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Research Article

Efficacy of SPH-24, a Proprietary Blend of Pomegranate (*Punica granatum*) peel and Holy Basil (*Ocimum sanctum*) Extracts on Skin Aging Parameters in Healthy Women: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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Abstract

The present randomized, double-blind, placebo-controlled clinical study evaluated the efficacy and safety of SPH-24, a proprietary botanical formulation containing standardized extracts of pomegranate (*Punica granatum*) peel and holy basil (*Ocimum sanctum*), on objective measures of skin aging and skin health in healthy women with mild-to-moderate facial wrinkles. Eighty women aged 30-55 years were randomized in a 1:1 ratio to receive SPH-24 (300 mg/day) or placebo once daily for 12 weeks. Forehead wrinkle number, wrinkle area, and wrinkle area percentage were assessed using New Visage-LS® imaging. Secondary assessments included skin firmness and elasticity, skin hydration, transepidermal water loss (TEWL), melanin index, erythema, and skin tone parameters (L*, a*, b*, and Individual Typology Angle [ITA]) using validated instrumental methods. Safety was assessed through adverse event monitoring, laboratory investigations, vital signs, and physical examinations. SPH-24 significantly reduced forehead wrinkle number, wrinkle area, and wrinkle area percentage compared with placebo after 12 weeks, with reduction in wrinkle number evident from Week 4. Significant improvements were also observed in skin hydration, elasticity, TEWL, melanin index, erythema, and the a* colorimetric parameter. No significant treatment differences were observed for skin firmness (R0), L*, or ITA values. SPH-24 was well tolerated, with no treatment-related adverse events, serious adverse events, or clinically significant laboratory or vital sign abnormalities reported. Daily supplementation with SPH-24 for 12 weeks significantly improved multiple objective measures of skin aging and skin health, particularly wrinkle burden, hydration, elasticity, epidermal barrier function, pigmentation, and erythema. These findings support the potential of SPH-24 as a botanical nutricosmetic for promoting healthy skin aging.

Keywords: Botanical nutraceutical; Facial wrinkles; Epidermal barrier; Skin elasticity; Pomegranate polyphenols; *Ocimum sanctum*

Introduction

Skin aging is a complex biological process influenced by intrinsic factors, including chronological aging and hormonal changes, as well as extrinsic factors such as ultraviolet (UV) radiation, environmental pollution, smoking, psychological stress, and dietary habits. These factors collectively contribute to structural and functional alterations in the skin, including the degradation of collagen and elastin fibers, impaired barrier function, increased transepidermal water loss (TEWL), pigmentation irregularities, reduced elasticity, wrinkle formation, and loss of overall skin radiance and hydration. As life expectancy increases and consumer interest in healthy aging continues to grow, interventions aimed at preserving skin structure and appearance have become an important focus of dermatological and nutritional research [1-3].

The global demand for scientifically validated nutricosmetic interventions has expanded substantially in recent years, driven by increasing recognition of the relationship between nutrition and skin health. Unlike topical products that primarily target the skin surface, oral nutraceuticals may influence multiple biological pathways involved in skin aging, including oxidative stress, inflammation, extracellular matrix remodeling, and epidermal barrier function [4]. Consequently, botanical ingredients rich in polyphenols and antioxidants have received considerable attention for their potential to support skin health and reduce visible signs of aging [5].

Oxidative stress is recognized as one of the principal mechanisms underlying cutaneous aging. Excessive generation of reactive oxygen species (ROS) induced by UV exposure and environmental stressors accelerates degradation of collagen and elastin through activation of matrix metalloproteinases (MMPs), impairs dermal extracellular matrix integrity, and promotes inflammatory responses that contribute to wrinkle formation, pigmentation abnormalities, erythema, and loss of skin elasticity [6-8]. Chronic low-grade inflammation further exacerbates these processes, leading to progressive deterioration of skin structure and function. Therefore, nutritional interventions capable of reducing oxidative stress and inflammation may offer a promising strategy for promoting healthy skin aging [6-9].

Pomegranate (*Punica granatum*) peel is a rich source of bioactive polyphenols, including ellagitannins, punicalagins, anthocyanins, and ellagic acid, which possess potent antioxidant and anti-inflammatory properties. Experimental and clinical studies have demonstrated that pomegranate-derived polyphenols can protect against UV-induced skin damage, reduce oxidative stress, inhibit collagen degradation, and improve skin appearance [10-12]. Pomegranate supplementation has been shown to enhance skin photoprotection, support collagen preservation, and modulate pigmentation pathways through inhibition of tyrosinase activity and melanogenesis [13-14]. In addition, ellagic acid and related metabolites have been reported to improve skin texture and reduce visible signs of photoaging through their effects on extracellular matrix homeostasis and inflammatory signaling pathways [11-14].

Holy basil (*Ocimum sanctum* Linn.), commonly known as tulsi, is an adaptogenic medicinal plant extensively used in traditional Ayurvedic medicine. The plant contains numerous bioactive constituents, including eugenol, ursolic acid, rosmarinic acid, apigenin, luteolin, and various flavonoids, which exhibit antioxidant, anti-inflammatory, immunomodulatory, and stress-modulating activities [15-18]. Pre-clinical investigations have demonstrated that tulsi extracts protect against oxidative tissue damage, suppress inflammatory mediators, and support cellular resilience under physiological stress conditions [16-18]. Emerging evidence suggests that these biological activities may also contribute to maintenance of skin integrity by reducing oxidative injury, improving microcirculation, and mitigating inflammation-associated skin aging processes [17-18].

The complementary mechanisms of pomegranate peel and holy basil provide a strong scientific rationale for their combined use as a nutritional intervention for skin health. Polyphenols derived from pomegranate peel may contribute to protection of dermal extracellular matrix components, reduction of pigmentation, and enhancement of skin appearance, while holy basil bioactives may support antioxidant defense systems, modulate inflammatory pathways, and promote overall skin resilience. Together, these mechanisms may help improve multiple manifestations of skin aging, including wrinkle formation, loss of elasticity, impaired hydration, altered pigmentation, and compromised skin barrier function [10-18].

SPH-24 is a proprietary botanical blend comprising standardized pomegranate peel and holy basil extracts, containing a total bioactive complex of punicalagins, ellagic acid, gallic acid, ursolic acid, and oleanolic acid standardized to not less than 12.5%. Based on the known biological activities of its constituent phytochemicals, SPH-24 is hypothesized to improve skin appearance and function through antioxidant, anti-inflammatory, photoprotective, and skin barrier-supportive mechanisms. However, clinical evidence evaluating the combined effects of pomegranate peel and holy basil supplementation on objective measures of skin aging remains limited.

