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Artichoke Leaf Extract Effectiveness on the Skin Aging Exposome: Efficacy and Safety Results of a Split-Face Study

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Abstract: The skin is a barrier organ subjected to lifelong exposure to internal and external factors influencing both its biological response and appearance. A randomized split-face study was carried out on 22 adult female outdoor workers using an artichoke leaf extract (Cynage^{TM}). The product's efficacy was measured before and after 28 days of use. The following skin parameters were measured: wrinkle depth, skin roughness, and radiance, to assess the product's effect on the skin appearance; Ferric Reducing Antioxidant Power (FRAP), to assess the total antioxidant capacity; and tumor necrosis factor-alpha (TNF-α) levels, to assess the anti-inflammatory efficacy. These parameters were also integrated by the evaluation of the subjective perception of product efficacy. After 28 days of product use, the skin's appearance improved as follows: wrinkle depth and skin roughness decreased by 5.2% and 7.0%, respectively, while the skin radiance increased by 19.0%. The total antioxidant capacity of the skin increased by 20.2%. The skin's TNF-α levels decreased by 8.2%. The product efficacy was also perceived by the subjects participating in the study. The product was well tolerated. Our findings demonstrate the active role of the ingredient in decreasing the skin damage induced by the exposome.

Keywords: skin exposome; clinical study; artichoke extract; oxidative stress; skin inflammation

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1. Introduction

Global pollution and exposure to hazardous chemical substances, driven by industrialization and modernization, have worsened over the last six decades. It has been estimated that since the 1960s, more than 350,000 chemical molecules have been introduced to our daily lives, and many of them have ended up as pollutants contributing to the human exposome [1]. Being a barrier organ, the skin is subject to lifelong exposure to a variety of environmental factors which undermine its barrier efficacy [2], influencing the physiology of the skin's response to them and its aging process [3].

The skin aging exposome was defined by Krutmann et al. [3] and "consists of external and internal factors and their interactions, affecting a human individual from conception to death as well as the response of the human body to these factors that lead to biological and clinical signs of skin aging". The external factors of the skin aging exposome include sun radiation (UV radiation, visible light, and infrared radiation), air pollution, climate change, and other factors falling into the lifestyle category (e.g., tobacco smoke, beauty routine, nutrition, lack of sleep, etc.) [4,5]. Interestingly, the impact of the exposome was quantified to be 80% [6], while that of genome-wide-associated diseases did not exceed

20% [7]. Exposome skin aging is driven by the generation of oxidative reaction species (ROS) [8–10] but also by the induction of the expression of genes related to the skin's protective capabilities against oxidation, such as heme oxygenase-1 (HO-1) and superoxide dismutase-2 (SOD2) [11–15].

In recent years, it has been demonstrated that the oxidative stress initiated by both UV and pollutant exposure contributes to the release of pro-inflammatory cytokines such as IL-2, IL-6, and TNF-α [16]. UV radiation also contributes to the activation of pathways involving lipoxygenase (LOX) and cyclooxygenase (COX) [17] which in turn produce ROS [18] and lipid mediators that induce inflammation [19]. The complex interplay between ROS and chronic levels of inflammation promotes aging, called inflammaging [20].

Cynara cardunculus L. var. *scolymus*, commonly known as globe artichoke, has been well known for its culinary and medicinal properties since ancient times. The most important bioactives found in artichoke are polyphenolic compounds (mainly flavonoids and phenolic acids) and sesquiterpenes such as cynaropicrin. It has been reported that the leaves have a higher concentration of sesquiterpenes and phenolic compounds [21]. In recent years, artichoke extracts have gained interest in topical applications, including cosmetics. Marques et al. investigated an artichoke extract containing chlorogenic acid and other bioactive molecules in topical formulations, observing ROS scavenging properties in the presence of H_2O_2 and after UVB radiation in a keratinocyte model. In the in vivo study, formulations containing the artichoke extract decreased the oxidant activity of the UVA radiation, evidencing a true in vivo antioxidant capacity [22]. In recent years, Elsebai et al. demonstrated the protective effect of cynaropicrin on UVB-induced skin aging in normal human keratinocytes [23]. The mechanism of action was related to a decrease in ROS production and the inhibition of pro-inflammatory cytokines (IL-6 and TNF-α) due to the activation of the AhR (aryl hydrocarbon receptor)-Nrf2 (nuclear factor E2-related factor 2)-Nqo1 (NAD(P)H:quinone oxidoreductase 1) pathway. Additionally, Tanaka et al. reported that cynaropicrin suppresses NF-κB-mediated transactivation of bFGF and MMP-1, preventing the photoaging of the skin induced by UV radiation [24].

