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Oral Supplementation with *Brassica oleracea* Dry Aqueous Extract (Purebkale™) Improves Skin Barrier Function, Dermis Density, and Wrinkle Appearance: A 56-Day Open-Label Clinical Study

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Abstract

Intrinsic and extrinsic factors compromise skin barrier function and dermal density over time. While *Brassica* vegetables are known for their bioactive glucosinolates, clinical data regarding their specific effects on skin structure are limited. This prospective, open-label exploratory study evaluated the anti-aging efficacy of Purebkale™, a *Brassica oleracea* dry aqueous extract. Fifty healthy women with mild-to-moderate signs of aging ingested the supplement daily for 56 days. Instrumental assessments included transepidermal water loss (TEWL), *stratum corneum* cohesivity (protein content), skin firmness, elasticity, wrinkle depth, and dermis density. This study met its primary objectives, indicating a significant improvement in barrier function: TEWL decreased by 7.5% and protein removal via tape-stripping was reduced by 27.8% ($p < 0.001$). Furthermore, dermal density increased by 12.8%, while wrinkle depth was reduced by 15.1%. Biomechanical parameters also showed significant improvements, with firmness increasing by 17.4% and elasticity by 9.7%. Although oxidative stress markers remained stable, participants' self-assessments reported high satisfaction with skin quality. These findings suggest that oral supplementation with *Brassica oleracea* extract effectively supports skin barrier integrity and dermal structure, offering a viable healthy aging strategy for skin.

Keywords: *Brassica oleracea*; glucoraphanin; oral supplement; skin barrier; wrinkles

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1. Introduction

Skin aging is a complex, multifactorial biological process resulting from the interplay of intrinsic (chronological) and extrinsic (environmental) factors, including air pollutants (particulate matter), smoking, unbalanced diet, and ultraviolet and infrared radiation [1–5]. This complex process accelerates skin aging and contributes to inflammatory or allergic conditions such as topic discomforts, with visible signs such as wrinkles, loss of firmness, and dryness, reflecting alterations in both the epidermis and dermis [1–3,6].

In the epidermis, a key alteration is the impairment of the *stratum corneum* (SC) barrier function [1–3,6,7]. The SC, composed of terminally differentiated corneocytes embedded in

a lipid-rich matrix, is the skin's primary defense against transepidermal water loss (TEWL) and environmental threats [7]. In aged skin, altered lipid composition and impaired differentiation processes compromise this barrier [8–10], leading to increased TEWL, which is widely regarded as a gold-standard objective measure of barrier integrity [11,12].

In the dermis, aging is defined by the progressive degradation and disorganization of the extracellular matrix (ECM) [3,6]. The structural integrity, tensile strength, and elastic recoil of youthful skin are provided by a dense, organized network of collagen and elastin fibers, synthesized and maintained by dermal fibroblasts [3,6,11]. With age, fibroblast function declines, collagen synthesis decreases, and the activity of matrix metalloproteinases (MMPs) increases, leading to the fragmentation and loss of collagen and elastin [3,13–16]. This net loss of structural support manifests in reduced dermal density, which can be measured using high-frequency ultrasound (HFUS) [17–19], and functional deficits in skin biomechanics, such as decreased firmness and elasticity, often quantified by cutometry [20]. Together, these dermal alterations underlie wrinkle formation and loss of facial firmness [3,6,13].

A central mechanism accelerating both epidermal and dermal aging is oxidative stress [14,15,21]. Reactive oxygen species (ROS), generated by cellular metabolism and massively amplified by extrinsic factors like environmental pollutants and UV radiation, overwhelm the skin's endogenous antioxidant defenses [14,15,21]. This oxidative imbalance triggers a cascade of damage: ROS attack cellular lipids, proteins, and DNA [14,21–23].

