

Efficacy and Safety of Phaseolus angularis L for. as Anti- aging Therapy in Healthy Skin

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Research Article

Keywords: Phaseolus angularis seed extract, anti-aging, health skin, cosmetic products

Posted Date: March 13th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2664226/v1>

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Abstract

Skin photoaging is caused by prolonged exposure to ultraviolet (UV) radiation from sunlight. UV-exposed skin appears rough, thick, dry, loose, and shows deep wrinkles, and thickening. Our previous research showed that a *Phaseolus angularis* seed extract (PASE) effectively inhibits photoaging through UVB protection in HaCaT cells. However, its efficacy has not been evaluated in clinical trials so far. In this single-centered, randomized, double-blind study, we investigated the anti-aging effect of PASE in eye wrinkle areas. To these 21 healthy adult women aged 30 to 59, a PASE cream was applied to the right eye wrinkle area and a placebo to the left eye wrinkle area twice a day (morning and evening) for 12 weeks. The change in thick, deep crease wrinkles around the eyes was confirmed by visual evaluation, skin measurements, and a questionnaire. As a result, the R1 (surface roughness), R2 (maximum roughness), R3 (average roughness), R4 (smoothness depth), and R5 (arithmetic mean roughness) values in the group using the PASE cream all decreased. Particularly, R1, R4, and R5 significantly decreased by 18.1%, 18.6%, and 25.0%. In addition, no participants reported side effects. Our study showed that PASE led to clinically significant levels of wrinkle improvement. In conclusion, as PASE is a natural food, safe, and with few side effects, it can be a good resource for natural anti-wrinkle cosmetics in the future.

1. Introduction

In a modern society that is aging, the proportion of the elderly is rapidly increasing, and by 2026, it is expected that the elderly population aged 65 or older will enter a super-aged society, accounting for more than 20% of the total population. With these changes in society, people's interest in anti-aging is gradually increasing, and many studies are being conducted to overcome aging [1]. In addition, because everyone has a longing for beauty that does not age outwardly even when they get older, the cosmeceutical industry is steadily increasing due to the increase in demand for anti-aging products as the aging population expands, and as the economic level increases, interest in appearance It is also expanding [2].

Skin aging is a process whereby intrinsic and extrinsic determinants progressively lead to a loss of structural integrity and physiological function. Intrinsic aging is an inevitable physiological process that results in thin, dry skin, fine wrinkles, and gradual dermal atrophy, whereas extrinsic aging is caused by external environmental factors resulting in coarse wrinkles, loss of elasticity, laxity, and a rough-textured appearance [3]. External factors of skin aging include reactive oxygen species (ROS), pollution, ultraviolet (UV) light, chemicals, toxins, and cigarette smoke. Among them, UV is considered to play the most important role in exogenous skin aging factors [4].

Human skin is exposed to UV repeatedly, and ROS are induced due to UV, resulting in oxidative stress [5]. Hydroxyl and superoxide radicals, important factors of skin damage due to UV, promote lipid and DNA or protein oxidative reactions in the skin, destruction of antioxidants, abnormal cross-linking of hyaluronic acids, collagen, and elastin, and accelerated skin aging by chain cutting [6–8]. Lipid peroxidation, DNA damage, and protein oxidation as a result of UV exposure cause aging and adult disease; moreover, cell components are damaged due to oxidative stress [9, 10]. In addition, the expression of matrix

metalloproteinases (MMPs) are enzymes that not only proteolyze ECM components but also play a major role in processing chemokines and altering other proteases' activity status. Especially among them, they decompose hyaluronic acid, collagen, and elastin, the substrates of the dermis, promotes melanin production and accelerates skin aging leading to skin hyperpigmentation, loss of skin elasticity, and wrinkles [11–13]. Wrinkles around the eyes are thinner and more sensitive than other tissues, so aging comes first. Wrinkles are formed around the eyes because this is the most sensitive tissue, so the skin's metabolic function decreases as the skin's elasticity are gradually lost, and the elastin and collagen tissues in the dermis are loosened, which is transformed into aging cells [14].

