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"Cosmetic composition for skin improvment comprising a novel biopolymer PHA microparticle derived from microbial strain"

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1. Introduction

Polyhydroxyalkanoates (PHA) are biodegradable polymers synthesized by microorganisms under restricted nutrient and excess carbon conditions. PHA, considered an ideal substitute for synthetic plastics, finds applications in packaging and biomedical fields.

The purpose of this study is to develop a biodegradable PHA new material that can replace microplastics related to marine pollution and to inform that it is an eco-friendly new material with excellent skin improvement effects through human efficacy evaluation.

In addition, by replacing PMMA materials, which are existing microplastic fine particle cosmetic materials, it is a new bio-new material that is safer for the skin and can improve the degree of skin improvement, and can be used in various skin improvement cosmetics.

This study is related to the development of eco-friendly new materials for various skin improvement in cosmetics by developing mass production of PHA from Halomonas xianhensis HN-1-3-2, a patented microbial strain.

Through this study, it was found that biodegradable PHA new materials can be produced from Halomonas xianhensis HN-1-3-2, a patented microbial strain, and can replace PMMA, Silica, Nylone Pwder, and Silcone Powder materials used in cosmetics, and can have a better spreadability, a better skin gloss expression function, a better selective sebum control function, and a better soft focus function.

In particular, it was found that the skin applicability of the PHA new material can be improved by first coating the PHA new material with polyurethane material, and the sebum adsorption effect can be enhanced by second coating with Trihydroxystearin, Polyglyceryl-10 stearate,

etc., and the skin flexibility can be enhanced and the soft focus effect can be improved by second coating with Hydrogenated Olive Oil Lauryl Esters, Jojoba Esters, Calendula Officinalis Flower Oil, etc.

2. Materials and Methods

2-1. Polyhydroxyalkanoate (PHA) is produced through fermentation of Halomonas xianhensis using glucose as the primary carbon source. The process involves harvesting, cell disruption, PHA recovery, and purification. PHA is a fully biodegradable polymer produced from renewable resources, making it an environmentally sustainable alternative to conventional petrochemical-based polymers. The microbial strain used for production is Halomonas xianhensis.

① Strain Information and Medium Composition

- Strain: Microorganism belonging to the genus *Halomonas*
- Medium Composition: Glucose 10 ~ 30 g/L, Artificial sea salt (Aquarium systems, Instant Ocean, USA) 30 g/L, Yeast extract 0.5 ~ 3 g/L, Soy peptone 3 ~ 7.5 g/L

② The recovery process can be carried out using a centrifuge or a microfiltration device and may include a washing step utilizing a washing solution during the recovery stage.

- Washing solution: Distilled water serves as the primary solvent, while auxiliary agents, including but not limited to, NaOH solution (≤ 0.5 M), NaClO solution (≤ 1%), ethyl alcohol (10-20%), and $NaHSO_3$ (≤ 200 ppm), can be used.

③ Cell disruption can be performed through either physical or chemical methods.

- Physical cell disruption can be conducted using a high-pressure homogenizer (HPH) or an ultra sonicator. The buffer solution used in this process can include, but is not limited to, distilled water, NaOH solution (≤ 0.2 M), and EDTA solution (≤ 2 mM).
- Chemical cell disruption can be carried out using a buffer solution comprising NaOH solution (≤ 0.5 M), SDS (≤ 0.5 M), and NaClO solution (≤ 3%).

2-2. Test materials are used Polyhydroxyalkanoate (PHA), Polyhydroxyalkanoate with wax coating (PHA(S)) and Polyhydroxyalkanoate with wax coating (PHA(C)).

① Polyhydroxyalkanoate (PHA) is used through 100% Polyhydroxyalkanoate.

