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Skin-Protective and Anti-Photoaging Potential of a Collagen-Based Bioactive Complex in Hs68 Dermal Fibroblasts

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Abstract

As we age, combined intrinsic and extrinsic aging processes lead to functional and structural alterations in the skin. Supplementation with nutraceuticals combining collagen peptides and plant-derived bioactive compounds represents a potential strategy to maintain skin health. In the present study, we aimed to investigate the skin-protective effects of collagen-based bioactive complex (CBC), which contains marine-derived bioactive peptides and plant-derived bioactive compounds, such as Amla, lingonberry, and other bioactive ingredients. Assessed via the ABTS radical scavenging assay, CBC exhibited dose-dependent antioxidant activity. In Hs68 cells, CBC significantly increased cell viability and showed a trend toward enhanced wound closure. Furthermore, in a UVB-induced photoaging cell model, CBC significantly reduced MMP-9 levels, suggesting a potential inhibitory effect on extracellular matrix degradation. Taken together, these findings suggest that CBC supports fibroblast viability and wound closure and attenuates UVB-induced responses associated with skin photoaging, supporting its potential as a multi-component bioactive nutraceutical formulation for maintaining skin health.

Keywords: *Collagen-Based Bioactive Complex; Hs68 Dermal Fibroblasts; Antioxidant Activity; Wound Healing; Matrix Metalloproteinase-9 (MMP-9); Skin Photoaging.*

INTRODUCTION

Skin aging is a complex biological process influenced by both intrinsic chronological aging and extrinsic environmental factors. These processes lead to functional and structural alterations in the skin, including wrinkle formation, loss of elasticity, hyperpigmentation, and impaired wound healing capacity. Consequently, there is growing interest in the use of exogenous nutrients as a potential strategy for maintaining skin health and mitigating age-related changes [1].

The reduced proliferative capacity of dermal fibroblasts is recognized as a major contributor to intrinsic skin aging. This decline may impair the maintenance and remodeling of the extracellular matrix (ECM), which is predominantly composed of collagen, elastin, adhesive glycoproteins, and proteoglycans [2]. Consequently, these ECM alterations can contribute to reduced skin elasticity and wrinkle formation. In contrast, extrinsic skin aging is predominantly driven by environmental factors such as solar radiation, air pollution, and smoking. Among these factors, ultraviolet (UV) radiation is a major contributor of extrinsic skin aging, a phenomenon commonly termed photoaging [3]. UV radiation has been shown to upregulate the expression of matrix metalloproteinases (MMPs), which can accelerate the degradation of ECM components and contribute to the loss of skin elasticity [4].

UV-induced oxidative stress is a primary contributor to the upregulation of MMPs. Upon exposure to UV radiation, cellular chromophores absorb radiant energy and subsequently generate reactive oxygen species (ROS) and other oxidation products. These ROS can activate downstream signaling pathways that promote MMP gene expression [3]. Among the various MMPs, matrix metalloproteinase-9 (MMP-9) plays an important role in extracellular matrix degradation and dermal matrix remodeling during photoaging [5,6]. Previous studies have demonstrated that exogenous antioxidants, such as rutin or ascorbic acid, can scavenge free radicals, attenuate oxidative stress, and enhance cell viability, thereby contributing to protection against UV-induced skin damage [7]. Taken together, reducing oxidative stress and MMP activity may represent an important strategy for attenuating UV-induced ECM degradation and skin photoaging.

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Among natural ingredients with potential benefits for improving skin health, marine-derived bioactive peptides have attracted increasing attention. Marine-derived bioactive peptides have been reported to exhibit antioxidant activity, highlighting their potential for application in anti-aging cosmeceuticals. In addition, accumulated evidence indicates that marine-derived bioactive peptides may support skin wound healing [8, 9]. Moreover, salmon nasal cartilage proteoglycan, a key extracellular matrix (ECM) component, and has been reported to improve skin elasticity and hydration and reduce the appearance of wrinkles and hyperpigmentation [10].