Cosmeceuticals stem from a hybrid approach between topical cosmetics and pharmaceuticals, intended to enhance health and beauty through ingredients that influence the skin's biological function [15]. Topical products that delay and/or reverse visible signs of aging are called anti-aging cosmeceuticals. Three primary structural components of the dermis, collagen, elastin, and glycosaminoglycans (GAGs), have been the focus of anti-aging skin research and formulation development [16]. For instance, antioxidants and cell regulators have been used in anti-aging cosmetics. The skin has an antioxidant defense network that can block and prevent oxidative damage from reactive oxygen species produced by ultraviolet rays. Enzymatic and non-enzymatic antioxidants directly remove reactive oxygen species or block chain reactions to protect cells and skin components from oxidative damage. Enzyme antioxidants include catalase, superoxide dismutase, glutathione peroxidase, glutathione S-transferase, etc. Non-enzyme antioxidants include vitamin C, vitamin E, β -Carotene, flavonoids, etc [17–19]. Existing antioxidant components in topical products include selenium, flavonoids, vitamin C, carotenoid, vitamin E, and coenzyme Q10 [20]. Recently, the increasing interest in using natural products had led to exploring extractable materials that exhibit antioxidant effects in natural products to replace synthetic materials.

Daily foods such as Aronia and blueberries have been described as newly active ingredients in the skin. *Phaseolus angularis* are the second most cultivated beans after soybeans in Korea. *P. angularis* contains many antioxidants such as saponin, catechin, procyanidine, and glycoside [21]. The known effects of *P. angularis* systemic effects include relieve edema, inflammation removal, fatigue recovery, and digestive absorption, and skin effects have antioxidant, anti-inflammatory, antibacterial, and anti-cancer properties [22–24]. In our previous research, *P. angularis* seed extract (PASE) could effectively inhibit photoaging by providing UVB cell protection by down-regulating the AP-1 signaling pathway and up-regulating the Nrf2/ARE signaling pathway in human keratinocyte cells.

Despite the numerous studies on the effectiveness of PASE, there are no clinical studies on its anti-aging effects in human skin. This single-center, randomized, double-blind, placebo-controlled study was conducted to confirm the anti-wrinkle effects of PASE.

2. Results

2.1. Participant demographics

The minimum number of participants should allow for >20 valid data for statistical comparison. Participants [=20/ (1–0.05)] were enrolled in consideration of the 5% dropout rate. No participants dropped out during the study; thus, data from a total of 21 participants were used for analysis. As shown in Table 1, 1 person in their 30s, 10 people in their 40s, and 10 people in their 50s participated in the experiment; the average age of the participants was 49.3 ± 6.1 years.

Table 1. Composition by age of participants

Total ^a	Age ^b	Enrolled	Drop out/Giving up halfway ^c	Completed (%)
21	30 - 39	1	0	1 (4%)
	40 - 49	10	0	10 (48%)
	50 - 59	10	0	10 (48%)

^a In consideration of the dropout rate of 5%, 21 subjects were selected, ^b Among adult males and females between the ages of 30 and 59, those who satisfied the selection criteria were selected, ^c Participants are allowed to voluntarily withdraw from the test at any time during the trial period.

2.2. Visual evaluation and photography

The visual evaluation showed no significant difference in wrinkle degree between PASE cream and placebo after 4 and 8 weeks of use ($p > 0.05$), but the degree of wrinkles decreased significantly after 12 weeks in the PASE group ($p > 0.05$). Therefore, the PASE cream resulted in a visual improvement in wrinkle appearance after 12 weeks of use compared to placebo (Table 2). Figure 1 shows images of the wrinkles around the eyes before (0 weeks) and 4, 8, and 12 weeks of product use. Since normality was not satisfied with respect to the initial value of visual evaluation between PASE cream and placebo, a non-parametric statistical method, Mann-Whitney U test, was performed to check the homogeneity between products (Table 3).