In this study, we aimed to investigate the antiaging, antioxidant and anti-inflammatory effect of the topical application of a standardized artichoke leaf extract (CYNAGE^{TM}, Bionap S.r.l., Piano Tavola Belpasso, CT, Italy). These putative mechanisms of action were tested on 22 human volunteers.

2. Materials and Methods

2.1. Trial Design

The trial was a single center, randomized (1:1 balanced randomization), split-face, double blind, placebo-controlled trial conducted in Complife Italia facility in San Martino Siccomario (PV, Italy) between September and November 2023. Complife is an independent international group of testing laboratories specialized in the in vitro and in vivo safety and efficacy assessment of cosmetics, food supplements and medical devices.

The study duration was 28 days. The study duration encompasses one complete stratum corneum turnover time and represents a cosmetic acceptable timing to observe any improvement in the skin. Subjects attended 3 clinical visits as follows: a screening visit followed by a basal visit (D0) and a follow-up visit after 28 days of products use. Subjects were informed of the study procedures, risks and benefits at the screening visit, while an informed consent form was obtained from all subjects at baseline. The study endpoints included the measurement of the following parameters: skin radiance (8° gloss parameter), skin profilometry (wrinkle depth and skin roughness), total skin antioxidant capacity (FRAP assay), and TNF-α cytokine dosage. These endpoints were chosen because of their correlation with the ageing process and to demonstrate the product's efficacy in supporting well-aging of the skin.

All the study procedures were carried out in compliance with the ethical principles for medical research (Ethical Principles for Medical Research Involving Human Sub-

jects, adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964) and its amendments.

2.2. Participants

Eligible subjects were all healthy female subjects aged between 40 and 65 years old showing both chrono- or photo-aging with dull skin and an outdoor career. For the study purposes, an outdoor career was defined as workers spending from 4 to 8 h of their workday doing tasks outside (e.g., traffic warden, warehousers, etc.). Exclusion criteria were acute or chronic diseases that were able to interfere with the outcome of the study or that are considered dangerous for the subject or incompatible with the study requirements; pregnant or breastfeeding women; pharmacological treatments that are considered incompatible with the study requirements by the investigator; and allergies or sensitivity to cosmetic products, drugs, patches, or medical devices. The complete inclusion and exclusion list is reported in the Supplementary Materials (Table S1).

2.3. Interventions and Randomization

Active and placebo products were randomly applied on the right or left side of the face. A restricted randomization list was computer-generated (PASS 11, version 11.0.8, PASS, LLC, Kaysville, UT, USA) using the "Efron's biased coin" algorithm by an external statistician. The active product was a cream containing 1.5% of a standardized artichoke leaf extract (CYNAGE^{TM}, Bionap S.r.l., Piano Tavola Belpasso, CT, Italy). The extract contained (*w*/*w*) 1–3% chlorogenic acid and its derivatives. The complete ingredient list of the active product is: AQUA/WATER, GLYCERIN, ISOAMYL COCOATE, ETHYLHEXYL STEARATE, GLYCERYL STEARATE CITRATE, HYDROXYETHYLACRYLATE/SODIUM ACRYLOYDIMETHYLTAURATE COPOLYMER, MALTODEXTRIN, POLYISOBUTENE, PEG-7 TRIMETHYLOLPROPANE COCONUT ETHER, CYNARA SCOLIMUS LEAF EXTRACT, PHENOXYETHANOL, ETHYHEXYLGLYCERIN, BENZOIC ACID, DEHYDROACETIC ACID, HYDROXYACETOPHENONE, XANTHAN GUM, SODIUM PHYTATE, ALCOHOL, TOCOPHEROL, SODIUM HYDROXIDE. The placebo formula was the same as the active formula without the active ingredients (CYNARA SCOLYMUS LEAF EXTRACT and MALTODEXTRIN). Both the active and the placebo products were applied twice a day in the morning and in the evening on cleansed skin.

2.4. Outcomes

2.4.1. Skin Profilometry

Wrinkle depth and skin roughness (Ra parameter) were measured in the periocular area ("crow's feet" wrinkles) using small-field PRIMOS^{CR} (Canfield Scientific GmbH, Bielefeld, Germany). The small-field PRIMOS^{CR} is a real 3D camera based on fringe projection, with a field of view of 45 × 30 × 25 mm (L × W × H) and a resolution of 20 × 20 × 2 µm (X,Y,Z). Subject repositioning was ensured by a stereotactic face device (Canfield Scientific GmbH, Bielefeld, Germany). The wrinkle depth was measured as the maximum length of the furrow over its transversal section. The Ra parameter was calculated as the average of the absolute values of profile heights of the lines within the measurement area.

