pISSN 1226-2587
J. Soc. Cosmet. Sci. Korea eISSN 2288-9507
Vol. 44, No. 4, December 2018, 363-373 http://dx.doi.org/10.15230/SCSK.2018.44.4.363

화장품에서 1,3-부틸렌 글라이콜 및 알칸디올계 조성에 따른 방부력에 관한 연구

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(2018년 7월 28일 접수, 2018년 12월 4일 수정, 2018년 12월 19일 채택)

Preservative Efficacies according to the Composition of 1, 3-Butylene Glycol and Alkane Diols in Cosmetics

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(Received July 28, 2018; Revised December 4, 2018; Accepted December 19; 2018)

요 약: 최근 화장품에서 방부제로 사용되는 파라벤류는 인체 안전성에 대한 문제가 이슈화되고 있다. 따라서 본 연구에서는 파라벤류를 대체할 수 있는 방부시스템으로 1,3-butylene glycol, 1,2-hexanediol 및 1,2-pentanediol의 함량에 따른 방부력 효능을 평가하고자 하였다. 화장품 크림에 1,3-butylene glycol을 5 - 25% 사이의 농도로 첨가하였다. 1,3-Butylene glycol의 방부력은 Personal Care Products Council (CTFA)의 M-3 시험법으로 측정하였다. 알칸 디올계인 1,2-hexanediol 및 1,2-pentanediol도 유사한 방법으로 평가하였다. 1,3-Butylene glycol의 함량에 따른 방부력 평가 결과, 25%를 첨가한 크림 처방에서 모든 시험 균주에 대하여 방부력을 나타내었으며, phenoxyethanol 0.3%와 ethylhexylglycerin 0.1%가 혼합된 처방에서 방부력을 나타내었다. 방부제인 phenoxyethanol의 0.3% 함량을 대체할 수 있는 대체 방부제로 alkane diol계인 1,2-hexanediol과 1,2-pentanediol을 선정하여 방부력 평가를 진행하였다. 1,2-Hexanediol과 1,2-pentanediol의 조성에 따른 방부력 평가 결과, 1,2-hexanediol 1%와 1,2-pentanediol 1%의 혼합 처방에서 방부력을 나타내었다. 결과적으로 본 연구에서는 25%의 1,3-butylene glycol과 0.1%의 ethylhexylglycerin, 1%의 1,2-hexanediol 및 1%의 1,2-pentanediol의 처방은 가장 우수한 방부력을 나타냄을 입증하였다. 따라서 이러한 처방은 화장품에서 사용되어 안전성의 이슈가 되어온 파라벤류 방부제를 대체할 수 있는 가능성이 있음을 시사한다.

Abstract: In recent years, parabens used as preservatives in cosmetics have become a problem of human safety. Therefore, in this study, we tried to evaluate the preservative efficacy of 1,3-butylene glycol, 1,2-hexanediol, and 1,2-pentanediol as a preservative system to replace parabens. 1,3-Butylene glycol was added to cosmetic creams at a concentration of between 5 and 25%. The preservative efficacy of 1,3-butylene glycol was determined using a M-3 challenge test, as recommended by the Personal Care Products Council (formally CTFA). The alkane diols, such as 1,2-hexanediol and 1,2-pentanediol, were assessed in a similar manner. An evaluation of the preservative efficacy of 1,3-butylene glycol

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revealed that it was effective against all tested microbial strains at a concentration of 25%. We also investigated the efficacy of combinations of 0.3% phenoxyethanol and 0.1% ethylhexylglycerin. Finally, we tested the alkane diols, including 1,2-hexanediol and 1,2-pentanediol, as an alternative to the preservative 0.3% phenoxyethanol. Both 1% 1,2-hexanediol and 1% 1,2-pentanediol demonstrated preservative efficacy. Taken together, our study demonstrated that the formulation of 25% 1,3-butylene glycol and 0.1% ethylhexylglycerin, 1% 1,2-hexanediol, and 1% 1,2-pentanediol had the best preservative efficacy of the compositions tested. Thus, this study suggests that the formulation is a possibility of substituting parabens preservatives, which has been used in cosmetics and has become a safety issue.

Keywords: *skin barrier, microbiology, formulation, preservative efficacy, 1, 3-butylene glycol, alkane diols*

