

## Article

# Effectiveness of a Complex Antioxidant Product Applied by Sonophoresis and Micro-Needle Mesotherapy

Anna Jaros-Sajda <sup>1</sup>, Elzbieta Budzisz <sup>1</sup>  and Anna Erkiert-Polgij <sup>2,\*</sup> 

<sup>1</sup> Department of Cosmetic Raw Materials Chemistry, Faculty of Pharmacy, Medical University of Lodz, Muszynskiego Street 1, 90-151 Lodz, Poland; elzbieta.budzisz@umed.lodz.pl (E.B.)

<sup>2</sup> Department of Cosmetology and Aesthetic Dermatology, Faculty of Pharmacy, Medical University of Lodz, Muszynskiego Street 1, 90-151 Lodz, Poland

\* Correspondence: anna.erkiert-polgij@umed.lodz.pl

**Abstract:** Antioxidants (including Vitamin C, ferulic acid, and phloretin) are among the compounds used in skincare products. The purpose of this study was to evaluate the effect of a combination containing L-ascorbic acid, ferulic acid, and phloretin. The delivery of active substances was facilitated by micro-needle mesotherapy and sonophoresis. The study was completed by 22 volunteers. The study resulted in a significant reduction of erythema and an improvement in skin hydration. According to participants, the therapy yielded visible anti-aging effects. The combination of up to three antioxidant-active ingredients and the use of transdermal application methods proved to be both safe and effective.

**Keywords:** ascorbic acid; ferulic acid; phloretin; anti-aging



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## 1. Introduction

Skin aging is a complex process influenced by the environment, lifestyle, and individual genetic factors [1,2]. One of the environmental factors accelerating aging is Reactive Oxygen Species (ROS). ROS damages cellular structures, including DNA and lipids. ROS especially leads to the degradation of collagen and elastin, as well as the formation of dysfunctional, disorganized clusters of collagens, elastic fibers, glycosaminoglycans, and glycoproteins [3,4]. In turn, these processes lead to the appearance of photoaging pigmentation, telangiectasias, deep wrinkles, rough skin texture, etc. [5].

Antioxidants are among the compounds used in skincare formulations [6]. They can delay skin aging associated with ultraviolet (UV) rays, environmental pollution, and infrared (IR) radiation [7,8]. Vitamin C is considered as one of the most potent antioxidants in combatting oxidative stress. Being an electron donor, it exerts a reducing effect, thereby protecting cellular elements from oxidation [9]. Ascorbic acid not only stimulates transcription factors involved in collagen synthesis and stabilizes procollagen mRNA, but it also inhibits collagen degradation [10]. Beneficial effects of Vitamin C also include protection against photocarcinogenesis, resulting from UV-induced erythema and thymine dimer mutation [5]. In addition, Vitamin C is also known for its anti-pigmentation properties as it inhibits the activity of tyrosinase, which is involved in the conversion of tyrosine into melanin, thus blocking the formation of melanin [11,12]. However, ascorbic acid is very unstable. It oxidizes easily and, thus, requires specific conditions, such as an acidic environment for better stability, as well as limited exposure to light and air. To increase its stability, ascorbic acid preparations may also contain ferulic acid [13]. In addition to acting as a stabilizer, ferulic acid also exhibits strong free-radical-sweeping properties, along with anti-inflammatory, anti-aging, anti-wrinkle, and tyrosinase expression-inhibiting effects [14,15], by which it reduces pigmentation and brightens the skin. The specific chemical structure of ferulic acid allows it to be a good antioxidant, even after it joins to the lipid bilayer membrane, providing protection against lipid peroxidation. It also takes part in angiogenesis by VEGF

(vascular endothelial growth factor) and PDGF (platelet-derived growth factor) [16,17]. Test results have shown that ferulic acid provides photoprotection as it can absorb UV radiation, occur in the regulation of repair after sun damage to the skin, and enhance SPF activity [18,19].

Interest in the flavonoid phloretin is due to its antioxidant, anti-aging, and depigmentation effects [20]. In addition, it exhibits antimicrobial and anticancer properties and is known for protecting the skin from damage caused by ultraviolet radiation [21–24]. It has been found to be beneficial in the treatment of melasma, photoaging, and acne. Like Vitamin C, phloretin inhibits elastase and extracellular matrix metalloproteinase 1 (MMP-1) activity, and it reduces both tyrosinase activity and melanin content by the chelation of copper ions, which are the key ions in the active site of tyrosinase [20].

