Open-Label Study to Evaluate the Efficacy of a Topical Anhydrous Formulation with 15% Pure Ascorbic Acid and Ginger as a Potent Antioxidant

Tamara Martínez-Valverde 1, Nuria Crespo 2 and Elisa Suñer 3,*

Abstract: Vitamin C is one of the naturally occurring antioxidants capable of reducing or preventing skin photoaging. Achieving a stable formulation with the optimal dose of ascorbic acid to ensure a biologically significant antioxidant effect is a challenge when developing cosmetic formulations. The objective of this study was to develop a stable formula in a non-aqueous media with 15% pure vitamin C supplemented with ginger and to study its efficacy, skin tolerance, and cosmetic assessment in 33 women. Vitamin C stability over time was determined via a high-performance liquid chromatography (HPLC) technique versus an aqueous option. Reactive oxygen species (ROS) determination was quantified to provide antioxidant effect. A 56-day in vivo study was performed to evaluate skin luminosity and hyperpigmentation reduction. Skin acceptability was verified by a dermatologist. The HPLC studies demonstrated a high stability of the anhydrous formula compared to an aqueous option. The in vitro studies showed a reduction in ROS of 93% (p-value < 0.0001). In vivo, luminosity increased by 17% (p-value < 0.0001) and skin tone became 10% more uniform (p-value < 0.007). Moreover, very good skin tolerance was determined as the dermatologist did not determine any clinical signs, and the subjects did not report any feelings of discomfort. We were able to develop an anhydrous formula of pure vitamin C that combines very good stability, consumer acceptance, and skin tolerance with a high level of efficacy.

Keywords: vitamin C; anhydrous formula; antioxidant; skin luminosity; uniform skin tone

1. Introduction

Vitamins are organic compounds that the body needs in small quantities to ensure its normal functioning and are typically obtained from the diet. Since they are essential nutrients, and some are involved in multiple biochemical processes, they are capable of exerting beneficial effects on a wide range of skin disorders. When applied topically for direct delivery to the skin, they are more likely to exert significant local effects than if ingested orally, when distribution of the vitamin is limited by circulation to the target site [1,2].

Some of the most widely used forms in dermatological preparations are L-ascorbic acid (AA), ascorbyl phosphate (sodium and magnesium salts), and other ascorbate derivatives (ascorbyl palmitate and ascorbyl glucoside), with AA being the most dermatologically active form.

Vitamin C, or AA, is a hydrophilic molecule composed of six carbon atoms derived from glucose metabolism. Humans do not have the ability to synthesize their own AA due to a deficiency of the enzyme L-gulono-gamma-lactone oxidase, which catalyzes the passage terminal in AA biosynthesis [3]. AA protects the skin from oxidative stress via a sequential donation of its electrons to neutralize free radicals. Further, it reduces the
production of reactive oxygen species (ROS) such as super-oxide radicals, hydroxyl radicals, and singlet oxygen molecules.

It can be found as AA (its reduced form) or in its oxidized form, called dehydroascorbic acid (DHA), which is a product of oxidation, meaning it loses two electrons to turn into AA. Oxidation not only results in the loss of the active compound, but also makes the product turn yellow [2,4].

The optimal concentration of AA depends on its formulation. In most cases, for a product to be of biological significance, it needs to have an AA concentration higher than 8%. Studies have shown that concentrations above 20% do not increase its biological significance and, conversely, might cause irritation [5,6]. Therefore, working between 10% and 20% would be considered optimal doses for a biological significance.

Unfortunately, the beneficial antioxidant effect of AA along with the optimal dosage for a biological significance also underscores the primary challenge in developing AA formulations, ensuring its stability. Oxidation of AA is mainly triggered by its ionization in an aqueous solution [7]. Moreover, light, pH changes, and high temperatures can affect product stability. These limitations can be controlled, for example, by packaging the products in amber glass ampoules, an inert material that guarantees that product–filler interactions are minimal and prevents degradation due to exposure to light [8]. Reducing the water content of formulations also helps to improve vitamin C preservation. Therefore, in an anhydrous vehicle, AA would inherently have greater stability [9]. However, this option entails two major challenges: (1) AA is appreciably soluble in water and its solubility in non-aqueous media is quite limited [10], and (2) to achieve attractive sensory properties for the consumer, formulating high doses of AA in an anhydrous vehicle.

Antioxidants such as AA play an important role in preventing signs of photoaging and hyperpigmentation [11–13]. Unlike chronological aging, which is predetermined by an individual’s physiological predisposition, photoaging depends primarily on the degree of sun exposure and the amount of melanin in the skin. Clinical signs of photoaging include wrinkles, mottled pigmentation (hypo- or hyperpigmentation), rough skin, loss of skin tone, dryness, sallowness, deep furrows, severe atrophy, telangiectasias, laxity, leathery appearance, solar elastosis, actinic purpura, precancerous lesions, skin cancer, and melanoma [14,15]. The sun is the main source of ultraviolet radiation (UVR) and the main contributor to photoaging and melanogenesis by creating ROS in the skin cells. ROS are related to melanin synthesis, DNA mutations, and upregulation of matrix metalloproteinases, among others [16].