In addition to marine-derived bioactive peptides, plant-derived bioactive compounds have also been investigated for their potential protective effects against UV-induced oxidative stress. Amla (*Phyllanthus emblica L.*), which is rich in polyphenols and vitamins, exhibits antioxidant activity and has been reported to inhibit MMP expression, potentially contributing to protection against UV-induced dermal damage [11]. Similarly, lingonberry (*Vaccinium vitis-idaea*), which contains various polyphenolic compounds, including arbutin, resveratrol, and coumaric acid, has been reported to support collagen production and skin elasticity and hydration, suggesting its potential application in skin health [12]. Likewise, tangerine (Citrus reticulata), which is rich in vitamin C and other bioactive compounds, exhibits antioxidant activity and has been associated with potential benefits for skin aging [13].

Emerging research indicates that collagen-based nutraceutical supplements combining multiple bioactive ingredients may provide complementary benefits for skin health. Formulations combining collagen peptides with antioxidant compounds have been reported to support fibroblast activity and attenuate the activity of matrix-degrading enzymes, suggesting that multi-ingredient nutraceutical formulations may offer broader biological effects than single-ingredient supplementation [14].

Based on previous findings, the combination of collagen-derived peptides and plant-derived bioactive compounds may provide complementary benefits for skin health. Therefore, in the present study, we aimed to evaluate the potential skin-protective effects of a collagen-based bioactive complex (CBC), composed of marine-derived bioactive peptides, Amla extract, lingonberry extract, and other bioactive ingredients. Using human dermal fibroblasts, we investigated the effects of CBC on cell viability, wound closure, and UVB-induced extracellular MMP-9 levels. Therefore, this study aimed to evaluate the potential skin-protective effects of CBC in human dermal fibroblasts by assessing cell viability, wound closure, and UVB-induced extracellular MMP-9 levels.

MATERIALS AND METHODS

Materials

The collagen-based bioactive complex (CBC; AquaGlow Hyaluronic Acid Drink, M. ARCHER) was provided by Lelan Co., Ltd. (Taiwan). CBC was dissolved in sterile deionized water at a concentration of 50 mg/mL and sonicated for 30 min at room temperature. The solution was then centrifuged at 6,000 rpm for 10 min, and the supernatant was collected and filtered through a 0.22 μm membrane filter. The sample stock solution was diluted with culture medium to the desired concentrations prior to use.

The human dermal fibroblast cell line Hs68 (BCRC No. 60038; corresponding to ATCC CRL-1635) was obtained from the Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan). Dulbecco's Modified Eagle's Medium (DMEM; #12800-017), phenol red-free DMEM (#31053-028), penicillin-streptomycin-neomycin antibiotic mixture (PSN; #15640055), and recombinant human epidermal growth factor (EGF; #PHG0311L) were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Fetal bovine serum (FBS; #89510-186) was obtained from Avantor (Radnor, PA, USA).

2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS; #SI-A1888), horseradish peroxidase (Type I, #SI-P8125), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox; #AL-238813), and hydrogen peroxide (30%, # 31642) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The Cell Counting Kit-8 (CCK-8; #I-HY-K0301) was purchased from MedChemExpress (Monmouth Junction, NJ, USA). Culture inserts (2 Well for self-insertion, #80209) were obtained from Ibidi (Gräfelfing, Germany). UVB irradiation was performed using a BIO-LINK® UV crosslinker (Vilber, Collégien, France). Enzyme-linked immunosorbent assay (ELISA) kits for human MMP-9 (#ab246539) were purchased from Abcam (Cambridge, UK).

Antioxidant Activity (ABTS Radical Scavenging Assay)

Antioxidant activity was evaluated using an ABTS radical scavenging assay with a modified radical generation system [15, 16]. The ABTS radical cation ($ABTS^{\bullet+}$) was generated by reacting ABTS with hydrogen peroxide in the presence of horseradish peroxidase. The reaction mixture was prepared at 4 °C and subsequently incubated with the samples at room temperature in the dark before the absorbance at 734 nm was measured. Trolox was used as a standard reference, and the antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC).

Cell Culture

Hs68 cells were cultured in high-glucose DMEM (4.5 g/L glucose) supplemented with 3.7 g/L sodium bicarbonate ($NaHCO_3$), 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin-neomycin (PSN). Cells were maintained in a humidified incubator at 37°C with 5% CO_2.

Cell Viability Assay

Hs68 cells were seeded into 96-well plates at a density of 1 x 10^4 cells/well and allowed to attach overnight. Cells were then treated with indicated concentrations of CBC for 24 h. Cell viability was subsequently evaluated using the Cell Counting Kit-8 (CCK-8) assay according to the manufacturer's instructions.