The wrinkle depth measurements were integrated in a calibration curve correlating age with the wrinkle depth to calculate the biological age or the "younger skin effect".

2.4.2. Skin Radiance

Skin radiance was measured by a spectrophotometer/colorimeter CM-700D (Konica Minolta, Tokyo, Japan). The measured parameter was the 8° gloss (gloss value with the specular reflection in the direction of 8°).

2.4.3. Biochemical Parameters

The total skin antioxidant capacity and skin inflammation were measured on using skin strippings. Non-invasive skin sampling was carried out using Corneofix® foils (Courage + Khazaka Electronic, Köln, Germany) (Figure S1). Ten strippings were collected under standard pressure conditions. The first stripping was discharged, while stripping no. 2 and 3 and stripping no. 2 to 11 were collected and stored at −80 °C for a Ferric Reducing Antioxidant Power (FRAP) assay and TNF-α dosage, respectively. The skin strippings for FRAP and TNF-a dosage tests were taken from two adjacent areas of the face.

Ferric Reducing Antioxidant Power (FRAP) assay. The total antioxidant capacity was measured via a Ferric Reducing Antioxidant Power (FRAP) assay as described by Benzie and Strain [25]. The FRAP assay is a colorimetric assay based on the reduction by antioxidants of a ferric-tripyridyltriazine (Fe^{III}-TPTZ) complex to the ferrous (Fe^{II}) form. The reduction of Fe^{III}-TPTZ to Fe^{II}-TPTZ forms an intense blue color. Briefly, 100 µL of distilled water and 500 µL of working FRAP reagent (200 mL acetate buffer, 20 mL TPTZ solution and 20 mL $FeCl_3$ $6H_2O$ solution) were added to 12 multiwell plates containing the skin strippings. The samples were then incubated for 30 min at 37 °C under continuous agitation using a microplate incubator/shaker (VWR® Microplate Shaker, VWR International, LLC, Milan, Italy). Absorbance was read at 595 nm using a microplate reader (BioTek Synergy LX Multimode Reader, Agilent Technologies, Inc., Santa Clara, CA, USA).

TNF-α dosage. The TNF-α concentration was measured via a commercially available ELISA kit (Catalog no. E-EL-H0109, Elabscience, Houston, TX, USA). Before the analysis, skin strippings were extracted in a 1% tween 20 (Merk Life Science S.r.l., Milan, Italy) water solution. The assay was carried out according to the manufacturer's instructions. Briefly, 100 µL of sample, blank or dilution of standard was added into the appropriate wells and incubated for 90 min at 37 °C (VWR® Microplate Shaker, VWR International, LLC, Milan Italy). After incubation, the liquid of each well was decanted and 100 µL of Biotinylated Detection Ab working solution (1× concentrated biotinylated detection Ab: 99× biotinylated detection Ab diluent) was added to each well and incubated for 60 min at 37 °C. The solution of each well was then decanted and 350 µL of wash buffer (30 mL of concentrated wash buffer with 720 mL of distilled water) was added to each well; after 1 min, the solution was aspirated and pat dried using clean absorbent paper 3 times and 100 µL of HRP conjugate working solution (1× Concentrated HRP Conjugate: 99× HRP Conjugate Diluent) was added to each well. The wells were incubated for 30 min at 37 °C, the solution from each well was then decanted and washed 5 times with 350 µL of wash buffer, and 90 µL of substrate reagent was added to each well and incubated for 15 min at 37 °C. A total of 50 µL of stop solution was then added to each well and the optical density was read at 450 nm using a microplate reader (BioTek Synergy LX Multimode Reader, Agilent Technologies, Inc., Santa Clara, CA, USA).

2.4.4. Skin Tolerability

Skin tolerability was assessed by a board-certified dermatologist (G.R. and E.C.). The occurrence of both physical (erythema, edema, desquamation, dryness, others) and functional (itching sensation, stinging sensation, burning sensation, skin tightness, others) skin local tolerance signs was scored as follows: 0. none, 1. very mild, 2. mild, 3. moderate, 4. severe. In the case of any local tolerance sign occurrence, the dermatologist was asked to record the duration of the sign, its localization on the face, and the frequency.