1. Introduction

The skin is the largest organ of the body and is directly exposed to the external environment. It is therefore vital to protecting the body from external hazards, such as ultraviolet rays (UV), microorganisms, and chemicals associated with smoking or pollution. Additionally, healthy human skin is colonized by commensal microorganisms which are *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Many yeasts also make up the skin microbiota, including *Candida albicans*. These are contribute to the immune system[1-4]. A normal microbiota is essential to skin, but overgrowth or invasion by harmful pathogens can lead to the skin becoming damaged and collapsed[1,5]. For example, the aerobic gram positive bacteria, *S. aureus* is typically found in the nose, axilla, and perineum of the skin in irregular clusters. However, excessive growth of *S. aureus* can lead to pustules forming in skin wounds and may cause furuncles (boils) or swelling of the skin surface[6]. In addition, *S. aureus* is found in less than 5% of normal skin, but in more than 90% of patients with atopic dermatitis, suggesting an association[7]. Gram negative bacteria, such as *E. coli* and *P. aeruginosa*, do not usually thrive in the dry environment of skin and are commonly found in humid conditions. In particular, immune-deficient patients can become infected with *Pseudomonas* spp.[8]. Finally, *C. albicans* is an opportunistic disease-causing yeast that can be found on skin and mucous membranes. It does not typically cause disease but if a health issue emerges that leads to a weakening of immunity, it can act as a pathogen and cause the disease candidiasis. This usually results in localized infections, such as thrush and female vaginitis, but can also cause systemic infections[9,10].

Cosmetics contain many ingredients, such as glycerin, sorbitol, amino acid derivatives, and proteins. Because of high water content of cosmetics, they are susceptible to colonization by microorganisms. In addition, cosmetics are generally stored at room temperature and often used repeatedly over long periods of time. This means they can be easily contaminated by airborne microbes or directly by consumers. If cosmetics are contaminated by microorganisms, it can result in skin infections and the value of an affected product will be decreased[11-14]. Therefore, it has become essential to use preservatives in cosmetics. The term ‘preservative’ refers to a component that acts to inhibit the growth and proliferation of microorganisms. There are several preservatives which already known as preservatives and commonly used in cosmetics such as parabens (methyl paraben), phenoxyethanol, isothiazole compounds (methylisothiazolinone), and imidazolidinyl urea. These ingredients are regulated by many global drug and safety administrations, including the Ministry Food and Drug Safety of Korea, as they can have safety complications, such as causing skin irritation[15-19]. For example, the parabens have long been used as preservatives in the cosmetic, food, and pharmaceutical industries due to a broad antimicrobial spectrum and high stability. However, some studies have suggested a link with breast cancer[20]. Contact dermatitis has also been reported as a side-effect of chloromethylisothiazolinone and methylisothiazolinone, commonly used as synthetic preserva-

Table 1. List of Strains and The Cultivation Conditions used for The Antimicrobial Experiments

Strains		Medium	Temperature (℃)	Time (h)
Gram (+) bacteria	*S. aureus* ATCC 6538	TSA[1)]	37	24
Gram (-) bacteria	*E. coli* ATCC 8739	TSA	37	24
	P. aeruginosa ATCC 9027	TSA	37	48
Yeast\Mold	*C. albicans* ATCC 10231	TSA	30	48
	A. niger ATCC 16404	PDA[2)]	30	72

[1)] TSA : Tryptic soy agar (Difco, USA) / [2)] PDA : Potato dextrose agar (Difco, USA)

tives[21]. That's the reason why research to identify alternative preservatives and preservative-free cosmetics have attracted increased attention[22]. Preservative-free cosmetics means that replaced preservative to components which have antibacterial activities but are not legislated as preservatives at Korea Cosmetic Ingredient.

The reagent 1,3-butylene glycol (1,3-BG) is a highly viscous, colorless, odorless, and transparent liquid. It has been used as a solvent for plant extracts and fragrances and can be found in wine, aged cheese, soil yeast, and sunflower oil. Due to its hygroscopicity, low volatility, antibacterial properties, and low toxicity with only mild irritation to skin, it is also commonly used as a moisturizer in cosmetics. Although many common glycols can be toxic to humans, 1,3-BG is minimally harmful and widely used. It has also been used as a carrier to evaluate antimicrobial activity. For example, in a comparison of the antimicrobial activity against six bacterial strains of 1,3-BG, propylene glycol, and hexylene glycol[23]. Other compounds are alkane diols include 1,2-pentanediol, 1,2-hexanediol, and 1,2-octanediol. These are all alkane diols that are used as moisturizing agents and viscosity modifiers in cosmetics. They can also reported to have low toxicity and some antimicrobial activity[24,25]. It has been reported that phenoxyethanol, a preservative, and 1,2-hexanediol and 1,2-octanediol, which are not classified as preservatives, are mixed in cosmetic formulations to evaluate the preservative efficacy[13]. However, an evaluation of the preservative efficacy of alkane diols in combination with 1,3-BG has not yet been performed for a potential use in cosmetics.

In this study, various cosmetic formulations were created with different compositions of 1,3-BG, 1,2-hexanediol, and 1,2-pentanediol. We then evaluated the preservative efficacies of these formulations against various microbes. As a result of this evaluation, we assess the possibility of constructing a preservative-free formulations for cosmetics based on using 1,3-BG and alkane diols.