Wound Healing Assay

Hs68 cells were seeded into 12-well plates at a density of 8 × 10^4 cells/well in the presence of 2-well culture inserts (Ibidi, #80209). After overnight attachment, the culture inserts were removed to create a uniform cell-free gap. The cells were gently washed once with Dulbecco's phosphate-buffered saline (DPBS), and fresh DMEM containing indicated concentration of CBC, vehicle control, or 30 ng/mL EGF (positive control) was added.

Images were captured immediately after insert removal (0 h) and after 24 h of incubation using a microscope. After 24 h, cells were washed once with DPBS, fixed with 10% formaldehyde (UniRegion Bio-Tech, #URH121) and stained with 0.05% crystal violet (Bio basic, #CB0331) to visualize cells within the wound area.

The wound area was quantified at 0 h and 24 h using ImageJ software. The percentage of wound closure was calculated relative to the initial wound area at 0 h using the following formula:

$$Migration\ rate\ (\%) = \frac{Area_{0h} - Area_{24h}}{Area_{0h}} \times 100\%$$

where $Area_{0h}$ and $Area_{24h}$ represent the wound area at 0 h and 24 h, respectively.

Determination of Photoaging Markers

Hs68 cells were seeded into 12-well plates at a density of 8 × 10^4 cells/well and allowed to attach overnight. The cells were treated with fresh DMEM containing the indicated concentrations of CBC, vehicle control, or 100 µM vitamin C for 24 h. Prior to UVB irradiation, the cells were gently washed once with DPBS and covered with DPBS (0.1 mL/well) to prevent dehydration during irradiation. UVB irradiation was performed at a dose of 30 mJ/cm^2 using a UV crosslinker. After irradiation, DPBS was removed, and the cells were incubated in phenol red-free DMEM containing 5% FBS for an additional 24 h. The culture supernatants were collected and centrifuged at 2,000 × g for 10 min to remove cellular debris. The levels of MMP-9 in the clarified supernatants were quantified using a commercially available ELISA kit (Abcam, #ab246539) according to the manufacturer's instructions.

Statistical Analysis

All data are presented as mean ± standard deviation (SD). Statistical analyses were performed using Microsoft Excel. Differences between groups were evaluated using Student's *t*-test. A p-value < 0.05 was considered statistically significant. Figures were generated using GraphPad Prism.

RESULTS

CBC Exhibits Dose-Dependent Antioxidant Activity.

Inhibition of oxidative stress is considered an important strategy for preventing UV-induced skin damage [17]. Therefore, the antioxidant activity of CBC was evaluated using the ABTS assay. As shown in Figure 1, CBC exhibited dose-dependent antioxidant activity within the detection range of the ABTS assay. At concentrations of 10, 50, 100, and 200 µg/mL, CBC exhibited TEAC values of 2.3, 5.7, 11.8, and 23.2 µg/mL, respectively. At higher concentrations of 400 and 1,000 µg/mL, the antioxidant capacity exceeded the upper detection limit of the ABTS assay, indicating a substantial radical scavenging ability.

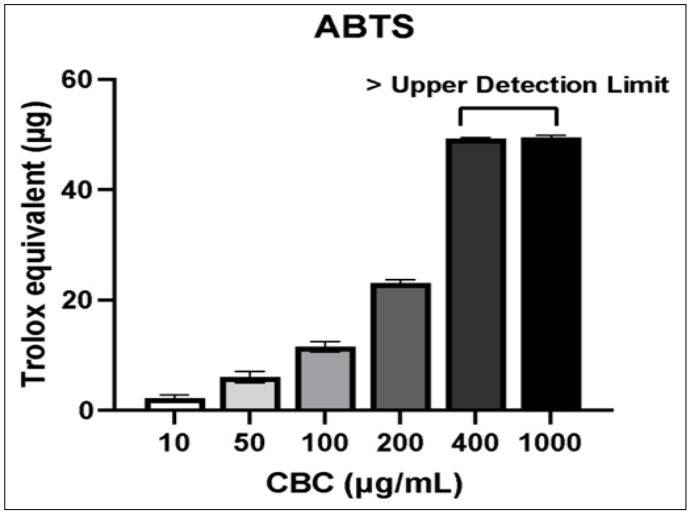


Figure 1. CBC exhibits dose-dependent antioxidant activity.

