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# *"Evaluating the Influence of Application Techniques on the Efficacy and Safety of Chemical Peeling Serum: A Study of Leave-On and Rinse-Off Methods"*

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## 1. Introduction

Regular epidermal skin cell turnover is essential to maintaining healthy skin. Skin cell proliferation, differentiation, and shedding are typically in a state of homeostasis [1]. However, factors like aging and exposome—such as pollution, UV radiation, and individual lifestyle—can disrupt this cycle, causing the stratum corneum to become thicker. The buildup of dead skin cells can lead to a dull skin appearance, clogged pores, and rough skin texture [2]. Cosmetics exfoliation serum could be used to address this issue.

Exfoliation is characterized based on its method, including physical, chemical, and enzymatic exfoliation. Physical exfoliation uses an abrasive agent such as scrub or loofah. Chemical exfoliation typically contains alpha-hydroxy acids (AHAs), beta-hydroxy acids (BHA), salicylic acid, polyhydroxy acids (PHAs), aldobionic acids, or other organic acids that could disrupt the desmosome bonds between epidermal cells [3]. Enzymatic exfoliation uses natural proteolytic enzymes which can catalyze the hydrolysis of protein bonds in epidermis layers [2].

Exfoliating product launch has constantly grown over the years. The trend starts with facial scrub format that now has been declining. Peeling gel and exfoliating serum are the formats that are currently rising in the market, especially chemical exfoliating products using AHA [4]. AHA is a carboxylic acid with a hydroxyl group attached to the alpha-position, such as glycolic, lactic, mandelic, malic, and tartaric acid. It was theorized that AHA disrupts cellular adhesion by reducing the calcium ion in the epidermis [5]. Clinical studies have shown that AHA effectively exfoliates the skin, promoting skin cell turnover, and improving skin pigmentation and photoaging [6].

Peeling formula often combines AHA with BHA and/or PHA to provide comprehensive exfoliation efficacy and targeting multiple skin concerns [7]. PHA is a carboxylic acid with two or more hydroxyl groups attached, such as gluconolactone. These multiple hydroxyl groups are responsible for its strong humectant properties. Because PHAs are larger than AHAs, the molecules penetrate weaker offering milder exfoliation [6]. Clinical studies showed that Gluconolactone exfoliates skin while improving skin hydration and maintaining the skin barrier integrity [8]. Aldobionic acids—PHAs with attached sugar moieties such as maltobionic acid

and lactobionic acid—offer additional benefits like skin plumping, antioxidant, and anti-aging by inhibiting the MMP-1 enzyme responsible for collagen degradation [9].

BHA is a carboxylic acid with a hydroxyl group attached to the beta-position. Some BHAs are also considered AHA as they also have one hydroxyl group attached to the alpha-position, such as malic and citric acid [6]. Salicylic acid is often referred as BHA, which is widely used in cosmetics as a keratolytic agent [10]. Because of its lipophilicity, salicylic acid is more suitable for oily skin. Salicylic acid also has anti-bacterial and anti-inflammatory properties, which makes it suitable for acne-prone skin [11]. Interestingly, succinic acid (a dicarboxylic acid) offers the same efficacy with salicylic acid, it has anti-bacterial and anti-biofilm properties [12-13]. It is a promising ingredient, but there is limited evidence regarding its efficacy.

Although generally acknowledged as safe to be used, there are some commonly reported side effects of peeling agents, such as stinging, tingling, or burning sensations; especially AHA, which is known to increase skin sensitivity to UV light [14]. Risk factors that could increase the side effects of peeling agents are the type of chemical used (AHA, BHA, or PHA), concentration, pH, and the application method [15-16]. To mitigate the risk factors, cosmetics manufacturers and dermatologists often recommend different modes of application, primarily leave-on and rinse-off methods [16]. However, evidence comparing the safety and effectiveness of these two methods remains limited. This study aims to address this gap by evaluating and comparing the efficacy and safety profiles of a chemical peeling serum containing 5% glycolic acid, 3% maltobionic acid, and 2% succinic acid, applied through both leave-on and rinse-off methods.

## 2. Materials and Methods

### Sample Preparation and Evaluation

A serum formulation was made with 0.4% xanthan gum and 1.5% propanediol using an IKA stirrer. The formula included 5% glycolic acid, 3% maltobionic acid, and 2% succinic acid as the exfoliating agent. Other additive agents such as moisturizers, soothing agents, preservatives, and fragrance were added to complete the formula. The formula was adjusted to pH 4–4.2.

