EFFECT OF HYDROGEL COSMECEUTICAL CONTAINING UNFERMENTED COCOA BEAN EXTRACT ON UNDER-EYE AREA TREATMENT

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ABSTRACT – *Besides being traditionally used for making chocolate and cocoa beverages, well fermented Theobroma cacao is highly prized in cosmeceuticals for its plentiful antioxidants and beneficial properties. Nonetheless, unfermented cocoa beans are preferred in cosmeceutical products over fermented ones due to their higher antioxidant content and superior skin benefits. Unfermented beans maintain a higher concentration of polyphenols, flavonoids, and other bioactive compounds that are frequently diminished during fermentation. Despite this, there is limited research on the specific use of unfermented cocoa beans in the under-eye area. Therefore, the primary objective of this research was to evaluate the UCBE's anti-ageing effects and antioxidant capacity. Regarding antioxidant capacity, the UCBE demonstrated significantly greater activity ($p<0.05$) in 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging compared to the standard ascorbic acid (AA) whereas no significant difference ($p>0.05$) was observed between UCBE and AA in ferric reducing antioxidant power (FRAP). In a skin efficacy study involving seven human subjects aged between 30 to 46 years, the application of approximately 500 mg of a 3% (w/v) UCBE formulation over one month resulted in a notable ($p<0.05$) increase in viscoelasticity parameters (Mpa), ranging from 13% to 87%. The findings suggest that UCBE is a promising active ingredient with antioxidant properties and demonstrated skin efficacy in vivo. This supports the primary objective of developing a cosmeceutical hydrogel enriched with unfermented cocoa bean extract that delivers significant benefits.*

Keywords: Hydrogel cosmeceutical, unfermented cocoa bean extract, under-eye, antioxidant, anti-ageing

INTRODUCTION

In term of cosmeceutical bioactives, phenolic compounds have been suggested to prevent collagenase and elastase degradation due to UV radiation in the skin's stratum corneum (Nisa *et al.*, 2024). Several studies have been conducted to analyze the phenolic compositions and antioxidant capacities including different cocoa bean extracts from various origins and hybrids. For example, Sulawesian cocoa beans extracted with 70% aqueous ethanol for 2 hours at 50°C exhibited the highest antioxidant capacity in FRAP assay, followed by Malaysian, Ghanaian, and Cote d'Ivoirian cocoa beans (Azizah *et al.*, 2010; Azizah *et al.*, 2007). Topical application of cocoa polyphenols has significantly enhanced skin elasticity and tonus by stimulating collagen I, III, and IV (Singh *et al.*, 2020). These findings suggest that the topical application of cocoa bean phenolics positively impacts skin health by protecting against oxidative damage.

Unfermented cocoa beans have been found to contain 12–18% phenolic compounds (dry weight), with 60% of the total phenolics consisting of epicatechin, catechin, and procyanidin oligomers (dimer to decamer) (Melo *et al.*, 2021). The reduction of polyphenol content from 100% to 10% during cocoa product processing undergoes various manufacturing processes, such as fermentation, drying, and roasting (Gil *et al.*, 2021). Phenolic compounds decreased by up to 35% during the initial 3 days of fermentation, and only 45% remained after 7 days of fermentation. Subsequently, the phenolic content continued to decrease during drying process, especially when using artificial heat sources of pressurized and heated air circulation on a wooden platform with temperature control at 60°C (Dzelagha *et al.*, 2020). Following this, the beans underwent roasting to determine the cocoa products' final color, aroma, and flavor. Exposure to high temperatures ranging from 130-150°C for 15-45 mins during convective roasting also led to significant loss of phenolic compounds due to oxidation and polymerization of phenolic compounds (Peña-Correa *et al.*, 2022). Therefore, due to the substantial loss of phenolic compounds during cocoa processing, well-fermented and roasted beans may not be suitable for cosmetic or cosmeceutical formulations.