The antioxidant capacity of CBC at the indicated concentrations (10, 50, 100, 200, 400, and 1,000 µg/mL) was determined using the ABTS radical scavenging assay and expressed as Trolox equivalent antioxidant capacity (TEAC). At 400 and 1,000 µg/mL, the antioxidant activity exceeded the upper detection limit of the assay. Data are presented as mean ± SD (n = 4).

CBC Enhances Hs68 Cell Viability without Cytotoxicity.

Prior to evaluating the effect of CBC on Hs68 cells, a CCK-8 assay was performed to assess its potential cytotoxicity. CBC exhibited no cytotoxic effects on Hs68 cells; instead, it significantly enhanced cell viability across a broad concentration range (Figure 2). At concentrations of 100, 200, 400, and 1,000 µg/mL, CBC significantly increased the cell viability of Hs68 cells compared with the control (p < 0.05). At lower concentrations of 25 and 50 µg/mL, CBC also showed a trend toward increased Hs68 cell viability. These results indicate that CBC supports fibroblast viability. Based on these findings, concentrations of 100, 200, and 400 µg/mL were selected for subsequent functional assays.

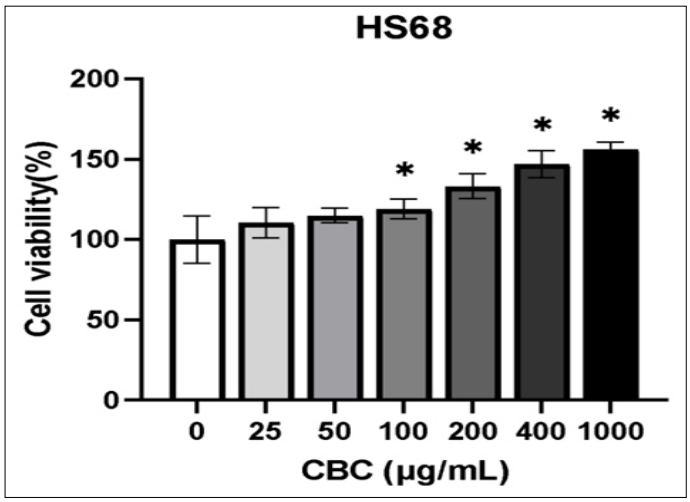


Figure 2. CBC enhances Hs68 cell viability without cytotoxicity.

Cells were treated with the indicated concentrations of CBC for 24 h, and cell viability was determined using the CCK-8 assay. Data are presented as mean ± SD (n = 5). *p < 0.05 compared with the control group.

CBC Shows a Trend toward Increased Wound Closure in Hs68 Cells.

To evaluate the effect of CBC on wound closure, an in vitro wound healing assay was performed using 2-well culture inserts. Representative images of the wound area stained with crystal violet demonstrated greater wound closure following CBC treatment compared with the control group (Figure 3A). Consistent with the visual observations, quantitative analysis of the wound area showed that 30 ng/mL EGF significantly increased wound closure compared with the control group ($p < 0.05$). Although CBC treatments at 100, 200, and 400 μg/mL did not reach statistical significance, a concentration-dependent upward trend toward increased wound closure was observed (Figure 3B). These findings suggest that CBC may support wound closure in Hs68 cells under the conditions tested.

