A combination of magnolia officinalis bark extract and heptyl undecylenate as a new multi-functional cosmetic ingredient

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Abstract

Background: Acne is a chronic inflammatory disease outbreak in the sebaceous glands within the hair follicle. The proliferation of *Cutibacterium acnes* (*C. acnes*, formerly known as *Propionibacterium acnes*) causes monocytes to stimulate secretion of inflammatory cytokines. A number of studies have been proposed the inhibitory effects of *C. acnes* and *C. acnes*-mediated inflammation by natural extracts.

Methods: In order to investigate the anti-acne activities of magnolia officinalis bark extract and heptyl undecylenate complex (MEHU), antimicrobial activity against C. acnes and nitric oxide (NO) inhibitory activity were tested. Sensory profile improvement experiments were investigated, and skin irritation level was determined by single patch test.

Results: In this study, we investigated the effeciency of novel cosmetic ingredient (complex of magnolia officinalis bark extract and heptyl undecylenate) in ant-*C. acnes* and anti-inflammation effect. In addition to its antibacterial properties, the anti-fungal reactivity were confirmed. Furthermore, the desired properties of oil balm such as spreadability, absorption, moisturizing, film formation, and feeling were improved by its applied with MEHU. It has provided same sensory profile as observed for oil balm containing synthetic silicone oils.

Conclusion: These excellent results are quite appealing, which suggest the new candidate for cosmetic ingredient that could be effective as anti-acne, that can be applicable to resolve the sensitive skin issues occurred due to the regular use of mask due to serious COVID-19 situation.

Keywords: Anti-acne; Anti-inflammation; *Cutibacterium acnes*; Magnolia officinalis bark extract; Heptyl Undecylenate

Introduction.

Preservation of personal care products containing water and organic/inorganic ingredients is necessary to prevent spoilage which subsequently can cause severe health risks for the consumers. One of the major concerns in personal care products is product spoilage causing microbial contamination. Personal care products have a nutrient-rich medium that favors the growth of microorganisms. Microbial contamination may occur while being manufactured or when consumers use the products. Therefore, the preservative system is used in personal care products to prevent microbial spoilage; improve the shelf life of the product, and protect consumers against adverse microbial infection [1]. One of the most common approaches used is the addition of preservatives such as paraben, which are esters of 4-parahydroxybenzoic acid (PHBA). Paraben, Formaldehyde releasers, Isothiazolinones, Triclosan, urea based preservatives, are commonly used preservative systems in personal care products. However, recently, the problem of the safety of popular preservatives has been suggested. Paraben is involved in endocrine disruptors and may make women more prone to developing estrogen positive breast cancer [2]. Formaldehyde releasers, which are added to shampoo, soaps, nail polishes, hair dyes, and conditioners have side effects of formadehyde exposure. Formaldehyde is considered as a strong allergen, and contact with this preservative may induce chronic allergic eczema. It has been observed that using skincare products containing a low amount of formaldehyde (2.5 ~ 400 ppm) in people with allergies led to dermatitis [3].

The debate has been further heightened by the growing popularity of pro-ecological and healthy lifestyle trends. It is not surprising that personal care product formulators have introduced alternative preservatives as safer than paraben [4]. The following strategies are implemented to efficiently control microbial contamination, and minimize adverse health effects from preservatives : (1) replacement of preservatives with other chemical or natural alternatives, (2) formulation of the mixtures of preservatives with other alternatives, (3) preparation of the composite mixtures of various alternatives to the preservative-free system. Consequently, preference for “preservative-free”, and “self-preserving” products is a rising trend among consumers these days. Multifunctional ingredients are defined as single ingredients that have more than one function in personal care product formulations. Multifunctional ingredients with antimicrobial activity are seen as potential alternatives. As these ingredients are not designed to be preservatives, they tend to be either antimicrobial or preservative boosters. These types of ingredients can help formulators reduce chemical inventory, reduce formula complications, and improve ease of manufacture. In recent years, therefore, formulators have taken a greater interest in multifunctional ingredients.