2. Materials and Methods

2.1. Reagents

The solvents used in this study, including ethanol and DMSO, were commercially purchased from Daejung Chemicals & Metals Co. (Seoul, Korea). The preservative phenoxyethanol was acquired from The Dow Chemical Co. (Midland, Michigan, USA), while 1,3-BG and ethylhexylglycerin, tested as alternatives to preservatives, were sourced from Oxea (Oberhausen, North Rhine-Westphalia, Germany) and Schulke & Mayr GMBH (Norderstedt, Hamburg, Germany), respectively. Finally, 1,2-hexanediol and 1,2-pentanediol were produced by M. I. Pharm Co. (Seoul, Korea).

2.2. Strain, Culture Medium and Incubation

The strains used for the challenge test were the aerobic gram positive bacterium *S. aureus* (ATCC6538), two aerobic gram negative bacteria, *E. coli* (ATCC8739) and *P. aeruginosa* (ATCC9027), and two fungi, *C. albicans* (ATCC10231) and *A. niger* (ATCC16404). These five strains were provided by the Korean Culture Center of

Table 2. Formulations for The Testing of 1,3-BG

Components		Content (%)					
		NC[6]	A	B	C	D	E
Water phase	DW	66.68	61.68	56.68	51.68	46.68	41.68
	Glycerin	5	5	5	5	5	5
	1,3-BG	-	5	10	15	20	25
	EDTA-2Na	0.02	0.02	0.02	0.02	0.02	0.02
	Tromethamine	0.2	0.2	0.2	0.2	0.2	0.2
	Carbopol ETD 2020 2%	5	5	5	5	5	5
	Carbopol 981 1%	10	10	10	10	10	10
Oil phase	Emergent JC 100[1]	2	2	2	2	2	2
	Stergent JC 300[2]	1	1	1	1	1	1
	Bergacare EM-CSO[3]	5	5	5	5	5	5
	Bergacare 88[4]	1	1	1	1	1	1
	DC 200 (20CS)[5]	2	2	2	2	2	2
	Shea butter	2	2	2	2	2	2
	Lavender oil	0.1	0.1	0.1	0.1	0.1	0.1

[1] Emergent JC 100 : Polyclyceryl-3 methylglucos disterate/glyceryl stearate SE/methyl glucose sesquistearate
[2] Stergent JC 300 : Cetearyl alcohol/hydrogenated vegetable oil/stearic acid/ behenyl alcohol
[3] Bergacare EM-CSO : Cetearyl ethylhexanoate
[4] Bergacare 88 : Ethylhexyl ethylhexanoate
[5] DC 200 (20CS) : Polydimethylsiloxane
[6] NC : Negative control

Table 3. Formulations Used for The Testing of Phenoxyethanol and Ethylhexylglycerin

Components		Content (%)			
		A	A-1	A-2	A-3
Water phase	DW	61.68	61.58	61.38	61.28
	Glycerin	5	5	5	5
	1,3-BG	5	5	5	5
	EDTA-2Na	0.02	0.02	0.02	0.02
	Tromethamine	0.2	0.2	0.2	0.2
	Carbopol ETD 2020 2%	5	5	5	5
	Carbopol 981 1%	10	10	10	10
	Phenoxyethanol	-	-	0.3	0.3
	Ethylhexylglycerin	-	0.1	-	0.1
	1,2-Hexanediol	-	-	-	-
	1,2-Pentanediol	-	-	-	-
Oil phase	Emergent JC 100[1]	2	2	2	2
	Stergent JC 300[2]	1	1	1	1
	Bergacare EM-CSO[3]	5	5	5	5
	Bergacare 88[4]	1	1	1	1
	DC 200 (20CS)[5]	2	2	2	2
	Shea butter	2	2	2	2
	Lavender oil	0.1	0.1	0.1	0.1

[1] Emergent JC 100 : Polyclyceryl-3 methylglucos disterate/glyceryl stearate SE/methyl glucose sesquistearate
[2] Stergent JC 300 : Cetearyl alcohol/hydrogenated vegetable oil/stearic acid/ behenyl alcohol
[3] Bergacare EM-CSO : Cetearyl ethylhexanoate
[4] Bergacare 88 : Ethylhexyl ethylhexanoate
[5] DC 200 (20CS) : Polydimethylsiloxane

Table 4. Formulations for The Testing of Alkanediols

Components		Content (%)					
		A-1	[6]E-1	E-2	E-3	E-4	E-5
Water phase	DW	61.58	60.58	60.58	60.58	60.18	59.58
	Glycerin	5	5	5	5	5	5
	1,3-BG	5	5	5	5	5	5
	EDTA-2Na	0.02	0.02	0.02	0.02	0.02	0.02
	Tromethamine	0.2	0.2	0.2	0.2	0.2	0.2
	Carbopol ETD 2020 2%	5	5	5	5	5	5
	Carbopol 981 1%	10	10	10	10	10	10
	Phenoxyethanol	-	-	-	-	-	-
	Ethylhexylglycerin	0.1	0.1	0.1	0.1	0.1	0.1
	1,2-Hexanediol	-	1	-	0.5	0.7	1
	1,2-Pentanediol	-	-	1	0.5	0.7	1
Oil phase	Emergent JC 100[1)]	2	2	2	2	2	2
	Stergent JC 300[2)]	1	1	1	1	1	1
	Bergacare EM-CSO[3)]	5	5	5	5	5	5
	Bergacare 88[4)]	1	1	1	1	1	1
	DC 200 (20CS)[5)]	2	2	2	2	2	2
	Shea butter	2	2	2	2	2	2
	Lavender oil	0.1	0.1	0.1	0.1	0.1	0.1