The constant search for improved methods of applying skincare preparations is associated with problems with the instability of the above-mentioned active substances, as well as the fact that these compounds should be administered to the deeper layers of the skin without reaching the systemic circulation to avoid adverse side effects [6]. The delivery of active substances is facilitated by various techniques, including micro-needle mesotherapy and sonophoresis. In the first method, the skin is punctured to improve the delivery of compounds to the living epidermis or dermis (in photoaging skin), but it also triggers the body's self-repair mechanisms by stimulating the production of collagen, hyaluronic acid, and elastin [25]. Because we decided to compare the effects of antioxidants, we chose 0.2 mm of needles to obtain only the effect of a better penetration of ascorbic acid, not to start a real skin regeneration after longer-needle microneedling [26–28]. Sonophoresis, on the other hand, uses low-frequency ultrasound to intensify the delivery of active ingredients deep into the skin.

The aim was to evaluate the effect on physiological skin parameters of a combination of L-ascorbic acid, ferulic acid, and phloretin, applied via mesotherapy (to the right side of the face) or sonophoresis (to the left side).

## 2. Materials and Methods

### 2.1. Volunteers

The study involved 30 healthy female volunteers aged 27–60 with visible erythematous lesions. Due to the COVID-19 pandemic, only 22 of them completed the therapy (Phototypes II–III, mean age 43). Exclusion criteria included pregnancy and lactation, active viral, and bacterial and fungal lesions, as well as participation in other dermatological therapies throughout the duration of the study and 6 months prior.

### 2.2. Applied Formulation

Formulation containing 10% L-ascorbic acid (pH = 3.0) stabilized with 0.5% ferulic acid and 2% phloretin (INCI: Water/Aqua/Eau, Ethoxydiglycol, L-Ascorbic Acid (Topical Vitamin C), SD Alcohol 40, Propylene Glycol, Glycerin, Phloretin, Triethanolamine, Ferulic Acid, D-Panthenol, Phenoxyethanol, Tetrasodium Edetate, Hyaluronic Acid) was applied to each participant's face through micro-needle mesotherapy (0.2 mm) (on the right side of the face) or sonophoresis (on the left side) (Figure 1).

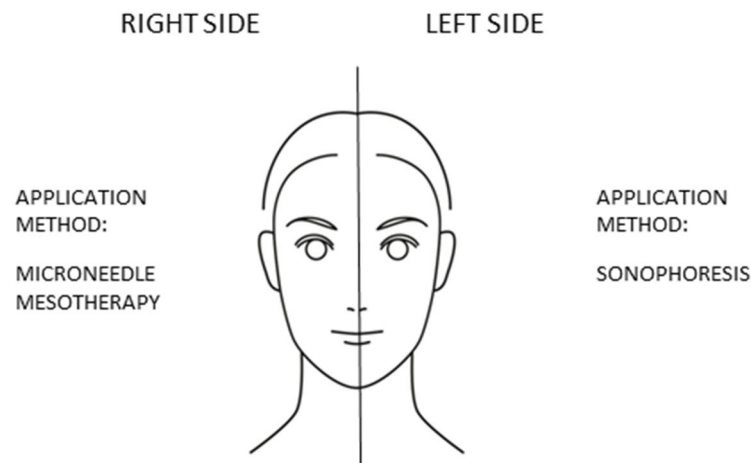


**Figure 1.** Diagram of the usage of sonophoresis/microneedle mesotherapy for antioxidant application.

### 2.3. Protocol

The therapy consisted of six series of treatments performed at 2-week intervals. Both techniques were applied to the same subjects—micro-needle mesotherapy on the right side of the face and sonophoresis on the left side, respectively; all the subjects were treated on whole face with the same cosmetic.

### 2.4. Microneedle Mesotherapy

The formulation was applied to the facial skin with microneedle mesotherapy (0.2 mm) on the right side of the face. The therapy consisted of six series of treatments performed at 2-week intervals. The microneedle mesotherapy was applied using a sterile tip. The microneedle equipment (Dr. Pen, Shanghai, China) was applied at 0.2 mm for 3–5 min to the slight erythema.