NRF2 (nuclear factor erythroid 2-related factor 2) is a central transcription factor that equips cells to withstand environmental stress. Once activated, it triggers a broad cytoprotective response involving antioxidant pathways, detoxification enzymes, and inflammatory and metabolic regulation. Its role in maintaining skin homeostasis and promoting cytoprotection has become increasingly evident in recent years [24]. Concurrently, there has been an increasing interest in "inside-out" beauty-from-within strategies, in which oral supplements provide bioactive compounds to the viable epidermis and dermis via the bloodstream [25–27]. A substantial body of evidence, including systematic reviews and meta-analyses, supports the efficacy of specific oral supplements [26,28,29]. For example, hydrolyzed collagen peptides have been shown to improve skin hydration, elasticity, and dermal density [26,28,30], while oral hyaluronan can significantly enhance skin moisture [31]. The Brassicaceae family of vegetables, which includes kale, broccoli, and cabbage (*Brassica oleracea* L.), has been identified as a promising source of phytochemicals (glucosinolates), such as glucoraphanin [32]. Glucoraphanin ($C_{12}H_{23}NO_{10}S_3$) is a glucosinolate characterized by a sulfur-bound β-D-glucopyranose/β-thioglucose moiety and a sulfonated oxime group. As with other glucosinolates, glucoraphanin is a relatively polar, water-soluble compound [33]. In the plant, it is stored until hydrolyzed by enzyme myrosinase into the isothiocyanate sulforaphane (SFN) [34,35]. *In vivo*, glucoraphanin can be converted into the biologically active form by gut microbiota with myrosinase-like activity (Figure 1) [32]. SFN has been shown to trigger the induction of phase II enzymes, activating the NRF2 signaling pathway or suppressing nuclear translocation of NF-κB, regulating xenobiotic detoxification, oxidative stress and inflammatory processes, with consequent impact on liver function and skin health [36]. Preclinical and early clinical data suggest that SFN-containing or *Brassica*-based preparations are bioavailable to the skin and may influence markers related to oxidative stress and ECM integrity [37–40].

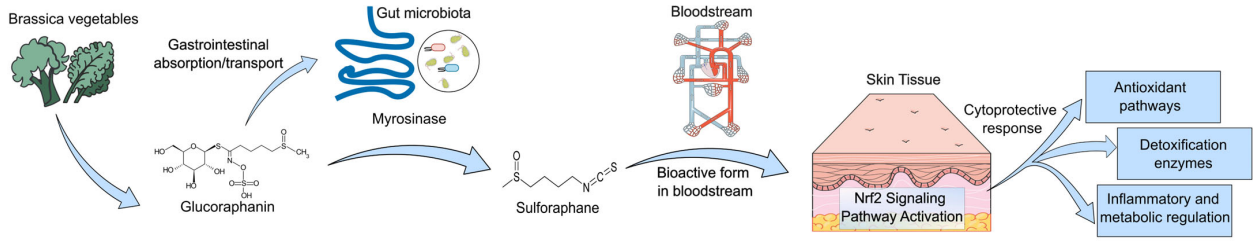

Figure 1. Proposed inside–out mechanism of action of glucoraphanin on skin via sulforaphane-mediated NRF2 activation.

While many nutricosmetic interventions have primarily focused on dermal- and appearance-oriented outcomes (e.g., hydration, elasticity, wrinkle-related parameters), there is a lack of comprehensive clinical–instrumental data on Brassica-derived supplements in skin aging, particularly regarding barrier function, dermis density, and wrinkles. To address this gap, we conducted a prospective, open-label clinical–instrumental study to explore the skin anti-aging efficacy of Purebkale™ (Indena S.p.A., Milan, MI, Italy), a dry aqueous extract of *Brassica oleracea* L. standardized to ≥17% glucoraphanin. The primary objective was to assess the product's effect on skin barrier function, evaluated by TEWL and SC cohesivity (protein content). Secondary objectives were to evaluate its effects on skin firmness (R0), elasticity (R2), "crow's feet" wrinkle depth, dermis density and subject-reported outcomes.

2. Materials and Methods

2.1. Study Design

This was a prospective, single-center, open-label, single-arm clinical–instrumental study conducted at Complife Italia S.r.l. (San Martino Siccomario, PV, Italy) on behalf of Indena S.p.A. (Milan, MI, Italy). This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. All participants provided written informed consent prior to participation.

The study duration for each participant was 56 days. Evaluations were performed at baseline (Day 0, T0) and after 56 days (T56) of product intake. A self-assessment questionnaire was also administered at an intermediate timepoint (Day 28, T28) and at T56.