[1)] Emergent JC 100 : Polyclyceryl-3 methylglucos disterate/glyceryl stearate SE/methyl glucose sesquistearate
[2)] Stergent JC 300 : Cetearyl alcohol/hydrogenated vegetable oil/stearic acid/ behenyl alcohol
[3)] Bergacare EM-CSO : Cetearyl ethylhexanoate
[4)] Bergacare 88 : Ethylhexyl ethylhexanoate
[5)] DC 200 (20CS) : Polydimethylsiloxane
[6)] E : Experimental cream

Microorganisms (KCCM, Seoul, Korea). The medium and culture conditions for each strain are indicated in Table 1. Briefly, *S. aureus* and *E. coli* were spread on tryptic soy agar plates (TSA, Difco, USA) and incubated at 37 ℃ for 24 h. *P. aeruginosa* and *C. albicans* were also plated on tryptic soy agar (TSA, Difco, USA) and incubated at 37 ℃ and 30 ℃ for 48 h, respectively. *A. niger* was plated on potato dextrose agar (PDA, Difco, USA) and incubated at 30 ℃ for 72 h.

2.3. Cosmetic Product

Cosmetic cream supplied by Aram Huvis Co., Ltd was used to evaluate the preservative efficacies of different concentrations of additives. The formulations of the different creams tested are shown in Tables 2-4.

2.4. Challenge Test

The challenge protocol used to assess preservative efficacy was based on the M-3 method recommended by the Personal Care Products Council (formally CTFA). Briefly, 30 g of cream was inoculated with a 1% microbial suspension (10^8 CFU/mL for bacteria or 10^7 CFU/mL for fungi). Next, a 1 g sample of each inoculated cream was serially diluted 10-fold with the liquid medium four times. The third and fourth dilutions were plated on solid medium and cultured with the growth conditions specific to each strain. Bacterial numbers were measured immediately after inoculation, and after 7 days, to establish antimicrobial activity. Bacterial counts were subsequently measured weekly for a total of 4 weeks.

Table 5. Log Reduction in Microbial Counts for Creams Containing Different 1,3-BG Concentrations

Strain	Log reduction					
	NC[1)]	A	B	C	D	E
S. aureus	0.8	6.1	6.1	6.0	6.1	5.8
E. coli	0.3	0.2	0.9	6.4	6.5	6.5
P. aeruginosa	0.2	2.5	6.4	6.5	6.6	6.5
C. albicans	+0.2	+0.1	5.7	5.8	5.8	5.7
A. niger	0.1	0.1	0.0	0.2	0.9	5.8