② Polyhydroxyalkanoate with wax coating (PHA(S)) is produced through 85% Polyhydroxyalkanoate (PHA) and 15% waxs (Trihydroxystearin, Polyurethane-15, Polyglyceryl-10 stearate)

③ Polyhydroxyalkanoate with wax coating (PHA(C)) is produced through 80% Polyhydroxyalkanoate (PHA) and 20% waxs & oils (Trihydroxystearin, Polyurethane-15, Polyglyceryl-10 stearate, Hydrogenated Olive Oil Lauryl Esters, Jojoba Esters, Calendula Officinalis Flower Oil)

2-3. Comparison materials are used PolymethylMethacrylate (PMMA-S), Methyl Methacrylate copolymer (PMMA-P) and Polymethylsilsesquioxane.

2-4. Human application test on skin tone of PHA

A total of 21 healthy Korean women volunteers (average age 49.762) were participated in the study in 02 ~ 03 December 2024. Skin tone was measured using VISIA-CR (Canfield Imaging Systems, USA) on the same facial area of the test subject before and after using the test product. The same left and right cheek areas of the captured images (Standard 2 mode) were analyzed for skin brightness (L*) values using Image-pro® plus (Media Cybernetics, USA). An increase in the analysis value indicates an improvement in skin tone. In order to determine the significance of the measurement values before and after using the test product, the statistical analysis program SPSS was used. Significance was confirmed when the significance probability was $p<0.05$ at the 95% confidence interval, and the significance probability was rounded to the third decimal place [1, 2, 3, 4, 5, 6, 7, 8, 9].

2-5. Human application test on sebum control effect of PHA(S)
A total of 21 healthy Korean women volunteers (average age 51.524) were participated in the study in 02 ~ 03 December 2024. Sebum was measured on the same cheek area of the test subjects before and after using the test and control products using Sebumeter SM815 (Courage+Khazaka electronic GmbH, Germany). The analysis parameter is ㎍/㎠ value, and a decrease in the value indicates a sebum improvement effect. In order to determine the significance of the measurement values before and after using the test product, the statistical analysis program SPSS was used. Significance was confirmed when the significance probability was $p<0.05$ at the 95% confidence interval, and the significance probability was rounded to the third decimal place [10, 11].

2-6. Human application test on wrinkles around the eyes of PHA(C)
A total of 21 healthy Korean women volunteers (average age 51.524) were participated in the study in 02 ~ 03 December 2024. Wrinkles around the eyes were measured on the same area around both eyes of the test subjects before and after using the test and control products using PRIMOS-CR Small Field (Canfield Imaging Systems, USA). The analysis parameter is the Ra (㎛) value, and a decrease in the value indicates an improvement in wrinkles around the eyes. In order to determine the significance of the measurement values before and after using the test product, the statistical analysis program SPSS was used. Significance was confirmed when the significance probability was $p<0.05$ at the 95% confidence interval, and the significance probability was rounded to the third decimal place.

2-7. Primary irritation test on human skin of PHA, PHA(S), and PHA(C)
A total of 30 healthy Korean volunteers (average age 45.033) were participated in the study in 04 ~ 06 December 2024. Skin irritation test (before, after 1 hour, 24 hours) was evaluated on the back area using the patch test methods(International Contact Dermatitis Research Group: ICDRG), expressed in grade of Mean score (grade 1: non-irritant (Mean Score 0.00~0.25), grade 2: slight irritant (Mean Score 0.26~1.00), grade 3: irritant (Mean Score 1.01~2.50), grade 4: severe irritant (Mean Score 2.51~4.00) [12, 13, 14, 15, 16].

