IFSCC 2025 full paper (IFSCC2025-1081)

Hippophae rhamnoides fruit oil improves skin condition by increasing oxygen levels in epidermal cells

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1. Introduction

As the largest organ of the human body, the health of the skin highly depends on an adequate oxygen supply [1]. Hypoxia is a significant contributing factor to various skin problems and commonly occurs in scenarios such as prolonged bed rest, sitting, clothing constriction, or external object compression [2]. These physical factors impede skin microcirculation, leading to a decrease in oxygen partial pressure in epidermal cells and triggering a series of pathological changes, including impaired barrier function, increased transepidermal water loss, and activation of the inflammatory response [3]. Clinical studies have shown that a hypoxic skin environment can induce abnormal expression of hypoxia-inducible factor-1α (HIF-1α), thereby inhibiting keratinocyte proliferation, reducing collagen synthesis, and promoting the release of pro-inflammatory cytokines [4]. However, there is a significant gap in the development of skincare products targeting skin hypoxia, especially in functional ingredients that can directly improve cellular oxygenation.

The fruit oil of *Hippophae rhamnoides*, a natural extract rich in unsaturated fatty acids (such as palmitoleic acid), carotenoids, and polyphenols, has been proven to possess biological activities such as antioxidant, anti-inflammatory, and tissue repair-promoting properties [5]. Notably, its unique fatty acid composition, characterized by high levels of ω-7 fatty acids, may have a positive impact on cellular functions in hypoxic microenvironments by regulating cell membrane fluidity, facilitating oxygen transmembrane transport, or interfering with hypoxic signaling pathways [6]. Although preliminary reports on the application of *Hippophae rhamnoides* fruit oil in skincare exist, research on its mechanism of action on hypoxic skin, particularly in regulating cellular oxygenation levels and improving related physiological functions, remains unexplored.

This study aims to investigate the regulatory effect of *Hippophae rhamnoides* fruit oil on the oxygen content of hypoxic epidermal cells and its underlying mechanisms, and to verify its improvement effect on compression-induced hypoxic skin through human efficacy tests. In vitro experiments involve culturing human keratinocytes (HaCaT) under hypoxic conditions to observe the impact of the fruit oil on cellular oxygen concentration and HIF-1α expression. In vivo experiments utilize a human skin hypoxia model induced by corrector compression to evaluate the improvement effect of the oil on transcutaneous oxygen pressure ($TcPO_2$), skin moisture content, barrier function and erythema. The findings of this study are expected to provide a novel natural active ingredient and target for the development of anti-hypoxic skincare products suitable for scenarios such as prolonged sitting and bed rest.

2. Materials and Methods

2.1 Cell culture and treatment

HaCaT keratinocytes purchased from the American Type Culture Collection (Manassas, VA, USA) were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1% antibiotics at 37°C and 5% CO_2. All materials needed for cell culture were obtained from Gibco (New York, USA). To simulate hypoxia condition, the cells were cultured at 10% O_2. Palmitic acid, Oleic acid and Palmitoeic acid (Sigma-Aldrich Chemical Co., St.Louis, MO, USA) were dissolved in dimethyl sulfoxide (Thermo Fisher Scientific, Waltham, MA, USA) to prepare stock solutions of 200 mM each. The stock solutions were diluted in the medium before utilzation. *Hippophae rhamnoides* fruit oil (Powdery Inc., Hubei, China) was dissolved in dimethyl sulfoxide to prepare stock slolution of 10% (v/v) and diluted in the medium before utilaztion.

2.2 Scratch wounding assay of HaCaT keratinocytes

Six-well plates were used for scratch wounding assay. The HaCaT keratinoctyes were plated at 6-well plates with the density of 1×10^7 / well and cultured overnight to allow cells to adhere to the wall. Asterile p10 pipette tip was used to create scratch wounds were created in confluent monolayers. The scratch strength should be moderate. Wash the suspended cells for three times, remove cell debris and impurities at the edges of scratches, and then put the wounded monolayers in a Nikon ECLIPSE Ts2 imaging system to acquire the scratch images. The NIH ImageJ image processing program was used to confirm the scale of the denuded area at each time point from the digital images, compared with the original wound.