2.4.5. Self-Assessment Questionnaire

Subjects were asked to give their opinions on products by answering a self-assessment questionnaire. The items of the self-assessment questionnaire are reported in the Supplementary Materials (Table S2). Completely agree, agree, very satisfied, satisfied and yes were considered as positive answers.

2.5. Statistical Methods

All the subjects participating in the study completed the study. The statistical analysis is then per protocol (PP) and includes all the randomized subjects.

Paired Student t-tests were used both for the intragroup (vs. baseline) and the intergroup (vs. placebo) statistical analyses. The intragroup statistical analysis was carried out on the raw data, while the intergroup statistical analysis was carried out on percentage variation. All the statistical analyses were two-sided at a 5% significance level ($p < 0.05$). All the statistical analyses were conducted in Microsoft Excel 365 for Enterprise (version 2312, build 17126.20132, Microsoft, Redmond, WA, USA) running on Microsoft Windows 11 Pro (version 23H2, build SO 22631.3085, Microsoft, Redmond, WA, USA). The level of statistical significance was reported as follows: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3. Results

3.1. Study Population

Thirty-one subjects were screened for eligibility; three declined to participate and six did not meet the inclusion criteria (Figure 1). Twenty-two subjects were then randomized to receive the active or the placebo products on the left or the right side of the face (split-face study design). The PP population consisted of 22 subjects. The study was completed by all the subjects without any lost to follow-up or drop-outs. The population was females aged 53.3 ± 1.4 (mean ± SE; min 41 years; max 45 years). The following skin types were represented: 31.8% ($n = 7$) normal, 27.3% ($n = 6$) mixed/oily, and 40.9% ($n = 9$) dry skin. Other demographics are reported in Table 1.

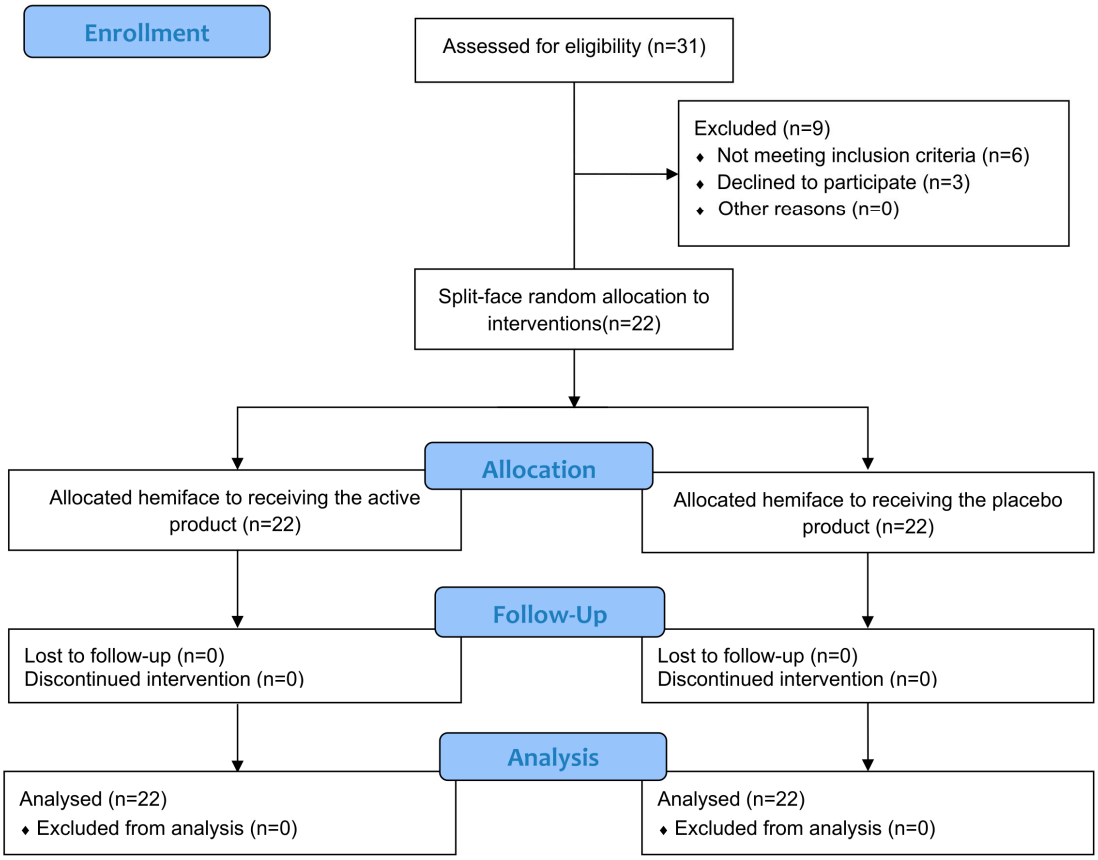

Figure 1. CONSORT flow diagram. The study design was split-face; the same subjects tested the active and the placebo product. Abbreviation: CONSORT, Consolidated Standards of Reporting Trials.