### Patch Test

A single patch test was carried out on 24 healthy adults to assess the skin irritation potential of the test product. The product was applied to patches placed on the upper back for 24 hours. Skin reactions were evaluated 30 minutes and 24 hours after removal using a standardized Draize-based scoring method, focusing on redness, dryness, wrinkling, and swelling.

### HRIPT

The Human Repeated Insult Patch Test (HRIPT) was conducted on 50 healthy adults to evaluate the skin sensitization potential of the test product. The product was applied to the upper back three times per week for three weeks during the induction phase, followed by a one-week rest and a single challenge application to a new area. Skin reactions were assessed at 24, 48, and 72 hours after removal using standardized dermatological scoring.

### Dermatologist Safety Evaluation

Safety assessment was conducted by a dermatologist through clinical observation of the 78 subjects throughout the testing period. Clinical signs such as irritation, allergic reactions, erythema, edema, and scaling were closely monitored. A product was considered safe if no

clinically or statistically significant signs of irritation, allergy, erythema, edema, or scaling were observed during the study period.

**Efficacy Testing**

The efficacy of the peeling serum were evaluated in a study involving 78 panelists, who were divided into two groups of 39 subjects each. The leave-on group applied the serum overnight, while the rinse-off group rinsed off the serum after 60 seconds. All participants met the inclusion criteria of being women with Asian skin types, aged between 21 and 33 years, and exhibiting clinical signs of skin roughness.

Skin texture, wrinkle formation, fine lines, and pore parameters were assessed utilizing the Antera 3D imaging system, with evaluations conducted at baseline and following 14 days of product use. Skin smoothness was analyzed through Visioscan, while skin brightness was measured using a spectrophotometer. Furthermore, the number of acne lesions was clinically evaluated and documented by a dermatologist.

**Statistical Analysis**

The collected data were analyzed using IBM SPSS Statistics software, version 29. Results are presented as mean values accompanied by standard deviations (SD). Normality of the data distribution was assessed using the Shapiro–Wilk test prior to conducting hypothesis testing. For data exhibiting normal distribution, paired t-tests were employed to evaluate differences between pre- and post-treatment values. In cases where the data were not normally distributed, the Wilcoxon signed-rank test was applied.

All analyses were performed with a 95% confidence level, and statistical significance was established at $p < 0.05$.

## 3. Results

In the experiment, the peeling serum was developed with pH 4 – 4.2 and viscosity 350 – 500 cPs. After 3 months of stability test, the serum could maintain its characteristics, such as pH and viscosity (Figure 1), so it considered as a stable serum.

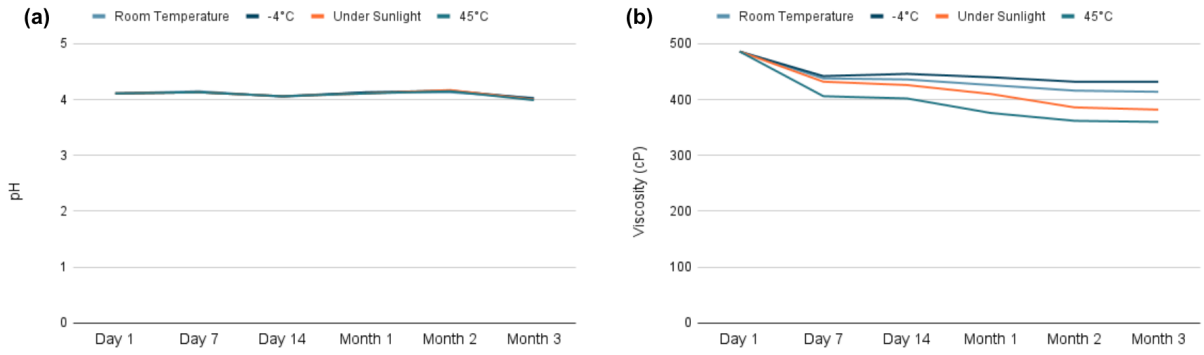


**Figure 1.** Stability profile of the peeling serum after 3 months (a) pH; (b) Viscosity

HRIPT (Human Repeat Insult Patch Test) results demonstrated that the product did not cause irritation during the induction phase and showed no signs of sensitization in the challenge phase. Additionally, the single patch test confirmed the product's non-irritant nature. These outcomes collectively support the product's safety for topical application.