2.3 Intracellular oxygen concentration assay

Intracellular oxygen concentration was determined using the Intracellular Oxygen Concentration Assay Kit (Biosen). The kit contains a red fluorescent probe BBoxiProbe®096 with an excitation wavelength of 456 nm and an emission wavelength near 612 nm. After treatment with a special conjugated complex, the probe can freely pass through the membrane of a living cell into the cell, and the red fluorescence increases as the oxygen concentration decreases. Based on the intensity of red fluorescence in living cells, changes in cellular oxygen concentration can be determined. Cells were seeded with the density of 1×10^4 / well in the 96-well plates. After diluting the probe according to 1:250, the cells were incubated with the probe in incubator for 60 minutes, washed 3 times with PBS (1×) phosphate buffer solution, and then the fluorescence was detected by flow cytometry (CytoFLEX, Beckman). The cells with low oxygen concentration were stained red and detected in PerCP fluorescent.

2.4 Cells metabolites extraction and for GC-MS untargeted metalomics analysis

Cells sample added with 400 µL methanol (HPLC grade) were centrifuged at 12 000 rpm for 10 min at 4°C. The supernatants were evaporated to dryness by concentrator under the nitrogen stream at 40°C. 50 µL O-methylhydroxylamine (MOX, Sigma-Aldrich) (15 mg/mL in pyridine solution) was added to the dried sample and vortexed for 5 min. samples were incubated at 40°C oven for about 1 h to oximation. Silylation reaction was performed by adding 40 µL N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA, Sigma-Aldrich) per sample and vortexed for 5 min, then incubated in the oven for 1h at 40°C. Samples were centrifuged at 12 000 rpm for 10 min at 4°C. 50 µL supernatant were transfer to GC glass vial equipped with a 200 µL insert for GC-MS analysis.

2.5 RNA Extraction and Real-Time Quantitative PCR

Cells RNAs were isolated with a QIAGEN RNeasy kit (QIAGEN). After reverse transcription into cDNA with a Reverse Transcription Kit (Bio-Rad), qPCR was then performed on QuantStudio 6 RT-PCR detection system (Applied Biosystems) with SYBR Green Supermix (Roche Diagnostics) and gene-specifific primers. We normalized gene expression amount to ACTB housekeeping gene and represented data as fold differences by the $2^{-\Delta\Delta Ct}$ method, where $\Delta Ct = Ct_{\text{gene of interest}} - Ct_{ACTB}$ and $\Delta\Delta Ct = \Delta Ct_{treated} - \Delta Ct_{control}$. The primer sequences of ACTB and HIF1A are: ACTB AAGGTGACAGCAGTCGGTT, TGTGTGGACTTGGGAGAGG; HIF1A AGAAACCACCTATGACCTGCT, CGACTGAGGAAAGTCTTGCTA

2.6 Cell immunofluorescence

Cells for immunofluorescence were fixed with 4% paraformaldehyde for 20 min at room temperature and permeabilized with 1% Triton X-100 in PBS on ice for 10 min. Samples were

blocked in PBS with 2% BSA for 1h at room temperature and incubated with antibodies specific for HIF-1α (1:50, 20960-1-AP, PTG) overnight at 4°C. Alexa Fluor conjugated secondary antibodies was incubated for 1h at room temperature. Hoechst 33342 was then used for counterstaining the nuclei, and images were obtained by Olympus BX53 microscopy.

2.7 Human efficacy test

Use wrist joint corrector to simulate skin pressure and hypoxia in the wrist skin area of 30 subjects. The subjects wore corrector on both hands simultaneously. The left hand served as the treatment group and applied *Hippophae rhamnoides* fruit oil after wearing, while the right hand served as the control group and did not apply oil. After wearing the corrector for 1 hour, each subject immediately applied *Hippophae rhamnoides* fruit oil to the compressed area and applied it once a day for 2 weeks. The transcutaneous oxygen pressure tester (TCM4), skin moisture content tester (Corneometer CM825), transcutaneous moisture loss tester (Tewameter TM300), and spectral imaging blood flow analysis tester (TiVi 700) were used to measure the transcutaneous oxygen pressure ($TcPO_2$), skin moisture content, transepidermal water loss (TEWL) rate, and skin hemoglobin content on the wrist skin of the subjects before wearing the corrector, after wearing the corrector, 30 minutes, 2 hours after the first application of *Hippophae rhamnoides* fruit oil, respectively.