### 2.5. Sonophoresis

On the left part of the face, sonication with examined cosmetics was conducted in the continuous mode, at an intensity of 1–1.5 W/cm<sup>2</sup> and a frequency of 2 MHz for 5 min (SY089, Zhong Shan Syou EiBeauty Instrument, Zhongshan, Guangdong, China). The therapy was also performed six times, every second week. During sonophoresis, ultrasonic waves improve the transfer of active substances deep into the skin by increasing cell permeability and the kinetic energy of the active substance particles. Ultrasounds of frequency 2 MHz were used. In high-frequency ultrasounds, cavitation bubbles seem to have the most important effect. These bubbles can interact and modify the skin barrier to form regions of increased permeability within the stratum corneum [29].

### 2.6. Skin Measurement Methods

Skin condition was assessed both before treatment and 2 weeks after the sixth session. Severity of erythema, pigmentation, the amount of sebum, as well as hydration and changes in transepidermal water loss (TEWL), were measured using an MPA 580 multi-probe system (Courage and Khazaka Electronic GmbH, Cologne, Germany). The used probes were:

- Mexameter MX18 probe—to measure the level of hemoglobin (erythema) and melanin;
- Sebumeter SM 815 probe—to measure sebum level on the skin;
- Corneometer CM825 probe to assess skin hydration by measuring water content in the stratum corneum;
- Tewameter TM300—to assess transepidermal water loss, evaluation of water barrier function;
- Cutometer MPA518 to evaluate skin elasticity and viscoelastic properties of the skin.

Measurements were conducted at the same location under constant conditions (temperature 20 °C and humidity 45% ± 5%) after participants were acclimatized to the envi-

ronment for 15 min. The participants were asked not to change their daily skincare routine. All of them were asked to use sunscreen every day and avoid the sun.

There was no control group. The results after the procedure were compared to the results obtained before the procedure.

Treatment effects were recorded with FotoMedicus system (equipped with Canon EOS camera with zoom control and a specially developed studio flash with high flash energy and constant color temperature) in the same room and under the same lighting during similar times of the day. FotoMedicus system because of special chair and technological functions let us take photos in the same face position after weeks.

In addition, the volunteers were asked to fill in a questionnaire evaluating the effects of the treatments that they observed. Also, in a questionnaire, the participants were asked to write about treatment's side effects. Also, during each visit, the person performing the study observed and discussed the side effects.

The study was conducted in accordance with the 1964 Helsinki Declaration, as amended, and was approved by the bioethics committee of the Medical University of Lodz. All women participating in the study signed an informed consent form.

### 2.7. Statistics

Mean and standard deviation (mean  $\pm$  SD) were used to describe normal distribution parameters. Differences in percentage changes in skin parameters from baseline values were calculated using the following formula:  $(([x(t_1) - x(t_0)]/x(t_0)) \times 100)$ .  $t_1$ - 2 weeks after the last session of treatment,  $t_0$ - beginning of the study. Variables that did not indicate normal distribution was expressed as median and quartile range (median (25%; 75%)). In order to evaluate differences in skin parameters over time, the  $t$ -test was used for data with a normal distribution and the Wilcoxon rank-sum test for data that deviated from normality. The Mann–Whitney test was used to statistically evaluate differences between independent samples with distributions deviating from normal. Statistical evaluation of categorical variables was performed using chi-square tests or Fisher's exact test.  $p$  values less than 0.05 were considered statistically significant.

### 3. Results

It was found that the treatment significantly reduced erythema on both the left and right sides of the face. Compared to the first measurement, erythema was reduced by 16% and 27% on the left side (forehead and cheek, respectively) and by 17% and 25% on the right side of the face (Figure 2).