This study was conceived as an exploratory investigation aimed at describing changes in skin parameters associated with daily intake of the supplement. To minimize potential confounding, participants were instructed to maintain their usual skincare routines and lifestyle habits throughout this study, and all efficacy endpoints were assessed using standardized, objective, non-invasive instruments.

2.2. Participants

Eligible participants were healthy women of Caucasian ethnicity, aged 35–55 years, with mild-to-moderate wrinkling and decreased elasticity/firmness. They were required to be in general good health based on medical history and clinical examination, and, where applicable, to have been on stable pharmacological therapy for at least one month before enrollment. Additional criteria included the ability and willingness to comply with study procedures and visit schedules, and a commitment not to change their usual daily routine or to initiate new anti-aging treatments, cosmetic procedures, or nutricosmetic supplements for the study duration. All participants provided written informed consent prior to inclusion.

Women were not eligible if they were pregnant or breastfeeding, or if they had acute, chronic or progressive systemic or dermatological diseases that could confound the

instrument-based endpoints, or pose a risk for participation. In particular, conditions associated with barrier disruption, inflammation, or skin lesions may directly alter TEWL and stratum corneum cohesivity, affect mechanical properties measured by cutometry, interfere with 3D wrinkle profilometry on lesional skin, or modify HFUS dermal density through inflammatory changes. Further non-inclusion criteria comprised known intolerance or hypersensitivity to any component of the test product or to cosmetic products, drugs, patches or medical devices used in this study, and recent aesthetic procedures (e.g., fillers, lasers, peels) in the periorbital area within the required wash-out period. Systemic treatments considered incompatible with this study (e.g., systemic corticosteroids or retinoids) within the relevant wash-out period, participation in another clinical trial within the restriction window, or any condition that, in the investigator's opinion, could limit the ability to attend visits or follow instructions also led to exclusion. Throughout this study, participants were instructed to maintain their usual skincare routine and to avoid introducing new anti-aging products or procedures.

2.3. Test Product and Administration

The test product was a food supplement duly notified to the Italian Ministry of Health by Scharper S.p.A. (Milan, Italy) and was administered as an oral film-coated tablet containing Purebkale™ (*Brassica oleracea* dry aqueous extract, Indena S.p.A) as the active ingredient. The extract is characterized by a defined phytochemical profile and is standardized to contain $\geq$17% glucoraphanin.

Film-coated tablets, each containing 150 mg of Purebkale™, consist of the following food grade ingredients: dicalcium phosphate, microcrystalline cellulose, crosslinked sodium carboxymethylcellulose, silicon dioxide, talc, magnesium salts of fatty acids and hydroxypropyl methylcellulose-based film-coating.

Film-coated tablets were characterized for appearance, average mass, uniformity of mass, HPLC content of the active ingredient, disintegration time and microbiological quality.

Subjects were instructed to ingest one tablet daily with a glass of water for 56 consecutive days. At T0, each participant received a sufficient quantity of the study product for the entire intervention period and was instructed to discontinue intake and promptly inform the investigator in case of intolerance or any adverse reaction. Adherence to the dosing directives was monitored at the end of the intervention using product accountability: returned tablets were counted and compared with the planned intake to compute quantitative compliance percentage, calculated as (tablets consumed/planned) $\times$ 100. Compliance with treatment acceptability was predefined as 80–120% of the planned intake. Participants with compliance outside this range were excluded due to poor adherence to the treatment regime.

2.4. Study Procedures and Timepoints

This study comprised three planned visits, as follows:

- T0 (baseline, day 0): eligibility confirmation; demographic and clinical data collection; baseline instrumental measurements (TEWL, skin firmness and elasticity, wrinkle depth, dermis density); collection of *stratum corneum* (SC) samples by tape-stripping for protein content; assignment of the test product and instructions for use.
- T28 (day 28 $\pm$ 2 days): administration of a self-assessment questionnaire on perceived product efficacy and tolerability; no instrumental measurements were taken.
- T56 (day 56 $\pm$ 2 days): repetition of all instrumental measurements and SC sampling performed at baseline; administration of the final self-assessment questionnaire; collection of used and unused product for compliance and safety evaluation.