Table 2. Reliability of visual evaluation results of two experts (HW vs KYG^a)

Time point	Spearman's Rank Correlation Coefficient ^b	
	Correlation coefficient ^c	<i>p</i> -value ^d
0 week	0.920	0.000
4 weeks	0.920	0.000
8 weeks	0.928	0.000
12 weeks	0.924	0.000

^a An abbreviation for the names of two researchers with extensive research experience in related fields, ^b a method of evaluating the reliability of two experts, ^c an indicator of the linearity of the variance situation between two variables, ^d *p*-value by Spearman's Rank Correlation Coefficient

Table 3. Statistical analysis results for the initial values of visual evaluation

Test	PASE cream	Placebo	Mann-Whitney's U	<i>p</i> -value ^a
	Average ± SD			
Visual Evaluation ^b	3.810 ± 1.167	3.905 ± 1.136	200.500	0.029*

^a *p*-value by Mann-Whitney test (*p* < 0.05*), ^b 12 weeks value

2.3. Skin measurement and analysis

Changes in wrinkle R1 around the eyes resulting from using PASE cream were measured 4, 8, and 12 weeks using the simulated plate. In the group using PASE cream (at 12 weeks), the R1–R5 values significantly decreased by 18.1%, 5.1%, 17.1%, 18.6%, and 25.0%, respectively, compared to before use (*p* < 0.05). In comparison, in the placebo group, R1, R4, and R5 significantly increased after 4 and 8 weeks compared to before use. Moreover, after 12 weeks of PASE cream use, there was a significant level of wrinkle improvement in R1, R4, and R5 compared to placebo use (*p* < 0.05; Table 4).

From these results, the cream containing PASE significantly decreased the R1, R2, R3, R4, and R5 indicators, which are direct wrinkle-related indicators, and for placebo, R1, R4, and R5 increased significantly after 4 and 8 weeks of use. When comparing between groups, it was confirmed that after 12 weeks of using PASE cream, there was a statistically significant level of wrinkle improvement effect compared to placebo in R1-R5 (Figure 2).

Table 4. Comparison of changes before and after each time point

Parameter	Time point (weeks)	PASE Cream	Placebo		
		Average ranking	<i>p</i> -value ^a	Average ranking	<i>p</i> -value ^a
R1 (Surface roughness)	0	2.74	-	1.98	-
	4	2.90	0.163	2.71	0.022*
	8	3.14	0.064	3.05	0.005*
	12	1.21	0.000*	2.26	0.593
R2 (Maximum roughness)	0	2.81	-	2.14	-
	4	2.52	0.308	2.57	0.109
	8	2.67	0.796	2.98	0.022*
	12	2.00	0.014*	2.31	0.480
R3 (Average roughness)	0	2.98	-	2.52	-
	4	2.71	0.248	2.69	0.480
	8	2.40	0.071	2.69	0.593
	12	1.90	0.003*	2.10	0.132
R4 (Smoothness depth)	0	2.64	-	2.02	-
	4	2.93	0.197	2.79	0.011*
	8	2.83	0.201	2.71	0.017*
	12	1.60	0.001*	2.48	0.130
R5 (Arithmetic mean roughness)	0	2.64	-	2.05	-
	4	2.76	0.414	2.62	0.014*
	8	2.81	0.480	2.81	0.005*
	12	1.79	0.004*	2.52	0.096

^a *p*-value by Wilcoxon signed-rank test ($p < 0.05^*$)

2.4. Self-satisfaction assessment after treatment

For survey evaluation, the general evaluation (feeling of use), efficacy (wrinkle improvement), product satisfaction, and adverse reactions of PASE cream and placebo were surveyed after 4, 8, and 12 weeks of use. There was no significant difference between PASE and placebo groups in the questionnaire results and statistical results on efficacy and feeling of use at each time point (Table 5).

Table 5. Statistical analysis results for feeling of use and efficacy

Group	Parameter	<i>p</i> -value ^b		
		4 weeks	8 weeks	12 weeks
Feeling ^a	Smell	0.626	0.479	0.776
	Color	1.000	1.000	1.000
	Viscosity	0.848	0.831	0.647
	Spreadability	1.000	0.575	0.813
	Absorption	0.489	1.000	0.814
	Freshness	0.597	0.524	1.000
Efficacy ^a	Soft	0.710	1.000	1.000
	Smoothing	1.000	1.000	1.000
	Shine	1.000	0.836	0.703
	Moist	1.000	0.830	0.813
	Elasticity	0.695	0.837	0.706
	Wrinkle	1.000	0.487	0.817

^a Five-point scale (very dissatisfied-dissatisfied-moderate-satisfied-very satisfied), ^b *p*-value by independent t-test

2.5. Safety results

Participants experienced no side effects of treatment, including edema, scaling, itching, erythema, tingling, burning, tightening, or skin tingling (data not shown).