Several strategies have been proposed for developing effective drug and bioactive delivery systems for skin-related pharmaceutical and cosmetic applications. Hydrogels are frequently regarded as ideal choices for treating the under-eye area due to their exceptional moisturizing properties. (Xiong *et al.*, 2021). Hydrogels, composed of water-soluble polymers cross-linked into a three-dimensional

network, are widely used in skincare products. They are advantageous for delivering active ingredients that target both the superficial layers and deeper layers of the skin. Furthermore, hydrogels are favored for their high water content and hydrophilic structure, which forms a matrix on the skin's surface, facilitating the effective delivery of these ingredients. Beyond their moisturizing and regenerative effects, hydrogels can also help regulate skin temperature and enhance physiological activity. As Khedkar and Aher (2022) noted, hydrogel-based cosmetics, which adhere well to the skin, are increasingly becoming staples in daily skincare routines. The delicate skin around the eyes, with fewer sebaceous glands, is particularly prone to dryness. However, hydrogel by itself may not be sufficient to address issues in the under-eye area, such as reducing dark circles, wrinkles, fine lines, and puffiness. The incorporation of unfermented cocoa beans could enhance the effectiveness of hydrogel-based cosmeceuticals for under-eye treatment, though this application is uncommon and still limited. Therefore, the study aims to evaluate the effectiveness of a hydrogel cosmeceutical infused with unfermented cocoa bean extract in treating the under-eye area, focusing on improvements in common concerns such as dark circles, wrinkles, fine lines, and puffiness.

MATERIALS AND METHODS

Preparation of Unfermented Cocoa Bean Extract (UCBE)

The extraction procedure for cocoa beans followed the method outlined by Azizah *et al.* (2007). Unfermented and dried beans, sourced from the Malaysian Cocoa Board's Cocoa Research and Development Centre in Jengka Pahang, were manually deshelled before being ground to a particle size of approximately 1 mm. To remove lipids and cocoa butter, 450 mL of hexane was used on 100 g of the ground beans. The remaining solids were then dried to yield about 45 g of fat-free mass. For the extraction process, polyphenol-rich extracts were obtained from the fat-free mass using 30–80% ethanol (v/v) for 2 hrs at 50°C in a shaker (Unimax 1010, Heidolph, Germany). The extracts were combined, and the organic solvent was evaporated using a rotary evaporator (IKA® RV 10 control, Staufen, Germany) under partial vacuum at 40°C. The aqueous extracts were then freeze-dried and stored in an airtight container.

Antioxidant Capacity of the UCBE

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH radical scavenging activity of the UCBE was assessed using a modified version of the method described by Azizah *et al.* (2007). An aliquot of UCBE (200 µL, 7.81 to 1,000 µg/mL in ethanol) or ascorbic acid (as a standard) was mixed with 800 µL of 100 mM Tris-HCl buffer (pH 7.4). Next, 1 mL of 500 µM DPPH, prepared in ethanol, was added to the mixture. The solution was vortexed thoroughly and allowed to stand for 20 minutes at room temperature in the dark. Ethanol (99.8%) served as the blank (control). Absorbance was measured at 517 nm using a filter-based microplate photometer (Thermo Scientific Multiskan® FC, Vantaa, Finland). The DPPH radical scavenging effect was calculated using the following equation:

$$\text{DPPH• Scavenging effect (\%)} = (1 - [A_0 - A_1]) \times 100 \quad \textbf{(1)}$$

Where A_0 was the absorbance of the control, and A_1 was the absorbance of the UCBE. The EC_{50} value was obtained from a graph plotting the scavenging effect versus UCBE concentration. This value represents the concentration of UCBE required to reduce the initial DPPH radical concentration by 50%. Scavenging effects were assessed based on the percentage of DPPH radicals significantly scavenged (p<0.05).