2.5. Instrumental and Biochemical Procedures

All instrumental measurements were performed by trained technicians in a dedicated, environmentally controlled room (temperature 18–26 °C; relative humidity 50 ± 10%) after subjects had acclimatized for 15–20 min. The same anatomical sites were used for measurements at T0 and T56.

2.5.1. Transepidermal Water Loss (TEWL)

SC barrier integrity was assessed using a Tewameter® TM 300 (Courage + Khazaka electronic GmbH, Cologne, Germany) in the cheek. TEWL is a widely used, validated marker of barrier integrity, with higher values indicating barrier impairment and lower values associated with intact barrier function [12]. Accordingly, a decrease in TEWL is interpreted as improved barrier function to water loss [11]. According to the technical guide, TEWL values ($g/h/m^2$) of 0–10 indicate very healthy condition, 10–15 healthy condition, 15–25 normal condition, 25–30 affected skin, and >30 critical condition [41]. These ranges are intended only as a guide for interpreting the results. A case-by-case evaluation is still necessary due to variability among instruments, subjects, body sites, environmental conditions, and the current lack of consensus in the scientific literature.

2.5.2. Skin Cohesivity (SC Protein Content)

Skin cohesivity was evaluated in the cheek using a tape-stripping technique using Corneofix® foil (Courage + Khazaka electronic GmbH, Cologne, Germany). Two consecutive tape-strips were collected; the first was discarded and the second was analyzed. Before analysis the skin tape-strips were stored at −80 °C. The total protein content removed on the strips was subsequently quantified using a colorimetric Lowry assay. Tape-stripping with subsequent protein quantification is an established method to assess SC cohesion and barrier properties. A lower amount of protein removed (in µg) signifies greater SC cohesivity and compactness [42].

2.5.3. Skin Firmness and Elasticity

Skin mechanical properties were measured in the cheek using a Cutometer® MPA 580 (Courage + Khazaka electronic GmbH, Cologne, Germany) [20,43]. A probe with a 2 mm aperture applied a constant negative pressure (450 mbar) for 2 s, followed by a 2 s relaxation period. The following parameters were analyzed:

- R0 (Uf): Maximum distension (mm), representing skin distensibility. A decrease in R0 indicates increased skin firmness [43].
- R2 (Ua/Uf): Gross elasticity (arbitrary units, a.u.), or the ability to return to the original position. An increase in R2 indicates improved overall elasticity [43].

2.5.4. Wrinkle Depth

Wrinkle morphology in the "crow's feet" region was quantified using a non-contact 3D optical profilometry system (Primos^{CR} SF, Canfield Scientific Europe BV, Utrecht, The Netherlands). The device uses fringe projection to reconstruct a 3D map of the skin surface, from which mean wrinkle depth (µm) was calculated before and after treatment.

2.5.5. Dermis Density

Dermal structure was assessed in the cheek using high-frequency ultrasound (HFUS) with a DUB® SkinScanner (tpm taberna pro medicum GmbH, Lüneburg, Germany) equipped with a 22 MHz probe. The system generates cross-sectional B-scan images, and the echogenicity of the dermal band was analyzed to determine dermis density (expressed as %).

2.6. Self-Assessment Questionnaire

At T28 and T56, subjects completed a structured self-assessment questionnaire addressing perceived changes in skin properties (suppleness, firmness, wrinkle appearance, radiance, complexion uniformity, perceived "detox"/balance) and product acceptability (ease of use, willingness to buy/recommend, overall satisfaction and tolerability).

Most items used a four-point Likert-type scale (e.g., "completely agree", "agree", "disagree", "completely disagree" or "very satisfied", "satisfied", "unsatisfied", "very unsatisfied"), while other items were yes/no questions. "Positive answers" were predefined as the sum of "completely agree" + "agree", "very satisfied" + "satisfied", or "yes", depending on the item. Global tolerability was rated as "very good", "good", "moderate", or "poor".

2.7. Statistical Analysis

All instrumental and biochemical data were analyzed using the per-protocol (PP) population (N = 49). Descriptive statistics (mean and standard error of the mean, SE) were calculated for each endpoint at T0 and T56. Intra-subject changes from baseline (T56 vs. T0) were analyzed using a paired Student's *t*-test. No formal adjustment for multiple comparisons was made, given the exploratory nature of this study. For the self-assessment questionnaire, the proportion of positive answers was calculated and analyzed using the chi-square test. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using Microsoft® Excel® for Microsoft 365 MSO (version 2511 at 64 bit, build 16.0.19426.20186).