3. Discussion

Aging refers to a change in its properties over time, and as the skin ages, wrinkle formation, loss of elasticity, and pigmentation appear [25, 26]. Among the factors that cause photoaging, exposure to ultraviolet (UV) light is the most important factor of exogenous aging [27].

Wrinkle-improving functional cosmetics alleviate wrinkles created by the skin aging process and delay their speed [28]. Recently, in the industry, through dermatological research, major factors involved in the function and activity change of proteins involved in skin aging in the skin are explored, and research on cell signal transduction systems is constantly being conducted. Substances that improve wrinkles in wrinkle-improving cosmetics include substances that promote the growth of fibroblasts that promote the formation of connective tissue in the dermis, substances that promote collagen synthesis in fibroblasts, and substances that remove active oxygen and free radicals [29].

In this explorative clinical trial, the efficacy and safety of PASE as an anti-wrinkle agent were evaluated by a single-center, randomized, double-blind, placebo-controlled. As a result, we found that when used locally in human subjects, PASE had a significant anti-wrinkle effect compared with placebo.

Recently, as problems with environmental hormones or cosmetics that cause genetic modification have emerged, interest in developing natural materials with various effects such as whitening, moisturizing, UV protection and absorption, harmful oxygen removal, collagen synthesis, and skin wrinkle prevention is increasing. In this explorative clinical trial, the efficacy of PASE various evaluation techniques, such as clinical trial evaluation by researchers, skin cloning measurement tool evaluation, and self-reported questionnaire, was used to determine whether PASE is effective and safe as an anti-wrinkle agent. As a result, we found that when used locally in human subjects, PASE has a significant anti-wrinkle effect compared with a placebo.

In this single-center, randomized, double-blind, placebo-controlled clinical study, compared with before use, the skin roughness (R1), smoothness depth (R4), and arithmetic average roughness (R5) values in the PASE cream treatment group were significantly reduced, while in the placebo group they significantly increased. Moreover, after 12 weeks of PASE cream use, the wrinkles of R1, R4, and R5 significantly improved compared with those of placebo ($p < 0.05$). A recent similar clinical study using a face cream containing *Bukkerese chrysanthemum* oil for eight weeks decreased R1 to Average roughness (R3) values, but R4 and R5 did not change [30]. In comparison, PASE cream could effectively improve wrinkles.

Korea patent no. 10-2152932 reported has shown that PASE contains a large amount of catechin and catechin-7-O- β -D-glucopyranoside, probably responsible for its excellent antioxidant activity. This is the core mechanism used in this experiment, and considering the cell line experiment, the above results suggest that the meaningful change related to collagen synthesis will be the core mechanism.

In particular, Lee et al. reported that the catechin of *Phaseolus calcaratus* had an excellent antioxidant activity of catechin glycosides [31]. Moreover, Arnao et al. reported that *Phaseolus vulgaris* could inhibit cardiac toxicity-related oxidative stress and inflammation in adriamycin-treated mice and Wang et al. reported that a *Phaseolus vulgaris* with enhanced catechin content can be used as a safe substance for the treatment of antibacterial and anti-inflammatory diseases [32, 33]. Park. reported that the in vitro DNA cleavage, DNA migration, and H2AX phosphorylation inhibitory activity of the *Phaseolus angularis* hydrothermal extract showed high activity, which effectively inhibited DNA damage by radicals. Also, looking at the effect of lipid peroxidation and p21 expression rate on cell oxidative damage, it is thought to protect cells from oxidative stress caused by radicals as it is very effective in inhibiting lipid peroxidation and p21 expression rate [34]. Therefore, *Phaseolus* species have fewer side effects and less harm to the Earth than chemical products and have excellent antioxidant properties, so they can be a good resource for cosmetics.