[1)] NC : Negative control

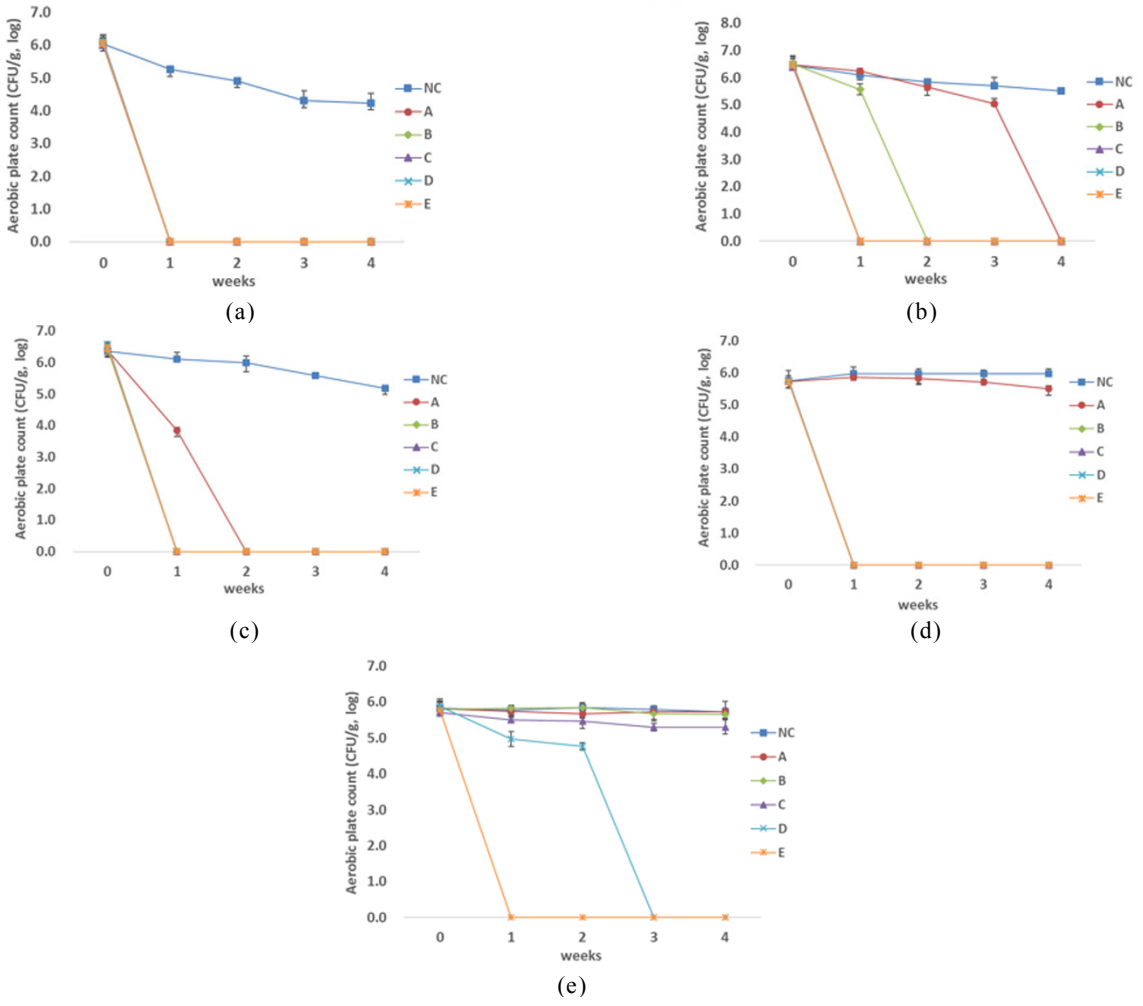

Figure 1. Efficacy of various 1,3-BG concentrations on the microbial counts in cosmetic cream. (a) *S. aureus*, (b) *E. coli*, (c) *P. aeruginosa*, (d) *C. albicans*, and (e) *A. niger*. NC (1,3-BG 0%); A (1,3-BG 5%); B (1,3-BG 10%); C (1,3-BG 15%); D (1,3-BG 20%); E (1,3-BG 25%).

2.5. Statistical Analysis

Results were conducted in triplicate and experimental data were calculated as mean ± standard deviation SD. SD did not exceed 0.3 logarithmic units.

Table 6. Log Reduction in Microbial Counts for Creams Containing Phenoxyethanol and Ethylhexylglycerin

Strain	Log reduction			
	A	A-1	A-2	A-3
S. aureus	6.1	6.1	6.5	6.0
E. coli	0.2	3.4	6.1	6.5
P. aeruginosa	2.5	6.5	6.3	6.5
C. albicans	+0.1	0.2	0.3	5.8
A. niger	0.1	0.0	0.4	1.2