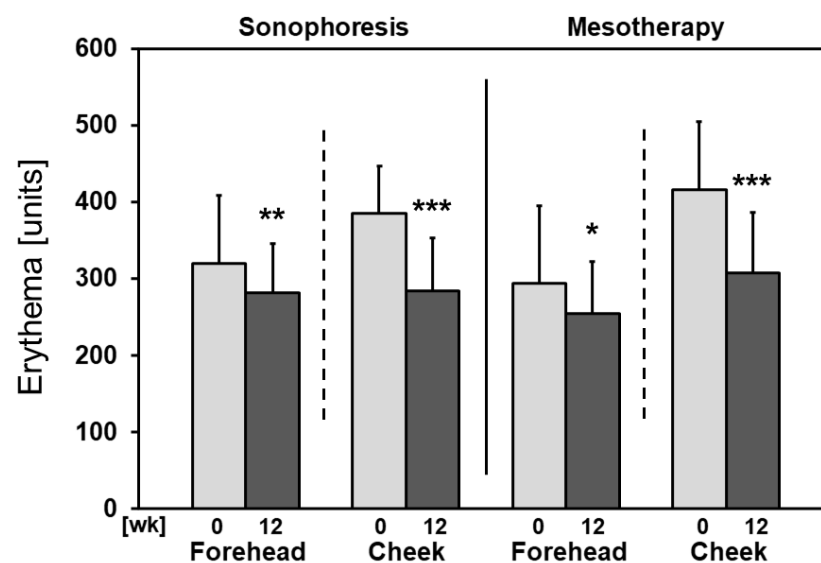


Figure 2. Effects of erythema reduction, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  compared to Day 0.

Significant improvements in skin hydration were observed on both cheeks (Figure 3). Hydration increased by 16% on the right side and by as much as 36% on the left side compared to the baseline. In contrast, the increase in hydration in the forehead was not statistically significant, but the baseline was within the range of normal results.

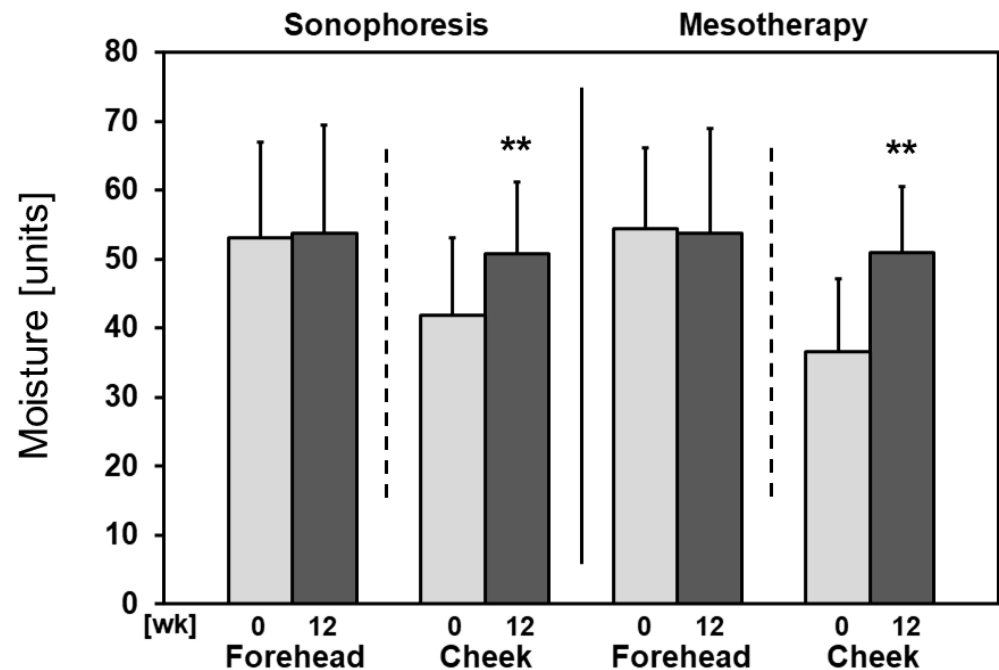


Figure 3. Effects of skin hydration enhancement, \*\*  $p < 0.01$  compared to Day 0.

The use of the tested preparations and both application methods significantly reduced transepidermal water loss (TEWL) in the entire facial area (Figure 4). Slightly better results were shown on the right side (reduction of 14% on the forehead and 21% on the cheeks on the right side versus 12% on the forehead and 14% on the left side) compared to baseline.