Vitamin C has a triple effect on the skin. It promotes collagen expression and also acts as a cofactor for proline and lysine, hydroxylases that establish the tertiary structure of the collagen molecule. Therefore, one of its roles is to participate in collagen synthesis. Second, it has a powerful antioxidant effect that can neutralize oxidizing agents like those found in environmental pollutants or those produced after exposure to UVR. What is more, it is especially effective at reducing oxidative skin damage when used together with oxidized vitamin E as a regenerative agent. Vitamin C returns oxidized vitamin E to its non-oxidized state, limiting the oxidative damage to structures of the cell membrane [17]. Lastly, it also acts as a depigmenting and brightening agent as it reduces melanin synthesis via its ability to interfere with tyrosinase function, a melanin synthesis-limiting enzyme [18,19].

The objective of this study was to develop a stable formula in a non-aqueous medium with 15% AA supplemented by another active component to enhance its antioxidant action, and to study its efficacy, skin tolerance, and cosmetic assessment in humans.

2. Materials and Methods

2.1. L-Ascorbic Acid Formulations and Content Determination

The aim of this part of the study was to observe the degree of degradation of AA in an aqueous vehicle versus an anhydrous one by means of high-performance liquid chromatography (HPLC) to demonstrate that the non-aqueous formula helps to preserve vitamin C properties better.
Two different formulas, aqueous and non-aqueous preformulas, were developed to determine the best stability option. The aqueous proposal (formula A) contained 15% AA (Ascorbic Acid Ultra Fine Powder, DSM Nutritional Products (UK) Ltd.) in water, thickeners (polyacrylate-13, polysorbate 20, sorbitan isostearate), and other components such as chelating agents, colorings, or buffer (disodium EDTA, CI 19140, CI 16255, sodium benzoate, potassium sorbate, citric acid, sodium hydroxide). Whereas the non-aqueous option (formula B) contained 15% micronized AA particles dispersed in a silicone/oil/glycol vehicles (oils (isododecane, dibutyl adipate, helianthus annuus seed oil), silicones (dimethicone), and glycols (ethoxydiglycol, caprylyl glycol)) and other components such as thickeners, colorings, or antioxidants (disteardimonium hectorite, polysilicone-11, propylene carbonate, acetyl zingerone, tocopheryl acetate, zingiber officinale root extract, tocopherol, parfum, silica dimethyl silylate, CI 47000, CI 26100 ethylhexylglycerin). For the HPLC determination, AA with purity greater than 99% was used as the standard. Phosphoric acids were used to prepare solvents, while potassium dihydrogen phosphate was used for the mobile phase. HPLC grade solvents are acetonitrile, methanol, isopropanol, and water.

The analyses were conducted using HPLC Waters Alliance 2695 equipment with a PDA 2998 detection module. The column used was a Luna C18 150 × 4.6 mm and 5 µm particle size and a 4 × 3 mm pre-column (Phenomenex). The chromatographic conditions for detecting AA in anhydrous and aqueous samples were as follows: the flow was isocratic 1 mL/min and the mobile phase was 0.02 M KH$_2$PO$_4$/methanol/acetoni trite (96:2:2% v/v). The 0.02 M KH$_2$PO$_4$ solution was prepared by weighing 3.48 g of KH$_2$PO$_4$ for one liter of water. The temperature of the column was 25 °C and the temperature of the sample was 5 °C. The injection volume was 5 µL and the detection wavelength was 250 nm.

For aqueous formula samples, a 0.1% H$_3$PO$_4$ (10 mL of H$_3$PO$_4$ for one liter of water) solvent was prepared. For the standard preparation, 150 mg of AA was measured and dissolved in 100 mL of solvent. Then, 1 ml aliquot was taken and dissolved in 10 mL of solvent. The sample was prepared in a 100 mL flask, and 1000 mg was weighed and brought up to volume with solvent. Both the standards and the samples were stirred with a magnetic stirrer.

2.2. In Tubo Study: Why Ginger as an Antioxidant Potentiator?

To enhance the antioxidant power of the main ingredients of the formula (vitamin C + E), four molecules with this property were preselected and tested (curcumin, rutin, glutathione, and ginger). To choose the best combination, a 2,2-diphenyl-1-picrylhydrazyl (DPPH) method of antioxidant assay based on Trolox equivalent antioxidant capacity (TEAC) was performed with the different options and the control (vitamin C + E) [20]. First, the sample was diluted in methanol (1 mg sample in 25 mL methanol) to obtain the stock solution from which seven additional serial dilutions (1:2) were made. To each dilution of the sample or Trolox, 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (1 mM) was added. After incubation in the dark for 30 min, the absorbance was measured at 517 nm.