Therefore, the present study was conducted to evaluate the efficacy and safety of SPH-24 in healthy women with mild-to-moderate facial wrinkles. The study investigated the effects of 12-week

supplementation on facial wrinkles, skin hydration, skin elasticity, skin firmness, pigmentation, erythema, overall skin tone, and skin barrier function using validated instrumental assessments in a randomized, double-blind, placebo-controlled clinical trial.

Materials and Methods

Study Design and Ethical Conduct

This prospective, randomized, double-blind, placebo-controlled, parallel-group clinical study was conducted to evaluate the efficacy and safety of a proprietary pomegranate peel and holy basil extract blend on skin health and skin aging parameters in healthy women presenting with mild-to-moderate facial wrinkles. The study was conducted at a single clinical research center in Bengaluru, India, following approval by the Institutional Ethics Committee of BGS Global Institute of Medical Sciences. The study was performed in accordance with the ethical principles outlined in the Declaration of Helsinki, International Council for Harmonization Good Clinical Practice (ICH-GCP) guidelines, and applicable local regulatory requirements. Written informed consent was obtained from all participants prior to the initiation of any study-related procedures. The study was registered with the Clinical Trials Registry of India (CTRI/2025/11/098223).

The study consisted of a screening period of up to 7 days followed by a 12-week intervention period. Eligible participants were randomized in a 1:1 ratio to receive either SPH-24 or placebo and attended study visits at baseline (Day 0), Week 4 (Day 28), Week 8 (Day 56), and Week 12 (Day 84).

Participants

Healthy female participants aged 30-55 years with visible mild-to-moderate periorbital wrinkles were recruited for the study. Eligible participants were required to have a Fitzpatrick wrinkle score between 4 and 6, Fitzpatrick skin type II-IV, and a body mass index (BMI) between 18.5 and 29.9 kg/m². Participants agreed to maintain their existing skincare routine, dietary habits, physical activity levels, and sun protection practices throughout the study period. They were also required to refrain from consuming oral supplements intended to improve skin health during study participation.

Participants were excluded if they had known hypersensitivity to the study product, were pregnant or lactating, had clinically significant systemic illnesses, uncontrolled hypertension or diabetes, dermatological disorders that could interfere with study assessments, recent cosmetic dermatological procedures, excessive ultraviolet exposure, sleep disorders requiring medication, or recent participation in another clinical trial.

Study procedures

Participants were enrolled only after providing written informed consent following a detailed verbal and written explanation of the study objectives, procedures, potential risks, benefits, and participant responsibilities during the screening period (Day-7 to Day-0). Adequate opportunity was provided for participants to ask questions and seek clarification before enrollment. Demographic information, medical history, concomitant medications, lifestyle factors, and physical examination findings were recorded during screening.

Eligibility was confirmed based on predefined inclusion and exclusion criteria. Screening assessments included anthropometric measurements, vital signs, clinical laboratory investigations, and evaluation of wrinkle severity using the Fitzpatrick Wrinkle Scale. Safety laboratory assessments included complete blood count (CBC), liver function tests [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)], renal function tests [serum creatinine and blood urea nitrogen (BUN)], and urine pregnancy testing for women of childbearing potential.

Eligible participants were randomized to receive either the proprietary pomegranate peel and holy basil extract blend or placebo. Participants were instructed to consume one capsule orally each morning after breakfast for 84 consecutive days and to maintain their usual skincare regimen, dietary habits, physical activity levels, and sun protection practices throughout the study period.

Efficacy assessments were performed at baseline (Day 0), Week 4 (Day 28), Week 8 (Day 56), and Week 12 (Day 84). Facial wrinkle parameters were evaluated using the New Visage-LS® imaging system. Skin hydration was assessed using a Corneometer®, skin firmness and elasticity using a Cutometer®, skin pigmentation, erythema and overall skin tone (L*, a*, b*, and Individual Typology Angle [ITA]) using a Skin Colorimeter®, and skin barrier function was evaluated Tewameter®.

Safety assessments, including physical examination, vital signs, clinical laboratory evaluations, concomitant medication review, and adverse event monitoring, were performed throughout the study. End-of-study safety evaluations were conducted at Week 12 (Day 84).

Randomization and blinding

Eligible participants were randomly assigned in a 1:1 ratio to receive either SPH-24 or placebo using a computer-generated randomization schedule. Randomization codes were maintained by an independent dispenser who was not involved in efficacy or safety evaluations.

The SPH-24 and placebo capsules were identical in appearance, packaging, labelling, and administration schedule. Participants, investigators, study personnel, outcome assessors, and statisticians remained blinded to treatment allocation until completion of database lock and statistical analyses.

Investigational product and dosing

The investigational product consisted of a proprietary botanical blend containing standardized extracts of pomegranate peel and holy basil, with a total bioactive complex comprising punicalagins, ellagic acid, gallic acid, ursolic acid, and oleanolic acid standardized to not less than 12.5%, as quantified by a validated high-performance liquid chromatography (HPLC) method. Each active capsule contained 300 mg of the proprietary botanical blend and 150 mg microcrystalline cellulose, providing a total capsule weight of 450 mg. The placebo consisted of 450 mg microcrystalline cellulose.

Participants were instructed to consume one capsule orally once daily in the morning after breakfast for 84 consecutive days. Compliance was assessed through capsule accountability and participant interview at each follow-up visit.

Study visits and procedures

Participants attended five clinic visits, including a screening visit (Day-7 to Day-0), baseline/randomization visit (Day 1), follow-up visit 1 (Week 4; Day 28 ± 2), follow-up visit 2 (Week 8; Day 56 ± 2), and end-of-study visit (Week 12; Day 84 + 2). Following confirmation of eligibility, participants were randomized in a 1:1 ratio to receive either the proprietary pomegranate peel and holy basil extract blend or placebo. Participants were instructed to consume one capsule orally each morning after breakfast for 84 consecutive days and to maintain their usual skincare regimen, dietary habits, physical activity levels, and sun protection practices throughout the study.

At baseline and each scheduled follow-up visit, efficacy assessments were performed under standardized environmental conditions following an acclimatization period. Facial wrinkle characteristics, including wrinkle line number, wrinkle area, and wrinkle area percentage, were evaluated using the New Visage-LS® imaging system. Skin hydration was assessed using a Corneometer®, skin firmness and elasticity was measured using a Cutometer®, and skin pigmentation, erythema and overall skin tone (L*, a*, b*, and Individual Typology Angle [ITA]) were evaluated using a Skin Colorimeter®. Skin barrier function was assessed by Tewameter®.