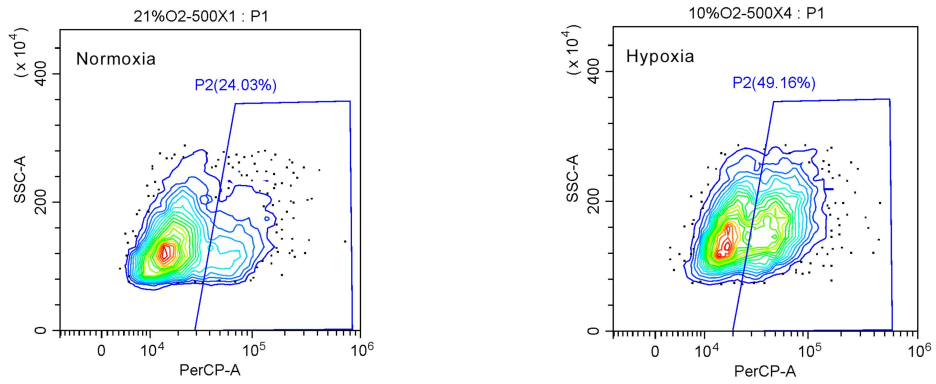
2.8 Statistical analysis

Statistical analyses were performed using SPSS v.13.0 (SPSS) or Prism GraphPad 7.0. For most of the in vitro and human experiments, independent sample t-tests were used to calculate the *P* values.

3. Results

3.1 Destabilized HIF-1α expression in HaCaT keratinocytes under hypoxia leaded to reduced migration and intracellular oxygen

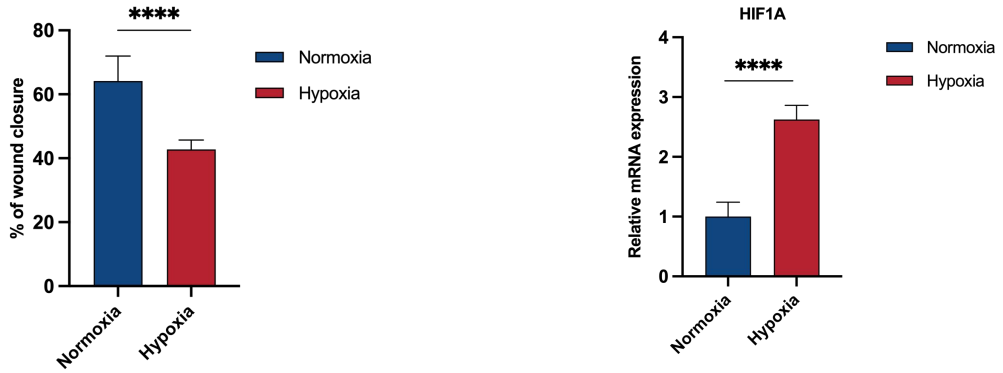
Result showed that intracellular oxygen decreased with 24h duration of hypoxia. Under normoxic conditions, the proportion of hypoxic cells is 24.03%. Under hypoxic conditions of 10% oxygen, the proportion of hypoxic cells increased to 49.16% (Figure 1).



(a) (b)

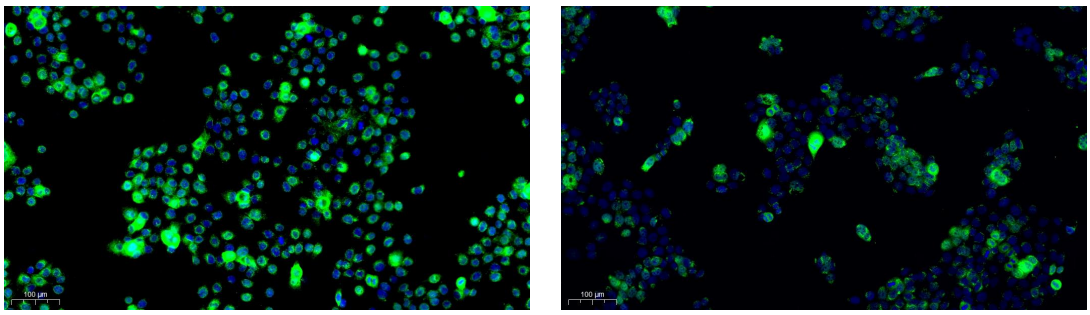
Figure 1. Intracellular oxygen flow cytometry detection of HaCaT cells cultured under (a) normoxic and (b) hypoxic conditions for 24 hours. Percentage represents the proportion of hypoxic cells.

The results of cell scratch test showed that compared with normoxic cells, the wound healing rate of hypoxic cells was significantly reduced. The wound closure rate of normoxic cells is about 62.09%, while the wound healing rate of hypoxic cells is about 41.82%. Real time quantitative PCR experiments showed that under 24-hour hypoxia conditions, the mRNA expression of HIF1A increased (Figure 2), but cell immunofluorescence showed that the protein expression of HIF-1α decreased compared to 0 hours (Figure 3).