3. Results

3.1. Participant Characteristics, Tolerability, and Compliance with Treatment

All subjects presented mild-to-moderate facial wrinkles and reduced elasticity/firmness, as clinically assessed by a board-certified dermatologist. One subject discontinued with this study before T56 for personal reasons unrelated to the product; therefore, forty-nine subjects completed the study and were included in the per-protocol instrumental analysis set. At baseline, the mean age was 47.3 ± 0.8 years (mean ± SE; range 36–55 years). Wrinkles were clinically graded as mild in 46.9% of participants and moderate in 53.1% (per-protocol population).

The tested product was well tolerated throughout the study period. No adverse events or clinically relevant reactions were observed by the investigator, and no tolerability issues were reported by the participants.

3.2. Primary Endpoints: Skin Barrier Function

This study met its primary objective, showing statistically significant improvements in both measures of skin barrier function (Table 1, Figure 2).

Table 1. Overall summary of instrumental study outcomes (per-protocol population, n = 49). Data are mean ± SE. Intra-subject changes were analyzed by paired Student's *t*-test.

Endpoint	Units/Method	T0	T56	Change (%)	*p*-Value
TEWL	g/h/m^2 (Tewameter, cheek)	17.8± 0.6	16.3 ± 0.5	–7.5	<0.001
SC protein removed	µg (tape-stripping, 2 strips)	36.93 ± 1.59	25.52 ± 1.41	–27.8	<0.001
Firmness (R0)	mm (Cutometer)	0.408 ± 0.007	0.335 ± 0.007	–17.4	<0.001
Elasticity (R2)	a.u. (Cutometer)	0.610 ± 0.009	0.667 ± 0.010	+9.7	<0.001
Wrinkle depth	um (Primos, crow's feet)	243.7 ± 11.6	207.8 ± 11.4	–15.1	<0.001
Dermis density	% (HFUS echogenicity)	17.29 ± 0.19	19.51 ± 0.27	+12.8	<0.001

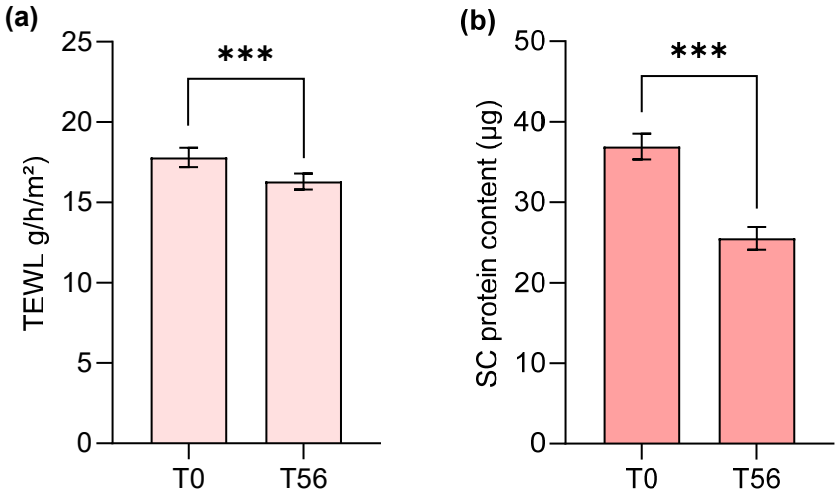

Figure 2. Primary endpoints of skin barrier function after 56 days of daily oral supplementation with Purebkale™: (**a**) transepidermal water loss (TEWL, g/m^2/h); (**b**) *Stratum corneum* (SC) protein content removed by standardized tape-stripping. Bars show mean ± SE at baseline (T0) and after 56 days (T56) (n = 49). *** $p < 0.001$ vs. T0.

3.2.1. Transepidermal Water Loss (TEWL)

Mean TEWL significantly decreased from a baseline of 17.8 ± 0.6 g/h/m^2 to 16.3 ± 0.5 g/h/m^2 at T56. This represents a mean intra-subject reduction of −7.5% ($p < 0.001$), indicating a strengthening of the skin's water barrier.