4. Materials And Methods

4.1. Preparation of Phaseolus angularis seed extract cream

PASE was prepared following a patented protocol [Korea patent no. 10-2152932 (in Snowwhitefactory Co., Ltd.), in press]. *P. angularis* seeds were purchased from Gyeongginongsan (Paju-si, Gyeonggi-do). A weight of 100 g *P. angularis* seed powder was extracted in 500 mL of 70% ethanol and shook for 24 hours, after the extract was filtered, concentrated, and frozen and lyophilized to yield extracts (yield: 10.44%). After separating the aqueous and oil phases, the oil phase was added to the aqueous phase until complete emulsification. Thereafter, the finished cream formulation was used for the experiment. The test group (PASE cream) used a cream prepared by adding 0.1% of PASE, the main ingredient, to the aqueous phase whereas the control group (placebo) used a cream formulation without the main ingredient. The composition of formulations is shown in Table S1.

4.2. Subjects and study design

In this randomized study, 21 healthy adult women aged 30–59, those who met the inclusion criteria were selected to exclude those who did not. Documented information was provided to the subjects to evaluate the improvement effects of the test product on wrinkles, and the lead investigator verbally detailed it and participated in the test after agreeing in writing to become the subject by the subject's free will. Before using the product, each subject's skin type and skin condition were directly assessed through a questionnaire (Table 6). All participants agreed to the written consent form before participating in the study. After completing registration, the study coordinator assigned subjects to each group according to the computer-generated random number table. In this study, neither the investigator nor the subject knew the group assignment. All subjects gave their informed consent for inclusion before they participated in the study. The Ethics Committee of Semyung University Korean Medicine Hospital (Jecheon, Korea) approved the experimental procedures, and the protocol was approved by the Ethics Committee of Semyung University Korean Medicine Hospital (SMCTC-033-21-023, SMCTC-059-21-004).

Table 6
Results of survey evaluation on skin condition

Items	Type	Number of People ^a
Skin type	Very Dry	1 (5%)
	Dry	10 (48%)
	Normal	9 (42%)
	Oily	1 (5%)
	Very Oily	0 (0%)
Skin Elasticity	Very Good	0 (0%)
	Good	1 (4%)
	Normal	16 (76%)
	Lack	2 (10%)
	Very Lack	2 (10%)
Skin surface condition	Very Soft	0 (0%)
	Soft	1 (5%)
	Normal	20 (95%)
	Roughness	0 (0%)
	Very Roughness	0 (0%)
Sensitivity	Sensitivity	0 (0%)
	Insensitivity	21 (100%)

^a At visit 0 week, participants were surveyed for skin type and skin condition prior to product use.

4.3. Treatment

The subjects were divided into two groups, and the positions using products PASE cream and placebo were used oppositely between the two groups. After washing, an appropriate amount of the PASE cream was applied to the right (left) eye area and placebo to the left (right) eye area twice a day (morning and evening) for 12 weeks. Until the end of the test, the use of similar functional cosmetics was stopped and packs or massages were prohibited.

4.4. Manufacturing of the replica

Using a specially made ruler, attach it to the center of one end of the adhesive plastic frame on both sides of the 1.0cm wide area. Drop 2 drops of each of the silicone solutions and the catalyst for the production of the replica into the mixing container, and mix the two solutions well for about 15 seconds under

reduced pressure by a vacuum pump (Fig. 3). The reduced pressure condition is to prevent the formation of air bubbles in the mixed solution during the mixing process. Drop 1 drop of the mixed solution on the part where the replica is made, put the cover plastic on it, and press it lightly so that the mixed solution is evenly applied to the part where the replica is produced. The above process proceeds quickly within 10 seconds.

The subject should maintain a comfortable posture without moving until the mixed solution applied to the replica is completely hardened. When the mixed solution is completely cured, carefully remove the silicone replica frame from the eye area, check the skin wrinkle replica state and the presence of air bubbles, and if there are any problems, re-manufacture the replica. The replica is made in the same way on the right and left. The completed replica is attached to a paper frame, and after recording the subject, management number, and date, etc., keep it organized and stored so as not to be dusty.