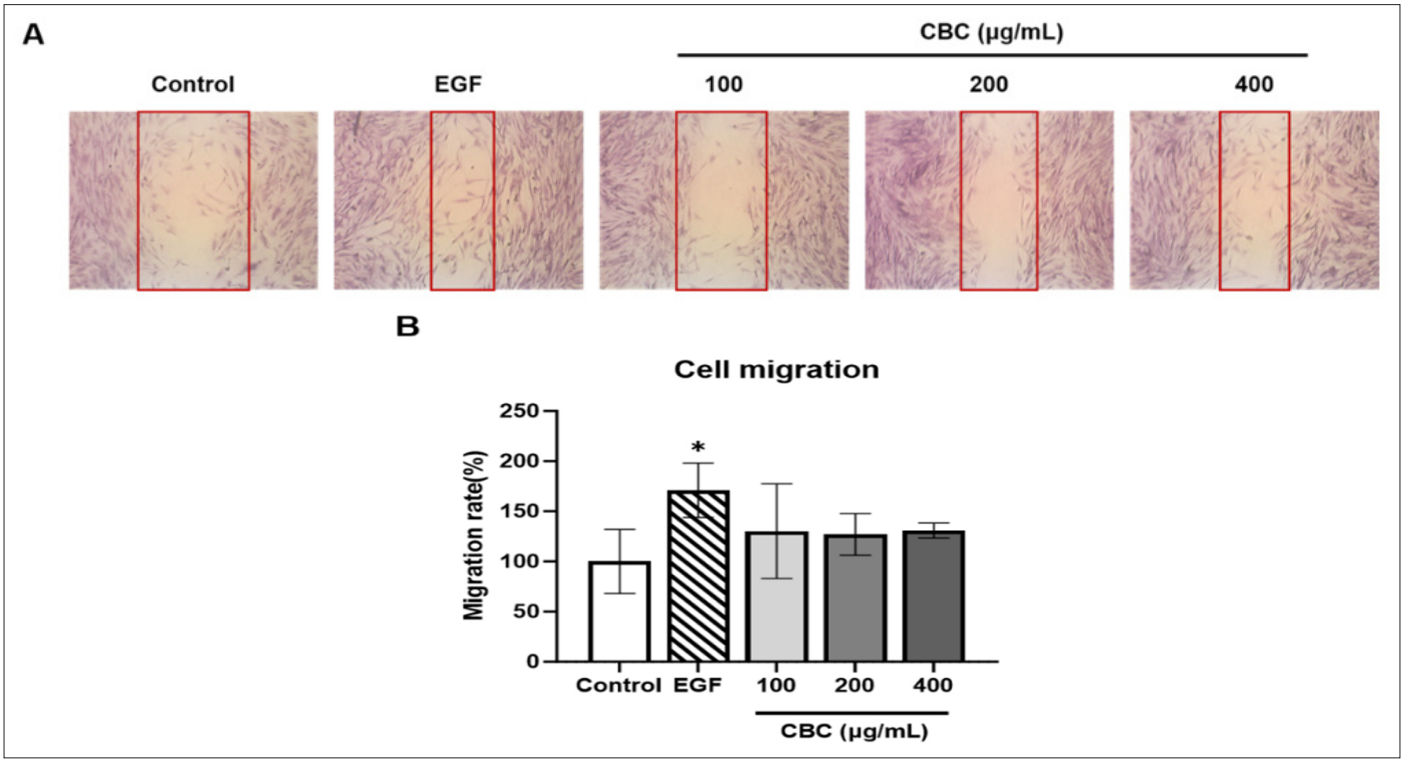


Figure 3. CBC shows a trend toward increased wound closure in Hs68 cells.

Representative images of wound closure in Hs68 cells, assessed by crystal violet staining after 24 h of treatment with CBC (100, 200, and 400 μg/mL) or EGF (30 ng/mL).

Quantification of wound closure based on the reduction in wound area relative to the initial wound area at 0 h. Data are presented as mean ± SD (n = 3). *p < 0.05 compared with the control group.

CBC Attenuates UVB-Induced Increases in Extracellular MMP-9 Levels in Hs68 Cells.

To evaluate the potential protective effects of CBC against UVB-induced photoaging, MMP-9 levels were examined in Hs68 cells treated with the indicated concentrations of CBC. As shown in Figure 4, UVB irradiation (30 mJ/cm^2) significantly increased MMP-9 levels compared with the non-irradiated control group, confirming the induction of a photoaging-related response. At the selected concentrations of 100, 200, and 400 μg/mL, CBC significantly reduced MMP-9 levels compared with the UV-irradiated group ($p < 0.05$). Notably, the inhibitory effects of CBC on MMP-9 was comparable to those of vitamin C (100 μM), suggesting that CBC may have protective potential against UVB-induced photoaging.