Compliance with study product consumption was assessed through capsule accountability and participant interviews at each follow-up visit. Concomitant medications and any changes in health status were reviewed throughout the study duration.

Safety monitoring

Safety assessments included physical examinations, vital sign measurements, laboratory evaluations, and monitoring of adverse events (AEs) and serious adverse events (SAEs). Vital signs included seated blood pressure, heart rate, and body temperature following a minimum of 5 minutes of rest. Laboratory evaluations included complete blood count (CBC), liver function tests [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)], renal function tests [serum creatinine and blood urea nitrogen (BUN)], and urine pregnancy testing for women of childbearing potential. Safety assessments were conducted at screening and at the end-of-study visit.

All AEs were documented in the case report form and evaluated for onset, duration, severity (mild, moderate, or severe), relationship to study intervention (definite, probable, possible, unlikely, or unrelated), action taken, and outcome. Any SAEs were reported according to applicable regulatory requirements and were followed until resolution, stabilization, or study completion.

Outcomes and Endpoints

The primary efficacy endpoint was the mean change from baseline in facial wrinkle parameters, including wrinkle line number, wrinkle area, and wrinkle area percentage, assessed using the New Visage-LS® imaging system and compared between the proprietary pomegranate and holy basil extract blend and placebo groups over the 12-week intervention period.

Secondary efficacy endpoints included changes from baseline in skin hydration measured using a Corneometer®, skin firmness and elasticity measured using a Cutometer®, skin pigmentation (melanin index), erythema and overall skin tone (L*, a*, b*, and Individual Typology Angle [ITA]) measured using a Skin Colorimeter®, and skin barrier function assessed through Tewameter®. Assessments were performed at baseline, Week 4, Week 8, and Week 12.

The safety endpoints for the study included clinically significant changes from baseline to the end of treatment in laboratory parameters, including complete blood count (CBC), liver function tests [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)], renal function tests [serum creatinine and blood urea nitrogen (BUN)], the incidence of adverse events (AEs) and serious adverse events (SAEs), and variations in vital sign parameters and physical examination findings.

Sample size determination

The sample size calculation was based on detecting a clinically meaningful difference between treatment groups for wrinkle-

related outcomes. Assuming a common standard deviation of 12.6 units, 35 evaluable participants per treatment arm were estimated to provide 80% statistical power at a two-sided significance level of 0.05. Allowing for an anticipated dropout rate of approximately 12.5%, a total of 80 participants were randomized into the study.

Statistical analysis

Statistical analyses were performed using R statistical software. Continuous variables were summarized using mean, standard deviation (SD) and standard error (SE), while categorical variables were summarized using frequency counts and percentages.

Changes from baseline and between-group differences were evaluated using analysis of variance (ANOVA) models incorporating treatment, visit, and treatment-by-visit interaction effects. Type III tests of fixed effects were used to evaluate statistical significance. All statistical tests were two-sided, and a p-value <0.05 was considered statistically significant. Safety analyses were performed on all randomized participants who received at least one dose of study product, while efficacy analyses were performed on modified intention-to-treat population defined as participants who completed the study at least till Day 56.

Results

A total of 84 subjects were screened for participation in the study, of whom 80 met the eligibility criteria and were randomized in a 1:1 ratio to receive either the proprietary extract blend of pomegranate peel and holy basil (SPH-24) or placebo (Figure 1).

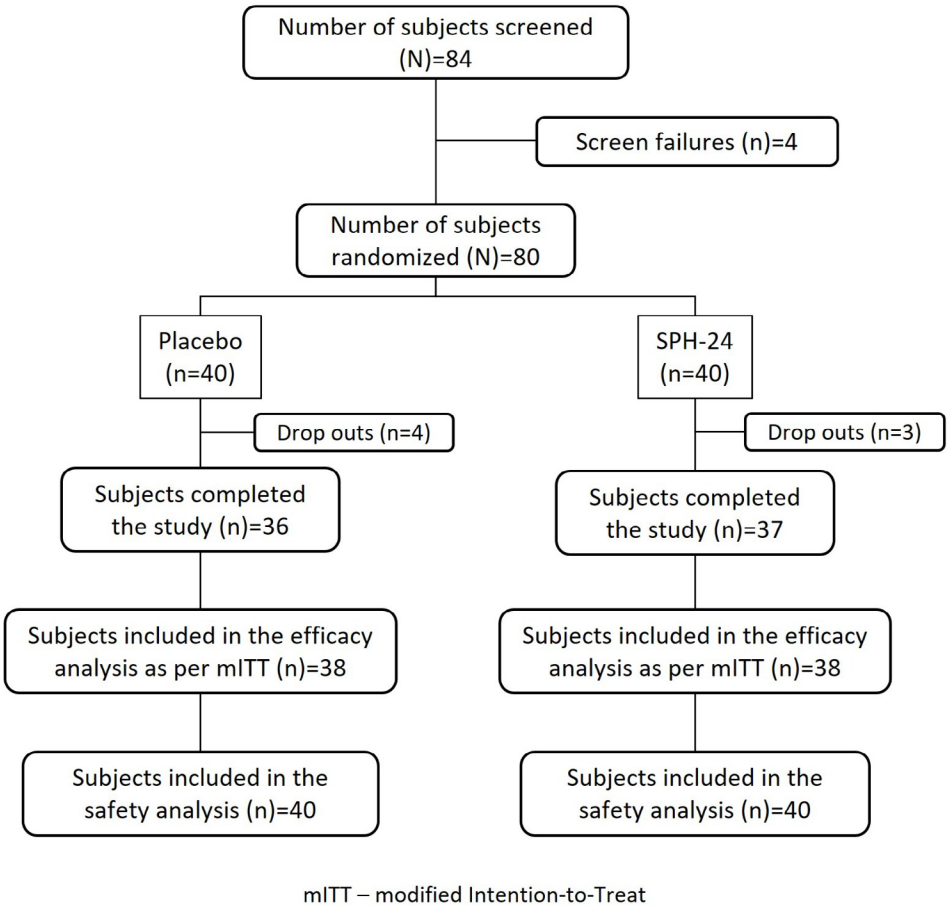


Figure 1: CONSORT diagram of participant flow.

Four subjects were excluded during screening and classified as screen failures. Of the 80 randomized subjects, 73 completed the study, while seven subjects discontinued before study completion. Four subjects discontinued from the placebo group (two at Visit 2 and two at Visit 4), while three subjects discontinued from the SPH-24 group (one at Visit 2, one at Visit 3, and one at Visit 4). A total of 76 subjects (38 per group) were included in the efficacy analyses based on modified ITT population, and all 80 randomized subjects were included in the safety analyses.