Ferric Reducing Power (FRAP) Assay

The FRAP value was assessed by measuring the reduction of ferric-2,4,6-tripyridyl-s-triazine (TPTZ) to blue ferrous-TPTZ at 593 nm, following the method outlined by Benzie and Strain (1996). To prepare the FRAP reagent, a mixture was made consisting of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM $FeCl_3$•$6H_20$, in a 10:1:1 ratio, and incubated at 37°C. A 3.0 mL aliquot of the FRAP reagent was placed in a test tube and incubated in a water bath at 37°C for 10 mins, where the absorbance was recorded as t0. Subsequently, 100 µL of the sample or standard and 100 µL of distilled water were added, mixed, and incubated for an additional 4 mins at 37°C, after which the absorbance was measured at 593 nm (t4). $FeSO_4$•$7H_20$ served as the standard. The FRAP value was calculated using the equation provided by Benzie and Strain (1996), with the reduction potential of the UCBE determined against a standard curve of $FeSO_4$•$7H_20$ (200–1000 mM). The FRAP value was expressed in mM $FeSO_4$•$7H_20$ equivalents per gram of dried sample.

Development of Hydrogel Formulation Incorporated with UCBE

The hydrogel was formulated using UCBE, pectin, distilled water, triethanolamine (TEA), glycerin, carbomer 940, and preservative. The UCBE concentration was maintained at 3% (w/w) due to its proven efficacy on the skin, as shown in the study by Norliza *et al.* (2019). To ensure a uniform aqueous phase and thorough mixing of the CBE, continuous

stirring was performed using a propeller-type Silverson L5M-A (Silverson Machines Inc., East Longmeadow, MA, USA

In vivo Skin Efficacy

In this study, the inclusion and exclusion criteria were based on the guidelines set by Azad *et al.* (2012). The research was conducted over 4 weeks starting in June 2023 and concluded in July 2023. Seven human subjects, aged 30 to 46, participated in the study, consisting of seven staff members from the Malaysian Cocoa Board, Cocoa Innovative & Technology Centre in Nilai, Negeri Sembilan. All participants were non-users of antioxidant cosmetics. The inclusion and exclusion criteria applied during the enrollment process are detailed in Table 1.

Table 1: The exclusion and inclusion criteria for *in vivo* studies involving humans

Exclusion Criteria	**1.**	Simultaneous participation in another clinical research study or any treatment that involved skin intervention of active cosmetic ingredients for sunscreens, moisturizers or anti-ageing purposes, for at least 2 months before the commencement of the study;
	2.	Cardiac pacemakers or implanted defibrillators;
	3.	A history of cancer, including malignant moles;
	4.	Diseases that could be activated by heat stimulation such as recurrent herpes simplex;
	5.	Impaired immune system caused by immunosuppressive diseases such as AIDS or the use of immunosuppressive medications;
	6.	Metallic implants in the treatment areas; and
	7.	Long-term steroid treatment
Inclusion Criteria	**1.**	Clinical symptoms of skin ageing such as decreased skin elasticity and turgor, dynamic and static wrinkles and folds and irregular skin texture;
	2.	Willingness to follow all recommendations and study instructions as explained by the investigators, including refraining from other anti-ageing corrective treatments and rejuvenation techniques before and during the study period; and
	3.	Willingness to sign a written consent form for treatment and participation in the study.

Source: Azad *et al.* (2012)

This study was classified as an *in vivo* study due to the involvement of human participants. The product application frequency was set at a minimum of three times per week (500 mg/skin area) (Najmee *et al.*, 2022). Participants were instructed not to use any other cosmetics on the application site throughout the study. Before starting, each subject underwent a basal skin assessment to establish baseline skin conditions (Azad *et al.*, 2016). Initial viscoelasticity (VE) assessments were performed using the DermaLab® Combo Series at 0 hours. An elasticity probe (Figure 1) was applied to three different locations under each eye (L1, L2, & L3) (Figure 2), with a vacuum applied to a consistent level of approximately 10 mm. The measurements, expressed in mega Pascals (MPa), reflect the skin's firmness, with higher vacuum strength indicating better firmness.