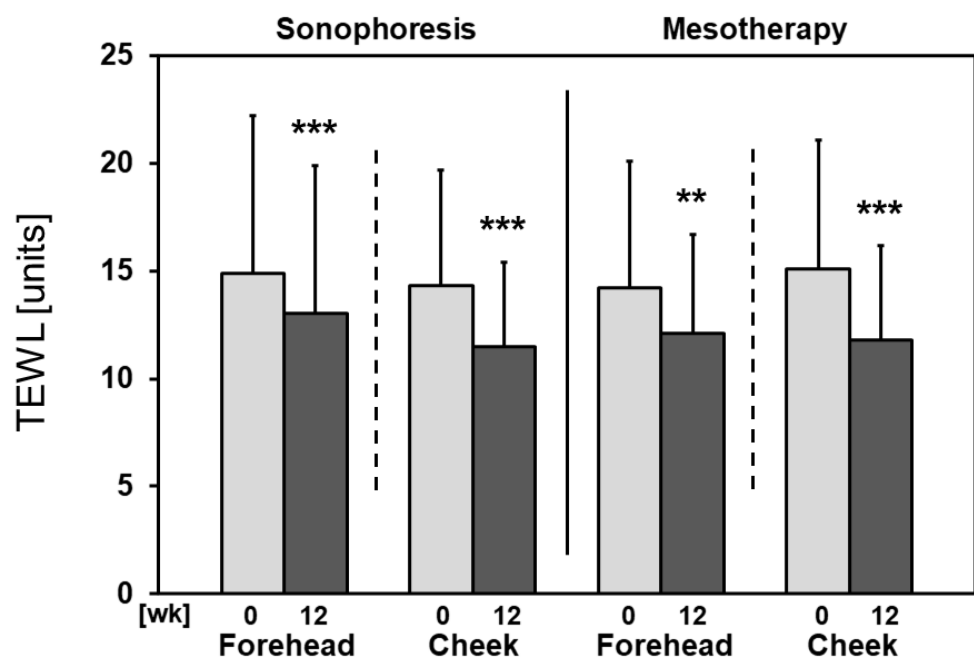


Figure 4. Effects of TEWL reduction, \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  compared to Day 0.

Results showed no significant effect on sebum secretion. Its level was normal for all participants, which may explain the lack of significant change in this parameter after a series of treatments.

Volunteers did not have significant hyperpigmentation. However, an evaluation of the photos showed a brightening and leveling of the skin tone (Figure 5). The results of measurements by the Mexametr probe and MPA 580 system (Courage and Khazaka) showed a slight reduction in melanin levels, but the difference was not statistically significant.

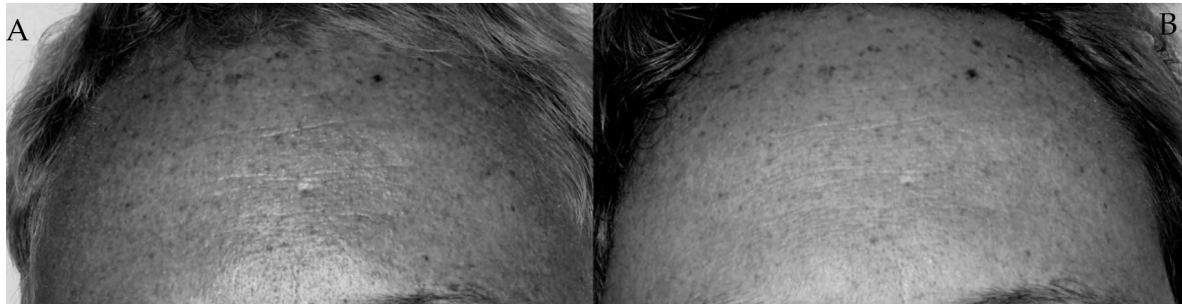


**Figure 5.** Brightening of skin hyperpigmentation after series of treatments. (A)-photo taken before treatment, (B)-photo taken after series of 6 treatments.

The applied therapy did not result in a significant change in the R2 and R5 parameters. The R2 parameter increased non-significantly on the right side compared to the left side and baseline value. The R5 parameter improved slightly in all areas except the left side of the forehead compared to the baseline value. (Table 1).

**Table 1.** Viscoelastic results. All the results were not statistically significant.

| R2             | Before Treatment     | After Treatment      |
|----------------|----------------------|----------------------|
| Left forehead  | 0.392 (0.346; 0.516) | 0.374 (0.295; 0.538) |
| Left cheek     | 0.436 (0.389; 0.680) | 0.491 (0.394; 0.643) |
| Right forehead | 0.459 (0.378; 0.481) | 0.632 (0.320; 0.935) |
| Right cheek    | 0.417 (0.323; 0.492) | 0.529 (0.429; 0.660) |
| R5             |                      |                      |
| Left forehead  | 0.184 (0.130; 0.400) | 0.167 (0.111; 0.182) |
| Left cheek     | 0.227 (0.200; 0.351) | 0.229 (0.150; 0.344) |
| Right forehead | 0.250 (0.205; 0.258) | 0.281 (0.158; 0.314) |
| Right cheek    | 0.186 (0.116; 0.476) | 0.197 (0.125; 0.376) |

A statistical comparison of the effects of measurements obtained between the sides did not show an advantage for either micro-needle mesotherapy or sonophoresis.