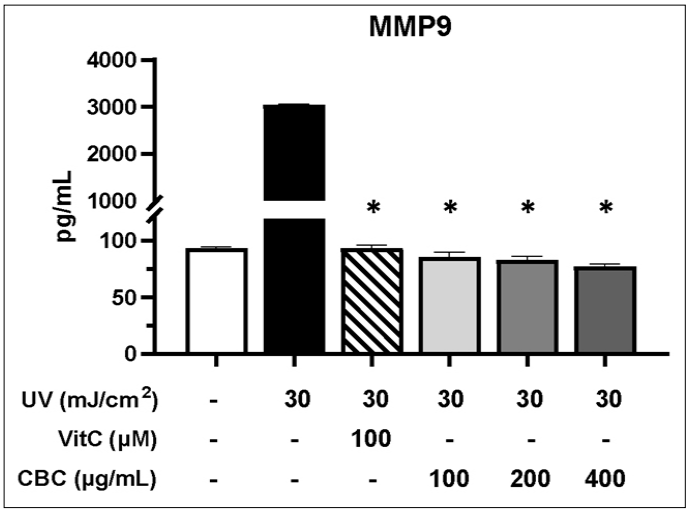


Figure 4. CBC attenuates UVB-induced increases in extracellular MMP-9 levels in Hs68 cells.

Cells were treated with the indicated concentrations of CBC (100, 200, and 400 μg/mL) or vitamin C (100 μM) for 24 h prior to UVB irradiation (30 mJ/cm^2). After an additional 24 h of incubation in phenol red-free DMEM, culture supernatants were collected and the levels of MMP-9 were measured by ELISA. Data are presented as mean ± SD (n = 3). *$p < 0.05$ compared with the UV-irradiated group.

DISCUSSION

Using the ABTS radical scavenging assay, CBC demonstrated concentration-dependent antioxidant capacity, which may contribute to its protective effects against UV-induced cellular damage. Previous studies have established that UV radiation induces the production of reactive oxygen species (ROS), and the subsequent accumulation of ROS can activate signaling pathways that upregulate matrix metalloproteinases (MMPs) [3, 17]. Therefore, the antioxidant activity of CBC may contribute to its ability to attenuate UVB-induced increases in MMP-9 levels; however, the underlying mechanisms remain to be determined.

Prior to evaluating the effects of CBC in the UVB-induced model, its effects on Hs68 cell viability were assessed. CBC exhibited no cytotoxic effects; rather, it significantly enhanced cell viability at concentrations ranging from 100 to 1,000 μg/mL, suggesting its role in supporting fibroblast metabolic activity and survival. Consistent with these observations, the in vitro wound healing assay revealed that CBC treatment led to a concentration-dependent trend toward accelerated wound closure, although these changes did not achieve statistical significance. Together, these findings indicate that CBC may foster a favorable microenvironment for dermal fibroblast viability and migration, thereby offering potential benefits for skin regeneration and tissue repair.

Photoaging is a major manifestation of extrinsic skin aging induced by chronic UV exposure [3]. To evaluate the protective effects of CBC against UV-induced cellular damage, we examined MMP-9 levels in dermal fibroblasts following UVB irradiation. Consistent with previous studies demonstrating that UV radiation upregulates MMP-9 levels, which can be attenuated by plant-derived antioxidants [6, 18, 19], our present results showed that UVB irradiation significantly increased MMP-9 levels. Notably, CBC treatment significantly reduced MMP-9 levels under UVB exposure, with an inhibitory effect comparable to that of vitamin C. These findings suggest that CBC may attenuate UVB-induced increases in extracellular MMP-9 and may therefore contribute to the attenuation of UVB-induced responses associated with skin photoaging.

Importantly, CBC is a multi-component formulation containing marine-derived bioactive peptides—such as salmon nasal cartilage proteoglycan—as well as plant-derived bioactive compounds, including Amla, lingonberry, and tangerine extracts, along with other functional ingredients. Previous studies have reported antioxidant and anti-aging activities for several of these individual components [8-13]. The combined presence of these bioactive ingredients may contribute to the overall antioxidant and skin-protective effects observed with CBC. However, the relative contribution of individual

components and potential synergistic interactions among them require further investigation.

In summary, the present study demonstrates that CBC supports dermal fibroblast viability, shows a potential to enhance wound closure, and attenuates UVB-induced MMP-9 levels. Specifically, CBC supports cell viability without cytotoxicity, exhibits a concentration-dependent trend toward accelerating wound closure, and effectively attenuates UVB-induced MMP-9 levels. Overall, these findings provide compelling evidence supporting the potential of CBC as a multi-component bioactive nutraceutical formulation for maintaining skin homeostasis and attenuating UVB-induced photoaging.

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