Figure 1: Suction device to measure skin elasticity, DermaLab Combo®

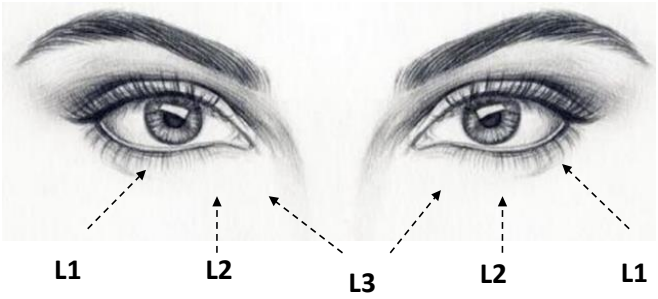

Figure 2: The area under the eyes (L1, L2 and L3) where the in vivo efficacy assessment was conducted

Each subject underwent a basal skin assessment using the DermaLab Combo to establish their baseline skin conditions before any intervention. Since the baseline skin conditions were objectively measured, there was no need to introduce a placebo, such as a hydrogel without the extract (Colvan *et al.*, 2018). The researchers relied on these baseline measurements to assess the effects of the treatment. Furthermore, the study had only 7 human volunteers, which limited the use of a placebo. The small sample size made it challenging to split participants into treatment and placebo groups without compromising the statistical power of the study. Thus, this study focused on directly comparing the treatment's effects against the established baseline skin conditions of the participants, instead of using a placebo-controlled, blinded study design.

Statistical Analysis

All data are presented as means ± SD of triplicate experiments. A one-way ANOVA (Minitab version 14.0) was performed to assess the mean differences in DPPH and FRAP with statistical significance set at $p<0.05$.

RESULTS AND DISCUSSIONS

Antioxidant Capacity of the UCBE

Scavenging Activity on DPPH

The EC_{50}, which can be obtained from the graph illustrating the correlation between scavenging activity (%) and extract concentration (Figure 3), refers to the amount of antioxidants needed to decrease the initial DPPH radical concentration by 50%. A lower EC_{50} value indicates a stronger ability of the extract to scavenge DPPH radicals.

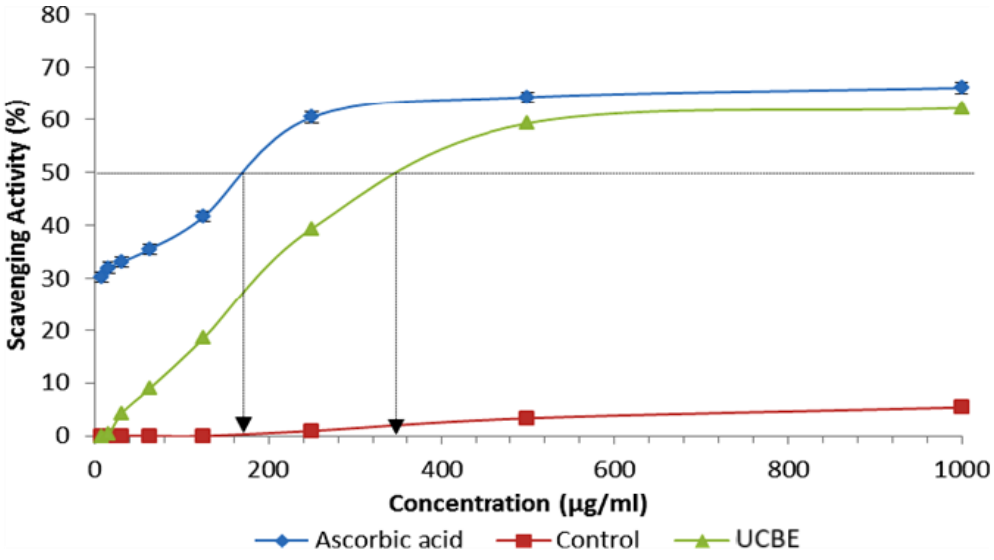

Figure 3: Scavenging activity of ascorbic acid standard and the UCBE on DPPH radicals. Values are expressed as mean ± standard deviation (n=3)