4.5. Visual assessment

The degree of wrinkles around the subject's eyes was evaluated in a double-blind fashion by two (HW, KYG) experienced researchers under a sufficiently bright light source. The degree of wrinkles around the eyes was evaluated based on the global photodamage score (0: none, 1: none/mild, 2: mild, 3: mild/moderato, 4: moderato, 5: moderato/severe, 6: severe, 7: very severe) before the start of the human application test and at 4, 8, and 12 weeks thereafter. If there was a difference in the experts' evaluation, a level with low efficacy was selected.

4.6. Photography

Photographs of the participants' face were taken at 0, 4, 8, and 12 weeks under the same conditions. A photograph was taken from the right and left side of the eye. All photographs were taken under the same environmental conditions and constant lighting with the same camera, camera settings, and camera placement.

4.7. Non-invasive skin measurements

All measurements were performed under standardized conditions at room temperature of $22 \pm 2^\circ\text{C}$ and humidity of 40–60%. To obtain skin replicas, two components (two drops of diluent and catalyst, respectively) were prepared at a ratio of 1:1 under reduced pressure. The mixture was then quickly applied to the skin surface. After drying and curing, the replica was analyzed (Fig. 4).

The skin viscometer SV600 (Courage & Khazaka, Cologne, Germany) was used for analysis. The Skin-Visiometer SV 600 is an exact, easy-to-use, most economical tool for the R&D laboratories or the test institutes for formulation and efficacy testing of anti-aging products. Random units (R1–R5) were given to each sample according to the shadow size, the brightness generated by lighting refraction, and the groove depth. The roughness parameters studied are R1 (surface roughness), R2 (maximum roughness), R3 (average roughness), R4 (smoothness depth), and R5 (arithmetic mean roughness). The analysis results of summarized by the values of R1–R5. As the values of R1 to R5 decrease, it means that skin wrinkles are improved and the depth of wrinkles is reduced.

4.8. Questionnaire study

After 4, 8, and 12 weeks of product use, a general evaluation (feeling of use), efficacy (wrinkle improvement), an affinity for the product, and adverse reactions to the preparation were evaluated, respectively. In each corresponding week, the condition of the subject is judged, and the corresponding score is recorded for the stimulation type and the stimulation site. Stimulation type and stimulation site are recorded for each sample. Four-point scale; 0: none (No adverse reactions), 1: mild, 2: severe, 3: very severe (serious adverse reactions).

4.9. Clinical assessment

The evaluation was conducted by two expert investigators at weeks 0, 4, 8, and 12, respectively. The left eye and the right eye corner were evaluated, respectively. If side effects such as edema, scales, erythema, itching, burns, or skin tingling occurred during the experiment, they were recorded and evaluated as none, mild, severe, or very serious. The evaluation on each visit was performed in the same location and environment.

4.10. Statistical analysis

The results were analyzed by using statistical analysis program (SPSS version 18.0 software). Significant differences within groups were determined using per protocol, using the Shapiro-Wilk, Mann-Whitney U, Spearman's Rank Correlation Analysis, Friedman test, and Wilcoxon signed-rank test methods. p -value < 0.05 was considered to indicate significance.

5. Conclusions

First, subjects in the PASE group experienced a decrease in their eye wrinkle scores after 12 weeks. Second, applying PASE locally could reduce skin roughness, which is a precursor to wrinkles. In conclusion, the changes of deep wrinkles in the eyes were confirmed by visual evaluation, skin measurement and questionnaire survey. As a result, R1, R2, R3, R4, and R5 were reduced in the group with PASE cream. These findings show that PASE is effective in inhibiting and alleviating the skin photoaging mechanism and it was confirmed that the cream containing PASE had a clinically significant level of wrinkle improvement effect.

6. Patents

Tae-Hoo Yi et al., Composition for improving skin conditions comprising catechin glycoside and method for improving skin conditions using the same. KR Patent 10-2152932, filed on 14 January 2014 and issued on 1 September 2020.