The study population consisted entirely of Indian women. The mean (± SD) age of subjects enrolled in the SPH-24 and placebo groups was 41.23 ± 6.76 years and 40.61 ± 5.42 years, respectively (Table 1). Mean (± SD) body weight was 62.83 ± 10.78 kg in the SPH-24 group and 68.22 ± 10.06 kg in the placebo group, while mean (± SD) height was 155.55 ± 6.33 cm and 157.56 ± 6.87 cm, respectively. The mean (± SD) BMI was 25.83 ± 3.22 kg/m² in the SPH-24 group and 27.34 ± 2.51 kg/m² in the placebo group. Overall, demographic and baseline anthropometric characteristics were comparable between treatment groups and indicated a well-balanced study population at baseline.

Parameter	SPH-24 (n=38)	Placebo (n=38)
Age (years)	41.23 ± 6.76	40.61 ± 5.42
Weight (kg)	62.83 ± 10.78	68.22 ± 10.06
Height (cm)	155.55 ± 6.33	157.56 ± 6.87
BMI (kg/m²)	25.83 ± 3.22	27.34 ± 2.51

Table 1: Baseline Demographic and Anthropometric Characteristics of Subjects; Data are presented as mean ± SD unless otherwise indicated. BMI: Body mass index.

Primary efficacy endpoints

Facial Wrinkles

Forehead Lines Number

As illustrated in Figure 2a, supplementation with SPH-24 resulted in progressive improvements in forehead lines number throughout the 12-week intervention period. Within-group analysis demonstrated significant reductions from baseline in forehead lines number at Days 28, 56, and 84 in the SPH-24 group ($p < 0.05$), whereas subjects receiving placebo exhibited significant increases in forehead lines number over the same period. The mean (± SD) change from baseline at Day 28 was -218.18 ± 443.32 in the SPH-24 group compared with 143.95 ± 353.42 in the placebo group, representing a statistically significant between-group difference ($p = 0.0002$). At Day 56, the mean (± SD) change from baseline was -191.71 ± 547.12 in the SPH-24 group compared with 146.24 ± 317.40 in the placebo group ($p = 0.0015$). By Day 84, the reduction in forehead lines number further increased to -270.45 ± 491.80 in subjects receiving SPH-24, whereas the placebo group demonstrated an increase of 254.87 ± 291.02, resulting in a highly significant between-group difference ($p = 0.0000$).

Forehead Lines Area

As shown in Figure 2b, supplementation with SPH-24 produced progressive reductions in forehead wrinkle area over the 12-week study period. At Day 28, the mean (± SD) change from baseline was −22.13 ± 226.76 mm² in the SPH-24 group compared with 77.47 ± 174.28 mm² in the placebo group. Although a statistically significant difference between treatment groups was observed ($p < 0.05$), this difference was not attributed to SPH-24 because the corresponding change from baseline was not significant. Similarly, at Day 56, the mean (± SD) change from baseline was -42.82 ± 186.85 mm² in the SPH-24 group compared with 69.74 ± 170.29 mm² in the placebo group. While a significant between-group difference was observed ($p < 0.01$), the change from baseline remained non-significant.

By Day 84, a clinically meaningful and statistically significant reduction in forehead wrinkle area was observed in subjects receiving SPH-24, with a mean (±SD) change from baseline of −97.26 ± 177.31 mm² compared with an increase of 89.00 ± 187.43 mm² in the placebo group ($p < 0.0001$). Within-group analysis revealed that SPH-24 produced a progressive and significant reduction in forehead wrinkle area by the end of the study, whereas placebo-treated subjects demonstrated significant increase in wrinkle area.

Forehead Lines Percentage Area

As illustrated in Figure 2c, supplementation with SPH-24 favorably affected the percentage area of skin occupied by forehead wrinkles during the study period. At Day 28, the mean (±SD) change from baseline was −0.25 ± 5.33% in the SPH-24 group compared with 1.79 ± 4.28% in the placebo group; however, the between-group difference was not statistically significant. At Day 56, the mean (±SD) change from baseline was −1.22 ± 4.53% in the SPH-24 group compared with 1.91 ± 4.60% in the placebo group. Although a significant between-group difference was observed ($p < 0.01$), this difference was not attributed to SPH-24 because the corresponding change from baseline was non-significant.

By Day 84, the reduction in wrinkle percentage area became more pronounced, with the SPH-24 group demonstrating a mean (± SD) change from baseline of −2.42 ± 4.26% compared with an increase of 2.07 ± 4.97% in the placebo group ($p < 0.001$). Within-group analysis demonstrated progressive and significant improvement in wrinkle percentage area at Day 84 among subjects receiving SPH-24, whereas placebo-treated subjects showed significant increase in wrinkle percentage area.

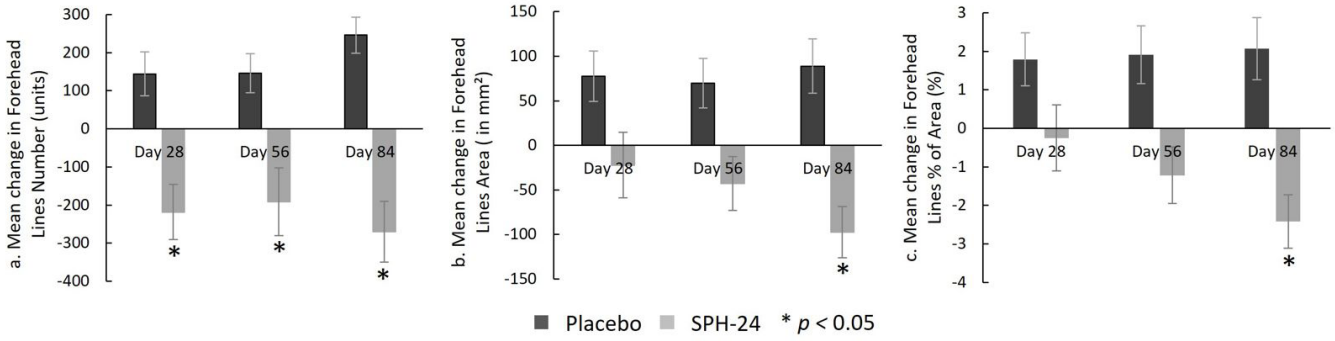


Figure 2: Mean (±SD) change from baseline in forehead wrinkle parameters following supplementation with SPH-24 or placebo over the 12-week study period. (a) Forehead wrinkle number (units); (b) Forehead wrinkle area (mm²); and (c) Forehead wrinkle area (%). Data are presented as mean ± SD. $p < 0.05$ versus placebo at the corresponding study visit.