2-8. Primary irritation test on human skin targeting sensitive skin of PHA, PHA(S), and PHA(C)
A total of 32 healthy Korean volunteers (average age 43.813) were participated in the study in 04 ~ 06 December 2024. First, a skin irritation test is conducted on a panel of selected subjects through the Lactic Acid Sting Test.
Lactic Acid Sting Test is conducted as dollows.
① The test was performed immediately after the hydration process in which a steam towel was applied to the face for 5 minutes while the subject was lying down comfortably.
② 50㎕ of 10% lactic acid was applied to the micropipette, dropped onto the nasolabial fold area, and gently rubbed with a cotton swab.
③ 1 minute after application, the test subjects were asked to evaluate their subjective stinging sensation on a scale of 0 to 3. (0=none, 1=weak, 2=moderate, 3=severe)
④ If a reaction of 1 or higher was observed even once, it was judged as a positive reaction.

3. Results

This study revealed that biodegradable PHA new materials can be produced from Halomonas xianhensis HN-1-3-2, a patented microbial strain, and can replace PMMA, Silcone Powder

materials used in cosmetics, and can have a better spreadability, a better skin gloss expression function, a better selective sebum control function, and a better soft focus function.

3.1. The Result of Polyhydroxyalkanoate (PHA) accumulated in the cells of H. xianhensis HN-1-3-2 strain in the form shown in Figure 1. Spherical PHA particles of various sizes were observed in the cytoplasm, with particle diameters ranging from 0.3 μm to a maximum of 1 μm, and these particles existed in a clearly distinguishable form in the cytoplasm (Figure 1).

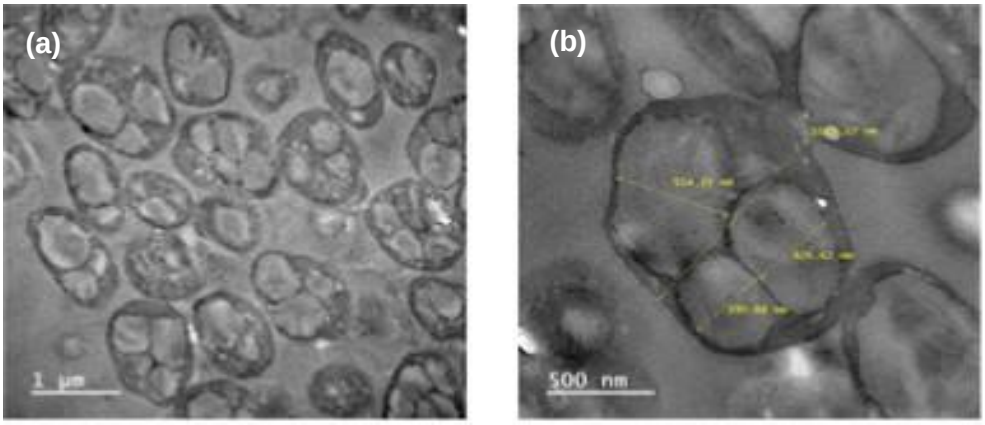

Figure 1. Cell growth and PHA production by *H. xianhensis* (Strain A) (a) Transmission electron microscope (TEM) pictures of 1 μm size. (b) Transmission electron microscope (TEM) pictures of 500 nm size surrounded by a homogeneous membrane.

3.2. The SEM analysis results showed that the extracted PHA was spherical in shape and ranged from 0.19 μm to 0.8 μm. This suggests that PHA accumulates in a fine spherical shape within the cell and maintains that shape during the extraction process. PHA formed into spherical particles is likely to be commercialized without a separate molding process. This can contribute to cost reduction and process simplification since it can be manufactured into a desired shape without using complex machines required for conventional plastic molding processes (extrusion, injection, etc.). In addition, spherical PHA particles have good fluidity, excellent processability, and can be easily applied to various polymer blends. This is expected to be advantageous for the mass production of PHA-based products in the future (Figure 2).