Natural extracts could be used as alternative preservatives. It has various properties in personal care products, including anti-inflammatory, anti-oxidant, and anti-microbial properties. In particular, the anti-microbial activity of natural extracts is more effective in inhibiting the growth of microorganisms due to various modes of action [5].

Therefore, we constructed research to develop plant-derived magnolia officinalis bark extract and heptyl undecylenate as multifunctional ingredients with various activities.

Materials and Methods.

<u>Materials</u>

All chemicals and solvent used were purchased from Daejung Chemical (Gyeonggi-do, Republic of Korea) or Sigma-Aldrich (St. Louis, MO, USA). The microbial strain used in this study were purchased from ThermoFisher Scientific Inc. (Waltham, MA, USA).

<u>Anti-microbial activity assay (agar well diffusion assay)</u>

As recommended by the 2013 Clinical and Laboratory Standards Institute guidelines, the concentration of the strain was adjusted to yield approximately 10^8 CFU ml^{-1} by direct colony suspension method. The microbial suspension was then evenly spread onto the agar surface. After that, 60 µL of the sample was pipetted onto a well. DMSO was used as a negative control. The inoculated agar plate of *S. aureus* was incubated at 35°C. The inoculated agar plate of *C. acnes* was incubated at 35°C under anaerobic conditions. In contrast, the inoculated agar plate of yeast (*C. albicans*) and mold (*A. brasiliensis*, *T. rubrum*) were incubated at 25°C.

<u>Growth inhibitory effect against *C. acnes*</u>

In order to investigate growth inhibitory effect of MEHU against *C. acnes*, modified preservative efficacy test was conducted. The preservative efficacy test (PET), also known as the challenge test, determines the effectiveness of the antimicrobial agent by calculating the logarithm of the number of viable microorganisms after microbial contamination of the product. In this study, the preservative efficacy test (PET) used PCPC (Personal Care Products Council) guidelines test method. *C. acnes* ATCC 6919 used for artificial contamination was inoculated to be 10^6 CFU g^{-1}. The preservative efficacy was determined by sampling 1 g from the formulation at each time-point.

<u>Inhibition of NO production assay</u>

RAW 264.7 macrophages at a density of 10^5 cells $well^{-1}$ were treated with the test sample in the presence of LPS (1 μg mL^{-1}) for 24h. Quercetin (25 μg mL^{-1}) was used as a positive control. The conditioned culture media was the collected using centrifuged. The supernatant was measured for nitrite levels by the Griess assay. The absorbance was performed at 550 nm to assess the NO production inhibition rate using the equation below:

$$\text{The NO production inhibition rate (\%)} = (N – S) / (N – C) \times 100$$

Where N is the absorbance of the negative control with LPS, C is the absorbance of the control without the LPS, S is the absorbance of the test sample with the LPS.

<u>Sensory analysis</u>

Sensory analysis was performed with 10 volunteers aged 20~50 years (5 male and 5 female). Oil balm containing MEHU was available to the volunteers that were oriented to apply on the back of the left hand in circular movement. Then, a questionnaire related to spreadability, absorption, moisturizing, film formation, feeling-after-use, and irritation was made available. Oil balm without MEHU and oil balm containing silicone oils (dimethicone) were used as a comparative group.

<u>Analysis of skin irritation (*in vivo*)</u>

In order to investigate the skin irritation of the magnolia officinalis bark extract and heptyl undecylenate complex (MEHU), patch test was conducted. The application of the MEHU was performed on the back, between the hips and the shoulders, free from any macroscopic trace of irritation or from any abnormality (scars, moles, freckles) which could interfere with the interpretation of the results. The patches were applied to the back of the subjects for 24 hours. Grading was performed at 30 min and 24 hours after patch removal, to the naked eye and a magnifying glass. For data analysis, the calculation of the Primary Cutaneous Irritation index (P.C.I.), calculated for 30 min and 24 hours allowed for determining the MEHU compatibility with the skin.

Results.