2.3. Formula Development and Composition

The formula used for in vivo studies contained: (a) 15% microfine AA particles to avoid a gritty feeling on the skin, dispersed in (b) a mixed anhydrous vehicle (silicones (dimethicone), oils (isohexadecane, isododecane, dibutyl adipate, caprylic/capric triglycery eride, helianthus annuus seed oil), glycols (propylene glycol, ethoxydiglycol, caprylyl glycol)) to ensure correct applicability, (c) emulsifiers and structurants to maintain the stability of the anhydrous vehicle and the correct suspension of vitamin C particles (cetyl peg/ppg-10/1 dimethicone, peg-12 dimethicone, disteardimonium hectorite, polysilicone-11, propylene carbonate), (d) antioxidants to minimize the oxidative effect (acetyl zingerone, tocopheryl acetate, zingiber officinale root extract, tocopherol, diethylhexyl syringyldene malonate), and (e) other components such us parfum and pigments to complete the formula (parfum, mica, titanium dioxide, CI 47000, CI 26100 ethylhexylglycerin).
The formula was developed in the R&D laboratory following this process and challenges. The first challenge for the laboratory was to find the most optimal anhydrous medium for formulation with 15% AA that could maintain the established stability parameters and sensorial feeling that could be acceptable to the consumer. It was formulated using three different mediums, an oil medium, a glycol medium, and a silicone medium. None of the three mediums was accepted by the consumer in terms of their sensory properties, so we continued to search for a formulation by finding a balance between the three mediums, ultimately finding the perfect combination with suitable sensory properties. Another challenge encountered during formulation was finding the optimal particle size to ensure consumers would hardly notice said particles. Once the appropriate medium (balance between oil/silicone/glycol) and optimal particle size were determined, we aimed to make the formula more robust to ensure it would meet our stability requirements. For this step, the stability of the formulas was monitored for 6 months at different temperatures (4 °C, 25 °C, 40 °C, and 55 °C), checking them every 15 days, to study both the physicochemical properties of appearance, color, smell, viscosity, and microscopic view as well as the AA content. The final formula was the one that remained stable for all the established parameters.

2.4. In Vitro Test: Antioxidative Effect

An in vitro analysis of the effects of the study product on counteracting oxidative stress (ROS reduction) induced by ultraviolet A (UVA) light after topical application on the EpiDermTM Reconstructed Human Epidermis 3D skin model (RHE) was performed. The RHE (size 0.6 cm\(^2\) in a 6-well plate) was acclimatized for 24 h after reception. A total of 4 mg/cm\(^2\) of the tested samples was topically applied to the surface of the RHE for 24 h. At the end of the incubation period, the RHEs were washed, and the ROS reaction mix was added. Then, the RHEs were irradiated with UVA light for 30 min (dose received = 3 J/cm\(^2\), at a wavelength of 350 nm) to induce oxidative stress and produce ROS accumulation. Non-irradiated controls were kept in the dark during the irradiation period. Two hours after starting the irradiation process, the RHEs were separated from the transwells and placed on a new plate for ROS measurement by spectrophotometry at \(E_x/E_m\): 490/525 nm. All data were statistically analyzed. Four technical replicates (RHE) were used for each condition. All data were normalized to the irradiated control and represented as mean ± standard error of the mean (SEM) and were statistically analyzed by comparing the irradiated vs non-irradiated samples. The tests used for the analysis were the ordinary one-way ANOVA plus Dunnett’s post hoc or T-test when only two groups were compared. Statistical significance was set at \(p < 0.05\), with a 95% confidence level.

2.5. Clinical Study: Efficacy, Skin Tolerance, and Cosmetic Acceptance

A clinical study was designed. All investigations were conducted in accordance with the rules of the 1975 Declaration of Helsinki in its current 2013 version, and an independent ethics committee approved the study protocol (code: PT.06.01). This study intended to (1) check skin compatibility and cosmetic acceptability and (2) objectively and subjectively assess the qualities and efficacy of the final product after application under normal conditions of use for 56 consecutive days.