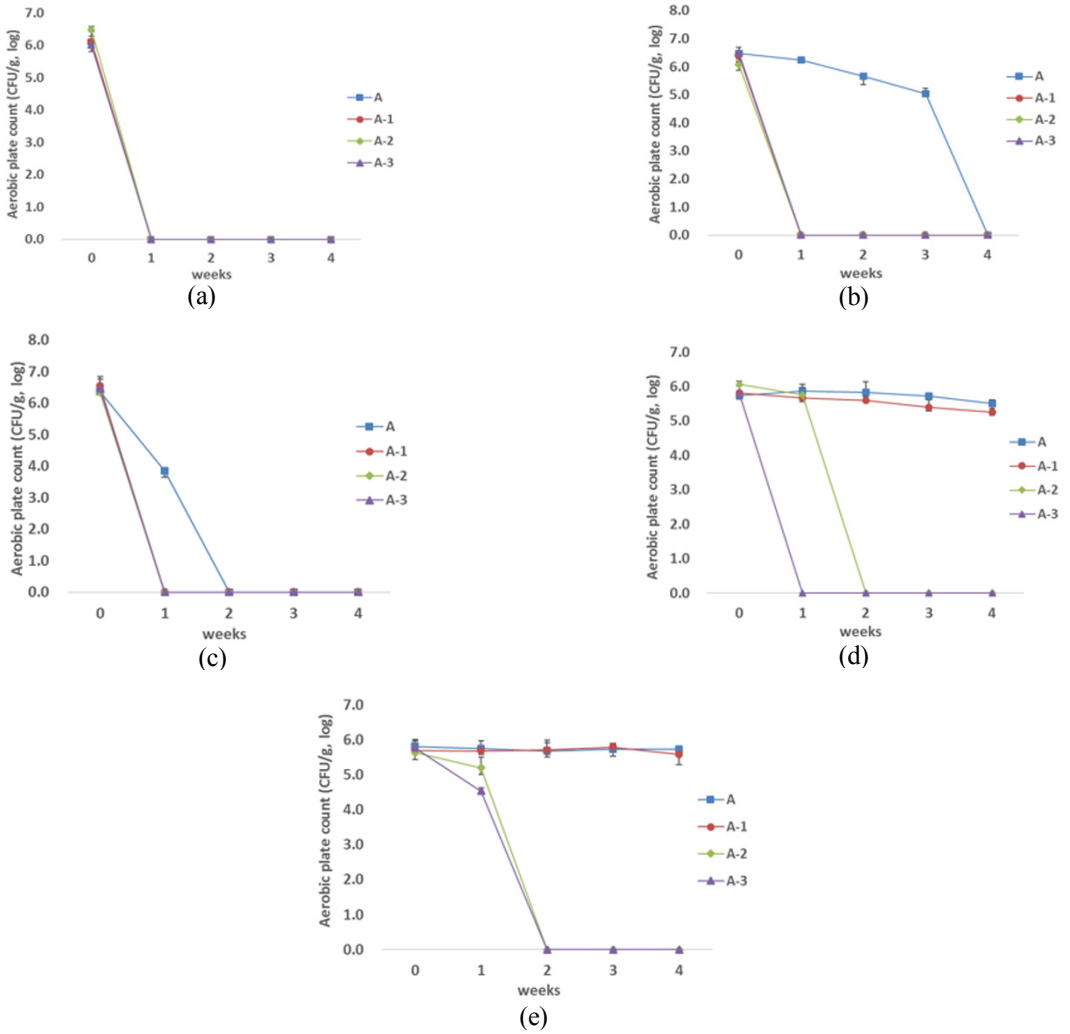

Figure 2. Efficacy on microbial counts of adding phenoxyethanol and ethylhexylglycerin to cosmetic cream. (a) *S. aureus*, (b) *E. coli*, (c) *P. aeruginosa*, (d) *C. albicans*, and (e) *A. niger*. A (1,3-BG 5%); A-1 (ethylhexylglycerin 0.1%); A-2 (phenoxyethanol 0.3%); A-3 (ethylhexylglycerin 0.1% + phenoxyethanol 0.3%).

Table 7. Log Reduction in Microbial Counts for Creams with Different Alkanediol Compositions

Strain	Log reduction					
	A-1	E-1	E-2	E-3	E-4	E-5
S. aureus	6.1	6.0	6.3	6.6	6.6	6.0
E. coli	3.4	6.4	6.5	6.6	6.6	6.4
P. aeruginosa	6.5	6.3	6.4	6.5	6.3	6.3
C. albicans	0.2	5.7	5.6	6.0	5.8	5.9
A. niger	0.0	0.6	0.0	0.0	0.4	1.4