Secondary Efficacy Endpoints

Skin Hydration

As illustrated in **Figure 3a**, supplementation with SPH-24 resulted in progressive improvements in forehead skin hydration throughout the 12-week intervention period. Within-group analysis demonstrated significant increases in skin hydration from baseline at Days 28, 56, and 84 in the SPH-24 group ($p < 0.05$), whereas placebo-treated subjects exhibited an initial worsening in skin hydration followed by a significant increase only at the end of the study. The mean (± SD) change from baseline at Day 28 was 8.65 ± 25.94 AU in the SPH-24 group compared with −10.31 ± 30.05 AU in the placebo group, representing a statistically significant between-group difference ($p < 0.01$). At Day 56, the mean (± SD) changes from baseline increased to 11.93 ± 23.85 AU in the SPH-24 group compared with −0.99 ± 28.12 AU in the placebo group, with a significant between-group difference ($p < 0.05$). By Day 84, the improvement in skin hydration further increased to 20.69 ± 23.71 AU in the SPH-24 group compared with 8.05 ± 23.90 AU in the placebo group, again demonstrating a statistically significant difference between treatment groups ($p < 0.05$).

Skin Elasticity

As illustrated in Figure 3b, supplementation with SPH-24 resulted in progressive improvements in forehead skin elasticity throughout the 12-week intervention period. Within-group analysis demonstrated a progressive and significant increase in skin elasticity from baseline across all post-baseline visits in the SPH-24 group ($p < 0.05$), whereas subjects receiving placebo exhibited non-significant increases throughout the study period. The mean (±SD) change from baseline at Day 28 was 5.47 ± 15.94 N/m in the SPH-24 group compared with 3.68 ± 19.75 N/m in the placebo group, with no significant between-group difference. At Day 56, the mean (±SD) changes from baseline increased to 12.45 ± 24.00 N/m in the SPH-24 group compared with 6.13 ± 20.43 N/m in the placebo group; however, the between-group difference remained non-significant. By Day 84, the improvement in skin elasticity further increased to 17.58 ± 21.58 N/m in the SPH-24 group compared with 4.63 ± 23.16 N/m in the placebo group, resulting in a statistically significant between-group difference ($p < 0.05$).

Transepidermal Water Loss (TEWL)

As illustrated in **Figure 3c**, supplementation with SPH-24 resulted in progressive improvements in epidermal barrier function, as demonstrated by reductions in forehead TEWL throughout the 12-week intervention period. Within-group analysis demonstrated a progressive and significant decrease in TEWL from baseline across all post-baseline visits in the SPH-24 group ($p < 0.05$), whereas subjects receiving placebo exhibited non-significant increases throughout the study period. At Day 28, the mean (±SD) change from baseline in TEWL was −4.83 ± 13.02 g/m²/h in the SPH-24 group compared with 0.31 ± 11.64 g/m²/h in the placebo group, with no significant between-group difference. At Day 56, the mean (±SD) change from baseline was −5.06 ± 14.45 g/m²/h in the SPH-24 group compared with 5.47 ± 17.50 g/m²/h in the placebo group, demonstrating a statistically significant between-group difference ($p < 0.01$). By Day 84, TEWL was further reduced to −6.33 ± 16.17 g/m²/h in subjects receiving SPH-24, whereas the placebo group exhibited an increase of 5.02 ± 16.33 g/m²/h, resulting in a significant between-group difference ($p < 0.01$).

Overall Skin Tone (ITA Value)

As illustrated in Figure 3d, supplementation with SPH-24 resulted in modest improvements in the Individual Typology Angle (ITA) of the forehead over the 12-week intervention period. Within-

group analysis showed non-significant differences from baseline at all visits for both the groups. At Day 28, the mean (±SD) change from baseline in ITA value was 1.37 ± 9.44° in the SPH-24 group compared with −3.43 ± 9.98° in the placebo group. Although a statistically significant between-group difference was observed ($p < 0.05$), this difference was not attributed to SPH-24 because the corresponding change from baseline was not statistically significant. At Day 56, the mean (±SD) change from baseline was 5.63 ± 13.76° in the SPH-24 group compared with 4.11 ± 17.78° in the placebo group, with no significant between-group difference. Similarly, at Day 84, the mean (±SD) change from baseline was 4.15 ± 18.61° in the SPH-24 group compared with −1.35 ± 12.51° in the placebo group, and the between-group difference remained non-significant.

Skin Pigmentation (Melanin Index)

As illustrated in Figure 3e, supplementation with SPH-24 resulted in progressive improvements in forehead skin pigmentation throughout the 12-week intervention period. Within-groups analysis indicated that SPH-24 provided a significant decrease in melanin index from baseline to Day 84 and non-significant differences at Days 28 and 56, whereas placebo group showed non-significant differences at all visits from baseline. At Day 28, the mean (±SD) change from baseline in melanin index was −3.74 ± 24.93 AU in the SPH-24 group compared with 3.97 ± 33.44 AU in the placebo group, with no significant between-group difference. At Day 56, the mean (±SD) change from baseline was −3.92 ± 32.99 AU in the SPH-24 group compared with 1.66 ± 32.73 AU in the placebo group, and the between-group difference remained non-significant. By Day 84, the reduction in melanin index further increased to −10.41 ± 29.65 AU in subjects receiving SPH-24 compared with an increase of 6.93 ± 37.82 AU in the placebo group, resulting in a statistically significant between-group difference ($p < 0.05$).

Skin Erythema

As illustrated in Figure 3f, supplementation with SPH-24 resulted in progressive reductions in forehead skin erythema throughout the 12-week intervention period. Within-groups analysis indicated that SPH-24 provided a significant decrease in erythema from baseline to Days 56 and 84 and non-significant difference at Day 28, whereas placebo group showed non-significant differences at all visits from baseline. At Day 28, the mean (±SD) change from baseline in erythema was −1.91 ± 10.33 AU in the SPH-24 group compared with 1.05 ± 14.49 AU in the placebo group, with no significant between-group difference. At Day 56, the mean (±SD) change from baseline was −4.33 ± 12.99 AU in the SPH-24 group compared with −0.03 ± 9.63 AU in the placebo group, and the between-group difference remained non-significant. By Day 84, the reduction in erythema further increased to −11.33 ± 17.95 AU in the SPH-24 group compared with −3.67 ± 12.94 AU in the placebo group, resulting in a statistically significant between-group difference ($p < 0.05$).