The questionnaire results indicate that all the participants recognized an improvement in skin condition, along with its brightening and reduction of erythema. In addition, most women experienced improved skin elasticity, increased skin density, and hydration. Skin became less reactive to changes during exercise under the influence of alcohol and spicy foods.

During the study, only three volunteers reported skin irritation on the left side, while four participants reported skin irritation on the right side, but this effect disappeared a few hours after treatment (Figures 6–10).

Evaluation of the effectiveness of the treatments by patients

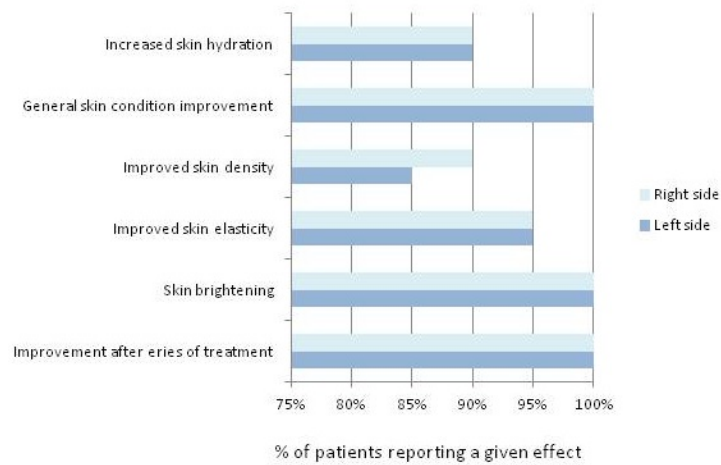


Figure 6. Evaluation of the effectiveness as assessed by volunteers.

Short-term side effects

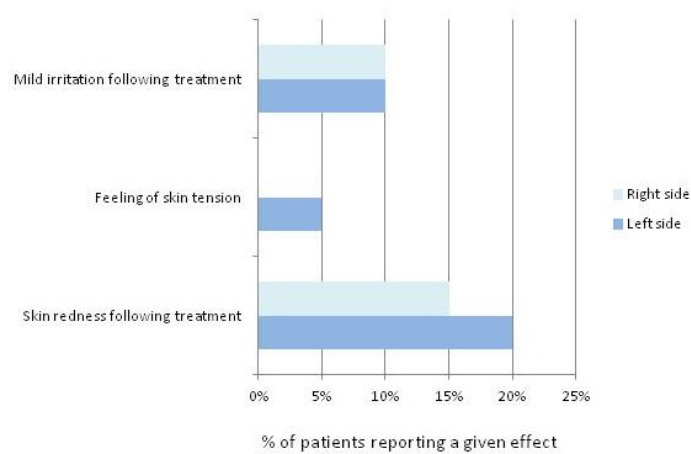


Figure 7. Volunteers’ reported short-term side effects of treatments.



Figure 8. Comparison of skin appearance before treatments (A) with appearance after a series of treatments (B)—erythema reduction, smoothing of skin structure and brightening of the skin.



Figure 9. Comparison of skin appearance before treatments (A) with appearance after a series of treatments (B)—erythema reduction.



**Figure 10.** Comparison of skin appearance before treatments (A) with appearance after a series of treatments (B)—anti-aging effect.

#### 4. Discussion

Changes in skin appearance, which represent the initial signs of aging, can negatively impact one's psychological well-being [30]. Identifying the mechanisms involved in this complex process will allow for the development of strategies aimed at preventing and combating skin aging. The group of women taking part in our study was special because of the erythema on their faces. It also can have an impact on one's well-being because it is visible and sometimes difficult to cover with make-up.

In this study, Vitamin C in the form of L-ascorbic acid was used combined with 0.5% ferulic acid and 2% phloretin, which could enhance the vitamin's properties. Other researchers have also observed that the antioxidant action of Vitamin C can be strengthened when combined with other antioxidants with different redox potentials, such as  $\alpha$ -tocopherol [5,31]. The study confirms other studies showing that the combination of 15% Vitamin C with 0.5% ferulic acid can significantly increase the effectiveness of Vitamin C, explaining the excellent reduction of erythema and anti-aging effects [13].

