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Research Article

Development of a Vitamin C Derivative Serum for Pre-Aging Skin: Raw Material Screening, Stability and Clinical Evaluation

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Abstract
Vitamin C (L-ascorbic acid) is a powerful antioxidant widely used in skin care. Its ability to counteract free radicals helps prevent signs of premature aging such as dullness, dark spots, sagging skin, enlarged pores, and wrinkles. However, pure form of vitamin C is difficult to formulate because of limitations in stability and penetration, making its derivatives a preferred alternative. This study aims to develop a pre-aging serum using a stable and potent vitamin C derivative. Screening of various vitamin C derivatives—sodium ascorbyl phosphate, ascorbyl glucoside, 3-*O*-ethyl ascorbic acid, and 3-glyceryl ascorbate—was conducted by evaluating antioxidant activity and stability. Antioxidant activity was assessed using the DPPH method, while stability was assessed under various conditions: room temperature, 45 °C, 50 °C, −4 °C, sunlight exposure, and xenon lamp irradiation. The most stable and potent derivative was incorporated into a serum formulation, which was further evaluated for its stability and efficacy. Efficacy test was conducted on 39 females under dermatological assessment using Mexameter® MX 18, Cutometer® Dual MPA 580, and Spectrophotometer CM600D, along with photography via Visia-CR and Antera ® 3D CS instruments after 28 days. The 3-*O*-ethyl ascorbic acid was selected as the best vitamin C derivative. Serum containing 10% 3-*O*-ethyl ascorbic acid demonstrated excellent stability and significantly reduced dark spots (4.25%), increased skin firmness (20.35%), skin elasticity (R2 3.08%, R5 15.19%, R7 11.55%) and skin brightness (4.49%), reduced pores (9.86%) and skin wrinkles (13.71%). The 3-*O*-ethyl ascorbic acid serum was proven to be stable, could brighten the skin, and reduced signs of pre-aging.

Keywords: vitamin C, serum, 3-*O*-ethyl ascorbic acid, aging skin, antioxidant

1. INTRODUCTION

Skin is the most visible organ that could display the most apparent indication of human aging. Skin aging is a complex pathophysiological process, as a result of damage accumulation induced by intrinsic and external factors [1]. Intrinsic skin aging is an inevitable risk factor attributed to genetic and chronological processes such as age, gender, and ethnicity that affect skin physiology [2]. Extrinsic skin aging is caused by exposome, such as solar radiation, air pollution, smoking, malnutrition, and microorganisms [3][4]. The term pre-aging has recently gained popularity, particularly in urban and modern society contexts. Pre-aging (premature skin aging) refers to aging which occurs earlier due to an exposome that accelerates the skin aging process [5]. Intense sun exposure is a main factor that can increase the risk of pre-aging skin [6]. Furthermore, exposome, such as poor air quality due to pollution and exposure of blue light as growing dependence on digital devices, exacerbates the risk of pre-aging [7][8]. Exposomes induce pre-aging by generating oxidative stress in skin, which leads to DNA damage, protein and lipid oxidation, and extracellular matrix (ECM) degradation [9]. The clinical signs of pre-aging skin are visible wrinkles, pigmentation, dryness, sagging, change of skin texture, and loss of skin firmness that would make someone appear older than their actual age [10]. Consequently, there is a growing awareness among young people about the importance of skincare, particularly anti-aging products.

Preventing pre-aging involves targeting these environmental and lifestyle factors, with antioxidants playing a crucial role in this strategy. Antioxidant works by scavenging reactive oxidation species (ROS), thereby reducing oxidative stress and preventing the degradation of collagen and elastin in the dermis [11]. Today there are numerous antioxidant ingredients available for skin care, among which vitamin C is very popular. Vitamin C is a water-soluble vitamin with strong antioxidant properties that can help protect skin cells from damage caused by free radicals. In

Table 1. Physicochemical properties of vitamin C derivatives.

Parameter	L-Ascorbic Acid	3-Glyceryl Ascorbate	Sodium Ascorbyl Phosphate	Ascorbyl Glucoside	3-*O*-Ethyl Ascorbic Acid
Appearance	Powder	Liquid	Powder	Powder	Powder
Color	White to slightly yellow	Colorless to light yellow	White to off-white	White to yellowish white	White crystalline
pH of solution	2.2–2.5	2.5–5.5	9.0–10.0	2.3–2.4	3.5–5.0
pH stability	< 3.5	2–5	≥ 6	5–7	3.0–5.0
Active content	100.4%	30.1%	99.1%	99.9%	100.0%
Molecular weight	176.13	250.2	322.05	338.26	204.18
Solubility	Soluble in water	Soluble in water	Soluble in water	Soluble in water	Soluble in water

Note: For this research, all vitamin C samples met the specifications and were within the range of their shelf life.