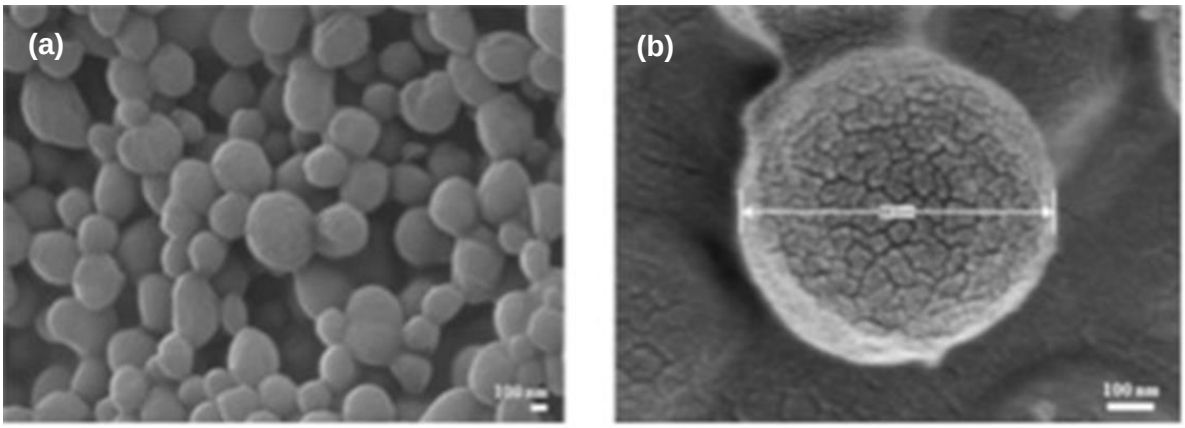

Figure 2. Characterization of the extracted PHA. (a) Analysis of the particle morphology of extracted PHA using Scanning Electron Microscope (SEM) through 30,000x, (b) Analysis of the particle morphology of extracted PHA using Scanning Electron Microscope (SEM) through 100,000x magnification images.

3-3. The Result of Human application test on skin tone of PHA and PMMA-S

The test product, Polyhydroxyalkanoate (PHA), is judged to be a product that helps improve skin tone compared to PolymethylMethacrylate (PMMA-S) with one use. Statistically significant improvement immediately after use compared to before use and in Inter-group comparison.

The improvement rate of skin tone was significantly increased immediately after use ($p<0.05$) (Table 1, Figure 3). The mean improvement rate of skin tone increased 0.826% for cosmetic with PHA (from 72.621 to 73.221, $p = 0.000$) and 0.401% for cosmetic with PMMA-S (from 72.572 to 72.863, $p = 0.000$). It was found that PHA was 2.05 times more effective than PMMA-S.

Table 1. Results of Human application test on skin tone of PHA and PMMA-S

Test-1		Skin tone measurement results, L*		Improvement rate(%)	*p*-value	
		before	Immediately after use		Intra-military comparison	Inter-military comparison
Comparison material	PMMA-S (PolymethylMethacrylate)	72.572 ± 2.005	72.863 ± 1.984	0.401	0.000#	0.022₸
Test material	Polyhydroxyalkanoates (PHA 100%)	72.621 ± 1.692	73.221 ± 1.764	0.826	0.000#	

※ Improvement rate(%)= | (after-before) | /before*100

: p<0.05 by Wilcoxon signed rank test, ₸ : p<0.05 by repeated measures ANOVA

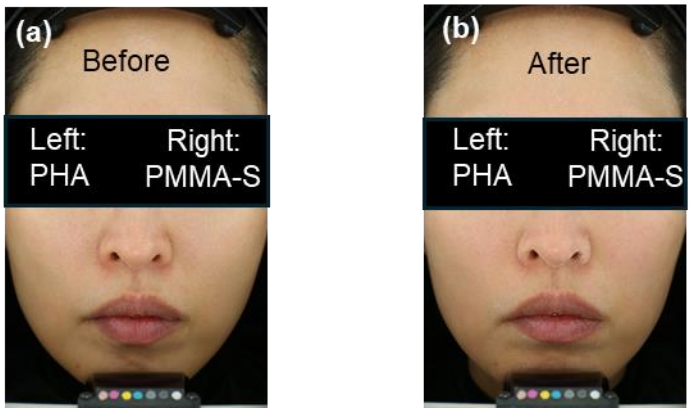

Figure 3. Skin tone measurement results after using cosmetics with PHA(left) and PMMA-S(right) (a) Before image of skin tone taken with VISIA-CR (Canfield Imaging Systems, USA) (b) After image of skin tone taken with VISIA-CR (Canfield Imaging Systems, USA).