Erythema is associated with the mechanical resistance of blood vessel walls. Therefore, it is crucial that ascorbic acid is involved in collagen synthesis at multiple stages, including gene transcription and post-translational modification, although it does not have a direct impact on translation processes [32]. It can directly activate transcription factors that control collagen gene expression, influencing cellular signal transduction processes [32]. This acid serves as an essential cofactor for prolyl and lysyl hydroxylase and plays a crucial role in collagen maturation at the post-translational stage. It has been shown that ascorbic acid stimulates the transcription of pro-collagen Genes I and III in cells, selectively increasing the synthesis of collagen proteins without affecting non-collagen proteins in human fibroblast cultures [33–36]. Alternatively, it may enhance the expression of TGF- $\beta$ 1 and activate signal transduction to ultimately increase collagen gene expression. The obtained results suggest that its level in skin cells is closely related to the quantitative and structural integrity of skin collagen. A deficiency results in skin aging [37,38].

Ferulic acid appears to enhance the action of Vitamin C, which, by stimulating collagen production, indirectly contributes to tightening blood vessels. Other studies have also confirmed the positive effect of ascorbic acid on maintaining the mechanical resistance of blood vessel walls and the integrity of their structure [39,40]. Some studies suggest that phloretin also protects blood vessels [41,42]. Oresajo et al. [24] demonstrated that a mixture containing Vitamin C, ferulic acid, and phloretin protects human skin from the harmful effects of UV radiation and prevents skin photodamage. The use of this antioxidant preparation reduced the formation of sunburned cells, the expression of matrix metalloproteinase-9, and p53 protein, as well as the formation of thymine dimers. The most effective method to reduce erythema was treating the cheeks, and there was more erythema on the cheeks than on the forehead at the beginning of the study. The effect is seen in the mexameter results, but it also can be seen in photos. The combined treatment is safe in erythema reduction, and side effects, which can easily appear on erythematous skin, were not a problem in our study.

What is worth emphasizing is the effect on the reduction of the reactivity of the skin—the temporary worsening of erythema. Due to the antioxidant effect of ingredients, and their impact on collagen synthesis, all participants noticed an improvement in their skin condition during physical exercises, alcohol consumption, or spicy food consumption. This effect is important in everyday life and improves one's psychological well-being.

Vitamin C has a beneficial impact not only on microcirculation, reducing the visibility of redness, but also on depigmenting processes. Vitamin C has also been shown to inhibit melanogenesis, which can be used to reduce skin pigmentation. As mentioned at the beginning of the discussion, ascorbic acid inhibits tyrosinase activity, thus limiting melanin production [43,44]. Although the participants did not have severe hyperpigmentation, an insignificant decrease in melanin content was shown by mexameter analysis, and all participants in the questionnaire declared brightening of the skin.

Transepidermal water loss (TEWL) is a skin parameter that describes the barrier function of the skin. Erythematous skin is often sensitive and can have an impaired barrier function. The use of 10% L-ascorbic acid in combination with 0.5% ferulic acid and 2% phloretin resulted in significantly reduced transepidermal water loss (TEWL) across the entire face and increased hydration of the cheeks. The skin on the cheeks was drier than on the forehead, and this can be probably the reason for the less pronounced improvement on the forehead. The improvement in TEWL and hydration may be due to the anti-inflammatory action of phloretin [45]. Skin with erythema often has a damaged hydrolipidic barrier, which predisposes it to inflammation. The use of phloretin reduces inflammation [45] and, therefore, allows the skin to rebuild its hydrolipidic barrier, which is reflected in improved TEWL and increased hydration. In many other studies with Vitamin C, no significant improvement in skin hydration or TEWL was observed, which was attributed to the exfoliation of epidermal cells due to the acidity of ascorbic acid, resulting in surface skin dehydration [46,47]. In this study, the delivery of the active substance deep into the skin may have prevented this effect. Differences in hydration between both sides of the face may be related to the fact that mesotherapy itself contributes to increased skin hydration, which in turn cannot be said for sonophoresis [14]. Different results were obtained in a study that evaluated the effects of applying ascorbic acid and its derivatives (magnesium ascorbyl phosphate (MAP) and ascorbyl tetraisoalmitate (ATIP)) to the forearm skin. After 4 weeks of use, all preparations increased the hydration level of the stratum corneum, but only ascorbic acid, which was also used in this study, improved TEWL values [16]. In another study on cell lines, it was shown that Vitamin C administration improves the lipid barrier and stimulates keratinocyte differentiation, which may contribute to the thickening of the stratum corneum while ensuring the integrity of the skin barrier and improving hydration retention [17,37]. These results suggest that the effects of ascorbic acid may be significantly influenced by the application site and technique.