3.2.2. Skin Cohesivity (SC Protein Content)

The mean amount of protein removed by two consecutive tape-strips significantly decreased from 36.93 ± 1.59 µg at T0 to 25.52 ± 1.41 µg at T56. As tape-strip protein content inversely reflects skin cohesion, the observed mean reduction of −27.8% ($p < 0.001$) after treatment suggests a marked improvement in SC cohesivity and compactness.

3.3. Secondary Endpoints

This study also fulfilled secondary endpoints, proving relevant changes in skin firmness and elasticity, wrinkle depth and dermis density after product application (Table 1, Figure 3).

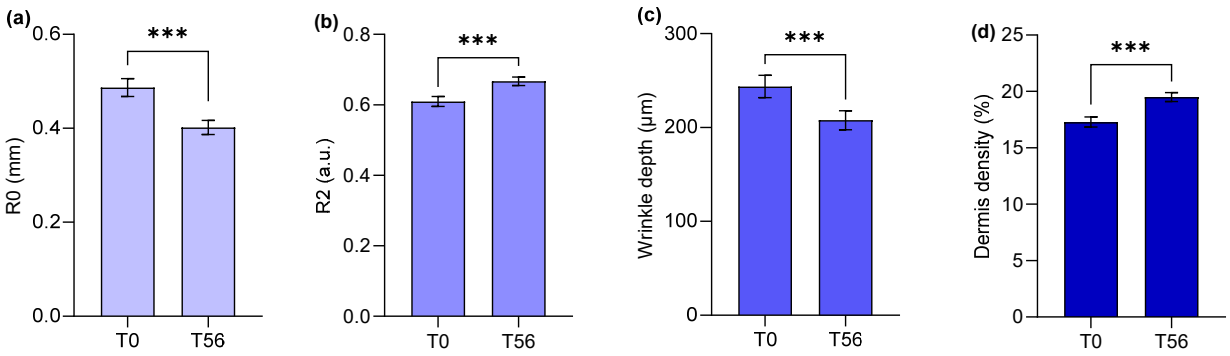

Figure 3. Secondary instrumental endpoints after 56 days of daily oral supplementation with Purebkale™: (**a**) Cutometer R0 (skin distensibility; lower values indicate greater firmness); (**b**) Cutometer R2 (gross elasticity; higher values indicate better elasticity); (**c**) wrinkle depth in the "crow's feet" area (µm) measured by 3D optical profilometry; (**d**) dermis density (%) assessed by high-frequency ultrasound. Bars show mean ± SE at baseline (T0) and after 56 days (T56) (n = 49). *** $p < 0.001$ vs. T0.

3.3.1. Skin Firmness (R0)

The mean R0 parameter (distensibility) decreased from 0.408 ± 0.007 mm at T0 to 0.335 ± 0.007 mm at T56. This mean change of −17.4% ($p < 0.001$) corresponds to a significant increase in skin firmness (Figure 3a).

3.3.2. Skin Elasticity (R2)

The mean R2 parameter (gross elasticity) increased from 0.610 ± 0.009 a.u. at T0 to 0.667 ± 0.010 a.u. at T56, representing a mean increase of +9.7% ($p < 0.001$) (Figure 3b). As the values were closer to 1, our findings indicate better skin elasticity over time [9].

3.3.3. Wrinkle Depth

3D optical profilometry of the "crow's feet" area showed a significant reduction in mean wrinkle depth, from 243.7 ± 11.6 µm at T0 to 207.8 ± 11.4 µm at T56. This represents a mean reduction of −15.1% ($p < 0.001$) (Figure 3c).

3.3.4. Dermis Density

HFUS analysis revealed a significant increase in dermal echogenicity, suggesting a denser, more structured dermis after treatment [17]. Mean dermis density increased from 17.29 ± 0.19% at T0 to 19.51 ± 0.27% at T56, corresponding to a mean increase of +12.8% ($p < 0.001$) (Figure 3d).

3.3.5. Self-Assessment Questionnaire

Self-assessment questionnaires were completed by 49 of 50 subjects at both T28 and T56 (Table 2). The single subject who discontinued with this study before T56 did so for personal reasons unrelated to the test product. Across all 20 items, the proportion of positive answers ranged from 91.8% to 100.0% at T28 and from 89.8% to 100.0% at T56.