The efficacy of the product was assessed by evaluating skin tone uniformity and increase in skin luminosity. Both parameters were objectively and quantitatively evaluated using instrumental measurements, including (1) skin tone uniformity on the face using a VISIA-CR system and (2) skin gloss using a Glossymeter\(^\circledR\) (Courage – Khazaka Electronic GmbH). Numerous publications support this methodology [21–23]. Measurements were taken before the start of the study (D0), after 28 days of application (D28), and at the end of the study (D56). The individual results were expressed as follows: (1) in absolute values of the parameter for each experimental time, (2) in variation of the parameter against D0 for each experimental time, and (3) in % of change of the parameter values for each intermediate day and against D0.
The acceptability was checked every day by the subjects themselves at home, monitored by the dermatologist (D0, D28 and D56), and after questioning the subjects after product application (D28 and D56). The skin tolerance was monitored via visual examination of the experimental area by the dermatologist under standard “daylight” light source and after questioning the subjects after product application.

The subjective qualities and efficacy of the product were assessed during and at the end of the study (D28 ± 2 and D56 ± 2) using a target questionnaire, according to the category and target market of the test product, using the following scale: totally agree (1), slightly agree (2), slightly disagree (3), and totally disagree (4).

The participants corresponded to the population likely to use the product. The main inclusion criteria were women aged 35–55 years, phototype I to IV, all skin types (normal and reactive), and the presence of signs of photoaging. The product was applied by the subjects after cleansing the skin, on dry skin using a gentle digital massage until complete absorption. The product was applied on the face twice a day (morning and night) from D0 to D 56 ± 2 consecutive days.

3. Results

3.1. AA Formulations and Content Determination

The results obtained showed that the AA content was better preserved in an anhydrous vehicle than in an aqueous vehicle, as can be seen in Table 1.

| Table 1. Ascorbic acid (AA) content in anhydrous and aqueous vehicle over the time. |
| --- |
| **Time (Months) | **Time (Months) | **AA Content (%) | **% Variation over the Time |
| **Time (Months) | **Time (Months) | **Anhydrous Vehicle | **Aqueous Vehicle | **Anhydrous Vehicle | **Aqueous Vehicle |
| 0 | 14.98 ± 0.050 | 15.18 ± 0.096 |
| 2 | 14.95 ± 0.058 | 11.00 ± 0.000 | −0.20 | 27.53 |
| 3 | 14.18 ± 0.050 | 9.83 ± 0.050 | −5.34 | 35.24 |

Results of AA content are expressed as mean ± SD.

As Table 1 shows, there was a 35.24% degradation of AA in an aqueous medium after three months of storage at room temperature while the anhydrous sample maintained about 95% of the initial content.

3.2. In Tubo Study

The standard line was obtained from the four concentrations, obtaining good linearity between them. The TEAC values of the samples were obtained by interpolating the sample absorbance into the linear Trolox regression equation. Table 2 shows the results obtained for each tested combination.

| Table 2. Trolox equivalent antioxidant capacity (TEAC) values for each combination. |
| --- |
| **Study Substance | **Concentration for Maximum Absorbance (µg/mL) | **Trolox Equivalent Concentration (µM) |
| Vitamin C + E (control) | 28.57 | 578 |
| Vitamin C + E + curcumin | 28.57 | 565 |
| Vitamin C + E + rutin | 28.57 | 550 |
| Vitamin C + E + glutathione | 0.029 | 609 |
| Vitamin C + E + ginger | 0.029 | 603 |

Both glutathione and ginger showed the best results for increasing the antioxidant effect of vitamin C + E (control). In light of these results, ginger was chosen as the best enhancer ahead of glutathione because of its complementary properties [24,25].
3.3. Preclinical Studies—In Vitro

The results (Figure 1) showed that UVA irradiation significantly induced ROS levels increase by 37.1 ± 4.3-fold, whereas topical treatment with the study product for 24 h significantly decreased ROS levels by 92.6 ± 9.2% compared to the untreated (and irradiated) control group.

![Figure 1](image)

Figure 1. Reactive oxygen species (ROS) levels. (A) ROS levels related to untreated (and irradiated) control. (B) ROS levels related to the UVA irradiated control. ** statistically significant.

The results were also expressed as the percentage of protection produced by the study product after the irradiation damage. This result was obtained by subtracting the mean of the non-irradiated controls from all the conditions and then relating the results to the mean of the irradiated control. Using this analysis, the study product produced a percentage of protection against ROS accumulation of 95.1 ± 11.6%.

3.4. Clinical Studies

The population included in the study had the characteristics specified in Table 3.

Table 3. Study population characteristics.

| Demographic Data | Skin | Subjects |
|------------------|------|----------|
| Number           | 33 (100%) |Skin reactivity:  | Included 33 (100%) |
| Female           | 33 (100%) | Normal: 20 (60.6%) | Analysed 30 (90.9%) |
| Mean age         | 44.8 | Reactive: 13 (39.4%) | Dropouts 3 (9.1%) |
| Age min          | 35 |Skin condition: | | |
| Age max          | 55 | Combined: 12 (36.4%) | | |
| Phototype I      | 1 (3.0%) |Normal: 9 (27.4%) | | |
| Phototype II     | 5 (15.2%) | Dry: 8 (24.4%) | | |
| Phototype III    | 24 (72.7%) |Oily: 4 (12.1%) | | |
| Phototype IV     | 3 (15.2%) | | | |

The dermatologist did not observe any skin reactions ascribable to the test product. Moreover, the included subjects did not report any sensations of discomfort ascribable to the study product. According to these results, the product has very good skin compatibility.

