tripeptide-1
Data were measured done in triplicate.
Data represent means ± standard deviation of each triplicate data.
ANOVA analysis results between periods (1 day~8 weeks), there was no statistically significant difference.

based on our pH results, we assumed that our maskne cosmetics could penetrate the skin. No significant pH changes were seen in any formulation over the 8 weeks, regardless of the storage duration, indicating that this formulation has suitable pH for topical formulation.

The patch test was conducted on 30 female volunteers over 3 weeks (Figure 4). CIT results are shown in Table 5. No skin reaction was observed in response to the prepared vehicle and maskne cosmetics.

The interpretation of patch test results is very important as it determines safety. The human patch testis not standardized, in contrast to in vitro tests or animal studies. Notably, various factors can affect the skin irritation response, such as personal characteristics and the climate. The human patch test is a useful primary test for irritation caused by direct contact with the skin (Tammela, Lindberg, Isaksson, Inerot, Rudel, & Berne, 2012). The most important step is to evaluate skin toxicity such as the potential of cosmetics to cause irritation and sensitization. In a previous report where lotions and creams are used on the face, the safety zone of these products (1.12) was high compared with that for other leave-on products. Z-score〈1 was determined as a safe zone for primary skin irritation for each cosmetic. As shown by their patch test result, these products were safe effective in most formulation with the

Table 5. *The results of the CIT for 3 weeks* (N=30)

	Reaction	1 week	2 weeks	3 weeks
Vehicle	Negative Positive	30 0	30 0	30 0
Sample A	Negative Positive	30 0	30 0	30 0
Sample B	Negative Positive	30 0	30 0	30 0
Sample C	Negative Positive	30 0	30 0	30 0

- : No irritant reaction
-/+ : Doubtful reaction
+ : Week Positive reaction
++ : Strong Positive reaction
+++ : Extreme Positive reaction

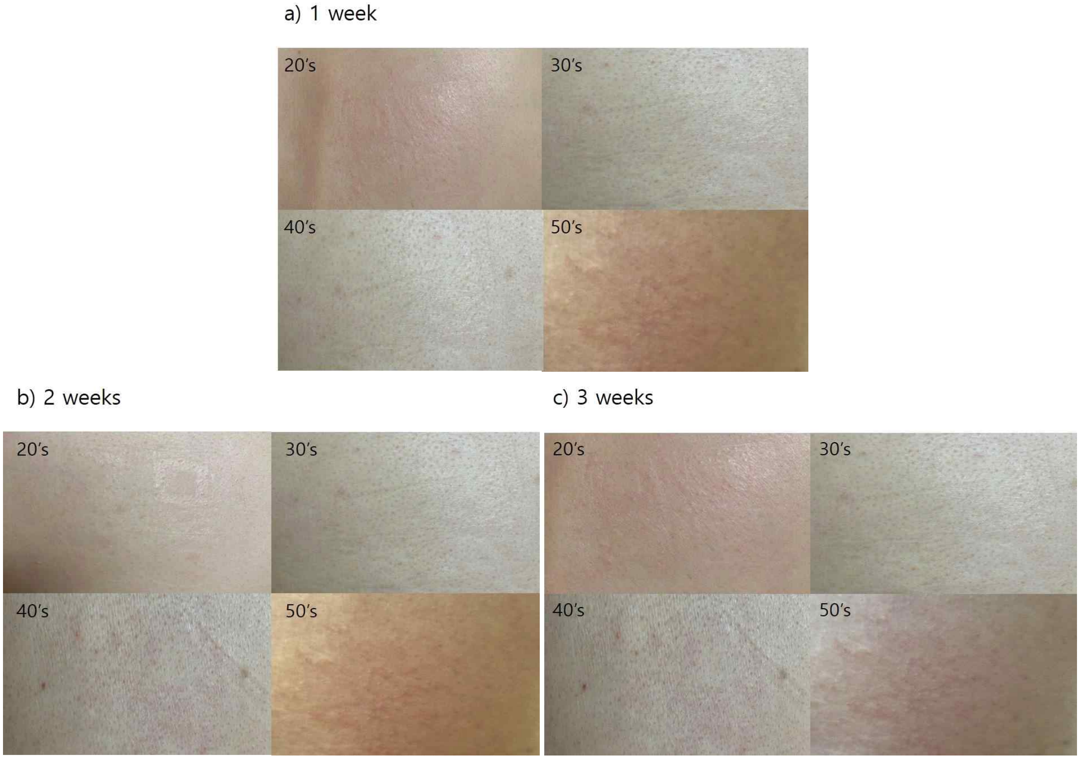

Figure 4. *Representative photographs of skin reaction by CIT*
a) 1 week b) 2 weeks c) 3 weeks

most various raw materials (An, Ham, Choi, Shin, An, Kim, & Koh, 2014).

Our results showed the prepared maskne cosmetics did not cause skin allergies when applied to the human body regularly.

Ⅳ. Conclusion

Face covering with masks has become a lifeline for humans to prevent the airborne transmission of highly infectious COVID-19. Masks are being used more often and longer than ever before. Meanwhile, an open concern surfaced about the safety of masks since new masks routinely display unpleasant scents that are related to the emissions of volatile organic compounds (VOCs). As a result, it causes a local acne eruption in the region covered by the mask, which is commonly referred to as "maskne" or "mask acne." There is a need for a maskne cosmetic that reduces the occurrence of skin problems. To prevent and treat acne, it is vital to create cosmetics based on active substances that are efficient at enhancing the skin barrier and acting as anti-inflammatory agents. Because they have excellent skin absorption and are anticipated to have anti-inflammatory and skin barrier-strengthening effects. In this study, *humulus lupulus* extract, and copper tripeptide-1 were selected as efficient ingredients for the preparation of maskne cosmetics.

We made the maskne cosmetics containing *humulus lupulus* extract and copper tripeptide-1. Further, we provides evidence for the stability and safety of *humulus lupulus* extract and copper tripeptide-1 as making maskne cosmetics. The maskne cosmetics containing *humulus lupulus* extract and copper tripeptide-1 were stable at low to high temperatures. Further, the prepared maskne cosmetics had constant viscosity and pH for 8 weeks. The patch test results showed that the maskne cosmetics did not cause a positive skin reaction. Maskne cosmetic containing *humulus lupulus* extract and copper tripeptide-1 can be formulated has a good stability and are safe use on human skin.

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