Table 2. Summary of self-assessment questionnaire at Day 28 (T28) and Day 56 (T56). Data are expressed as the percentage of subjects ($n = 49$) giving a positive judgment. For items 1–15, positive answers were "completely agree" or "agree". For items 16–19, positive answers were "yes". For item 20, positive answers were "very satisfied" or "satisfied". Percentages are rounded; therefore, totals may not equal 100%. For all items and both timepoints, the proportion of positive answers was significantly greater than 50% (chi-square goodness-of-fit test; all $p < 0.001$).

No.	Item	Positive Answers T28 (%)	Positive Answers T56 (%)
1	The skin is more supple	98.0	98.0
2	The skin is firmer	98.0	98.0
3	The appearance of wrinkles and fine lines is reduced	93.9	91.8
4	The skin is plumped	91.8	89.8
5	The skin looks healthier	98.0	98.0
6	The skin looks purified	93.9	95.9
7	The skin is more radiant and the complexion has improved	98.0	98.0
8	Overall, the appearance of the skin has improved	98.0	98.0
9	I consider this product a great addition to my skincare routine alongside creams and serums	98.0	95.9
10	The product provides a detox effect	98.0	93.9
11	My skin feels less oily or less dry, as if it has been internally rebalanced	98.0	98.0
12	The product supports healthy skin	98.0	98.0
13	Overall, I feel a better balance in my body thanks to a detoxifying effect	98.0	91.8
14	Taking the product once a day is acceptable	100.0	95.9
15	The product is easy to take	100.0	98.0
16	Do you like the idea of taking an oral product to improve your skin?	93.9	93.9
17	Was the product well tolerated?	100.0	100.0
18	Would you buy the product?	98.0	89.8
19	Would you recommend the product?	98.0	98.0
20	Overall, are you satisfied with the product you used?	98.0	98.0

4. Discussion

This 56-day, open-label clinical study suggested that daily oral supplementation with a *Brassica oleracea* dry aqueous extract (Purebkale™) was associated with significant and consistent improvements in multiple skin parameters (barrier function, firmness, elasticity, wrinkle depth and dermis density) in women with mild–moderate signs of facial aging. Participants' diet was not restricted for cruciferous vegetables, which could add baseline variability in glucoraphanin exposure. However, all subjects maintained their usual diet with instructions to avoid starting new supplements, and compliance data suggest dosing was consistent. All measurements were taken in a controlled environment; nonetheless, it is recognized that uncontrolled factors (ambient humidity, sun exposure) could influence skin parameters throughout the study period. However, the impact of this variability was mitigated by instructing subjects to avoid voluntary sun exposure, refrain from lifestyle changes, and maintain their usual dietary habits throughout the study period.

In this trial, the primary endpoint was reached, as both barrier-related measures showed statistically significant changes. TEWL is widely used to measure permeability barrier function, although absolute values can vary by anatomical site and may not be consistent across the lifespan [44]. In healthy adults, TEWL is often <10 $g/m^2/h$, whereas values >25 $g/m^2/h$ are commonly associated with skin barrier dysfunction [8,11]. According to previous evidence on *Brassica oleracea*-based extract [45], our exploratory findings indicate that TEWL decreased by 7.5% (from 17.8 to 16.3 $g/m^2/h$) after Purebkale™ intake, which is consistent with improved skin barrier integrity. These data are complemented by a 27.8% decrease in protein removal during standardized tape-stripping. As this endpoint serves as a proxy for SC cohesivity, a lower protein yield from a fixed number of strips suggests tighter adhesion between corneocytes [42]. Together, these changes suggest a trend towards a more resilient and functionally efficient epidermal barrier after oral supplement intake, capable of retaining moisture more effectively.

Dermal echogenicity typically demonstrates changes in dermal structure and its main source is represented by well-arranged collagen bundles [46]. The increase in dermis density that was observed after oral supplementation reflects collagen remodeling but can be in part influenced by the skin water content [47]. Consistently, cutometric measurements indicated a 17.4% increase in firmness (R0 reduction) and a 9.7% increase in elasticity (R2 increase) after product intake, adding values to previous evidence on animal models that support the role of *Brassica oleracea* extracts in inhibiting skin aging effects [20,48]. These instrumental changes are consistent with the 15.1% reduction in mean wrinkle depth observed by 3D profilometry, an effect that appears to fall within the range reported for other oral supplements, including collagen peptides and hyaluronan [26,28,30,31].