Table 4 shows the mean values and percentages of variation with respect to the initial determination (D0) from the instrumental evaluations. Regarding skin luminosity, the study product presented a 9.5% and 16.5% increase after 28 and 56 days of application, respectively. For skin tone, the irregularities count and irregularities area improved. The irregularities count presented a 5.6% and 9% reduction after 28 and 56 days of application, respectively. The irregularities area was reduced by 10% after 56 days of application. These results were statistically significant.
Table 4. Instrumental efficacy evaluations.

| Parameter                                      | D0   | D28  | D56  |
|------------------------------------------------|------|------|------|
| Skin luminosity evolution                      | 6.2  | 6.7  | 7.1  |
| Skin luminosity variation                      | 9.5% | 16.5%|      |
| Skin luminosity, statistical significance a     | 0.019| <0.0001|
| Skin tone irregularities count evolution        | 70.5 | 64.2 | 64.1 |
| Skin tone irregularities count variation        | -5.6%| -9.0%|      |
| Skin tone irregularities count, statistical    | 0.04 | 0.007|      |
| significance b                                  |      |      |      |
| Skin tone irregularities area evolution         | 12.7 | 11.9 | 11.1 |
| Skin tone irregularities area variation         | -1.8%| -10.0%|      |
| Skin tone irregularities area, statistical      | 0.289| 0.022|      |
| significance a                                 |      |      |      |

D0: pre-treatment evaluation; D28: during treatment evaluation, after 28 days of application; D56: post-treatment evaluation, after 56 days of application. a Results from Wilcoxon Signed-Rank Test. b Results from paired sample test. The results are expressed as the mean value and p-value for statistical significance.

Figure 2 illustrates the results of a representative case where an increase in luminosity and even skin tone can be observed.

Figure 2. Representative case. D0: pre-treatment evaluation; D28: during treatment evaluation, after 28 days of application; D56: post-treatment evaluation, after 56 days of application.

Table 5 shows the subjective efficacy of the product, according to the subject’s evaluation. The results are expressed as the percentage of satisfied subjects and the mean score (1 being the top score).
Table 5. Subjective efficacy.

| Question about Cosmetic Efficacy | D28 Satisfied Subjects | D56 Satisfied Subjects |
|----------------------------------|------------------------|------------------------|
| It revitalizes the skin          | 100.0 (1.43)           | 100.0 (1.43)           |
| It leaves the skin more radiant  | 93.3 (1.53)            | 96.7 (1.43)            |
| It leaves the skin more luminous | 96.7 (1.50)            | 96.7 (1.47)            |
| It provides a more even skin tone| 93.3 (1.50)            | 96.7 (1.40)            |
| It improves the appearance of frown lines | 96.7 (1.57)    | 96.7 (1.50)            |
| It reduces fine wrinkles         | 93.3 (1.70)            | 96.7 (1.60)            |
| It improves skin texture         | 90.0 (1.47)            | 93.3 (1.43)            |
| It leaves the skin softer and smoother | 96.7 (1.30)    | 90.0 (1.40)            |
| It improves overall appearance of the skin | 96.7 (1.50)    | 93.3 (1.50)            |

D28: during treatment evaluation, after 28 days of application; D56: post-treatment evaluation, after 56 days of application. Results expressed as percentages and (medians).

4. Discussion

As previously described, vitamin C is a potent antioxidant that exerts different effects on the skin. To enhance these effects, diverse formulations were prepared with other antioxidant substances to study its antioxidant power and to choose the best formulation. The effects were determined by DPPH analysis. The effects of antioxidants on DPPH radical scavenging are believed to be due to their ability to donate hydrogen. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The decrease in absorbance of the DPPH radical caused by antioxidants, due to the reaction between the antioxidant molecules and the progressed radical, results in the elimination of radicals by hydrogen donation. The change could be observed visually as a color change from purple to yellow. Lastly, if the antioxidant exerts such an effect, the absorbance will be lower. The results were compared to those of Trolox, which is a water-soluble vitamin E analogue with high antioxidant capacity. The obtained results show that the addition of ginger to the formula enhances the effect of AA by three orders of magnitude compared to other tested combinations. Moreover, previous studies performed with ginger formulations demonstrated its efficacy in preventing cell damage and signs of cutaneous photoaging [24,25].