addition, vitamin C is essential to various biochemical functions, including collagen synthesis, melanogenesis inhibition, proline hydroxylation, and the detoxification of histamine [12]. With those properties, vitamin C could be a good choice for preventing the signs of aging. Formulation of vitamin C for skincare products is challenging due to its chemical structure. Vitamin C is a weak organic acid and can be easily degraded by changes in temperature, exposure to sunlight, oxygen concentration, alkaline, copper, and heavy metals [13]. Penetration of vitamin C through skin encounters limitations due to its hydrophilic property, its poor stability and the strong barrier properties of skin. Skin barrier resides in the stratum corneum and is composed of differentiated keratinocytes embedded in a lipid matrix. This structure acts as a strong barrier that prevents the penetration of chemical compounds and pathogens as well as hydrophilic molecules through skin [14]. Therefore, vitamin C derivatives become alternative options when it comes to formulation of skincare. The use of structurally modified vitamin C in skincare products could allow efficient penetration of the vitamin, thus allowing better protection from the damaging effects of free radicals produced by UV irradiation [13]. This study aims to compare various vitamin C derivatives, namely sodium ascorbyl phosphate, ascorbyl glucoside, 3-*O*-ethyl ascorbic acid, and 3-glyceryl ascorbate. The selected vitamin C derivative was formulated in water-based serum formula and evaluated for stability, safety and efficacy for reducing the signs of pre-aging.

2. MATERIALS AND METHODS

2.1. Materials

Vitamin C and its derivatives used in this research were L-ascorbic acid (DSM, France), sodium ascorbyl phosphate (DSM, France), ascorbyl glucoside (Hayashibara, Japan), 3-*O*-ethyl ascorbic acid (Corum, Taiwan), and 3-glyceryl ascorbate (Seiwa Kasei, Japan). Methanol, 1,1-diphenyl-2-picrylhidrazyl (DPPH), and aquadest were used for antioxidant activity assay. The serum formula featured xanthan gum and ammonium acryloyldimethyltaurate/VP copolymer as thickeners, enriched with moisturizing ingredients

like hyaluronic acid and biosaccharide gum-1. It also included soothing agents such as aloe vera extract and allantoin, along with other additives like preservatives, chelating agents, and sensory modifiers. The physicochemical properties of vitamin C and its derivatives used in this research are presented in Table 1, based on the certificates of analysis (CoA) data of the raw materials. The pH stability data were obtained from raw material stability data and previous research [15][16].

2.2. Methods

2.2.1. Vitamin C Derivatives Screening

2.2.1.1. Antioxidant Actvity Assay

The antioxidant activity of vitamin C derivatives was measured by DPPH assay [17]. Test sample was diluted in methanol:aquadest (1:1 v/v) with 5 points of concentrations which are 40.08, 80.17, 160.34, 240.51, and 320.68 mg/L. Into the test tube, 2 ml of the sample was placed and the 100 mL DPPH solution was added at 50 mg/L concentration, then kept in the dark. After 30 minutes, the absorbance was measured by an UV-Vis spectrophotometer at 517 nm. The antioxidant activity was expressed as IC_{50} which stands for the amount of the sample needed to decrease the initial DPPH concentration by 50%.

2.2.1.2. Stability Test Screening

Each of vitamin C derivatives with concentration equivalent to 1% L-ascorbic acid were added to simple serum base containing 0.35% thickener (xanthan gum) and 0.6% preservative (phenoxyethanol). Each derivatives were tested under optimum pH for its activity. The optimum pH for 3-glyceryl ascorbate and 3-*O*-ethyl ascorbic acid are around pH 4.5, while the optimum pH for sodium ascorbyl phosphate and ascorbyl glucoside are around pH 7. The samples were then placed in several conditions: room temperature, 45 °C, 50 °C, -4 °C, as well as under sunlight exposure and a xenon lamp. After 1 month, product performances such as pH (by Mettler Toledo S220 Sevencompact pH meter), color (by Datacolor 200 Benchtop Spectrophotometer), odor, and sensory profile were evaluated.

2.2.2. Serum Formulation and Evaluation

The serum was made by swelling thickener with water followed by addition of 10% vitamin C derivatives and other ingredients. The mixing procedure was performed with IKA Stirrer. Stability test was done for 3 months in room temperature, 45 °C, -4 °C, as well as under sunlight exposure and xenon lamp. Both fresh and stability test samples were evaluated for several parameters: pH (by Mettler Toledo S220 Sevencompact pH meter), viscosity (Brookfield DVE Viscometer),

Table 2. Scale for classifying the degree of irritation based on the MII.

MII	Classification of the Irritation Degree
From 0.00 to 0.2	Non-irritant
From 0.20 (non-included) to 0.50	Slightly irritant
From 0.50 (non-included) to 2.00	Moderately irritant
From 2.00 (non-included) to 3.00	Very irritant
From 3.00 (non-included) to 4.00	Severely irritant

Table 3. Antioxidant activity (IC_{50}) of raw vitamin C derivatives.