In this study, the EC_{50} of UCBE was determined to be 342.0±0.10 µg/mL, whereas the EC_{50} of the ascorbic acid standard was 170±0.24 µg/mL (Figure 3). Based on Azizah *et al.* (2007), the scavenging activity of 70% (v/v) ethanolic cocoa bean extracts from Malaysia, Ghana, and Ivory Coast were noted as 1300.0±0.01, 1300.0±0.01, and 1500.0±0.1 µg/mL, respectively**.** It is evident that when utilizing the same extraction medium, i.e., 70% (v/v) aqueous ethanol, the DPPH scavenging effect of the studied UCBE (EC_{50}=342.0±0.10 µg/mL) was relatively greater than those reported by Azizah *et al.* (2007). This is potentially attributable to the substantial amounts of preserved phenolic compounds, as no fermentation was involved, thereby preventing the breakage of phenolic compounds.

FRAP Activity

The FRAP assay assesses the antioxidant's ability to reduce the ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex, resulting in the formation of a colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ). The reduction of the Fe^{3+}-TPTZ complex to the blue-colored Fe^{2+}-TPTZ occurs favorably under low pH conditions (Benzie and Strain, 1996, 1999). In this study, both UCBE and ascorbic acid showed an apparent increase in dose-response characteristics with rising concentrations, attributed to the generation of the Fe^{2+}-TPTZ complex. The reducing power of ascorbic acid and UCBE exhibited a dose-dependent increase, yielding values of 1008.21±10.16 and 822.09±10.16 mMFeSO4•7H2O/g DW, respectively, with no significant difference ($p>0.05$) (Figure 4).

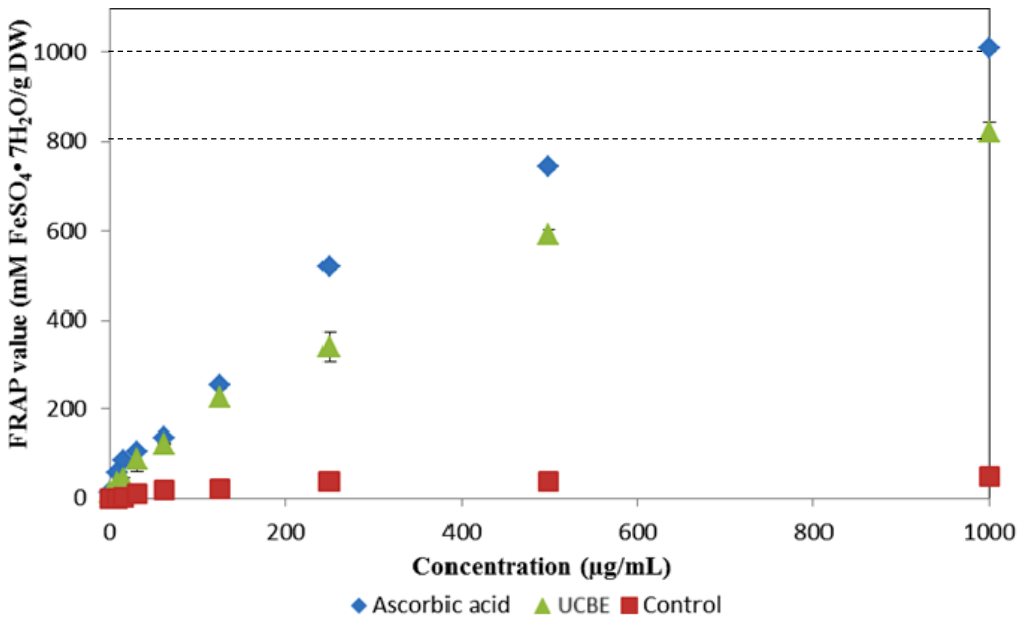

Figure 4: FRAP activity of ascorbic acid standard and the unfermented CBE on the reduction of Fe^{3+}-TPTZ to Fe^{2+}-TPTZ. Values are expressed as mean ± standard deviation (n=3).