Skin Lightness [L value (L*a*b)]

As illustrated in Figure 3g, supplementation with SPH-24 showed numerical improvements in forehead skin lightness (L* value) over the 12-week intervention period; however, no statistically significant differences were observed between treatment groups. Within-group analysis showed non-significant differences from baseline at all visits for both the groups. At Day 28, the mean (±SD) changes from baseline in L* value was −0.28 ± 2.36 in the SPH-24 group compared with −1.18 ± 3.03 in the placebo group, with no significant between-group difference. At Day 56, the mean (±SD) change from baseline was 0.29 ± 3.01 in the SPH-24 group compared with −0.57 ± 1.72 in the placebo group, and the between-group difference remained non-significant. Similarly, at Day 84, the mean (±SD) change from baseline was 0.49 ± 2.64 in the SPH-24 group compared with −0.79 ± 2.94 in the placebo group, with no statistically significant difference between treatment groups.

Skin Redness–Greenness [a value (L*a*b*)]

As illustrated in Figure 3h, supplementation with SPH-24 resulted in progressive reductions in forehead a* values over the 12-week intervention period, indicating an improvement in skin redness-greenness. Within-groups analysis indicated that SPH-24 provided a significant decrease in forehead a* value from baseline to Day 84 and non-significant differences at Days 28 and 56, whereas placebo group showed non-significant differences at all visits from baseline. At Day 28, the mean (±SD) changes from baseline in the a* value was 0.12 ± 2.15 in the SPH-24 group compared with −0.25 ± 2.07 in the placebo group, with no significant between-group difference. At Day 56, the mean (±SD) change from baseline was −0.59 ± 1.88 in the SPH-24 group compared with −0.38 ± 1.83 in the placebo group, and the between-group difference remained non-significant. By Day 84, the reduction in the a* value increased to −1.80 ± 2.08 in the SPH-24 group compared with −0.43 ± 2.00 in the placebo group, resulting in a statistically significant between-group difference ($p < 0.01$).

Skin Yellowness–Blueness [b Value, (L*a*b*)]

As illustrated in Figure 3i, supplementation with SPH-24 resulted in progressive increases in forehead b* values over the 12-week intervention period. Within-groups analysis indicated that SPH-24 provided a significant increase in forehead b* value from baseline to Day 84 and non-significant differences at Days 28 and 56, whereas placebo group showed non-significant differences at all visits from baseline. At Day 28, the mean (±SD) changes from baseline in the b* value was 0.07 ± 2.80 in the SPH-24 group

compared with −0.29 ± 2.47 in the placebo group, with no significant between-group difference. At Day 56, the mean (±SD) change from baseline was 0.55 ± 3.08 in the SPH-24 group compared with −0.20 ± 2.94 in the placebo group, and the between-group difference remained non-significant. By Day 84, the mean (±SD) changes from baseline increased to 1.05 ± 2.98 in the SPH-24 group compared with −0.40 ± 3.14 in the placebo group, resulting in a statistically significant between-group difference ($p < 0.05$).

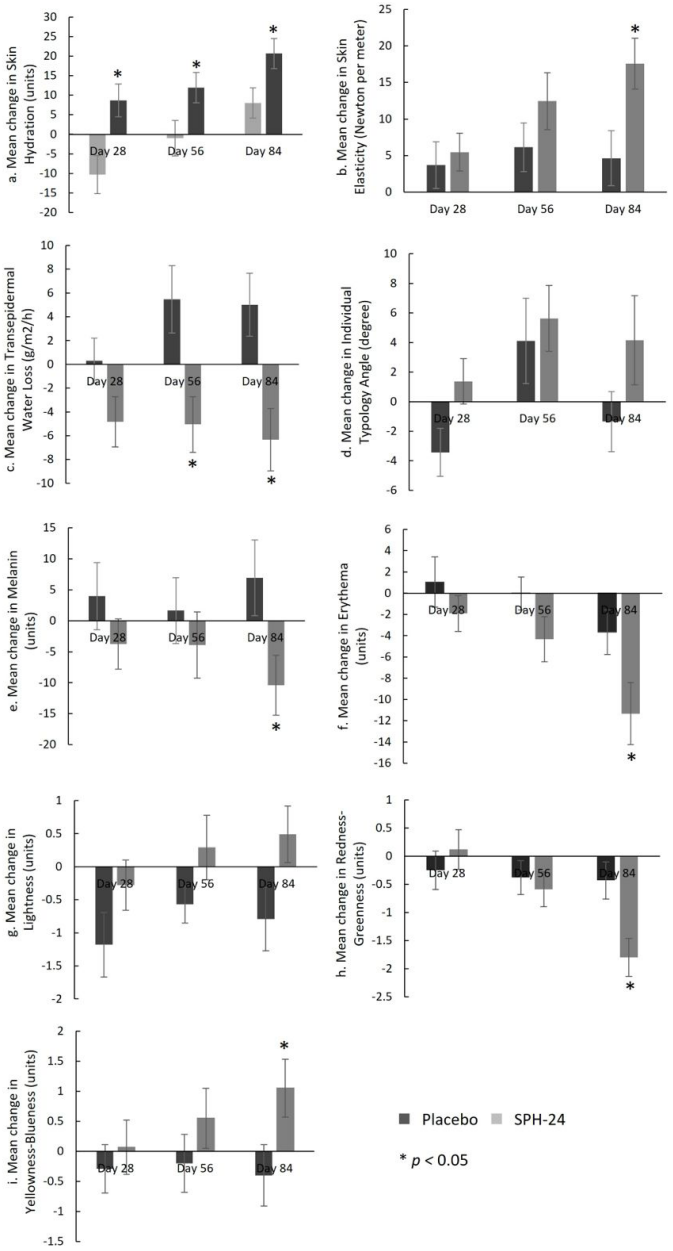


Figure 3: Mean (±SD) change from baseline in secondary skin parameters following supplementation with SPH-24 or placebo over the 12-week study period. (a) Skin hydration (arbitrary units); (b) Skin elasticity (Newton per meter); (c) Transepidermal water loss (g/m²/h); (d) Individual Typology Angle (degrees); (e) Melanin index (arbitrary units); (f) Erythema (arbitrary units); (g) Skin lightness (L*); (h) Skin redness–greenness (a*); and (i) Skin yellowness–blueness (b*). Data are presented as mean ± SD. $p < 0.05$ versus placebo at the corresponding study visit.

Safety and Tolerability

A total of 12 adverse events (AEs) were reported during the study (Table 2). Eleven subjects (13.75%) experienced at least one AE, including five subjects (12.5%) in the SPH-24 group and six subjects (15.0%) in the placebo group. The total number of AEs reported was five in the SPH-24 group and seven in the placebo group. Headache was the most commonly reported adverse event. Overall, nine subjects received at least one concomitant medication during the study, including four subjects in the SPH-24 group and five subjects in the placebo group. Paracetamol was the most frequently used concomitant medication.