Antimicrobial activity of magnolia officinalis bark extract

To evaluate the anti-microbial activity, the agar well diffusion assay was performed. The degree of anti-microbial activity was shown by measuring the diameter of the inhibition zone formed on the plate (Fig. 1, Table 1). According to analysis results, magnolia officinalis bark extract was strongly antimicrobial and antifungal activity against skin-related microorganisms, such as *Staphylococcus aureus*, *Cutibacterium acnes*, *Candida albicans*, *Aspergillus brasiliensis*, and *Trichophyton rubrum*. It was found that the extract showed the strongest inhibitory effect against *C. acnes*, followed by *S. aureus*. In addition, concentrations greater than or equal to 0.1% of magnolia officinalis bark extract completely inhibited the growth of gram-positive bacteria, yeast and mold, *S. aureus* and *C. albicans*, whereas greater than or equal to 0.02% of magnolia officinalis bark extract inhibited the growth. However, it did not show an inhibitory effect on gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*).

Anti-*C. acnes* activity of MEHU

The antibacterial activity of salicylic acid (SA) and *Centella asiatica* extract (CE), commonly used ingredients for acne treatment, as compared with complex of magnolia officinalis bark extract and heptyl undecylenate (MEHU). To evaluate the anti-microbial activity of *C. acnes* (*C. acnes* ATCC 6919), an agar well diffusion assay was performed. The degree of anti-microbial activity was shown by measuring the diameter of the inhibition zone formed on the plate (Fig. 2, Table 2). In this study, the concentrations of SA, CE, and MEHU required to inhibit cell growth of *C. acnes* were >0.5%, >2%, and 0.6%, respectively.

To evaluated the growth inhibitory effect of MEHU against *C. acnes* in oil balm. For analysis, *C. acnes* was contaminated with more than 10^6 CFU g^{-1}, and viable *C. acnes* was accumulated on days 1, 2, 3, 7, 14, 21, and 28 (Table 3). As a result, *C. acne*, which was contaminated in the oil balm containing 1~3% MEHU, was inhibited by 99.999% after 2 days. In contrast, negative control without MEHU showed low inhibitory effect against *C. acnes* for 28 days.

Anti-inflammation activity of MEHU

In order to investigate the effect of the MEHU on cell viability using the MTT assay. Treatment of RAW 264.7 cells stimulated by LPS (1 μg mL^{-1}) with the MEHU at a concentration of 0.0125% ~ 0.05% exerted no effect on cell viability as shown in Figure 3. Therefore, the MEHU was used at a concentration of 0.0125 ~ 0.05% to investigate its effect on inflammation induced by LPS (1 μg mL^{-1}) in RAW 264.7 cells (Fig. 3). The rate of NO production inhibition exhibited a statistically significant ($p < 0.05$) decrease following stimulation of the cell by LPS. The MEHU at a concentration of 0.05% accomplished a statistically significant increase ($p < 0.05$) of NO production inhibition activity in cells stimulated by LPS (63.30 ± 0.29%) compared with the LPS treated cells group.

Effect of MEHU on sensory profile improvement

We conducted a blind sensory test to compare the spreadability, absorption, moisturising, film formation, and feeling of MEHU and silicone oil (Fig 4). The volunteers were guided to choose between 1 to 5 (very good to very bad) which sensory experience they had with the oil balm. As showed on the sensory map of oil balm containing MEHU presented, all parameters evaluated showed good evaluation compared oil balm without MEHU. Spreadability, absorption, moisturizing and film formation were improved compared to placebo oil balm. In addition, a similar level of feeling-after-use was confirmed as compared to the same amount of silicone oils (dimethicone).

Safe of MEHU

To verify the good cutaneous compatibility of the MEHU, after single application, patch test was conducted on 33 adult volunteer aged 20-60s [6]. The skin irritation analysis of MEHU was compared with squalene. As a result, MEHU was a no-irritant and safe ingredient even at a concentration of 100% (Table 4). Therefore, MEHU was very low irritation compared to the commonly used preservative boosting ingredients such as 1,2-alkanediol or acne treat ingredients such as salicylic acid.

Discussion.