3-4. The Result of Human application test on sebum control effect of PHA(S) and PMMA-P

The test product, Polyhydroxyalkanoates with wax coating PHA(S) is judged to be a product that helps improve sebum control effect compared to Methyl Methacrylate copolymer (PMMA-P) with one use. The improvement rate of sebum control effect was significantly increased immediately after use ($p<0.05$) (Table 2). The mean improvement rate of sebum control effect increased 92.606% for cosmetic with PHA(S) (from 74.714 to 5.524, $p = 0.000$) and 77.643%

for cosmetic with PMMA-P (from 74.333 to 16.619, p = 0.000). It was found that PHA(S) was 1.19 times more effective than PMMA-P.

Table 2. Results of Human application test on sebum control of PHA(S) and PMMA-P

Test-2		Sebum amount measurement results, μg/㎠		Improvement rate(%)	p-value	
		before	Immediately after use		Intra-military comparison	Inter-military comparison
Comparison material	PMMA-P (Methyl Methacrylate copolymer)	74.333 ± 16.619	16.619 ± 5.962	77.643	0.000*	0.048‡‡
Test material	Polyhydroxyalkanoates with wax coating (PHA 85%, Trihydroxystearin, Polyurethane-15, Polyglyceryl-10 stearate)	74.714 ± 22.696	5.524 ± 2.462	92.606	0.000#	

※ Improvement rate(%)= | (after-before) | / before*100
: p<0.05 by Wilcoxon signed rank test, * : p<0.05 by Paired t-test, ‡‡ : p<0.05 by Generalized Estimation Equation

3-5. The Result of Human application test on wrinkles around the eyes of PHA(C) and Polymethylsilsesquioxane

The test product, Polyhydroxyalkanoates with wax & oils coating PHA(C) is judged to be a product that helps improve wrinkle decreasing effect to Polymethylsilsesquioxane with one use. The improvement rate of wrinkle decreasing effect was significantly increased immediately after use (p<0.05) (Table 3). The mean improvement rate of wrinkle decreasing effect increased 9.761% for cosmetic with PHA(C) (from 22.416 to 20.228, p = 0.000) and 4.289% for cosmetic with Polymethylsilsesquioxane (from 22.404 to 21.443, p = 0.000). It was found that PHA(C) was 2.27 times more effective than Polymethylsilsesquioxane.

Table 3. Results of Human application test on wrinkles around the eyes of PHA(C) and Polymethylsilsesquioxane

Test-3		Results of eye wrinkle measurement, Ra(㎛)		Improvement rate(%)	p-value	
		before	Immediately after use		Intra-military comparison	Inter-military comparison
Comparison material	Polymethylsilsesquioxane	22.404± 3.941	21.443± 3.949	4.289	0.005#	0.021‡‡
Test material	Polyhydroxyalkanoates with wax & oil coating (PHA 80%, Trihydroxystearin, Polyurethane-15, Polyglyceryl-10 stearate, Hydrogenated Olive Oil Lauryl Esters, Jojoba Esters, Calendula Officinalis Flower Oil)	22.416 ± 4.361	20.228 ± 3.962	9.761	0.000#	

※ Improvement rate(%)= | (after-before) | / before*100
: p<0.05 by Wilcoxon signed rank test, ‡‡ : p<0.05 by Generalized Estimation Equation