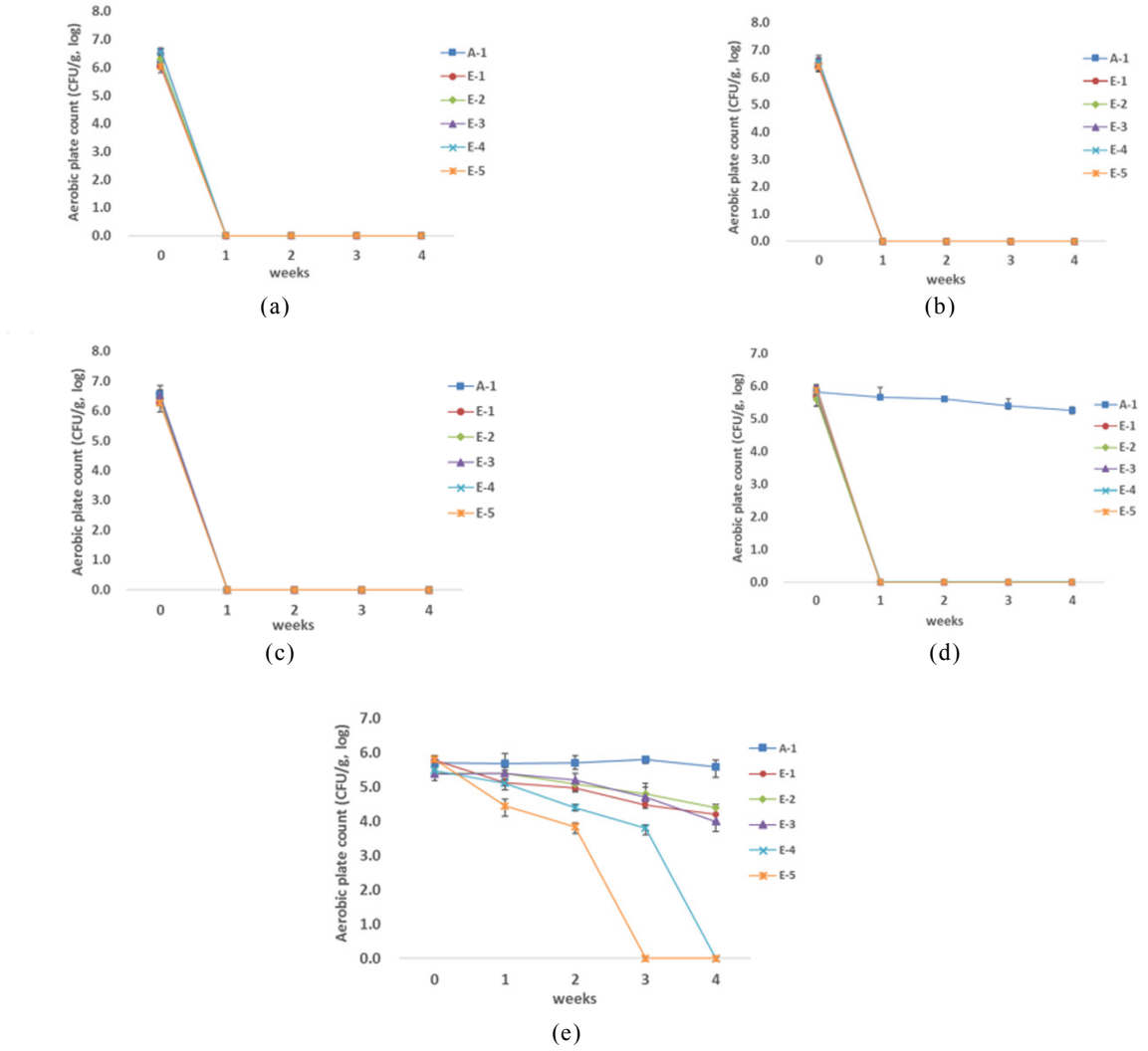

Figure 3. Efficacy on microbial counts for various alkanediol compositions. (a) *S. aureus*, (b) *E. coli*, (c) *P. aeruginosa*, (d) *C. albicans*, and (e) *A. niger*. A-1 (ethylhexylglycerin 0.1%), E-1 (ethylhexylglycerin 0.1% + 1,2-hexanediol 1%), E-2 (ethylhexylglycerin 0.1% + 1,2-pentanediol 1%), E-3 (ethylhexylglycerin 0.1% + 1,2-hexanediol 0.5% + 1,2-pentanediol 0.5%), E-4 (ethylhexylglycerin 0.1% + 1,2-hexanediol 0.7% + 1,2-pentanediol 0.7%), and E-5 (ethylhexylglycerin 0.1% + 1,2-hexanediol 1% + 1,2-pentanediol 1%).

3. Results

3.1. Challenge Test according to the 1,3-BG Content

Figure 1 demonstrates the preservative efficacy that different concentrations of 1,3-BG had on five microbial strains, while Table 5 reveals the log reduction values for the first week after inoculation. These data reveal that 99.9% of *S. aureus* were killed by formulation A (1,3-BG 5%), B (1,3-BG 10%), C (1,3-BG 15%), D (1,3-BG 20%), and E (1,3-BG 25%), relative to NC (1,3-BG 0%). For *E. coli*, there was preservative efficacy in formulation C (1,3-BG 15%), D (1,3-BG 20%), and E (1,3-BG 25%) but no effect in NC (1,3-BG 0%), nor formulations A (1,3-BG 5%) and B (1,3-BG 10%). For *P. aeruginosa* and *C. albicans*, there was preservative efficacy shown by formulation B (1,3-BG 10%), C (1,3-BG 15%), D (1,3-BG 20%), and E (1,3-BG 25%) but not NC (1,3-BG 0%) and formulation A (1,3-BG 5%). Only formulation E (1,3-BG 25%) showed any preservative efficacy against *A. niger* and there was no effect in the other formulations. Based on the M-3 method, the cream with the highest preservative efficacy was formulation E, containing 25% 1,3-BG.

3.2. Challenge Test according to the Composition of Phenoxyethanol and Ethylhexylglycerin.

Figure 2 displays the efficacies of the preservative phenoxyethanol and ethylhexylglycerin, a commonly used alternative preservative. Table 6 shows the log reduction values for the first week after inoculation. Formulation A (1,3-BG 5%) showed a preservative efficacy against *S. aureus*, but not against the gram negative bacteria *E. coli* and *P. aeruginosa*. The addition of 0.1% ethylhexylglycerin and 0.3% phenoxyethanol to formulation A resulted in preservative efficacy against all bacteria (*S. aureus*, *E. coli*, and *P. aeruginosa*). However, adding 0.1% ethylhexylglycerin (A-1) and 0.3% phenoxyethanol (A-2) individually did not have any preservative efficacy against yeast nor fungi according to the M-3 method, although a combination of 0.1% ethylhexylglycerin and 0.3% phenoxyethanol (A-3) did demonstrate preservative efficacy against these microorganisms. Therefore, the formulation with the best preservative efficacy based on the M-3 method was A-3 (5% 1,3-BG, 0.1% ethylhexylglycerin and 0.3% phenoxyethanol).