Magnolia officinalis bark extract is an important herb that contains diverse biological active compounds, including magnolol and honokiol. According to results of anti-microbial activity against various microorganisms, magnolia officinalis bark extract was strongly antimicrobial and antifungal activity (Fig. 1, Table 1). Especially, *C. acnes* and *S. aureus* are the Gram-positive bacteria that are mostly related to promoting follicular inflammation or inflammatory acne. In this study, the anti-acne bacterial properties of magnolia officinalis bark extract were screened by using agar well diffusion assay.

Although the antibacterial activity against gram negative bacteria such as *E. coli* and *P. aeruginosa*, the antimicrobial activity against gram positive bacteria, yeast, and mold was excellent. Therefore, if used with other ingredients with high antibacterial activity against gram negative bacteria, this extract can potentially be used as an antimicrobial agent for personal care products.

Acne is a chronic inflammatory disease outbreak in the sebaceous glands within the hair follicle. The proliferation of *Cutibacterium acnes* (formerly known as *Propionibacterium acnes*) causes monocytes to stimulate the secretion of inflammatory cytokines. *C. acnes* hydrolyses neutral lipids into free fatty acids, which promotes oxidative stress, inflammatory reaction, and tissue destruction. *C. acnes* also interacts with various components of the immune system. In addition, the aerobic bacteria *Staphylococcus aureus* proliferates in acne lesions and causes inflammatory skin diseases. SA, which is generally used less than 0.5% due to skin dryness and irritation as an ingredient in personal care products for acne treatment, showed no anti-*C. acnes* effect at 0.5% concentration. In addition, the anti-*C. acnes* activity of CE (less than 2%) was not detected. In contrast, MEHU was inhibited the cell growth of *C. acnes* at 0.6% concentration (Fig. 2, Table 2). In this study, MEHU was able to effectively inhibit *C. acnes* at a low concentration without causing side effects such as skin dryness or skin irritation. In addition, oil balm applied MEHU has powerful protect ability against contaminated *C. acnes* (Table 3). It is expected that effective acne care products can be developed by applying MEHU to the products.

Inflammation is one of the mechanisms and aspects that induce acne formation. Normal sebocytes, the major cell of sebaceous glands, produce a low concentration of NO and TNF-α, but these cytokines significantly increased when activated by *C. acnes* or LPS. According to previous studies, inflammatory cytokines can promote the emergence of skin adhesion molecules and hypersensitivity response with the production of protease, hyaluronidase, and chemotactic factors [7]. According to the results, MEHU inhibits the NO production in RAW 264.7 cells stimulated by LPS (Fig. 3). Therefore, MEHU has anti-inflammatory effects induced by *C. acnes*.

Silicone oil is one of the most widely used ingredients in personal care products. Silicone oils, such as dimethicone, offer excellent spreading properties and give end-use products a soft, pleasant and non-sticky after-feel. Silicones have exceptional physicochemical and sensory properties but their high chemical stability results in very low biodegradability. Consumers are becoming increasingly sensitive to environmental issues and demand more environmentally friendly personal care products. This recognition strongly encourages the personal care products industries to develop plant derived biodegradable alternatives to silicone oils [8]. In the sensory comparison analysis, MEHU showed performance attributes required for enhancing the sensory profile of oil balm formulation (Fig 4). Therefore, these sensory results show that MEHU can be used as plant derived biodegradable ingredient to replace synthetic silicone oils.

Conclusion.

In conclusion, examined MEHU inhibits the growth of microorganisms including fungi and acne causing bacteria. In addition, MEHU showed excellent anti-inflammatory effects induced by *C. acnes*. Therefore, MEHU can be recommended as a candidate for effective an alternative preservative system and also as an anti-acne ingredient. MEHU can be simultaneously replaced the use of synthetic preservatives or silicone oils. It was analyzed by sensory test and patch test which leads to an excellent feeling-after-use and no-irritation, respectively. These interesting results demonstrate that MEHU is a new multifunctional ingredient for personal care products that is effective as an anti-acne and this can be feasible to resolve the sensitive skin issues occurring by the mask regularly used in COVID-19 situations.