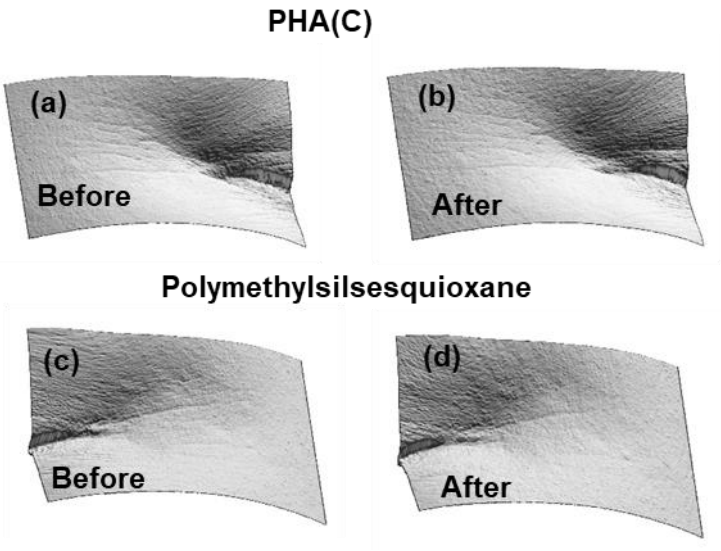

Figure 4**. Eye wrinkle decreasing effect measurement results taken with PRIMOS-CR Small Field(Canfield Imaging Systems, USA) after using cosmetics with PHA(C) and Polymethylsilsesquioxane (a) Before image of eye using PHA(C), (b) After image using PHA(C), (c) Before image of eye using Polymethylsilsesquioxane (d) After image using Polymethylsilsesquioxane**

3-6. The Result Primary irritation test on human skin of PHA, PHA(S), PHA(C), PMMA-S, PMMA-P, and Polymethylsilsesquioxane.
In the 24 hours occlusive patch test, the mean skin reaction score of the tested of PHA, PHA(S), PHA(C), PMMA-S, PMMA-P, and Polymethylsilsesquioxane was shown in Table 4. PHA, PHA(S), and PHA(C) show that they can be used safely on the skin without irritation.

Table 4. Results of human patch test

No.	Sample name	Mean score	Grade
1	Polyhydroxyalkanoates (PHA)	0.00	non-irritant
2	Polyhydroxyalkanoates with wax coating (PHA(S))	0.01	non-irritant
3	Polyhydroxyalkanoates with wax & oil coating (PHA(C))	0.00	non-irritant
4	PolymethylMethacrylate (PMMA-S)	0.01	non-irritant
5	Methyl Methacrylate copolymer (PMMA-P)	0.00	non-irritant
6	Polymethylsilsesquioxane	0.00	non-irritant

3-7. The Result Primary irritation test on targeting sensitive skin of PHA, PHA(S), PHA(C), PMMA-S, PMMA-P, and Polymethylsilsesquioxane.
In the 24 hours occlusive patch test, the mean skin reaction score of the tested of PHA, PHA(S), PHA(C), PMMA-S, PMMA-P, and Polymethylsilsesquioxane was shown in Table 5. PHA, PHA(S), and PHA(C) show that they can be used safely on the skin without irritation.

Table 5. Results of Primary irritation test on targeting sensitive skin of PHA, PHA(S), PHA(C), PMMA-S, PMMA-P, and Polymethylsilsesquioxane.