Ingredients	IC_{50} (mg/L)
L-ascorbic acid	21.3
Ascorbyl glucoside	21.74
3-*O*-ethyl ascorbic acid	25.01
3-glyceryl ascorbate	185.09
Sodium ascorbyl phosphate	613.14

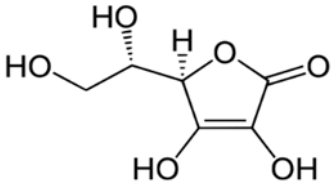

Figure 1. Chemical structure of L-ascorbic acid.

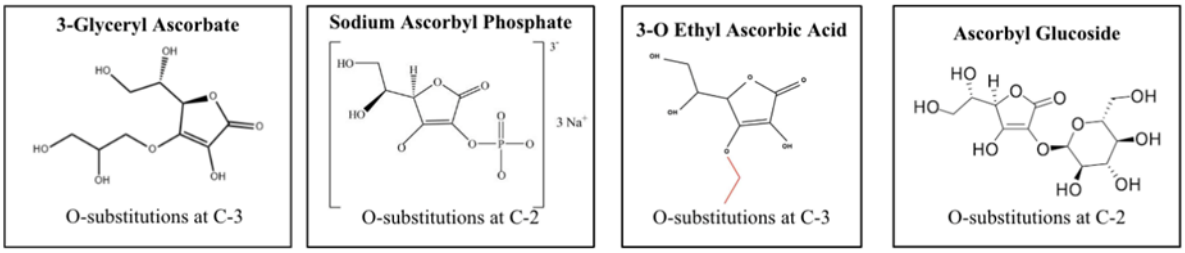

Figure 2. Chemical structure of each vitamin C derivative and position of O-substitution.

visual observation (under ColorPro Light Box CAC60), and sensory evaluation.

2.2.3. Safety and Efficacy Test

Safety tests were conducted for the serum, including single patch test, Human Repeat Insult Patch Test (HRIPT), and dermatological evaluation. Single patch test involved 33 healthy male and female volunteers aged 19 to 36 years old, meanwhile, HRIPT was performed on 66 healthy male and female subjects aged 20 to 60 years old. For single patch test, the product was applied pure (20 µL/mg) on the back for 48 hours under occlusion. An empty occlusive patch was also applied as the negative control. The study design applies single-blind and non-comparative study. At baseline (T0), occlusive patches containing the test products (one patch for each test product and one additional patch for the negative control) are applied to the subjects' backs and left in place for 48 hours. The Mean Irritation Index (MII) is calculated by dividing the total sum of the scores by the number of volunteers and evaluated according to the classification of the irritation degree of the test item as presented in Table 2. HRIPT includes several phases, namely induction, rest, and challenge phase. The test began with the induction phase, where the test product was applied repeatedly to assess whether it had the potential to sensitize the skin. This was followed by a rest phase, during which no product application was made, allowing the immune system to develop a potential response. Lastly, the challenge phase was conducted by applying test product to other areas of the skin to evaluate whether an allergic reaction occurred, indicating sensitization.

In the induction phase, occlusive patches containing the test product were applied on the back by a medical doctor on days T+2, T+4, T+7, T+9, T+11, T+12, T+14, T+16, and T+18. Cutaneous irritation for each day is classified based on the daily MII scale presented in Table 2. There are no clinical assessments during the rest phase. Furthermore, dermal responses during the challenge phase of the study are evaluated based on the criteria established by the International Contact Dermatitis Research Group (ICDRG). All measurements and evaluations were performed after 30 minutes rest period in a controlled environment (20–24 °C, 40% < RH < 60%). Additionally, dermatological safety evaluation was conducted on 39 healthy female subjects aged 18 to 36 years old. The product was applied twice daily on the face for 28 days. The evaluation was performed on day 0, before product application, as baseline and after 28 days of use.

Meanwhile, the efficacy test was done on 39 healthy female subjects who used the serum twice daily on their face for 28 days. After usage of 7, 14, and 28 days, panelists' skin improvements (brightness, dark spot, pore, and wrinkle) were assessed by dermatologist and measured by several instruments: Cutometer® Dual MPA 580 (elasticity and firmness), Mexameter® MX 18 (dark spot),

reflectance by Spectrophotometer CM600D (skin brightness and dark spot), photography by VISIA-CR, and Antera® 3D CS (brightness and pores).

3. RESULTS AND DISCUSSIONS

3.1. Vitamin C Derivatives Screening

Vitamin C is a reducing agent and a good free radical scavenger. As a reducing agent, vitamin C will donate its electron (H^+ or e^-), cycling between its fully reduced form and the radical anion, monodehydroascorbate [18]. The DPPH assay was used to assess the antioxidant activity of vitamin C derivatives. The DPPH assay uses a stable free radical, that can change its color upon reduction by an antioxidant. This change is measured by UV-Vis Spectrophotometer to determine the scavenging activity of the test compounds [19]. L-ascorbic acid showed the best antioxidant activity with IC_{50} of 21.3 mg/L, followed by ascorbyl glucoside, 3-*O*-ethyl ascorbic acid, 3-glyceryl ascorbate, and sodium ascorbyl phosphate, as described in Table 3.