The outcome of FRAP reducing power demonstrated by UCBE was strongly backed by Jinap *et al.* (1995) study, which indicated that the naturally low pH of Sulawesian and Malaysian bean cotyledons likely played a major role in their higher antioxidant capacity compared to beans from Cote d'Ivoire and Ghana.

Azizah *et al.* (2010) also discovered a strong correlation between epicatechin and the FRAP reducing power of cocoa bean extracts. These results align with our findings, as the high FRAP value of UCBE may have been influenced by the significant presence of phenolic compounds preserved due to the absence of fermentation.

In vivo Efficacy Study

This study examined the consistency of skin elasticity measurements conducted using the DermaLab Combo® on all participants' skin beneath the eye area. The conclusions draw from three area measurements: L1, L2, and L3 (see Figure 2). Regarding the assessment of under-eye skin ageing based on quality, it was observed that all identified skin areas (L1, L2, and L3) of all participants exhibited significant improvement in elasticity. Compared to the initial skin assessment (Figures 5A1 and 5B1), apparent enhancement in terms of firmness and reduction in wrinkles were noted in the close-up images of both under-eye areas (Figures 5A2 and 5B2). This observation aligns with the quantitative data demonstrating a percentage increase in skin elasticity (Wan Sulaiman *et al.*, 2016). Moreover, the viscoelasticity (VE) parameter has demonstrated various results at different measured areas.

Increasing VE values measured at three under-eye skin locations of all participants varied from 13% to as high as 87% and were consistent with the improvements in skin shown in Figures 5A2 and 5B2. In this study, the skin tone and moisturizing effects were improved by enhancement of the skin's viscoelasticity as evidenced in Table 2, leading to firmer, healthier, and more resilient skin. When the skin is well-hydrated and has an even tone, it maintains better structural integrity. Improved hydration boosts the skin's moisture barrier, while even skin tone reflects overall skin health and balance. These findings suggest that the eye hydrogel containing UCBE possesses anti-ageing effects, as evidenced by the remarkable results of antioxidant capacity (Figures 3 and 4). Future studies could focus on recruiting older participants and extending the study duration to enhance the results' accuracy and reliability.