Declarations

Author Contributions: Conceptualization, T.H.Y. and S.O.; methodology, S.O., and S.Z.; software, S.O. and J.J.; validation, S.O. and J.J.; formal analysis, S.O., S.Z., and M.J.K.; investigation, T.H.Y., S.O. and X.J.; resources, J.J. and M.J.K.; data curation, S.O., X.J., and S.Z.; writing—S.O., and X.J.; writing—review and editing, T.H.Y. and S.O.; visualization, S.Z., X.J., and M.J.K.; supervision, T.H.Y., Y.M.K., and J.J.; project administration, T.H.Y., Y.M.K., and J.J.; All authors have read and agreed to the published version of the manuscript.

Funding: Please add: This study was carried out with the support of ‘2021 Startup Growth Technology Development Project (Project No. S3160102) provided by Ministry of SMEs and Startups.

Data Availability Statement: The data presented in this study are available in this paper.

Acknowledgments: This research was kindly supported by Snowwhitefactory co., Ltd.

Conflicts of Interest: The authors declare no conflict of interest.

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Figures



Figure 1

This is a figure. Schemes follow the same formatting.

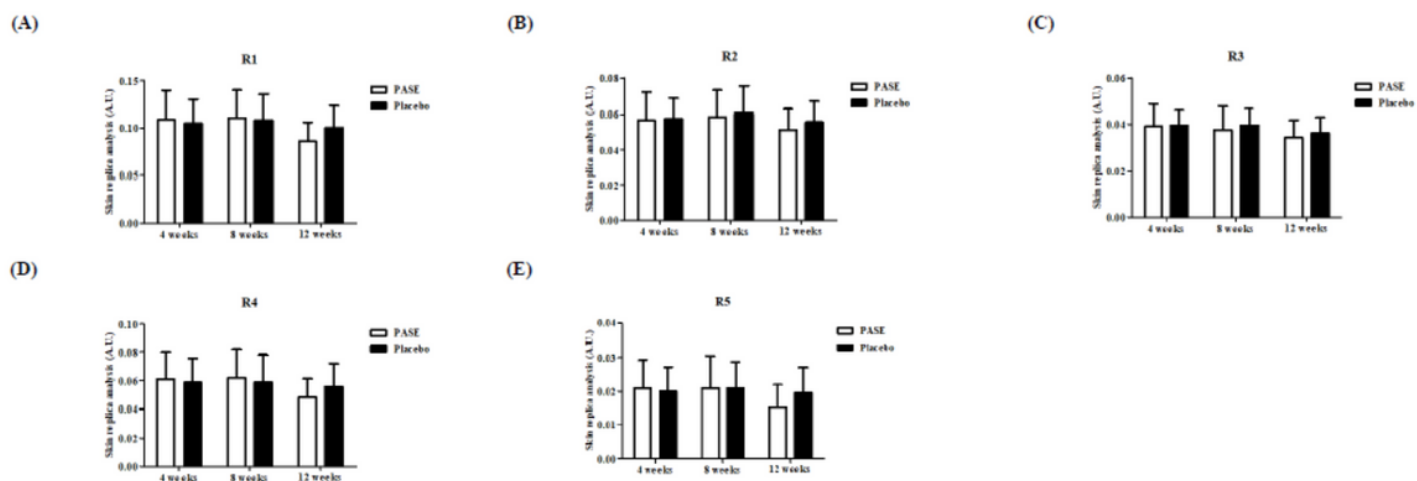


Figure 2

Results of image analysis change between PASE cream and placebo after product use.

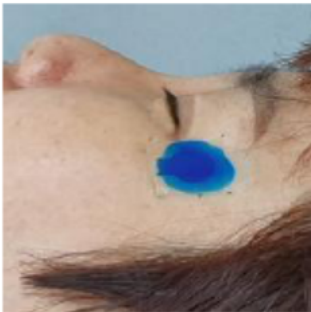
(A)



(B)



(C)



(D)

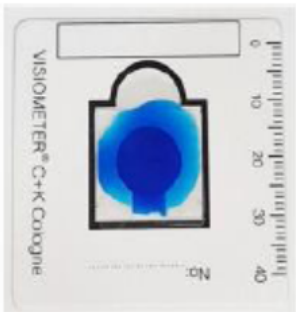


Figure 3

Manufacturing of the replica.