Table 1. Baseline and demographic characteristics. Data are means ± SE. a.u.: arbitrary units.

		Active	Placebo	Units
Sex				
	Male	0% (0)	0% (0)	% (no.)
	Female	100% (22)	100% (22)	% (no.)
Age		53.3 ± 1.4	53.3 ± 1.4	Years
Skin type				
	Normal	31.8% (7)	31.8% (7)	% (no.)
	Mixed/Oily	27.3% (6)	27.3% (6)	% (no.)
	Dry	40.9% (9)	40.9% (9)	% (no.)
Wrinkle depth		260.9 ± 13.9	252.4 ± 15.3	µm
Skin roughness		33.5 ± 1.7	32.7 ± 1.2	µm
8° gloss (skin radiance)		12.0 ± 0.6	12.7 ± 0.7	a.u.
FRAP (antioxidant capacity)		44.6 ± 2.0	46.7 ± 2.9	µmol Fe^{II}
TNF-α		11.7 ± 0.8	11.6 ± 0.6	pg/mL

3.2. Skin Tolerability

Both the active and the placebo products were well tolerated. No physical (erythema, edema, desquamation, dryness, others) or functional (itching sensation, stinging sensation, burning sensation, skin tightness, others) local tolerance signs were reported.

3.3. Skin Profilometry

The wrinkle depth at baseline was 260.9 ± 13.9 µm in the active-treated hemiface and 252.4 ± 15.3 µm in placebo-treated hemiface. After 28 days of product use, the wrinkle depth on the active-treated side statistically significantly ($p < 0.001$) decreased by 5.2%, while it remained unchanged ($p > 0.05$) on the placebo-treated side (Figure 2a). A decrease in the wrinkle depth of 5.2% corresponds to a calculated biological age decrease of 2.3 ± 0.5 years (from 50.8 ± 2.3 years at D0 to 48.5 ± 2.2 years at D28).

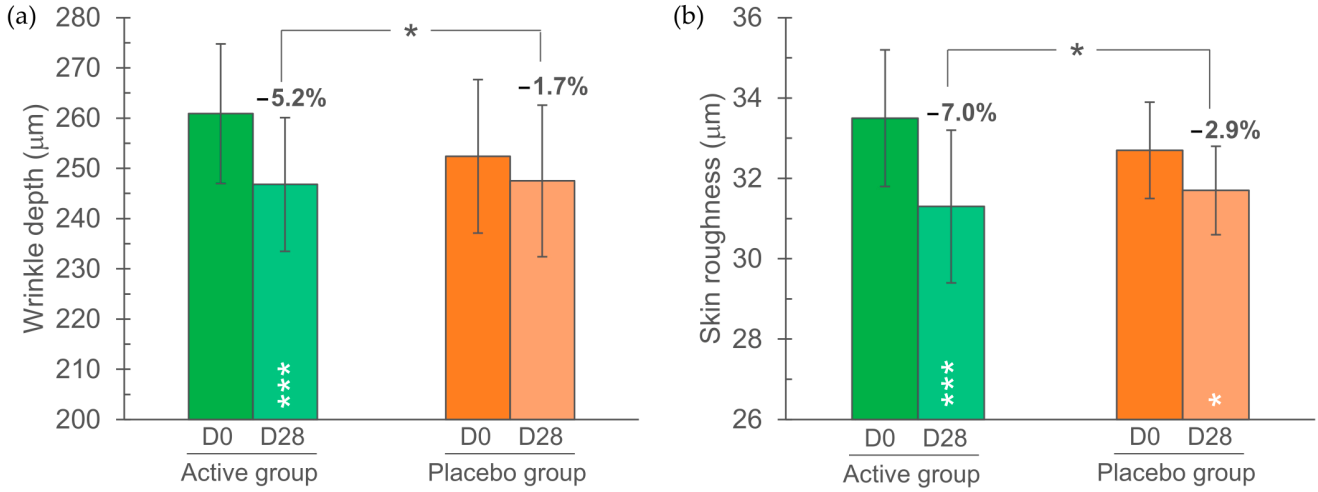

Figure 2. Skin profilometry. (**a**) Wrinkle depth. (**b**) Skin roughness (Ra parameter). The intragroup (vs. D0) statistical analysis is reported inside the bars, while the intergroup (active vs. placebo) statistical analysis is reported above the bars. * $p < 0.05$; *** $p < 0.001$.