(a) (b)

Figure 2. Migration ability and HIF1A mRNA expression of HaCaT cells cultured under low oxygen conditions for 24 hours: (a) The migration ability of HaCaT cells decreases during hypoxia; (b) Increased expression of HIF1A mRNA in HaCaT cells during hypoxia.



(a) (b)

Figure 3. Immunofluorescence of HIF-1α protein in HaCaT cells after 24 hours of low oxygen culture: (a) Immunofluorescence of HIF-1α protein in HaCaT cells at baseline (0 hour); (b) Immunofluorescence of HIF-1α protein in HaCaT cells after 24 hours of hypoxia.

3.2 Untargeted metabolomics revealed decreased fatty acids in hypoxic HaCaT keratinocytes

Cells metabolites changes under hypoxia may impact the stability of HIF-1α. Untargeted metabolomics were used to analyze the metabolites and metabolic pathway changes that occur in cells under low oxygen conditions. The results showed significant changes in lipid metabolism in hypoxic cells, particularly in pathways related to fatty acid synthesis and oxidation, which were abnormal compared to normoxic conditions. The most significant decrease was observed in the content of palmitic acid, palmitoleic acid, and oleic acid （Figure 4).

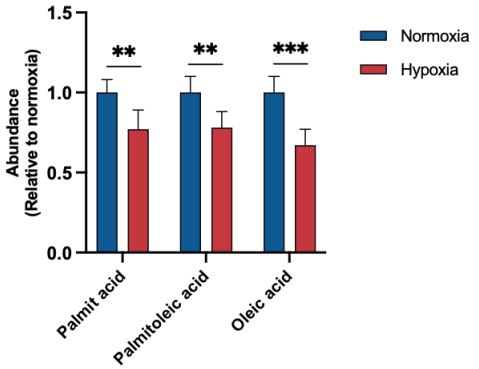


Figure 4. palmitic acid, palmitoleic acid and oleic acid decreased in HaCat cells after 24 hours hypoxia

3.3 Palmitoleic acid promoted hypoxic HaCaT keratinocytes migration

In order to observe the effects of palmitic acid, palmitoleic acid, and oleic acid on cell migration ability under low oxygen conditions, these three fatty acids were co cultured with cells under low oxygen conditions and subjected to cell scratch tests. The concentrations of these three fatty acids in the cell culture medium were set as 100 uM. The results indicate that all three types of fatty acids can promote the migration of hypoxic cells. Palmitoleic acid can significantly promote wound healing rates of hypoxic cells from 45% to 60% (Figure 5). Next are palmitic acid and oleic acid, but there is no significant difference in the effect of these two fatty acids on promoting wound healing rate.

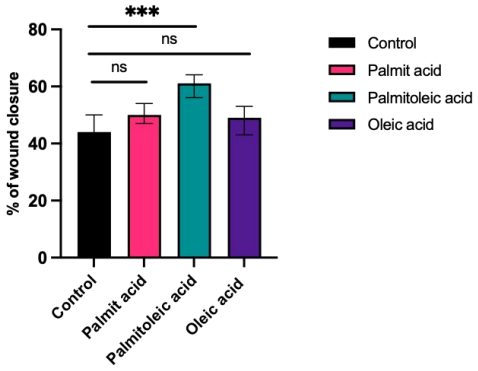
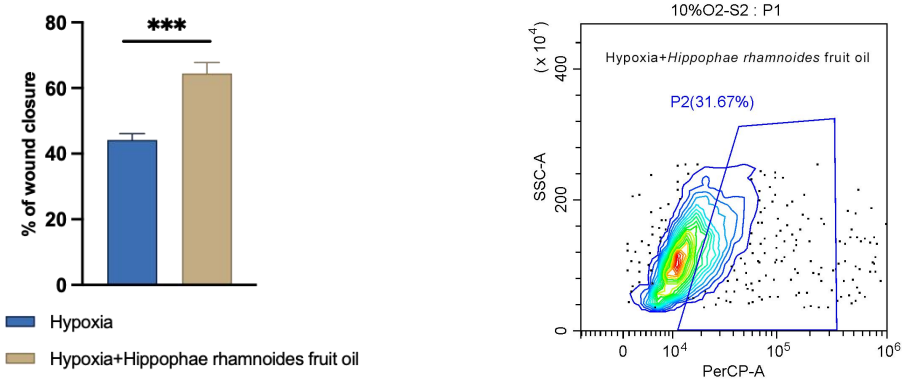


Figure 5. Palmitic acid, palmitoleic acid and oleic acid promoted HaCaT cells migration under hypoxia