As previous authors indicate, clinical studies on the efficacy of topical AA formulations remain limited, and the challenge is to find the most stable and permeable formulation to achieve optimal results [26]. In this study, we present a stable anhydrous formula that contains a high dose of AA. In vitro and in vivo studies demonstrate its efficacy as an antiaging skin product.

The most relevant aspect for the applicability and efficacy of topical vitamin C is the proposal formulation. As previously described, in an aqueous environment, AA is rapidly degraded to different oxidation products, which concomitantly result in brownish discolorations and loss of efficacy. The stability of AA and its derivatives in emulsified systems have been investigated by other authors [27–29]. Our proposal is a water-free formula that protects the vitamin C from oxidation and degradation, as we have demonstrated in the HPLC studies conducted. Our option is presented as the extreme, an anhydrous formula that retains the effectiveness of topically applied AA to improve the appearance of photoaged skin. This type of vehicle has allowed us to work with high doses of pure vitamin C in combination with ginger, which proved to be highly effective in reducing oxidative stress caused by UVA radiation in the RHE 3D skin model by 93%. Our results are in line with previous studies that have concluded that oxidative stress increases the formation of ROS, which may be implicated in skin aging and pigmentation. Thus, improving the capacity of antioxidant defenses, using topical products, can reduce oxidants to cope with oxidative insults and stimulate natural antioxidation [13].
Vitamin C is one of the most powerful antioxidants in the skin, and there is a significant body of scientific research supporting the use of cosmeceuticals containing this ingredient due to its diverse biological effects, including antioxidant, photoprotection, antiaging, and depigmentation of the skin [13]. Regarding the antiaging and depigmenting effect, after 56 days of application, we obtained a 16.5% increase in skin luminosity and a 9% and 10% reduction in skin tone irregularities count and area, respectively. The in vivo study was carried out for 56 days; however, statistically significant results were determined from the first control point after 28 days of use. The volunteers’ self-assessment reported fine wrinkle reduction, improvement in skin texture and overall appearance of the skin, and more even skin tone, among others.

Several mechanisms have been reported to be associated with the depigmenting effect of vitamin C. It has been demonstrated that vitamin C can interact with copper ions at the active site of tyrosinase and inhibit its enzymatic function, which further decreases melanin formation [30]. Other evidence, more aligned with our in vitro results, has shown that vitamin C can protect cells against UVA-dependent melanogenesis by improving the antioxidant defense capacity and inhibiting nitric oxide (NO) production by downregulating eNOS and iNOS mRNA [31]. Many studies have found that vitamin C can increase collagen production, protect against damage from UVA and UVB rays, correct pigmentation problems, and improve inflammatory skin conditions [32]. Moreover, previous studies demonstrated significant improvement with active treatment greater than the control for fine wrinkles, tactile roughness, coarse rhytids, skin laxity/tone, sallowness/yellowing, and overall features [2]. In a clinical study examining the effect of a topical formulation containing 25% vitamin C and a chemical penetration enhancer, Hwang et al. reported a significant decrease in pigmentation caused by melasma after 16 weeks [33]. Moreover, Traikovich et al., in a 3-month daily regimen of topical AA, noted significant objective and subjective improvement in photodamaged facial skin [2]. All these previous studies indicate that topical application of AA improves skin appearance related with hyperpigmentation and aging.

Topical vitamin C is safe to use on a daily basis for long durations. It can safely be used in conjunction with other common topical antiaging agents such as sunscreens, tretinoin, other antioxidants, and alfa hydroxy acids. Minor adverse reactions are associated with the topical use of AA and its derivates. Rarely, stinging, erythema, and dryness are observed after use, which can easily be treated using a moisturizer [2,34]. These tolerance data are in line with the results obtained in our in vivo study.

As Kim et al. showed in their study, the combination of topical antioxidants and medical treatments improves efficacy [35]. Thus, our results seem to indicate that the formula studied could be also used as an adjuvant treatment in medical-aesthetic protocols to improve the signs of skin aging as well as hyperpigmentation.

5. Study Limitations

As a main limitation of this study, it should be noted that the enhancing antioxidant action of ginger has only been tested in preclinical studies as a way of selecting the active ingredient with greater antioxidant power. Further studies are needed to demonstrate the synergic action in vivo.

6. Conclusions

In conclusion, we were able to develop an anhydrous stabilized formula of pure vitamin C and ginger that combines very good stability, consumer acceptance, and skin tolerance with a high level of efficacy. Its activity includes protection against oxidative stress (ROS reduction), increase in skin tone uniformity, and improved skin luminosity for a rejuvenated skin appearance.
Author Contributions: Conceptualization, T.M.-V. and E.S.; methodology, T.M.-V. and N.C.; validation, T.M.-V., N.C. and E.S.; formal analysis, T.M.-V.; investigation, T.M.-V., N.C. and E.S.; resources, T.M.-V. and N.C.; data curation, T.M.-V. and N.C.; writing—original draft preparation, T.M.-V., N.C. and E.S.; writing—review and editing, T.M.-V., N.C. and E.S.; visualization, T.M.-V., N.C. and E.S.; supervision, E.S.; project administration T.M.-V.; funding acquisition, E.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Martiderm S.L., Cervellón, Spain. Project name and code: VITAMIN C ANTIOX, 202.