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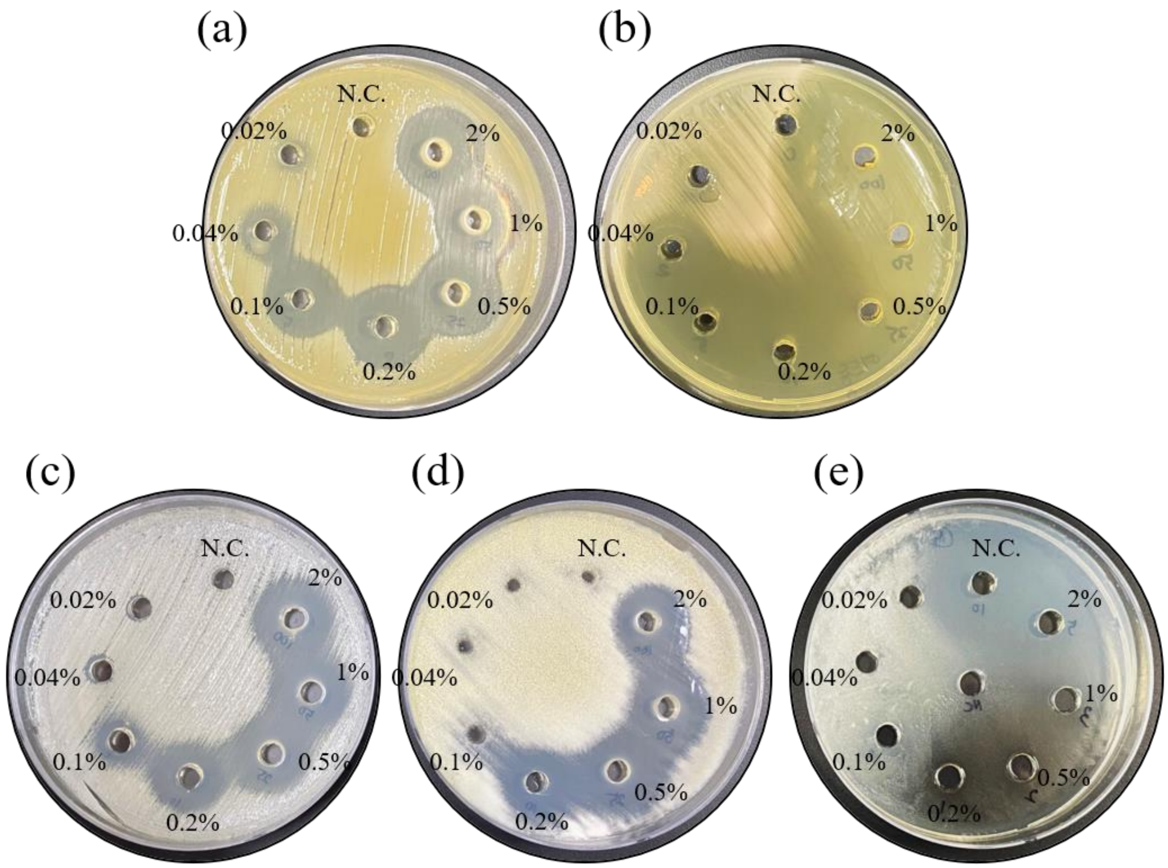

Fig. 1. Inhibition zone of magnolia officinalis bark extract against various microorganisms.

(a) *Staphylococcus aureus* ; (b) *Cutibacterium acnes*; (c) *Candida albicans*; (d) *Aspergillus brasiliensis*; (e) *Trichophyton rubrum*. N.C., negative control with DMSO.

Table 1. Diameter of the inhibition zone against various microorganisms.