Dermatological evaluation indicated that both leave-on and rinse-off serum applications were classified as safe, as no signs of irritation or allergic reactions were observed. However, a

trend of increased comedone formation was noted on Day 1 following leave-on serum application, which showed improvement by Day 7. In contrast, the rinse-off product demonstrated a consistent decreasing trend in comedone formation throughout the observation period. Detailed results are presented in Figure 2.

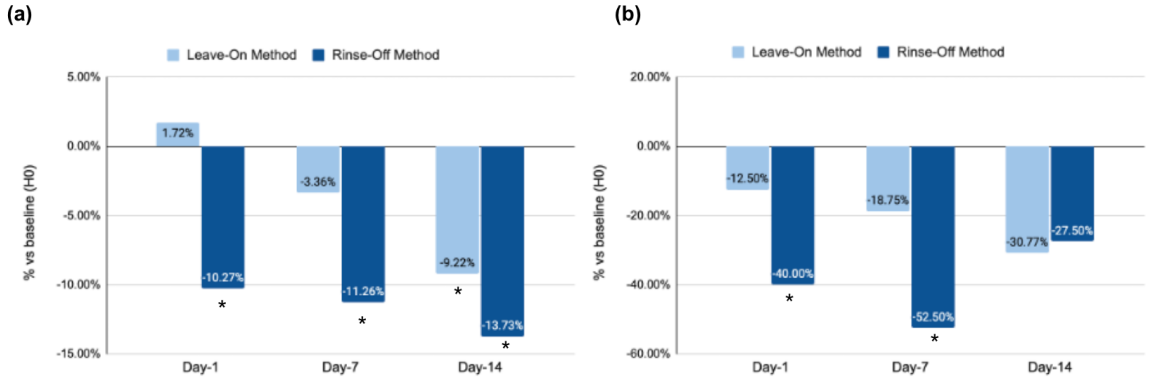


**Figure 2.** Evaluation by dermatologist of (a) non-inflammatory acne lesions and (b) inflammatory acne lesions formation at the time of application with different methods of product application *(*p<0.05; indicates a statistically significant difference compared to baseline)*.

Furthermore, the leave-on application method was associated with increased squama formation after 7 days, as evaluated dermatologically, and greater transepidermal water loss (TEWL) (measured by Tewameter), redness (measured by Antera 3D), and scalliness (measured by Visioscan) after 14 days of application. These findings are further illustrated in Figure 3.

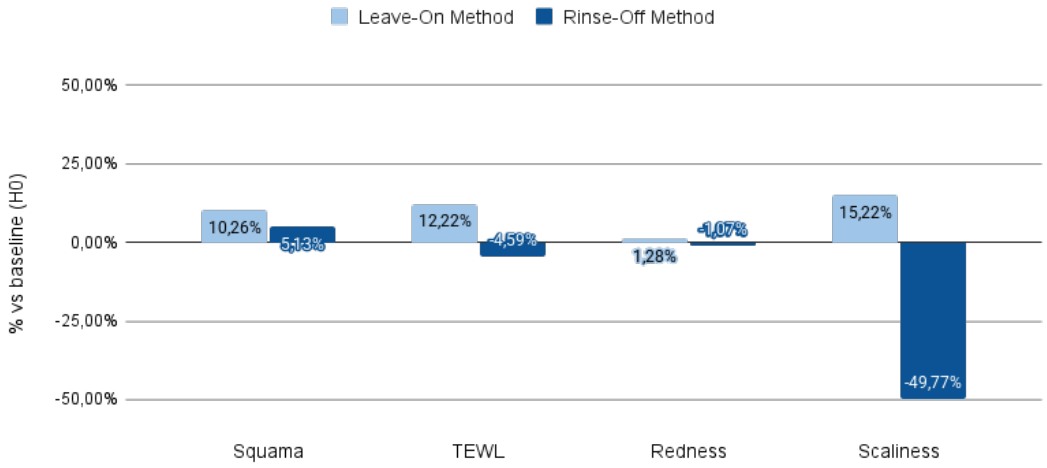


**Figure 3.** Observation of squama formation (evaluated by dermatologist), TEWL (measured by Tewameter), redness (measured by Antera 3D), and scalliness (measured by Visioscan) with different methods of product application.