Ascorbic acid is a dibasic, unsaturated lactone with a five-membered ring containing an electron-rich enediol group at positions C2 and C3 [20]. The chemical structure of L-ascorbic acid is illustrated in Figure 1. The main challenge with ascorbic acid is its susceptibility to oxidation and conversion to either monodehydroascorbate radical (Asc•−) or dehydroascorbate (DHA) when exposed to light, heat, transition metal ions, and alkaline conditions [21]-[23]. This is due to the enediol group at C2 and C3 that is critical for electron donation [20]. This reaction can be seen during the stability test based on color, odor, or other performance changes. Structural modification at positions 2, 3, 5, or 6 of the ascorbic acid rings, as well as variations in the length of alkyl chain substitutions, can enhance its stability by reducing its reactivity [24], as shown in Figure 2. Therefore, the stability test for each vitamin C derivative had to be conducted.

The stability screening of each vitamin C derivative was conducted using an equivalent concentration of 1% ascorbic acid. The first parameter that was examined was color stability. Based on visual observation, 3-glyceryl ascorbate, 3 -*O*-ethyl ascorbic acid, and ascorbyl glucoside maintained good stable color. Meanwhile, evaluation of color change by Datacolor 200

Table 4. Parameter evaluation data of vitamin C derivatives during stability screening at 1 month in oven 50 °C.

Parameters	Vitamin C Derivatives			
	3-Glyceryl Ascorbate	Sodium Ascorbyl Phosphate	Ascorbyl Glucoside	3-O-Ethyl Ascorbic Acid
CIE2000 ΔE	0.613±0.02	9.463±0.02	0.800±0.01	0.480±0.01
Odor Change	No	Yes	Yes	No
Sensorial Change	No	No	No	No
Initial pH	4.68	7.05	7.51	4.50
pH after 1 month	3.59	6.57	6.40	3.91
Visual Observation				

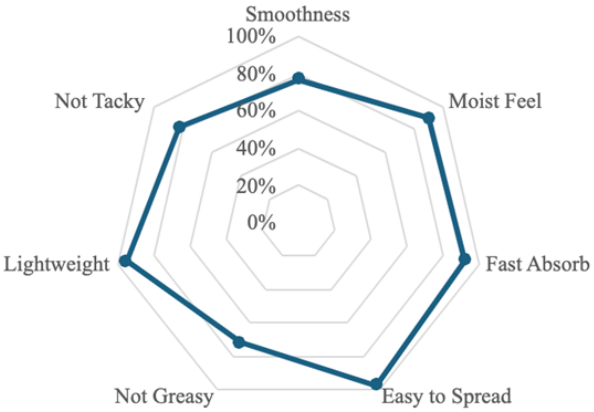

Figure 3. Sensory acceptance of vitamin C Serum based on questionnaire to 39 subjects.

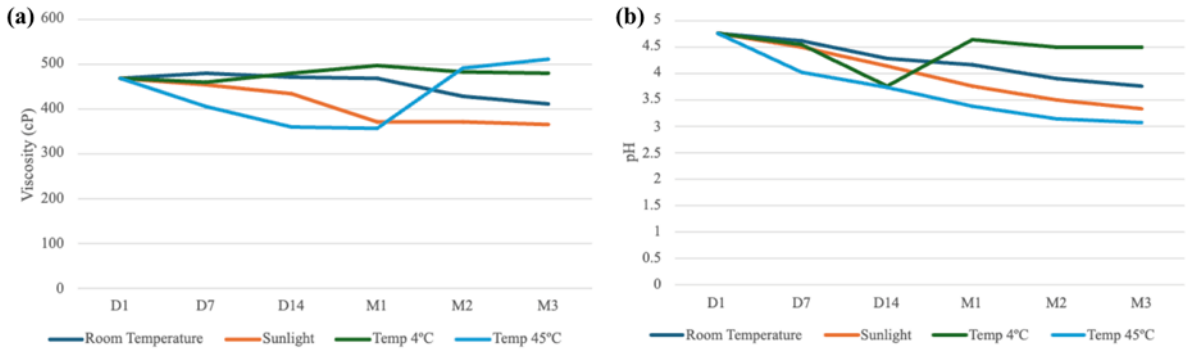

Figure 4. The (a) viscosity and (b) pH stability profiles of vitamin C serum over a 3-month stability test under various storage conditions.

Benchtop Spectrophotometer showed that there is a different CIE2000 ΔE value among all derivatives. Sodium ascorbyl phosphate showed the worst stability with very high CIE2000 ΔE value of 9.51 indicating the extreme yellow color. Other derivatives have less than 1 CIE2000 ΔE value. From lower to higher, these values correspond to 3-*O*-ethyl ascorbic acid, 3-glyceryl ascorbate, ascorbyl glucoside. Complete data can be seen in Table 4. The second parameter evaluated in this stability test was odor change. Two derivatives, namely sodium ascorbyl phosphate and ascorbyl glucoside, exhibited odor changes in the oven condition after one week. The other parameter, which is product sensory, remains stable for all vitamin C derivatives tested. Meanwhile, the pH value of all derivatives is decreased but still in their optimal pH recommendation range.