No.	Sample name	Mean score	Grade
1	Polyhydroxyalkanoates (PHA)	0.01	non-irritant
2	Polyhydroxyalkanoates with wax coating (PHA(S))	0.01	non-irritant
3	Polyhydroxyalkanoates with wax & oil coating (PHA(C))	0.01	non-irritant
4	PolymethylMethacrylate (PMMA-S)	0.01	non-irritant
5	Methyl Methacrylate copolymer (PMMA-P)	0.01	non-irritant
6	Polymethylsilsesquioxane	0.01	non-irritant

4. Discussion

Polyhydroxyalkanoates (PHAs) are biodegradable polymers synthesized by microorganisms under restricted nutrient and excess carbon conditions. In our study, PHA derived from *H. xianhensis* is synthesized in the form of relatively spherical fine particles without additional shaping processing. Thus, *H. xianhensis* offers a novel PHA manufacturing platform by generating diverse nano- and micro-particles (500~2000 nm) within cells, applicable for biomedical and cosmetic purposes. In particular, it was found that the skin applicability of the PHA new material can be improved by first coating the PHA new material with polyurethane material, and the sebum adsorption effect can be enhanced by second coating with Trihydroxystearin, Polyglyceryl-10 stearate, etc., and the skin flexibility can be enhanced and the soft focus effect can be improved by second coating with Hydrogenated Olive Oil Lauryl Esters, Jojoba Esters, Calendula Officinalis Flower Oil, etc.

5. Conclusion

This study is related to the development of eco-friendly new materials for various skin improvement in cosmetics by developing mass production of PHA from Halomonas xianhensis HN-1-3-2, a patented microbial strain. Through this study, it was found that biodegradable PHA new materials can be produced from Halomonas xianhensis HN-1-3-2, a patented microbial strain, and can replace PMMA, Silica, Nylone Pwder, and Silcone Powder materials used in cosmetics, and can have a better spreadability, a better skin gloss expression function, a better selective sebum control function, and a better soft focus function.

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References.

1. Bjarnason B, Flosadóttir E, Fischer T. Objective non-invasive assessment of patch tests with the laser Doppler perfusion scanning technique. Contact Dermatitis. 1999;40(5):251-60.
2. Bornmyr S, Svensson H, Lilja B, Sundkvist G. Skin temperature changes and changes in skin blood flow monitored with laser Doppler flowmetry and imaging: a methodological study in normal humans. Clin Physiol. 1997;17(1):71-81.

3. Daly CH, Odland GF. Age-related changes in the mechanical properties of human skin. J Invest Dermatol. 1979;73(1):84-7.
4. Garrel C, Fontecave M. Nitric oxide: Chemistry and biology. Analysis of Free Radicals in Biological Systems. 1995;21-35.
5. Gilchrest BA. Skin aging and photoaging: an overview. J Am Acad Dermatol. 1989;21(3 Pt 2):610-3.
6. Hogg N. Pro-oxidant and antioxidant effects of nitric oxide. Analysis of Free Radicals in Biological Systems. 1995;37-49.
7. O'Donoghue MN. Cosmetics for the elderly. Dermatol Clin. 1991;9(1):29-34.
8. Ramos-e-Silva M, Celem LR, Ramos-e-Silva S, Fucci-da-Costa AP. Anti-aging cosmetics: facts and controversies. Clin Dermatol. 2013;31(6):750-8.
9. Rice-Evans CA, Burdon RH. Free radical damage and its control. Elsevier. 1994;28:131-151.
10. Thanaroat Timudom et al., Anti-Sebum Ecacy of Phyllanthus emblica L.(Emblica) Toner on Facial Skin. Apploed sciences. 2020;10;8193
11. P. Meetham et al. Development and clinical efficacy evaluation of anti-greasy green tea tonner on facial skin. Revista Brasileira de Farmacognosia. 2018;28; 214–217
12. Association of Food and Drug Officials of the United States. Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. USA, 1965.
13. Cosmetic, Toiletry and Fragrance Association. CTFA Safety Testing Guideline. USA, 1991.
14. Draize J, Woodard G, Calvery H. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharm. Exp. Ther. 1994;82:377-90.
15. Fischer T, Maibach H. Finn chamber patch test technique. Contact dermatitis. 1984;11(3):137-40.
16. Frosch PJ, Kligman AM. The soap chamber test. A new method for assessing the irritancy of soaps. J Am Acad Dermatol. 1979;1(1):35-41