Magnolia officinalis bark extract conc. (%, w/w)	Inhibition zone size (mm)				
	Bacteria		Yeast	Mold	
	Gram (+)				
	S. aureus	*C. acnes*	*C. albicans*	*A. brasiliensis*	*T.rubrum*
2.00	22	>30	19	20	30
1.00	22	>30	19	20	30
0.50	22	>30	18	20	20
0.20	21	>30	16	17	20
0.10	18	>30	10	8	15
0.04	11	18	8	-	-
0.02	8	-	7	-	-

Well size., 6 mm; -., no antimicrobial activity

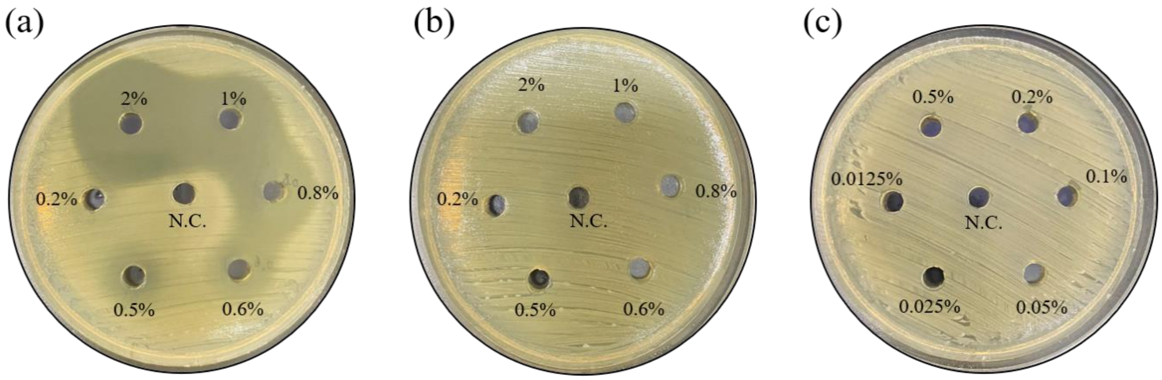

Fig. 2. Inhibition zone of MEHU, *Centella asiatica* extract, and salicylic acid against *Cutibacterium acnes*.

(a) MEHU; (b) *Centella asiatica* extract; (c) Salicylic acid. N.C., negative control with DMSO.

Table 2. Diameter of the inhibition zone against *Cutibacterium acnes*.

Inhibition zone size (mm)				
Sample conc. (%, w/w)	Magnolia officinalis bark extract	Centella asiatica extract	Sample conc. (%, w/w)	Salicylic acid
2.0	32	-	0.5	-
1.0	22	-	0.2	-
0.8	18	-	0.1	-
0.6	10	-	0.05	-
0.5	-	-	0.025	-
0.2	-	-	0.0125	-

Well size., 6 mm; -., no antimicrobial activity

Table 3. The growth inhibition effect of MEHU against *C. acnes* in oil balm.

		Log reduction (CFU/g)							
		Day 0 (innoculation)	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28
MEHU conc. (%, w/w)	1%	6.5	0.5	6.5	6.5	6.5	6.5	6.5	6.5
	2%	6.5	0.6	6.5	6.5	6.5	6.5	6.5	6.5
	3%	6.5	1.0	6.5	6.5	6.5	6.5	6.5	6.5
Without MEHU		6.5	0.1	0.5	0.4	0.5	0.7	1.0	1.6