Ascorbic acid derivatives with *O*-substitutions at the C2 position, such as ascorbyl glucoside and sodium ascorbyl phosphate exhibited lower stability compared to other derivatives, as evidenced by noticeable color (high CIE2000 ΔE values) and odor changes during one month storage at 50 °C. In contrast, derivatives with *O*-substitutions at the C3 position, including 3-glyceryl ascorbate and 3-*O*-ethyl ascorbic acid, showed better stability, with minimal discoloration and smaller pH shifts, although odor change is still observed. The improved stability of C3-substituted derivatives is attributed to the chemical properties of the C3 position, which is more acidic (pKa ≈ 4.25) than the C2 position (pKa ≈ 11.57), making C3 more easily ionized. In contrast, C2 is not acid enough to be significantly ionized unless the 3-monoanion (refer to ionized form of C3) donates an electron to it. By protecting the C3 position through *O*-substitution, the C2 position is indirectly stabilized as well, resulting in greater overall molecular stability [20] [25]. In conclusion, considering results from physicochemical characterization, antioxidant activity testing, and stability assessments, 3-*O*-ethyl ascorbic acid was selected as the optimal vitamin C derivative for the serum formulation.

3.2. Serum Formulation and Stability Test

The 3-*O*-ethyl ascorbic acid was further formulated into a serum formulation. For a product containing ascorbic acid to have the desired effect, the concentration of ascorbic acid should be greater than eight percent, but less than 20 percent due to its irritation potency [13]. Reputable products of vitamin C available today are in the range of 10% to 20% [26]. Therefore, 10% concentration of 3-*O*-ethyl ascorbic acid was chosen to be incorporated into serum base for targeting pre-aging signs such as dark spot, wrinkle, and pore. Since the concentration of the vitamin C derivative is different from the one in the screening, another comprehensive stability test should be conducted meticulously. On the other hand, the optimum pH of 3-*O*-ethyl ascorbic acid is in the acidic range; therefore, the serum was designed to be within a pH range of 4.5 to 5.0 [15]. A combination of xanthan gum and ammonium acryloyldimethyltaurate/VP copolymer were added as thickeners. These excipients are stable at acidic pH. The final viscosity of the serum was 350–550 cP.

Skincare products with low pH may cause undesirable skin reactions such as rash, pruritus, burning, and other irritation symptoms [27]. To overcome this issue, vitamin C serum needs to be enriched with other active ingredients like soothing agents and moisturizers. In this research, allantoin and aloe vera extract were added as soothing agents, meanwhile hyaluronic acid and biosaccharide gum-1 were chosen as moisturizers. The sensory feel of the serum was adjusted for a comfortable daily use. The serum appeared to be clear to hazy, transparent to yellowish color, pH 4.5 –5.0, and viscosity 350–550 cP. Thirty-nine subjects were asked to try the serum and evaluate the parameters shown in Figure 3. The percentage of subjects who agreed with each property of the serum is presented in the corresponding spider web chart.

To ensure the performance of products did not decrease over time, stability test was performed in several conditions for 3 months. Heat and light are the factors that can trigger and accelerate the instability reaction [28], thus the serum was stored in the temperature 45 °C, -4 °C, as well as under sunlight and xenon lamp [29]. As shown in Figure 4

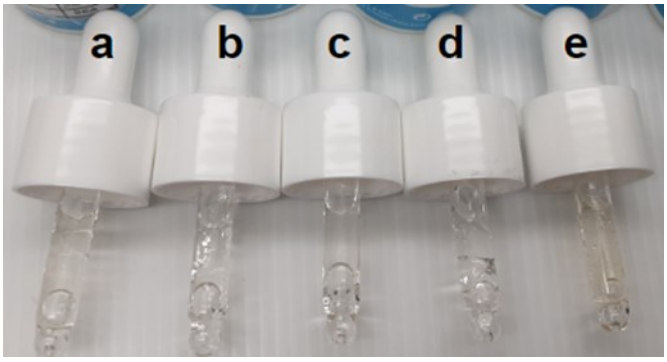

Figure 5. The vitamin C serum's color after 3 months stability test at (a) room temperature, (b) xenon lamp, (c) temperature of -4°C, (d) under sunlight, and (e) temperature of 45°C.

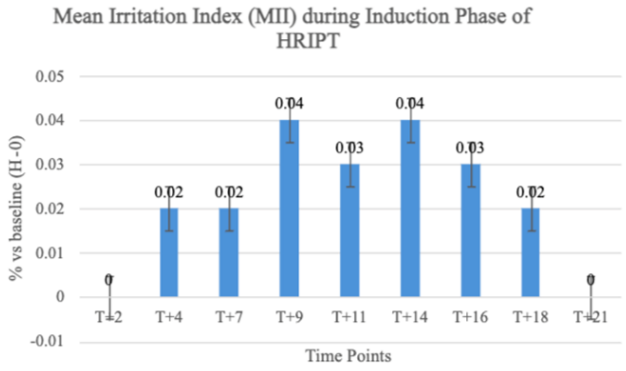

Figure 6. MII at each time point during induction phase of HRIPT.