Institutional Review Board Statement: All investigations were carried out following the rules of the 1975 Declaration of Helsinki revised in 2013, and an independent ethics committee (Saúde Clínica Carlos Ramos, Lousado, protocol study code: PT.06.01) approved the study protocol.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful for the support received from the Martiderm laboratory, the research sponsor who has contributed to its financing and to providing all the necessary technical resources.

Conflicts of Interest: Author E.S. holds a position on Scientific Advisory Boards for the MartiDerm Group.

References
1. Draelos, Z.D. Dermatología Cosmética, Productos y Técnicas; Grupo Aula Médica: Toledo, Spain, 2011.
2. Traikovich, S.S. Use of topical ascorbic acid and its effects on photodamaged skin topography. Arch. Otolaryngol. Head Neck Surg. 1999, 125, 1091–1098. [CrossRef] [PubMed]
3. Nishikimi, M.; Fukuyama, R.; Minoshima, S.; Shimizu, N.; Yagi, K. Cloning and chromosomal mapping of the human non-functional gene for L-gulono-gamma-lactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man. J. Biol. Chem. 1994, 269, 13685–13688. [CrossRef]
4. Colven, R.M.; Pinnell, S.R. Topical vitamin C in aging. Clin. Dermatol. 1996, 14, 227–234. [CrossRef]
5. Tsao, C.S.; Young, M.A. stabilized ascorbic acid solution. Med. Sci. Res. 1996, 24, 473–475.
6. Al-Niaimi, F.; Chiang, N.Y.Z. Topical Vitamin C and the Skin: Mechanisms of Action and Clinical Applications. J. Clin. Aesthet. Dermatol. 2017, 10, 14–17.
7. Stamford, N.P. Stability, transdermal penetration, and cutaneous effects of ascorbic acid and its derivatives. J. Cosmet. Dermatol. 2012, 11, 310–317. [CrossRef]
8. Pinnell, S.R.; Yang, H.; Omar, M.; Monteiro-Riviere, N.; DeBuys, H.V.; Walker, L.C.; Wang, Y.; Levine, M. Topical L-ascorbic acid: Percutaneous absorption studies. Dermatol. Surg. 2001, 27, 137–142. [CrossRef]
9. Heber, G.K.; Markovic, B.; Hayes, A. An immunohistological study of anhydrous topical ascorbic acid compositions on ex vivo human skin. J. Cosmet. Dermatol. 2006, 5, 150–156. [CrossRef]
10. Ribeiro Neto, A.C.; Pires, R.F.; Malagori, R.A.; Moilhon, R.F. Solubility of Vitamin C in Water, Ethanol, Propan-1-ol, Water + Ethanol, and Water + Propan-1-ol at (298.15 and 308.15). J. Food Sci. Technol. 2012, 55, 310–317. [CrossRef]
11. Farris, P.K. Topical vitamin C: A useful agent for treating photoaging and other dermatologic conditions. Dermatol. Surg. 2005, 31, 814–817. [CrossRef]
12. De Dormael, R.; Bastien, P.; Sextius, P.; Gueniche, A.; Ye, D.; Tran, C.; Chevalier, V.; Gomes, C.; Souverain, L.; Tricaud, C. Vitamin C Prevents Ultraviolet-induced Pigmentation in Healthy Volunteers: Bayesian Meta-analysis Results from 31 Randomized Controlled versus Vehicle Clinical Studies. J. Clin. Aesthet. Dermatol. 2019, 12, E53–E59. [PubMed]
13. Chen, J.; Liu, Y.; Zhao, Z.; Qiu, J. Oxidative stress in the skin: Impact and related protection. Int. J. Cosmet. Sci. 2021, 43, 495–509. [CrossRef][PubMed]
14. Yaar, M.; Eller, M.S.; Gilchrest, B.A. Fifty years of skin aging. J. Investig. Dermatol. Symp. Proc. 2002, 7, 51–58. [CrossRef]
15. Gilchrest, B.A. Skin aging and photoaging. Dermatol. Nurs. 1990, 2, 79–82. [PubMed]
16. Pandel, R.; Poljšak, B.; Godic, A.; Dahmane, R. Skin photoaging and the role of antioxidants in its prevention. Int. Sch. Res. Not. 2013, 12, 930164. [CrossRef]
17. Burke, K.E. Interaction of vitamins A, C and E as better cosmeceuticals. Dermatol. Ther. 2007, 20, 314–321. [CrossRef]
18. Pullar, J.M.; Carr, A.C.; Vissers, M.C.M. The Roles of Vitamin C in Skin Health. Nutrients 2017, 9, 866. [CrossRef]
19. Gaspar, L.R.; Campos, P.M. Photostability and efficacy studies of topical formulations containing UV-filters combination and vitamins A, C and E. Int. J. Pharm. 2007, 343, 181–189. [CrossRef]
20. Kedare, S.B.; Singh, R.P. Genesis and development of DPPH method of antioxidant assay. J. Food Sci. Technol. 2011, 4, 412. [CrossRef]
21. Bae, E.J.; Seo, S.H.; Kye, Y.C.; Ahn, H.H. A quantitative assessment of the human skin surface using polarized light digital photography and its dermatologic significance. Ski. Res. Technol. 2010, 16, 270–274. [CrossRef]