Adverse Event	SPH-24 (n = 40), n (%)	Placebo (n = 40), n (%)	Total (N = 80), n (%)
Subjects with ≥ 1 AE	5 (12.5)	6 (15.0)	11 (13.75)
Total AEs, n	*5*	*7*	*12*
Headache	2 (5.0)	3 (7.5)	5 (6.25)
Fever	2 (5.0)	2 (5.0)	4 (5.0)
Cough	1 (2.5)	0	1 (1.25)
Body pain	0	1 (2.5)	1 (1.25)
Loose stools	0	1 (2.5)	1 (1.25)

Table 2: Summary of Treatment-Emergent Adverse Events (Safety Population); AE, adverse event; n, number of subjects; N, total number of subjects. Data are presented as n (%). Percentages are based on the safety population. Subjects may have experienced more than one adverse event.

All reported AEs were mild in severity, transient in nature, and resolved completely before the end of the study. According to the investigator's causality assessment, none of the reported AEs were considered related to the investigational product. No serious adverse events (SAEs) were reported, and no subject discontinued the study because of an AE or SAE. Furthermore, no clinically significant changes were observed in physical examination findings or vital signs, and all vital sign measurements remained within normal physiological limits throughout the study. Overall, SPH-24 was well tolerated following 12 weeks of oral supplementation.

Discussion

The present randomized, double-blind, placebo-controlled clinical trial demonstrated that 12 weeks of oral supplementation with a proprietary blend of pomegranate peel and holy basil extracts significantly improved multiple objective indicators of skin aging in healthy women with mild-to-moderate facial wrinkles. Compared with placebo, SPH-24 produced significant reductions in forehead wrinkle number, wrinkle area, and wrinkle area percentage, accompanied by improvements in skin hydration, elasticity, barrier integrity, erythema, and melanin index. Importantly, improvements in wrinkle number were evident as early as Week 4, whereas significant improvements in elasticity, pigmentation, and erythema became more pronounced after sustained supplementation, suggesting both early and cumulative biological effects of the botanical formulation. Overall, these findings indicate that SPH-24 positively influences several complementary aspects of skin health rather than acting on a single cosmetic endpoint.

Reduction in wrinkle formation represented the principal finding of the present study. SPH-24 significantly improved all three wrinkle-related endpoints at Day 84, while placebo-treated subjects demonstrated progressive worsening or minimal improvement. Wrinkle formation is a multi-factorial process driven by intrinsic aging and cumulative environmental exposures, particularly ultraviolet (UV) radiation, which induces oxidative stress, chronic inflammation, and extracellular matrix remodeling through activation of matrix metalloproteinases (MMPs), resulting in progressive degradation of collagen and elastin fibers. Bioactive polyphenols present in pomegranate, including punicalagins and ellagic acid, have been shown to attenuate these processes by reducing reactive oxygen species generation, suppressing inflammatory signaling, inhibiting MMP expression, and preserving collagen homeostasis [19]. Likewise, holy basil contains bioactive phytochemicals such as eugenol, ursolic acid, rosmarinic acid, and flavonoids that exhibit potent antioxidant and anti-inflammatory activities, promote collagen synthesis, inhibit MMP-mediated extracellular matrix degradation, and provide protection against UV-induced skin damage [20,21]. These complementary mechanisms provide a biologically plausible explanation for the significant reduction in forehead wrinkle number and wrinkle area observed following SPH-24 supplementation. The observed reductions in wrinkle number, wrinkle area, and wrinkle percentage therefore suggest that the botanical blend may attenuate these underlying biological processes rather than merely improving the cosmetic appearance of the skin.

The wrinkle-related findings of the present study are consistent with previous clinical investigations evaluating oral pomegranate supplementation for skin health [22]. In a prospective,

randomized, double-blind, placebo-controlled trial, Chakkalakal et al. demonstrated that four weeks of supplementation with a standardized punicalagin-enriched pomegranate extract significantly reduced facial wrinkle severity and improved several skin biophysical properties in healthy adults. The study also reported favorable modulation of the gut–skin axis, with participants exhibiting higher abundances of Eggerthellaceae showing greater reductions in TEWL and wrinkle severity, suggesting a potential role of gut microbial metabolism in mediating the skin benefits of pomegranate polyphenols [22].

Similar improvements have been reported with fermented pomegranate extract. In a randomized placebo-controlled clinical study, oral supplementation significantly improved skin hydration, elasticity, brightness, and dermal collagen-related parameters, supporting the anti-photoaging effects of pomegranate-derived polyphenols [23]. Collectively, these studies, together with the findings of the present trial, provide growing clinical evidence that oral pomegranate polyphenols can improve objective measures of skin aging, including wrinkle appearance, skin hydration, elasticity, and epidermal barrier function.

Several complementary biological mechanisms may explain the observed wrinkle improvements. Pomegranate is rich in ellagitannins, particularly punicalagins, together with ellagic acid, anthocyanins, and other polyphenolic compounds possessing potent antioxidant and anti-inflammatory activities [19,24]. Experimental studies have demonstrated that these phytochemicals attenuate UV-induced oxidative stress, suppress inflammatory signalling, inhibit matrix metalloproteinase (MMP) expression, preserve collagen homeostasis, reduce oxidative DNA damage, and maintain extracellular matrix integrity [12,24,25]. In addition, pomegranate-derived polyphenols have been shown to stimulate hyaluronic acid metabolism and collagen synthesis in dermal fibroblasts while protecting against collagen degradation, thereby contributing to improved skin structure and reduced wrinkle formation [12,25,26]. These mechanistic findings provide biological plausibility for the progressive reduction in wrinkle number and wrinkle area observed following SPH-24 supplementation. One of the most consistent findings in the present study was the significant improvement in skin hydration accompanied by reduced TEWL, indicating enhancement of epidermal barrier integrity. Skin hydration improved significantly from Week 4 onwards, whereas improvements in TEWL became significant during the latter half of the intervention, suggesting gradual restoration of barrier function with continued supplementation. Maintenance of epidermal hydration depends largely on the structural and functional integrity of the stratum corneum and its extracellular lipid matrix, which together regulate TEWL and preserve skin barrier homeostasis. Chronic oxidative stress and persistent low-grade inflammation disrupt epidermal barrier function by impairing lipid organization, keratinocyte differentiation, and extracellular matrix homeostasis, resulting in increased water loss, reduced skin elasticity, and accelerated skin aging. Consequently, the concomitant improvement in skin hydration and reduction in TEWL observed in the present study represents a clinically meaningful enhancement of epidermal barrier function that extends beyond cosmetic improvement [27-29]. These findings are in agreement with previous studies demonstrating that pomegranate-derived bioactives enhance hyaluronan synthesis, collagen metabolism, and skin moisture while simultaneously improving barrier function.