3.3. Challenge Test according to the Composition of Alkane Diols

Figure 3 shows the preservative efficacy of different 1,2-hexanediol and 1,2-pentanediol alkanediol compositions. The log reductions of microbial counts one week after inoculation are shown in Table 7. A formulation of 0.1% ethylhexylglycerin (A-1) was used as a base to investigate the preservative efficacy of 1,2-hexanediol and 1,2-pentanediol. Compositions E-1 (ethylhexylglycerin 0.1% + 1,2-hexanediol 1%), E-2 (ethylhexylglycerin 0.1% + 1,2-pentanediol 1%), E-3 (ethylhexylglycerin 0.1% + 1,2-hexanediol 0.5% + 1,2-pentanediol 0.5%), E-4 (ethylhexylglycerin 0.1% + 1,2-hexanediol 0.7% + 1,2-pentanediol 0.7%), and E-5 (ethylhexylglycerin 0.1% + 1,2-hexanediol 1% + 1,2-pentanediol 1%) all showed preservative efficacy against the bacteria *S. aureus*, *E. coli*, and *P. aeruginosa*, and the yeast *C. albicans*. However, only formulation E-5 demonstrated preservative efficacy against the fungus *A. niger*. Therefore, based on the M-3 method, the formulation with the best preservative efficacy was E-5 (ethylhexylglycerin 0.1% + 1,2-hexanediol 1% + 1,2-pentanediol 1%).

4. Discussion and Conclusion

In this study, we have investigated the preservative efficacies of 1,3-butylene glycol and various compositions of the alkanediols 1,2-hexanediol and 1,2-pentanediol. These ingredients are typically used as moisturizers in cosmetics. Although evaluating preservative efficacy can be performed using different methods and standards across countries, our experiments used the M-3 method proposed by the Personal Care Products Council (formally CTFA)[26]. As any ingredient can affect final preservative efficacy, it is essential to verify the overall preservative efficacy of a new formulation after it changes. Our study was therefore conducted to aid development of a

formulation free from known as preservatives that instead used safer alternatives, although the results of our evaluation are only applicable to the precise formulations described in the study.

Our study initially assessed the preservative efficacy of 1,3-butylene glycol (1,3-BG) by preparing creams with 5%, 10%, 15%, 20%, and 25% 1,3-BG. Efficacy was evaluated against five different microbial strains (*S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans*, and *A. niger*). This revealed antibacterial activity against all strains for a formulation containing 25% 1,3-butylene glycol (formulation E). In addition, we found that formulations containing 15% and 20% 1,3-butylene glycol had preservative efficacy against all strains, except the fungus *A. niger*. This suggests that it is possible to create cosmetic formulations that are free of known as preservatives. We conclude that, although 1,3-butylene glycol is currently used primarily as a moisturizer in cosmetic manufacturing, also it could be an application in preservative-free formulations.

Using a base formulation of 5% 1,3-butylene glycol, we next assessed the preservative efficacy of creams containing 0.1% ethylhexylglycerin and 0.3% phenoxyethanol. This revealed that low concentrations of preservatives were only effective against bacterial strains when used individually, and not against fungal strains. However, when mixed, they showed preservative efficacy against all microbial strains. This demonstrated that phenoxyethanol can be used in combination with the alternative preservative ethylhexylglycerin at lower effective concentrations than if used individually, reducing the compounding limit prescribed by the Food and Drug Administration FDA) by 70%. Therefore, formulations containing low concentration mixtures of preservatives may reduce the overall concentration of preservatives required in a cream. Finally, using a base formulation containing 0.1% ethylhexylglycerin, we investigated whether phenoxyethanol could be substituted by safer alternatives. We assessed the preservative efficacy of two alkanediols, 1,2-hexanediol and 1,2-pentanediol. This revealed that a formulation containing 1% 1,2-hexanediol and 1% 1,2-pentanediol with 0.1% ethylhexylglycerin was effective against all microbes, using the M-3 test.

In conclusion, we have identified two novel preservative-free formulations, E (25% 1,3-butylene glycol) and E-5 (1% 1,2-hexanediol and 1% 1,2-pentanediol with 0.1% ethylhexylglycerin). Based on our results, we suggest that could be applied to the creation of new preservative-free formulations for use in the cosmetic industry.

Acknowledgement

This work was supported by the National Strategic R&D Program for Industrial Technology (10043869, Development of service platform for Personalized Quasi-drug & Cosmetic to individual skin & hair), funded by the Ministry of Trade, Industry and Energy (MOTIE), Republic of Korea.

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