The skin roughness (Ra parameter) in the active-treated side statistically significantly ($p < 0.001$) decreased by 7.0% (31.3 ± 1.9 µm at D28 vs. 33.5 ± 1.7 µm at D0). A small, statistically significant ($p < 0.05$) decrease (−2.9%) was also observed in the placebo-treated side (Figure 2b).

Differences between the variation in both the wrinkle depth and the skin roughness in the active side were statistically significant ($p < 0.05$) compared to the placebo-treated side.

3.4. Skin Radiance

Skin radiance (8° gloss) in the active-treated side statistically significantly ($p < 0.001$) increased by 19.0%. A statistically significant ($p < 0.01$) increase of 14.0% was observed also in the placebo-treated side versus baseline. The gloss variation in the active-treated side was higher ($p < 0.05$) than the variation in the placebo-treated side (Table 2). Differences between skin radiance in the active side were statistically significant ($p < 0.05$) compared to the placebo-treated side.

Table 2. Skin gloss. The percentage variation vs. baseline is reported in brackets. The intragroup (vs. D0) statistical analysis is reported near the raw data as follows: ** $p < 0.01$; *** $p < 0.001$. The intergroup (active vs. placebo) statistical analysis is reported near the percentage variation as follows: ‡ $p < 0.05$. Data are arbitrary units.

Radiance (8° Gloss)	Active	Placebo
D0	12.0 ± 0.6	12.7 ± 0.7
D28	13.8 ± 0.4 *** (+19.0%) ‡	13.9 ± 0.5 ** (+14.0%)

3.5. Biochemical Parameters

The total skin antioxidant capacity at baseline was 44.6 ± 2.0 µmol Fe^{II} in the active-treated hemiface and 46.7 ± 2.9 µmol Fe^{II} in placebo-treated hemiface. The daily use of the active product increased ($p < 0.001$) the skin antioxidant capacity by 20.2% (53.4 ± 2.3 µmol Fe^{II}) versus the baseline. A similar statistically significant ($p < 0.001$) increase of 12.3% (52.4 ± 3.3 µmol Fe^{II}) was seen in the placebo-treated side (Figure 3a) versus the baseline. Despite an increase being observed in both active- and placebo-treated side versus the baseline, the increase in total skin antioxidant capacity was greater in the active-treated side, with statistically significant differences ($p < 0.001$) compared to placebo-treated side.