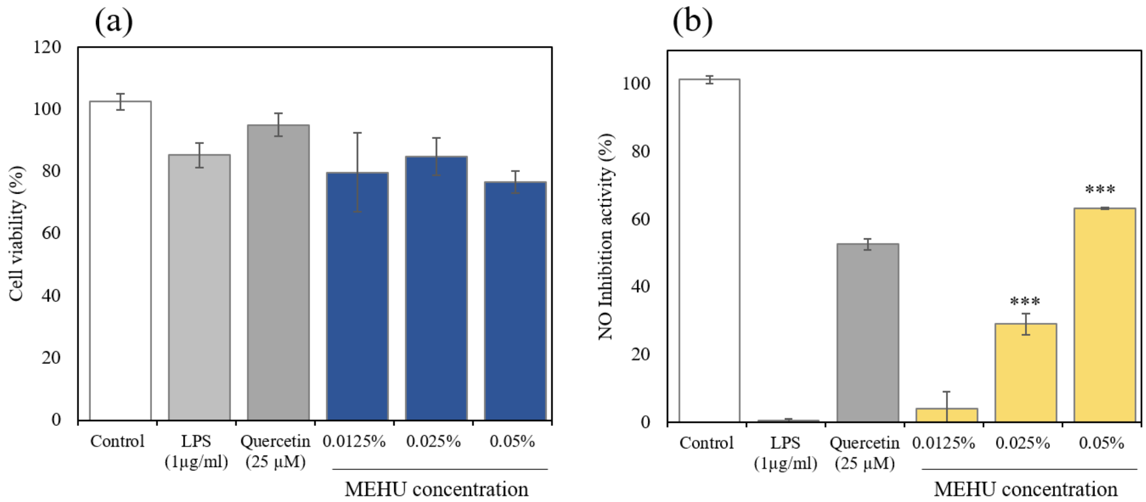

Fig. 3. Anti-inflammation activity of MEHU.

(a) MTT assay of MEHU; (b) NO production inhibition activity of MEHU. ***$p < 0.005$

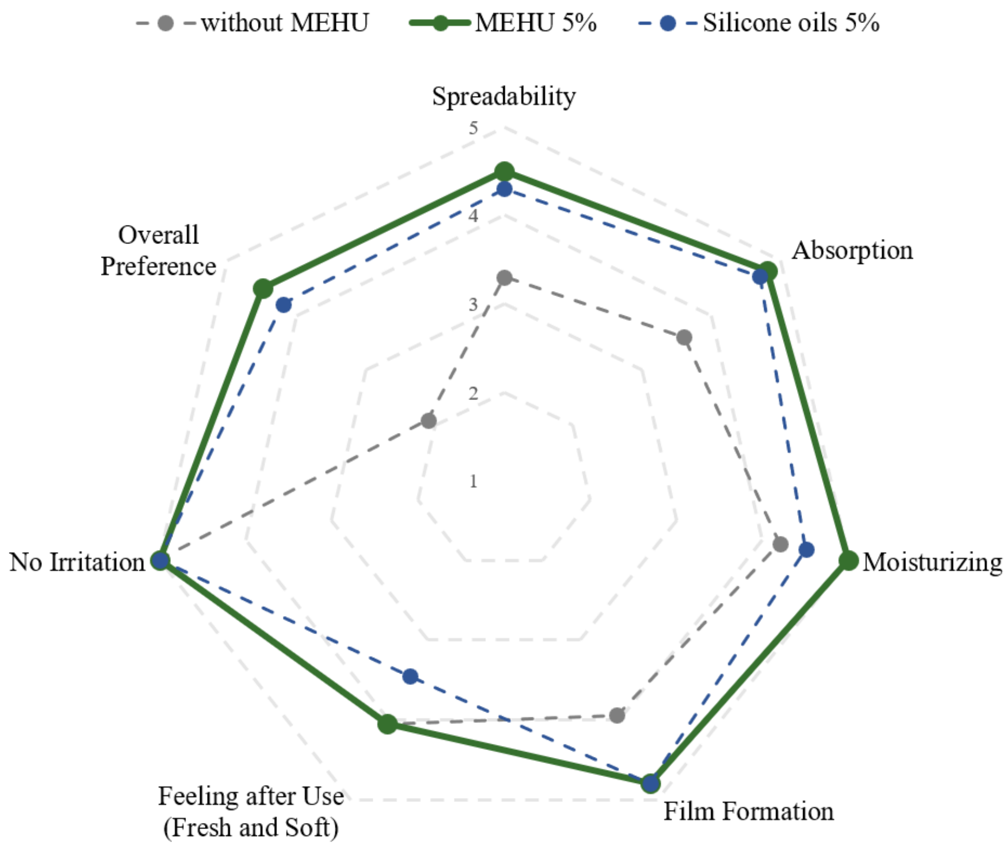

Fig. 4. Sensory profile obtained using volunteers questionnaires.

Table 4. The skin irritation analysis of MEHU.

		P.C.I.	Conclusion
MEHU conc. (%, w/w)	2% (in squalane)	0.00	No-irritant
	10% (in squalane)	0.00	No-irritant
	100%	0.02	No-irritant
Squalane		0.00	No-irritant