22. Callaghan, T.M.; Wilhelm, K. A review of ageing and an examination of clinical methods in the assessment of ageing skin. Part 2: Clinical perspectives and clinical methods in the evaluation of ageing skin. Int. J. Cosmet. Sci. 2008, 30, 323–332. [CrossRef] [PubMed]

23. Nkengne, A.; Roure, R.; Rossi, A.B.; Bertin, C. The skin aging index: A new approach for documenting anti-aging products or procedures. Skin Res. Technol. 2013, 19, 291–298. [CrossRef] [PubMed]

24. Chaudhuri, R.K.; Meyer, T.; Premi, S.; Brash, D. Acetyl zingerone: An efficacious multifunctional ingredient for continued protection against ongoing DNA damage in melanocytes after sun exposure ends. Int. J. Cosmet. Sci. 2020, 42, 36–45. [CrossRef] [PubMed]

25. Dhaliwal, S.; Rybak, I.; Pourang, A.; Burney, W.; Haas, K.; Sandhu, S.; Crawford, R.K.; Sivamani, R. Randomized double-blind vehicle controlled study of the effects of topical acetyl zingerone on photoaging. J. Cosmet. Dermatol. 2021, 20, 166–173. [CrossRef] [PubMed]

26. Ravetti, S.; Clemente, C.; Brignone, S.; Hergert, L.; Allemandi, D.; Palma, S. Ascorbic Acid in Skin Health. Cosmetics 2019, 6, 58. [CrossRef]

27. Gallarate, M.; Carlotti, M.E.; Trotta, M.; Bovo, S. On the stability of ascorbic acid in emulsified systems for topical and cosmetic use. Int. J. Pharm. 1999, 188, 233–241. [CrossRef]

28. Spiclin, P.; Gasperlin, M.; Kmetec, V. Stability of ascorbyl palmitate in topical microemulsions. Int. J. Pharm. 2001, 222, 271–279. [CrossRef]

29. Raschke, T.; Koop, U.; Düsing, H.J.; Filbry, A.; Sauermann, K.; Jaspers, S.; Wenck, H.; Wittern, K.P. Topical activity of ascorbic acid: From in vitro optimization to in vivo efficacy. Skin. Pharmacol. Physiol. 2004, 17, 200–206. [CrossRef]

30. Sanadi, R.M.; Deshmukh, R.S. The effect of Vitamin C on melanin pigmentation—A systematic review. J. Oral Maxillofac. Pathol. 2020, 24, 374–382. [CrossRef]

31. Shindo, Y.; Witt, E.; Han, D.; Epstein, W.; Packer, L. Enzymic and non-enzymic antioxidants in epidermis and dermis of human skin. J. Investig. Dermatol. 1994, 102, 122–124. [CrossRef]

32. Poljsak, B.; Dahmane, R. Free radicals and extrinsic skin aging. Dermatol Res. Pract. 2002, 2012, 135206.

33. Hwang, S.W.; Oh, D.J.; Lee, D.; Kim, J.W.; Park, S.W. Clinical efficacy of 25% L-ascorbic acid (C’ensil) in the treatment of melasma. J. Cutan. Med. Surg. 2009, 13, 74–81. [CrossRef] [PubMed]

34. Telang, P.S. Vitamin C in dermatology. Indian Dermatol. Online J. 2013, 4, 143–146. [CrossRef]

35. Kim, J.; Lee, Y.I.; Almurayshid, A.; Jung, J.Y.; Lee, J.H. Effect of a topical antioxidant serum containing vitamin C, vitamin E, and ferulic acid after Q-switched 1064-nm Nd: YAG laser for treatment of environment-induced skin pigmentation. J. Cosmet. Dermatol. 2020, 19, 2576–2582. [CrossRef] [PubMed]