On the other hand, the leave-on application method showed improvement in skin texture and a reduction in fine lines and pores based on Antera 3D measurements, while the rinse-off application method did not show these improvements, as demonstrated in Table 1.

**Table 1.** Evaluation of skin texture, fine line, and pore parameter at Day 14 of application compared to baseline.

| Parameter | % Improvement at Day 14 compared to baseline (Day 0) | | | |
|---|---|---|---|---|
| | Leave-On Method | | Rinse-Off Method | |
| | % | *p* value** | % | *p* value** |
| Skin Texture (Ra) | -1.37% | 0.708 | +1.93% | 0.650 |
| Fine Line Width | -1.50% | 0.880 | +1.26% | 0.909 |
| Fine Line Depth | -9.97% | 0.439 | +1.60% | 1.000 |
| Fine Line Count | -20.98% | 0.232 | +4.65% | 0.829 |
| Pore Volume | -11.55% | 0.438 | +17.62% | 0.178 |
| Pore Area | -10.85% | 0.291 | +15.69% | 0.181 |
| Pore Density | -8.91% | 0.299 | +6.07% | 0.538 |
| Pore Count | -8.91% | 0.299 | +6.07% | 0.538 |

***p* value < 0.05 indicates statistical significance.

However, both the leave-on and rinse-off methods showed improvement in skin smoothness, indicated by a decrease in %SEsm compared to baseline as measured by Visioscan, along with improved skin brightness as assessed by the Spectrophotometer CM600D. In addition, a reduction in the width and depth of wrinkles was also noticed after 14 days of product application, as measured by Antera 3D. These findings are illustrated in Figure 4.

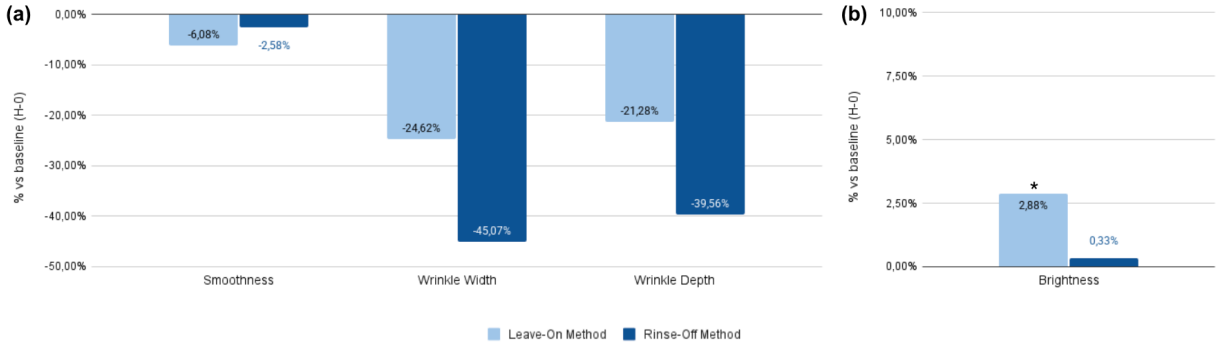


**Figure 4.** Comparison of the efficacy of different serum application methods, based on evaluation of (a) skin smoothness assessed by Visioscan and wrinkle parameters assessed by Antera 3D, and (b) skin brightness measured using a Spectrophotometer CM600D *(*p<0.05; indicates a statistically significant difference compared to baseline).*

## 4. Discussion

This study examined the safety and efficacy potential of a peeling serum in different ways of application: leave-on and rinse-off. The peeling serum was formulated with 3 kinds of acid, glycolic acid, maltobionic acid, and succinic acid. Glycolic acid is an Alpha Hydroxy Acid

derived from sugarcane. It was chosen as the main exfoliant in the formula because of its effective penetration, promotion of skin cell turnover, and enhancement of skin texture, due to its small molecule size [3,17]. Maltobionic acid is a type of aldobionic acid with a relatively large molecular size, allowing it to stay on the surface of the skin. Due to its functional groups and its capacity to form hydrogen bonds, it can provide moisturizing and moisture-absorbing properties on the skin. Furthermore, its hygroscopic properties make it give high-water uptake, enhancing skin hydration. This hydrating effect may help to overcome or prevent skin dryness [18]. Although bionic acids are bigger molecular size than AHAs, their molecular weight is still within the range that allows effective skin penetration. In addition, their pKa value is comparable to AHA [19]. Meanwhile, succinic acid acts like BHA who can unclog the pore, reduce comedone, and is known for its ability to downregulate *C. acnes* induced inflammation [20]. These three acids were designed to work synergistically to deliver powerful efficacy with minimal risk of irritation.