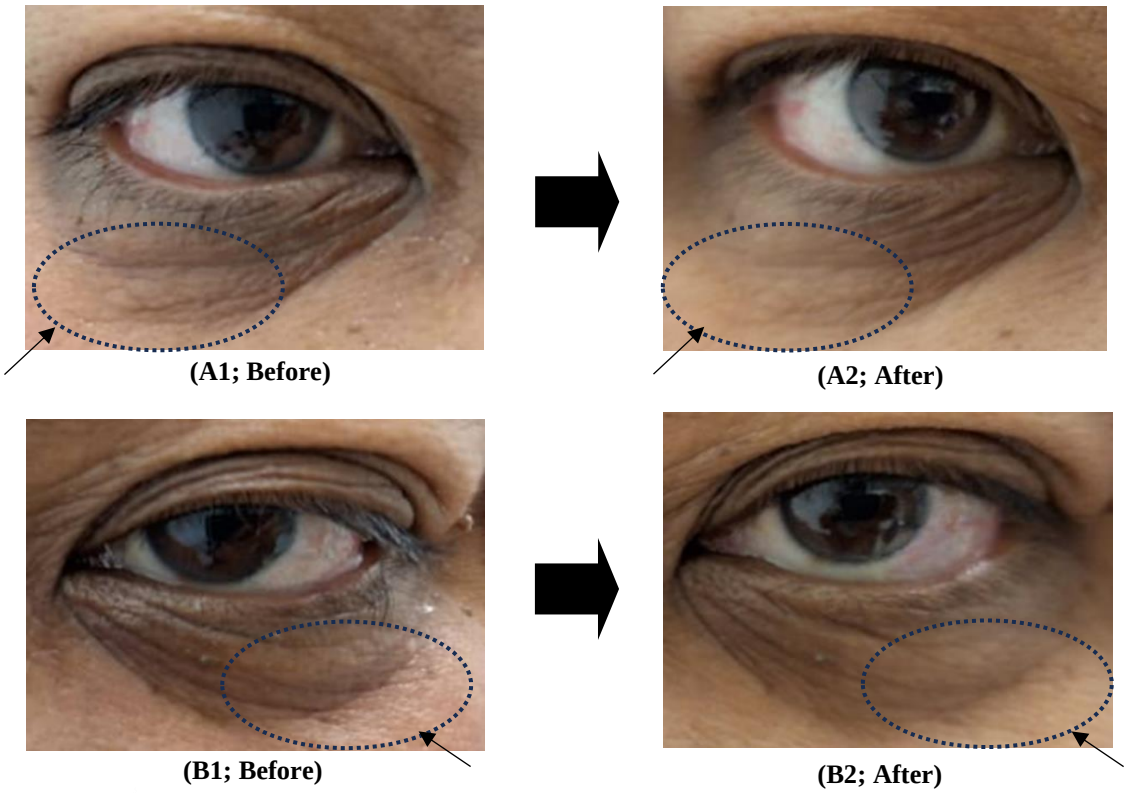

Figure 5: The areas under left and right eyes of a human subject assessed at initial (0 hour) (A1 and B1) and after 1 month of hydrogel cosmeceutical product application (A2 and B2)

Table 2**:** The VE parameter of under-eye skin area measured with DermaLabCombo®

Subject	Under-eye skin area	Before (Initial – Baseline)			After (1 month)			VE increased (%)		
		L1 (MPa)	L2 (MPa)	L3 (MPa)	L1 (MPa)	L2 (MPa)	L3 (MPa)	L1	L2	L3
1	Left	1.3	2.0	1.6	2.1	2.8	2.2	38%	29%	27%
	Right	1.4	1.8	1.8	2.2	2.5	2.4	36%	28%	25%
2	Left	4.7	4.2	4.5	5.4	5.5	5.7	13%	24%	21%
	Right	4.6	4.4	4.6	6.0	6.1	6.3	23%	27%	27%
3	Left	2.6	2.6	2.4	4.5	4.6	4.0	43%	43%	40%
	Right	2.0	2.4	2.0	4.0	3.8	4.2	50%	39%	52%
4	Left	3.2	3.0	3.4	4.1	4.0	4.5	22%	25%	24%
	Right	3.2	3.5	3.5	4.3	4.6	4.8	26%	24%	27%
5	Left	1.7	1.8	1.5	2.8	3.0	2.9	39%	40%	48%
	Right	1.4	1.8	1.6	2.0	2.4	2.8	30%	25%	43%
6	Left	3.5	3.8	3.4	5.3	6.3	5.4	34%	39%	37%
	Right	3.8	3.8	3.5	5.9	6.0	5.7	36%	37%	38%
7	Left	1.8	2.0	2.3	6.0	6.8	6.8	70%	71%	66%
	Right	1.6	1.0	1.2	5.3	5.4	5.6	87%	81%	78%

CONCLUSIONS

The biological analyses reveal that UCBE, which is rich in polyphenol compounds, has significant potential as a natural antioxidant due to its strong antioxidant capabilities, including radical scavenging and the reduction of ferric to ferrous ions. Additionally, the research shows that when UCBE is included at a 3% (w/v) concentration in hydrogel cosmeceutical formulation, it exhibits anti-ageing properties, enhancing under-eye skin elasticity and reducing fine lines and wrinkles. Consequently, UCBE could be a key source of natural antioxidants in cosmetics for skin ageing protection. This study also highlights the effectiveness of non-invasive biophysical techniques in assessing the anti-ageing effects of topical applications. Moreover, the CBE formulation was found to be safe *in vivo* and offers a cost-effective option for anti-ageing treatments.

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