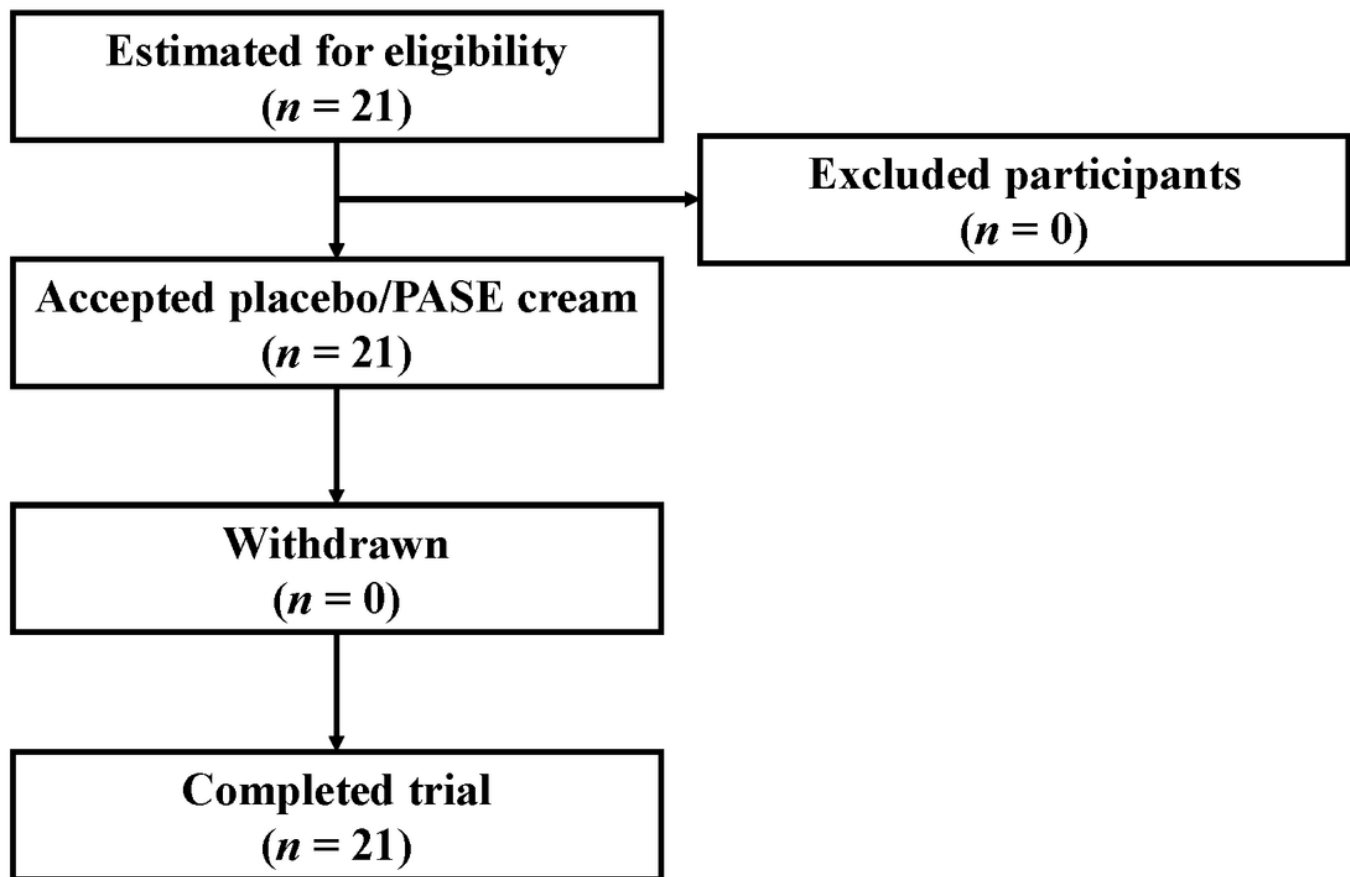


Figure 4

Study flowchart of the participants describing trial progress.

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