Besides the type of acid used, selecting the appropriate pH range is essential for peeling products to achieve optimal efficacy while minimizing potential skin irritation. It is widely known that AHAs show optimal efficacy at a lower pH range which is at range from 3 to 5. This acidic environment promotes desquamation, thereby facilitating the efficient removal of corneocytes and enhancing the penetration of AHAs into the deeper layer of the skin [3]. However, according to the National Agency of Drug and Food Control of Indonesia, cosmetic products intended for consumer use containing AHAs are considered safe when formulated at concentrations up to 10% with a minimum pH of 3.5 [21]. Therefore, this peeling serum was formulated at a pH range of 4.0 to 4.2 to balance exfoliation efficacy and minimize the risk of skin irritation, while also ensuring compliance with regulatory safety guidelines. In addition to pH, the concentration of acids also differs based on the intended efficacy and individual skin sensitivity. In this peeling serum formulation, combination of glycolic acid, maltobionic acid, and succinic acid were formulated at concentrations of 5%, 2%, and 3%, respectively. The selection of these concentrations was based on previous studies that demonstrated these doses remain effective for daily exfoliation in skincare products [3, 9, 13].

The result of the stability test for this formulation, with a combination of these acid doses in the pH range of 4.0 to 4.2, showed that the pH and viscosity remained stable over time. This pH stability is crucial to ensure the product's efficacy remains consistent throughout its shelf life. Despite the type of acid, concentration, and pH, the application method can also be adjusted to accommodate individual skin sensitivity tolerance- such as using rinse-off or leave-on method. The rinse-off method is applying the product to the face and leaving it on for a specified duration before rinsing. In contrast, the leave-on method is applied product to the face until its fully absorbed without rinsing. For peeling serum, the rinse-off method is generally considered milder than leave-on method, as the product's contact time with skin is limited. On the other hand, the leave-on method allows for prolonged activity on the skin, which may offer better bioavailability but also give a higher risk of irritation. Products intended for rinse-off application often contain higher concentrations of acids and are formulated at a low pH to help minimize the potential for irritation.

To investigate the different effects of leave-on and rinse-off application methods for a peeling serum, the efficacy test was conducted on 78 panellists divided into two groups. The first group used the peeling serum with the rinse-off method, while the second group applied it with the leave-on method. The peeling serum was applied at night every two days to minimize skin irritation by allowing a rest day in between. This application was followed by the application of a placebo moisturizer to help reduce potential skin barrier damage, as AHAs exfoliate dead skin cells and may cause adverse effects such as skin irritation, redness, or burning sensation

[3]. The use of a moisturizer supports the skin's barrier function through its emollient components, which enhance hydration and reduce TEWL [22]. Furthermore, since the peeling serum contains AHA that can increase photosensitivity of skin to UVB-irradiation [15], this serum should be only used at night, followed by the application of a sunscreen with at least SPF 15 the next morning [21].

Based on this research, application methods do influence the result of peeling properties, both for safety and efficacy point of view. In the leave on method, it was demonstrated that panelists showed measurable improvement in multiple skin parameters, including increased smoothness (6.08%), brightness (2.88%, $p<0.05$), and texture (1.37%). It was a sign that the exfoliant agent worked by penetrating the stratum corneum, the outermost layer of the epidermis, and interacting with desmosomes, thus promoting desquamation [3]. This exfoliative action facilitates the removal of dead skin cells, resulting in a brighter complexion and smoother skin texture [3]. Additionally, glycolic acid can enhance skin cell turnover rate [23].

Furthermore, interesting result is shown in the parameter of wrinkle and pore. Panelists showed reductions in wrinkles (2.76%), wrinkle width (24.62%), wrinkle depth (21.28%), fine line width (1.5%), fine line depth (9.97%), fine line count (20.98%) based on Antera 3D measurement. AHA was known for its effectiveness for diminishing fine lines and wrinkles by stimulating collagen synthesis in the dermis, a deeper layer of the skin, and facilitating skin cell turnover [15,24]. It can activate fibroblasts and upregulating the production of collagen and other extracellular matrix proteins [15]. Enhanced collagen deposition contributes to improved skin elasticity, firmness, and the attenuation of fine lines and wrinkles [15,24]. Additionally, reduction in pore volume (11.55%), pore area (10.85%), pore density (8.91%), and pore count (8.91%) were also observed in the panelists. Besides of the glycolic acid's exfoliating properties which can unclogging the pores [25], succinic acid was also contribute to the pore refining and sebum reduction [12]. These improvements in wrinkle, fine lines, and pore also contribute in skin smoothness.