While Brassica bioactives have been widely investigated for systemic benefits, their effects on skin have been scarcely explored in human studies. Most of the available evidence comes from preclinical works, primarily *in vitro* studies and murine models [49–51]. This work offers new clinical evidence supporting the role of a standardized *Brassica oleracea* extract in improving the skin barrier function to transepidermal water loss (TEWL) and in reducing the signs of skin aging (e.g., wrinkles, loss of skin elasticity and alteration in the extracellular matrix) [52].

Although mechanistic endpoints were not assessed in this study, the pattern of barrier, dermal, and biomechanical changes is consistent with the known biological actions of glucosinolate-rich *Brassica* extracts. Notably, Purebkale™ extract is designed to provide a standardized minimum dose of 17% glucoraphanin, promoting consistent intake per capsule. There is multiple evidence proving that, following oral ingestion, glucoraphanin undergoes enzymatic hydrolysis driven by the activity of the gut microbiota [53]. According to our data, a barrier-oriented effect is mechanistically plausible for a glucoraphanin-

standardized Brassica extract. Although the present study did not measure pathway activation directly, glucoraphanin-derived SFN is a well-established and potent activator of the NRF2 signaling pathway, and has been shown to reach human skin and modulate protective enzymes *in vivo* [32,36–40,54]. It is therefore reasonable to hypothesize that regular supplementation with glucoraphanin-related substances may modulate epidermal barrier-related outcomes, indirectly contributing to the instrumental improvements observed here. Activation of the NRF2 pathway, in fact, could reduce oxidative stress-driven barrier disruption, support epidermal differentiation and cornified envelope formation, improve barrier lipid homeostasis, and counteract pro-inflammatory signaling leading to skin barrier disruption. However, this remains speculative in the present study and should be confirmed by future trials specifically designed to measure mechanistic biomarkers.

Although the results are promising, this study was limited by its open-label, single-arm design without a placebo control, and the relatively short 56-day intervention period. Furthermore, the homogenous sample (middle-aged Caucasian women) limits the generalizability of the results. While positive controls strengthen methodological validation, our aim was exploratory (proof of concept). Future studies recruiting male and female subjects with longer follow-up (e.g., 12 weeks or more) and randomized, double-blind, placebo-controlled designs are warranted to confirm and extend these findings. Additionally, mechanistic investigations incorporating biomarkers of antioxidant defense and extracellular matrix remodeling would help clarify the *in vivo* pathways.

5. Conclusions

In this 56-day, single-center, open-label clinical–instrumental study, daily oral supplementation with a glucosinolate-rich *Brassica oleracea* L. dry extract (Purebkale™) was associated with statistically significant and directionally consistent improvements in several markers of skin aging. The primary objective was met, with improved barrier function reflected by reduced transepidermal water loss and increased *stratum corneum* cohesivity. In parallel, secondary endpoints showed gains in skin firmness and elasticity, a clinically relevant reduction in "crow's feet" wrinkle depth, and increased dermis density on high-frequency ultrasound. The supplement was very well tolerated, and participant-reported outcomes were in line with instrumental findings, with most subjects perceiving smoother, firmer and healthier-looking skin.

While these multi-parametric, convergent findings are encouraging, they derive from an open-label, non-controlled study and should be interpreted accordingly. Overall, the data support the potential of this *Brassica* extract as a promising ingredient for "inside-out" support of skin barrier function and anti-aging appearance.

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Abbreviations

The following abbreviations are used in this manuscript:

ECM	Extracellular matrix
GCP	Good clinical practice
HFUS	High-frequency ultrasound
MMPs	Matrix metalloproteinases
NF-κB	Nuclear factor kappa B
NRF2	Nuclear factor erythroid 2-related factor 2
ROS	Reactive oxygen species
SC	*Stratum corneum*
SE	Standard error
SFN	Sulforaphane
TEWL	Transepidermal water loss

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