In this study, no significant difference was observed in the effectiveness of therapies performed using sonophoresis or micro-needle mesotherapy (0.2 mm). This is likely due

to the fact that both methods have the ability to increase the penetration of the active substance and, on the basis of our results, it should be concluded that both of them are effective. The literature suggests that micro-needle mesotherapy also stimulates the regeneration of collagen and elastin fibers [48,49], but in this study, it did not show a better improvement in skin elasticity compared to sonophoresis. This is probably due to the use of too-shallow needle punctures, which did not affect collagen production but only increased the penetration of ascorbic acid and other substances through the epidermal layer [50]. In contrast, in the case of sonophoresis, it is ultrasonic waves, not micro-needles, that increase cell permeability and the kinetic energy of active substance particles, thus improving their penetration through the epidermal layer [50].

The applied therapy did not significantly improve the R2 and R5 parameters. However, it seems that gross elasticity (R2) improved more with microneedle mesotherapy. The impact of both techniques on net elasticity (R5) appears to be similar. Despite significant changes in the aforementioned parameters, each study participant reported an improvement in skin condition, skin brightening, and a reduction in redness. Moreover, most participants observed improved skin elasticity, increased density, and hydration. The skin became less reactive following physical exercise, alcohol consumption, or eating spicy foods. The effect of three combined antioxidants was seen on participants' faces, as well as in the above-mentioned aspect of decreasing skin reactivity, which was important for our participants. The anti-aging benefits of Vitamin C have been confirmed in clinical studies on humans. The lack of statistically significant differences in the results of R2 and R5 parameters may be due to the small size of the study group or their relatively young age. In a double-blind, placebo-controlled study, topical application of 10% Vitamin C for a period of 12 weeks significantly reduced signs of photoaging and wrinkles compared to placebo [51]. It was found that the local application of a cream containing 3% Vitamin C for 4 months significantly increased the density of skin papillae, probably through angiogenesis [52]. Additionally, Sauermann et al. [52] pointed to the partial restoration of the anatomical structure of the epidermal–dermal junction in young skin, as well as an increase in the nutritive loops of blood vessels in the papillary dermis in the aging skin of postmenopausal women. Xu et al. [53] demonstrated a reduction in surface roughness and fine wrinkles in subjects treated with locally applied 23.8% L-ascorbic acid serum, which also inhibited elastin synthesis *in vitro* [54]. It has also been shown that ferulic acid plays a protective role in important skin structures, such as collagen, keratinocytes, and elastin fibroblasts [14].

Finally, our study has potential limitations. Firstly, there could be a greater group of volunteers. There is also no control placebo group. The placebo group could be understood in two ways. Firstly, adding a group with no cosmetic, or the blank cosmetic, could add value to our paper on the effect of only the method of application to the skin. Secondly, the placebo group could use only cosmetic topically with no special application method. The face skin is quite specific in our body. Because of the impact of external factors, we did not decide to add extra places of body skin to our protocol. We controlled our post-treatment results to before-treatment ones.

## 5. Conclusions

In summary, the study demonstrated a significant impact on redness and visible signs of skin aging. At the same time, the therapy was not associated with important or long-lasting side effects. The innovative solution, combining three active ingredients and using the transdermal application method, proved to be both safe and effective. The effectiveness of the therapy is confirmed by subjective and objective measurement methods. The results of this study indicate that the combination of antioxidants can multiply the anti-aging effects.

**Author Contributions:** Conceptualization, A.J.-S. and A.E.-P.; methodology, A.J.-S.; software, A.J.-S.; validation, A.E.-P. and E.B.; formal analysis, A.E.-P.; investigation, A.J.-S.; resources, A.J.-S. and A.E.-P.; data curation, E.B.; writing—original draft preparation, A.J.-S.; writing—review and editing, A.E.-P. and E.B.; visualization, A.J.-S.; supervision, E.B.; project administration, A.E.-P.; funding acquisition, E.B. All authors have read and agreed to the published version of the manuscript.

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

**Data Availability Statement:** Data available on request.

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

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