Not only the leave-on method but also the rinse-off method demonstrated improvements, despite its shorter contact time. It showed increases in skin smoothness (2.58%) and brightness (0.33%), as well as reductions in skin scaliness (49.77%), wrinkle width (45.07%), and wrinkle depth (39.56%). Although not as significant as the leave-on method, the rinse-off method still provided noticeable improvements, particularly in pore appearance, fine lines, and overall skin texture. An additional noteworthy finding was the improvement in skin scaliness, indicating that the serum helped to smooth the skin without causing dryness or flakiness. Based on this study, both the leave-on and rinse-off methods are effective, though the leave-on method delivers faster and more pronounced results. It is possible that the rinse-off method could achieve comparable efficacy with longer-term use, as contact time influences the exfoliating effects [26]. Some parameters like fine lines and wrinkle need a longer time, up to two months because it include the process of remodeling the elastin and collagen [27].

However, the leave-on method was associated with higher incidences of side effects, including squama formation (10.26% vs. 5.13% of panelists in the rinse-off group), increased trans-epidermal water loss (+12.22% vs. -4.59%), and redness (+1.28% vs. -1.07%). It high possibly because of the longer contact time, contributing to more acid deposition and deeper penetration [28]. Acids, influenced by its concentration, pH, and contact time, could give potential adverse reaction, including dryness, redness, burning, and pruritus [15]. The exfoliating properties could thin the skin layer and leads to the presence of side effect including squama, redness, and skin dryness, as observed in the panelists. To reduce this risk, occlusive

topical moisturizing agent is needed to give moire intense hydrating properties, and board spectrum sunscreen should be used meticulously [29].

Furthermore, the leave-on method demonstrated a tendency to increase comedone formation (1.72%) at the beginning of the application. Nevertheless, comedone levels decreased after 7 and 14 days of usage (3.36% and 12.50% respectively), indicating that the comedones were already present beforehand and the exfoliant accelerated their elimination. The phenomenon did not occur in the rinse-off method, as the comedone decreased since day 1.

This study showed that both leave-on and rinse-off methods provide exfoliation benefits. While leave on method show more potent and faster result, rinse-off method offer the milder action while keeping the skin in the low risk of irritation. This make the rinse off method is suitable for who has sensitive and dry skin, or a beginner user of peeling product, while leave-on applications may be appropriate for individuals seeking stronger, targeted results. Efficacy effect of chemical peels categorized based on their depth of skin penetration (from light to medium to deep), and could be influenced by several factors, including pH, concentration, skin condition, as well as application technique and contact time [26,28]. In the leave-on method, the prolonged exposure of the exfoliants enhance their bioavailability and penetration, resulting in greater skin cell turnover and collagen stimulating effects, compared to the rinse-off method.

This study also underscores the role of combination acids—glycolic acid, maltobionic acid, and succinic acid—in offering synergistic benefits. Glycolic acid with its small molecule size penetrate deeper to epidermis and promoting desquamation as well as stimulating the collagen production [3] but also has higher potential to give adverse reaction such as dryness, redness, burning, and pruritus [15]. The inclusion of maltobionic acid, the higher molecule of acid with hydrogen bonds, give moisturizing properties and reduce the irritation potential, while it also has exfoliating benefits as well [18]. Succinic acid complete the efficacy with its ability to penetrate deeper and unclog pore, and reduce the excess sebum [12].

This study was limited by a short testing period of only 2 weeks. Therefore, it would be valuable to assess the long-term benefit of the serum in the longer duration in the future study. Additionally, it will be interesting to investigate how long the rinse-off method could achieve the same level of efficacy as the leave-on method. Testing to the larger and more diverse group of subjects also can be beneficial to validate the observed skin improvements by the serum.

## 5. Conclusion

This study showed that both leave-on and rinse-off methods provide exfoliation benefits. However, the leave-on method offers greater exfoliation effectiveness, while the rinse-off method is associated with a lower risk of side effects compared to the leave-on application.

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