Table 5. Clinical reaction after serum application during HRIPT test.

Time Point	Induction Phase Site	Challenge Phase Site
T+37	3 IRs and 1 weak reaction	1 IR and 1 weak reaction
T+38	2 IRs and 1 weak reaction	3 IRs and 1 weak reaction
T+39	2 IRs and 1 doubtful reaction	2 IRs and 1 doubtful reaction

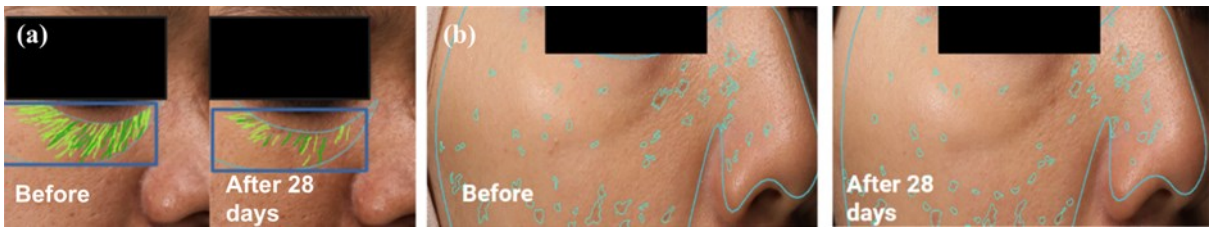

Figure 7. Skin improvement after 28 days of using a serum containing 10% 3-*O*-ethyl ascorbic acid compared to baseline: (a) wrinkle reduction and (b) dark spots reduction.

(a), the serum's pH decreased under all tested conditions, with the most significant reduction observed under heat exposure (45 °C and direct sunlight). The lowest recorded pH was 3.07. According to Golonka [15], ethyl ascorbic acid demonstrates stability within the pH range of 3.0–5.0. Thus, the formulation remains within an acceptable range, despite the observed decrease in pH. Additionally, the viscosity profile of the serum, as shown in Figure 4(b), remained within the target range. The lowest viscosity of the serum, 365 cP, still providing acceptable sensory characteristic and functional performance.

Oxidation of vitamin C not only can reduce its antioxidant activity but also affect the aesthetic aspect because of the color change. When vitamin C gets oxidized, it will change into dehydroascorbic acid (DHAA), which imparts a yellow color [30]. To prevent the oxidation, serum needs to be protected from air, heat, and light exposure, by choosing the right packaging. Based on this research, a slight color change was still observed under high-temperature conditions when using dropper bottle packaging (Figure 5). Therefore, it is strongly recommended to use airless and opaque packaging to help prevent instability reactions in the serum and keep the serum stable.

3.3. Safety and Efficacy Test of 10% Vitamin C Serum

A cosmetic product must be safe and should not cause damage when applied on skin [31]. To assess the topical toxicity effect, safety test was conducted by single patch test and HRIPT. The single patch test was used to assess primary skin irritancy [32], while HRIPT was used to assess skin irritancy and sensitizing potential of the formulation [33]. Based on the single patch test, this serum has an MII of 0.03. According to MII classification for the single patch test, a product with an MII below 0.20 can be considered as non-irritant. During the challenge phase of HRIPT, this serum is considered non-irritating when applied in its pure form under occlusion, as the MII of the product remained below 0.20 at all time points, as illustrated in Figure 6. Additionally, this serum did not show significant clinical reaction when applied in its pure form and assessed under occlusion, as shown in Table 5. Therefore, this serum is substantiated as hypoallergenic.

In addition, the serum passed the dermatological safety assessment, showing a reduction of 38.08% ($p < 0.05$) in non-inflammatory acne lesions and 39.29% (ns) for inflammatory acne lesions. These results prove that 10% vitamin C combined with other ingredients in the formula is safe to be used in regular use. Skin aging is driven by both intrinsic and extrinsic factors, leading to the deterioration of skin integrity and structural degradation. Overexposure to UV radiation enhances the ROS production which, at elevated concentrations, can damage key proteins in the skin, such as collagen and elastin. This results in a loss of structural integrity and elasticity, contributing to skin aging which can lead to the formation of wrinkles and pores in the skin [34][35]. In addition, the

Table 6. Skin wrinkle evaluation comared to baseline (Day 0, N = 39).