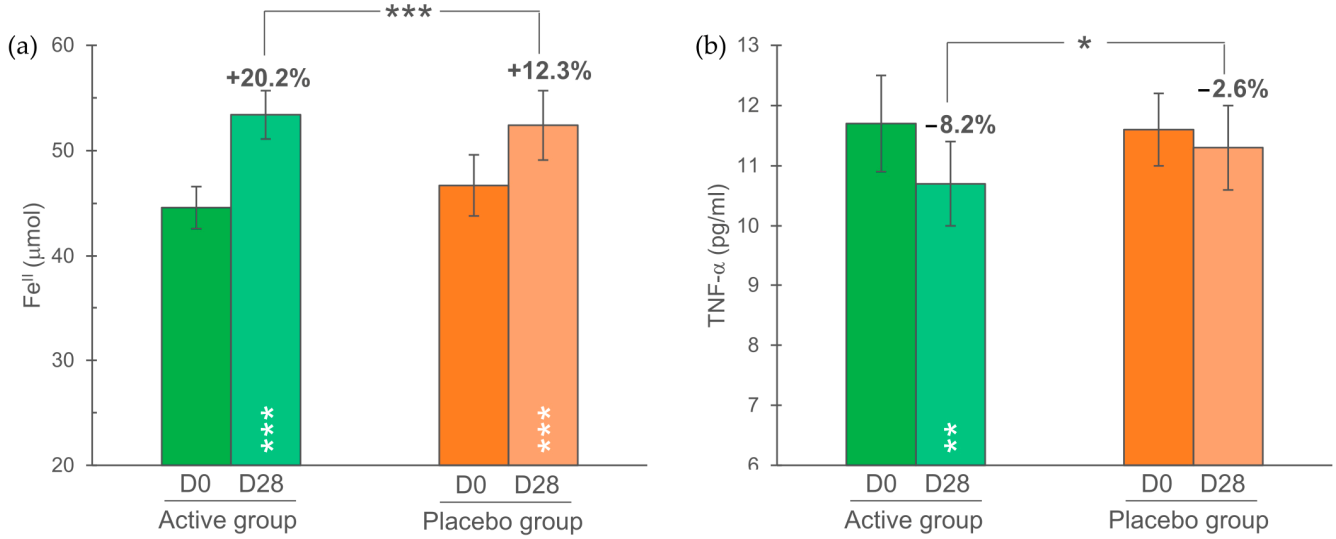

Figure 3. Biochemical parameters. (**a**) Skin antioxidant capacity (FRAP assay). (**b**) TNF-α dosage. The intragroup (vs. D0) statistical analysis is reported inside the bars, while the intergroup (active vs. placebo) statistical analysis above the bars is reported. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Skin inflammation decreased ($p < 0.01$) in the active-treated side by 8.2% (10.7 ± 0.7 pg/mL at D28 vs. 11.7 ± 0.8 pg/mL at D0). The variation in the placebo-treated side (−2.6%) was not statistically significant ($p > 0.05$). The difference between the active- and placebo-treated side (Figure 3b) was statistically significant ($p < 0.05$).

3.6. Self-Assessment Questionnaire

The self-assessment questionnaire was completed, independently, by the panelists at the end of the study (D28). The questionnaire output (Figure 4) was most favorable for the active group. In the active group, the percentage of subjects who gave positive answers to all items was higher than 75%.

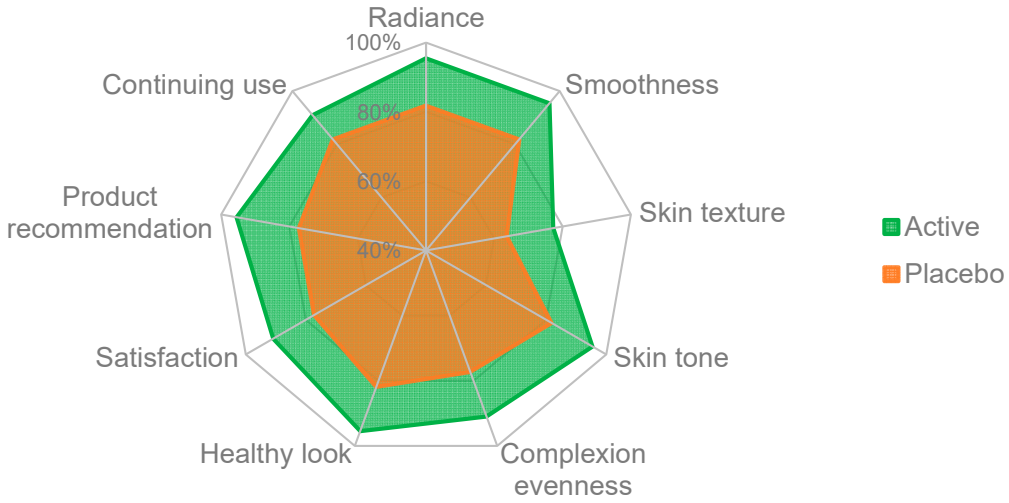

Figure 4. Self-assessment questionnaire. The graph reports the percentage of positive answers (i.e., "completely agree" and "agree", "very satisfied" and "satisfied" or "yes").

4. Discussion

Throughout life, the skin is subjected to internal and external factors influencing both its biological response and its appearance. All these factors are known as the exposome and play a pivotal role in premature aging. Among them, solar radiation and air pollution are exposomal factors that dramatically accelerate skin aging [3,26] by inducing oxidative stress and inflammaging [27–29]. As the average human life expectancy is increasing, supporting the skin during the aging process has become a growing concern [30]. This new concept called well-aging involves both lifestyle and the beauty routine and aims to support the natural beauty of the skin at all ages. In the cosmetic sector, well-aging involves the development of cosmetic ingredients with proven beneficial effects on skin homeostasis and resiliency with a good skin tolerability profile [31]. In this study, we aimed to demonstrate for the first time the efficacy of a standardized artichoke leaf extract (CYNAGE^{TM}) in improving skin parameters related to aging caused by pollution and UV exposure. Color change and loss of skin radiance; decreased skin elasticity associated with loss of underlying tissue and skin wrinkling; and impaired barrier functions to oxidative stress were chosen as endpoints since they represent the clinical hallmarks of skin aging [32,33].