Skin elasticity also improved significantly following SPH-24 supplementation, whereas changes in skin firmness (R0) remained non-significant. Although both parameters are obtained using the Cutometer®, they represent distinct biomechanical properties. Skin elasticity reflects the ability of the skin to recover following deformation and is strongly influenced by collagen and elastin organization, whereas R0 primarily reflects passive mechanical extensibility. Consequently, significant improvements in elasticity without corresponding changes in R0 are biologically plausible, particularly over a 12-week intervention.[30] Experimental studies have demonstrated that pomegranate polyphenols promote collagen preservation and reduce oxidative degradation of extracellular matrix proteins [12,19,24], while holy basil has been shown to stimulate collagen deposition and wound repair in pre-clinical models.[15,16] Together, these mechanisms may contribute to the improved dermal biomechanical properties observed in the present study.

The present study also demonstrated significant reductions in melanin index and erythema following 12 weeks of supplementation.

Hyper-pigmentation and erythema are recognized manifestations of chronic ultraviolet (UV) exposure, oxidative stress, and cutaneous inflammation, all of which contribute to photoaging and uneven skin appearance [31,32]. Pomegranate-derived polyphenols, particularly ellagic acid and punicalagins, have been shown to inhibit tyrosinase activity and melanogenesis while attenuating oxidative stress and inflammatory pathways, thereby reducing melanin synthesis and UV-induced pigmentation [19,31,33]. These mechanisms provide biological plausibility for the significant reductions in melanin index and erythema observed following SPH-24 supplementation. Clinical studies evaluating ellagic acid-rich pomegranate extracts have demonstrated reduced UV-induced pigmentation and improvements in facial brightness, supporting the pigmentation-related findings observed in the present investigation [19,23,31]. Likewise, reductions in erythema may reflect attenuation of inflammatory signalling through the antioxidant and anti-inflammatory actions of both pomegranate

and holy basil polyphenols. Improvement in pigmentation together with reduction in erythema suggests enhanced skin clarity and a more uniform complexion following supplementation [34].

In contrast, overall skin tone assessed by L* and ITA values did not demonstrate sustained statistically significant differences between treatment groups despite favorable numerical trends. These findings indicate that instrumental measures of pigmentation, such as the melanin index, may reflect greater specificity for quantifying epidermal melanin than global colorimetric parameters such as L and ITA, which are influenced by multiple optical and physiological factors [35-36]. Skin tone is influenced by multiple physiological factors, including vascular perfusion, baseline pigmentation, environmental exposure, and individual variability, which may partially explain the absence of consistent treatment effects [37]. Importantly, the lack of significance in these exploratory endpoints does not diminish the clinically relevant improvements observed in objective wrinkle, hydration, elasticity, pigmentation, and barrier function outcomes.

The favorable efficacy profile observed in this study is likely attributable to the complementary mechanisms of the two botanical ingredients. Pomegranate peel primarily contributes potent polyphenolic antioxidants that protect against oxidative stress, collagen degradation, pigmentation, and photoaging, whereas holy basil provides adaptogenic, antioxidant, anti-inflammatory, and immunomodulatory phytochemicals that may further reduce chronic inflammatory responses and support tissue homeostasis. Although human clinical evidence evaluating holy basil specifically for skin health remains limited, its established biological activities provide a plausible complementary mechanism that may enhance the overall efficacy of the botanical combination [16]. Future mechanistic investigations evaluating inflammatory biomarkers, oxidative stress markers, collagen turnover, and skin microbiome composition would help further elucidate these interactions.

The investigational product demonstrated an excellent safety profile. All reported adverse events were mild, transient, and resolved without sequelae. No serious adverse events, clinically significant laboratory abnormalities, or treatment-related safety concerns were identified. These findings are consistent with previous clinical investigations demonstrating the favorable safety profiles of both pomegranate extracts and holy basil supplementation, supporting their suitability for long-term oral use in healthy adults.

The study possesses several strengths, including its randomized, double-blind, placebo-controlled design, high treatment compliance, objective instrumental assessment of multiple skin parameters, and comprehensive evaluation of wrinkle characteristics, pigmentation, hydration, elasticity, erythema, and barrier function. Nevertheless, certain limitations should be acknowledged. The study was conducted at a single center and included only healthy women with mild-to-moderate facial wrinkles, which may limit generalizability to other populations, including men or individuals with more advanced photoaging. The intervention duration of 12 weeks may also have been insufficient to detect maximal improvements in slower-remodeling parameters such as skin firmness and global skin tone. Future multicenter studies with larger populations, longer supplementation periods, biomarker analyses, and direct comparisons with established nutricosmetic ingredients would further clarify the long-term clinical benefits and mechanisms of SPH-24.

Conclusion

Daily supplementation with a proprietary blend of pomegranate peel and holy basil extracts for 12 weeks significantly improved multiple objective indicators of skin health and skin aging in healthy women. SPH-24 produced significant reductions in facial wrinkle number, wrinkle area, wrinkle area percentage, melanin index, erythema, and TEWL while significantly improving skin hydration, elasticity, and epidermal barrier function compared with placebo. The formulation was well tolerated, with no treatment-related safety concerns identified during the study. Collectively, these findings support SPH-24 as a safe and effective botanical nutraceutical for improving multiple dimensions of skin appearance and function and provide a strong rationale for larger multicenter studies to further confirm its long-term clinical benefits and underlying mechanisms.

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Ethics Approval Statement

This study was conducted in accordance with the study protocol, the ethical principles of the Declaration of Helsinki, the International Council for Harmonization–Good Clinical Practice (ICH-GCP) guidelines, and applicable local regulatory requirements. The study protocol was reviewed and approved by the Institutional Ethics Committee of BGS Global Institute of Medical Sciences, Bengaluru, India, prior to the initiation of any study-related procedures. The study was prospectively registered with the Clinical Trials Registry of India (CTRI) (Registration No. CTRI/2025/11/098223). Written informed consent was obtained from all participants before enrolment and prior to the conduct of any study-related procedures.

Conflict of Interest

The author(s) report no conflicts of interest in this work. The study was sponsored by Samriddh Nutractive Pvt. Ltd.

Data Sharing Statement

Data supporting the findings of this study are available upon request from the corresponding author.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Clinical Trial Registration Number: CTRI/2025/11/098223

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