Parameter	% Improvement Compared to Baseline (Day 0)					
	Day 7		Day 14		Day 28	
	%	*p* value*	%	*p* value*	%	*p* value*
Fine lines on forehead	+16.67%	0.317	-50.00%	0.059	-50.00%	0.083
Eye (crow's feet)	0.00%	1.000	-33.33%	0.180	0.00%	1.000
Eye (drooping of the upper outer eyelid)	NA	0.317	0.00	1.000	0.00	1.00
Eye (underneath eye)	+2.27%	0.655	-11.11%	0.075	-10.87%	0.197
Nasolabial fold	-3.57%	0.564	-7.14%	0.441	-10.34%	0.317
Small folds on nasolabial zone	+53.33%	0.131	-53.33%	0.029	-12.50%	0.676
Cheek sebaceous pores	-6.15%	0.482	-4.55%	0.228	-15.94%	0.017
Density of pigmentary spots	-9.09%	0.046	-8.89%	0.086	-13.04%	0.014
Localized pigmentary spots on the cheek	-6.98%	0.083	-11.36%	0.020	-13.33%	0.014
Contrast of isolated pigmentary spot of the face	-6.98%	0.083	-13.64%	0.011	-13.33%	0.014
Size of an isolated spot	-6.98%	0.083	-11.36%	0.020	-13.33%	0.014
Wrinkle of the corner of the lips	0.00	1.000	0.00	1.000	0.00	1.000
Total	**-1.80%**	**0.382**	**-12.35%**	**0.000**	**-13.71%**	**0.001**

* *p* value < 0.05 indicates statistical significance.

production of ROS, mainly H_2O_2, can stimulate melanogenesis-related proteins, such as cAMP-responsive element-binding protein, MITF, TYR, and PAH. PAH is an enzyme responsible for generating L-tyrosine, the initial substrate for tyrosinase [36]. The human body has a strong antioxidant system to preserve the redox balance. Oxidative stress occurs when the equilibrium between oxidants and antioxidants is disrupted [37]. Therefore, it is suggested that the skin antioxidant defenses be enhanced using agents that can scavenge and neutralize oxidants and free radicals, which can be damaging to skin.

Antioxidants are essential for neutralizing the ROS generated by UV exposure. Vitamin C, known as L-ascorbic acid, protects the skin from oxidative stress by reducing the production of ROS [10]. Research has demonstrated that vitamin C significantly reduces UVB induced erythema and sunburn cell formation. Furthermore, vitamin C regulates collagen synthesis by directly activating the transcription of collagen synthesis and stabilizing procollagen mRNA. In addition, it can decrease melanin formation by interacting with copper ions at the tyrosinase-active site [30]. Those effects were demonstrated by 28 days of twice-daily application of a 10% 3-*O*-ethyl ascorbic acid serum formulation, which effectively treated signs of premature aging. Skin wrinkles, as evaluated by dermatologists and VISIA imaging, decreased from the baseline after 7, 14, and 28 days of application, as shown in Table 6 and Figure 7(a). The improvement in skin dark spots was measured using Mexameter® MX 18, Spectrophotometer CM600D, Antera® 3D CS, dermatological evaluation, and VISIA-CR imaging, as shown in Table 7 and Figure 7(b). Additionally, the improvement in skin pores was assessed using Antera 3D and dermatology evaluation, as presented in Table 7.

Skin firmness and elasticity as the implication of collagen and elastin content in the skin were measured by Cutometer® Dual MPA 580. The study demonstrated an improvement in skin firmness as illustrated in Figure 8(c). Meanwhile, there are several parameters describing skin elasticity. The R2 parameter is the gross elasticity/ viscoelasticity, which is the skin's resistance to the mechanical suction force versus its ability to recover. R5 represents the net elasticity, meaning

Table 7. Dark spot and skin pore evaluation by different assessment types compared to baseline (Day 0, N = 39).

Parameter	Assessment Type	% Improvement compared to baseline (Day 0)					
		Day 7		Day 14		Day 28	
		%	*p* value*	%	*p* value*	%	*p* value*
Dark Spot	Mexameter	-1.25%	0.624	-2.12%	0.435	-4.25%	0.017
	Spectrophotometer CM600D (b*)	1.23%	0.121	2.53%	0.002	3.85%	0.000
	Antera 3D (color)	1.23%	0.121	2.53%	0.002	3.85%	0.000
	Dermatologist grading	-14.27%	0.000	-13.62%	0.000	-18.45%	0.000
	Antera 3D (density)	-13.70%	0.041	-17.42%	0.02	-8.21%	0.341
Pore	Antera 3D (count)	-14.43%	0.036	-17.55%	0.02	-8.01%	0.353
	Dermatologist grading	-2.99%	0.157	-7.35%	0.025	-9.86%	0.008

* *p* value < 0.05 indicates statistical significance.