The results from this study demonstrate the efficacy of the tested active ingredient (*Cynara scolymus* leaf extract) in improving skin appearance and in decreasing both the oxidative stress and the inflammatory processes underlying the clinical manifestation of skin aging. Both skin wrinkles and skin roughness decreased after 28 days of product use (-5.2% and -7.0%, respectively), leading to a reduction in the calculated biological age of 2.3 ± 0.5 years. A worsening of wrinkle depth and skin roughness is correlated with a decrease in the dermis thickness [34], loss of elastic fibers, and degeneration of collagen bundles in the dermis [35,36]. The degeneration of collagen (mainly collagen I, III and VII) during extrinsic aging is further aggravated by elastases produced by neutrophils migrating to the dermis after inflammation or UV exposure and by the activation of matrix metalloproteases (MMPs) [37] and accelerated by ROS production after UVA/B exposure [18,38]. The active ingredient may protect, indirectly, collagen and elastic fibers from UV- and pollution-induced degradation by strengthening the cutaneous intrinsic antioxidant defenses and decreasing skin inflammation.

The skin radiance showed a significant increase after using the active ingredient. Enhanced skin radiance can contribute to a more youthful and healthy appearance, indicating an improvement in skin tone and texture. Moreover, participants in the study also reported positive perceptions of the product's effectiveness, as indicated by the responses in a self-assessment questionnaire. This subjective feedback aligns with the objective improvements observed in various skin parameters, with both confirming the clinical relevance of results.

The potential of artichoke leaf extract as a skincare ingredient to combat the effects of the skin aging exposome is a fairly new concept for this ingredient. There are only a few studies, mainly in vitro and one in vivo, on mouse models linking artichoke extract to antioxidant and anti-inflammatory activities together with anti-aging and UV protection [22–24,39]. By comprehensively evaluating its efficacy and safety profile, we aimed to advance the field of skincare research and provide valuable evidence-based results for the first time, as far as we know, in the literature on human subjects. This study evaluated a wide range of skin parameters, including wrinkle depth, skin roughness, radiance, antioxidant capacity, and inflammation levels, thoroughly assessing the product's efficacy in promoting skin health and vitality and emphasizing its clinical outcomes and mechanism of action. The objective measurements were then integrated with subjective assessments to enhance the reliability and validity of our findings, providing valuable insights into both the physiological and perceptual effects of artichoke leaf extract on skin aging. Finally, our research adopted a rigorous study design, including a randomized, split-face, double-blind, placebo-controlled trial, which minimizes bias and ensures the robustness of the obtained results. Additionally, advanced methodologies such as skin profilometry and biochemical assays were employed to accurately quantify changes in skin parameters, demonstrating a commitment to methodological rigor and scientific evidence.

In conclusion, CYNAGE^{TM} was effective after 28 days of use in improving the clinical appearance of aging signs (wrinkles depth, skin roughness and dullness), in improving the skin antioxidant capacity, and in decreasing the levels of the pro-inflammatory cytokines such as TNF-α induced from pollution and environmental exposure. As a result of these variations, the skin appeared younger by 2.3 years. The ingredient was also well tolerated since no local intolerance signs were observed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cosmetics11030069/s1, Table S1: Inclusion and exclusion criteria; Figure S1: Skin stripping technique; Table S2: Self-assessment questionnaire.

Author Contributions: Conceptualization, V.N. and V.Z.; methodology, V.N. and V.I.; validation, V.N., E.C. and G.R.; formal analysis, V.N.; investigation, E.C., G.R. and A.P.; resources, V.N. and V.Z.; data curation, V.N. and F.D.G.; writing—original draft preparation, V.N.; writing—review and editing, V.N.; visualization, V.N. and F.D.G.; supervision, V.N.; project administration, V.N.; funding acquisition, V.N. and F.D.G. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. Ethical review and approval were waived for this study. According to the EU cosmetic Regulation no. 1223/2009, the cosmetic product must not cause damage to human health when applied under normal or reasonably foreseeable conditions of use and must be assessed for its safety of use before human subjects are exposed to it, and as such, further ethical approval is not required.

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

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available since they are the property of the sponsor of the study (Bionap Srl, 95032 Piano Tavola Belpasso, CT, Italy).

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Conflicts of Interest: G.R., E.C., F.D.G., A.P. and V.N. are Complife S.r.l. employees. V.Z. is a Bionap S.r.l. employee. This does not alter the author's adherence to all the journal policies on sharing data and materials. The other authors declare no conflicts of interest. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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