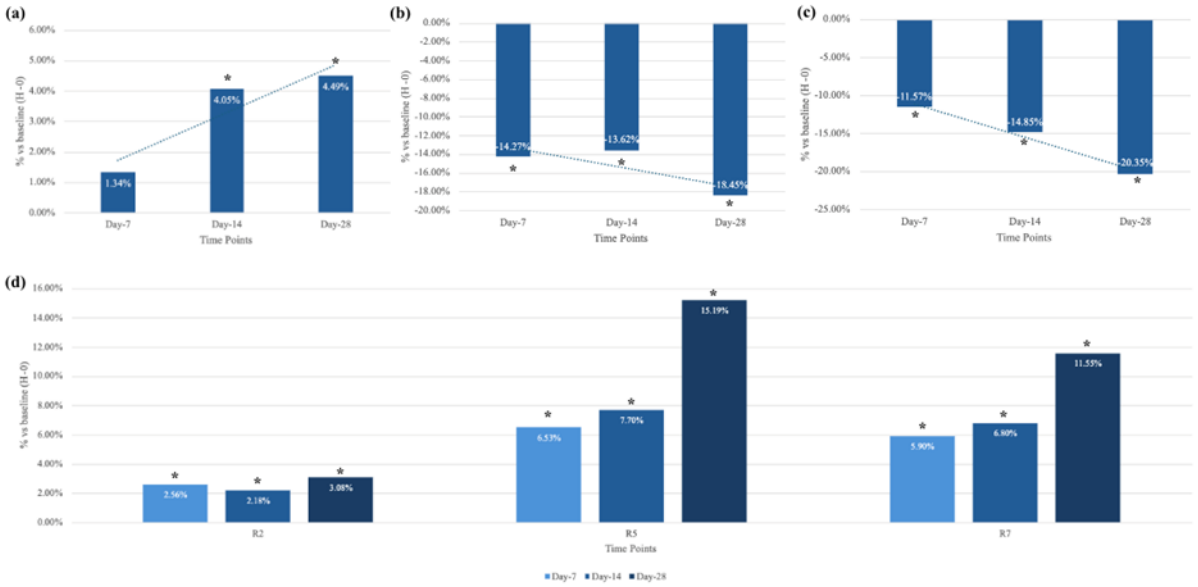

Figure 8. Skin improvement after using a serum containing 10% 3-*O*-ethyl ascorbic acid, evaluated on days 7, 14, and 28 across multiple parameters: (a) skin brightness measured using Spectrophotometer CM600D based on the b* value; (b) skin brightness evaluated by dermatologist assessment; (c) skin firmness assessed by cutometer using the R0 parameter; and (d) skin elasticity measured by cutometer using the R2, R5, and R7 parameters.

Figure 9. Skin brightness improvement after 28 days using 10% 3-*O*-ethyl ascorbic acid serum.

the higher the value the more elastic the skin. R7 is the immediate recovery in the first 0.1 seconds compared with the amplitude after suction and can be interpreted as another marker of elasticity [38]. The improvement in R2, R5, and R7 could be observed in Figure 8(d). Additionally, the b* value was measured with Spectrophotometer CM600D to assess the improvement in skin brightness. Figures 8(a) and 8(b) displayed the improvement in skin brightness, as measured by the Spectrophotometer CM600D and dermatologist grading, respectively. Improvement of skin brightness was photographed using VISIA as shown in Figure 9.

The action of vitamin C is further supported by the whole formulation system of the serum. Two important aspects such as stability of vitamin C and the appropriate excipients in the formulation being ensured in this study. Stability of vitamin C is important to make sure the active content remains stable in formula during the shelf life. This closely relates to the screening of vitamin C derivatives that was done in the first step of the study. Meanwhile, the excipients affect the ability of effective penetration of vitamin C, since human skin functions as a barrier to prevent the penetration of external molecules [37]. The serum formulation contains penetrant enhancer that facilitates the better penetration to target sites. The vitamin C derivative (3-*O*-ethyl ascorbic acid) chosen in this study also possesses semi-polar characteristics that allow penetration into the skin. This study developed and demonstrated the effectiveness of a

vitamin C serum, aligning with consumer demands for genuinely efficacious and safe cosmetic products [39].

4. CONCLUSIONS

Based on this study, the best vitamin C derivative for pre-aging serum formulation among sodium ascorbyl phosphate, ascorbyl glucoside, 3-*O*-ethyl ascorbic acid, and 3-glyceryl ascorbate is 3-*O*-ethyl ascorbic acid, due to its superior antioxidant activity and excellent stability under various conditions. The 10% 3-*O*-ethyl ascorbic acid serum was proven to be stable, safe for topical use, and showed significant efficacy to prevent pre-aging signs. After 28 days of application, the serum increased skin firmness (20.35%), skin elasticity (R2 3.08%, R5 15.19%, R7 11.55%), and skin brightness (4.49%), while reducing dark spots (4.25%), pores (9.86%), and skin wrinkles (13.71%). These findings suggest that 3-*O*-ethyl ascorbic acid is a potent active ingredient for anti-aging and skin-brightening formulations. Further studies are recommended to assess skin penetration and the clinical performance of other vitamin C derivatives, with particular focus on long-term efficacy and broader population groups. In addition, future studies may also explore innovative delivery systems to enhance product performance.

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Conflicts of Interest

All the authors are employees of PT Paragon Technology and Innovation, which paid 100% of the cost of this research.

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