3.4 Hippophae rhamnoides fruit oil increased HIF-1α expression, migration and intracellular oxygen of hypoxic HaCaT keratinocytes

Due to the rich content of palmitoleic acid in Hippophae rhamnoides fruit oil, it was selected for subsequent experiments and research. In the Hippophae rhamnoides fruit oil used in the experiment, the content of palmitoleic acid was determined to be approximately 28.4% of all fatty acids in the oil according to the peak area normalization method. Dilute Hippophae rhamnoides fruit oil to 0.1% in cell culture medium and co culture with hypoxic cells. The cell scratch test showed that Hippophae rhamnoides fruit oil promoted the migration ability of hypoxic cells from 44.18% to 64.41%, and the proportion of hypoxic cells decreased from 49.16% to 31.67% (Figure 6). The mRNA expression of HIF1A significantly increased, and cell immunofluorescence assay also showed a significant increase in protein expression of HIF-1α (Figure 7).



(a) (b)

Figure 6. Hippophae rhamnoides fruit oil (a) promoted HaCaT cells migration and (b) Intracellular oxygen under hypoxia

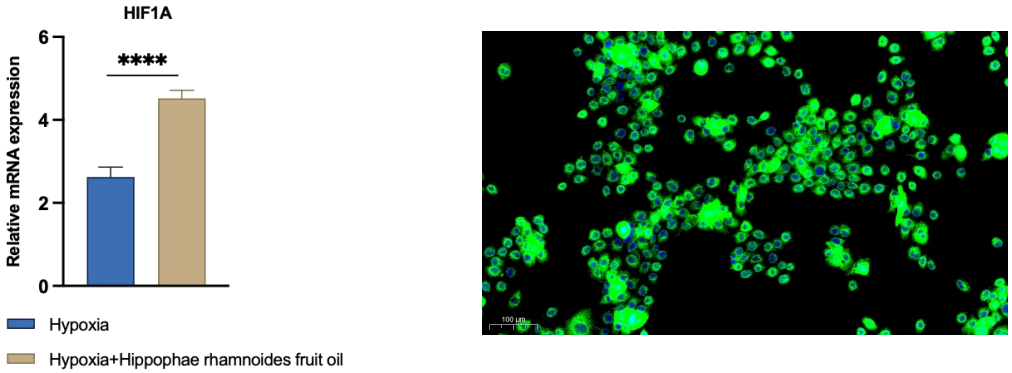


Figure 7. Hippophae rhamnoides fruit oil (a) increased HIF1A mRNA expression and (b) HIF-1α protein expression under hypoxia

3.5 Hippophae rhamnoides fruit oil improved human hypoxic skin conditions

The results of human efficacy test showed that after 1 hour of corrector compression, the transcutaneous oxygen partial pressure ($TcPO_2$) in the wrist decreased by 20%. After applying *Hippophae rhamnoides* fruit oil for 30 minutes, the $TcPO_2$ in the wrist increased by about 19% and returned to normal levels. The subjects who did not apply *Hippophae rhamnoides* fruit oil only showed a 6% increase in $TcPO_2$ in the wrist. After 2 hours of compression, the subjects who applied *Hippophae rhamnoides* fruit oil maintained normal levels of $TcPO_2$, while those who did not apply *Hippophae rhamnoides* fruit oil only increased their $TcPO_2$ by 10%, still significantly lower than normal levels. The skin moisture content of the subjects who applied oil increased by 16% and 44% at 30 minutes and 2 hours after application, respectively. The transepidermal water loss (TEWL) rate decreased by 31% and 37%, respectively, and the skin hemoglobin content decreased by 18% and 27%, respectively. Skin moisture content, TEWL and skin hemoglobin content continue to improve after 2 weeks of use the oil. These results indicate that Hippophae rhamnoides fruit oil has the effect of increasing oxygen content in hypoxic skin and has the effects of moisturizing, repairing, and soothing hypoxic skin.

Table 1. The change rate (%) of $TcPO_2$, Skin moisture content, TEWL and skin hemoglobin content in different time points compared with baseline

Indicator	30 min	2 hour	2 weeks
$TcPO_2$	19%(6%)※	20%(10%)※	20%(19%)※
Skin moisture content	16%	44%	42%
TEWL	-31%	-37%	-41%
Skin hemoglobin content	-18%	-27%	-25%

※Note: The values in parentheses represent the data of the control group

4. Discussion

This study is the first to demonstrate that *Hippophae rhamnoides* fruit oil can significantly increase the oxygen content in hypoxic epidermal cells and exhibit multiple beneficial effects in improving skin oxygenation, barrier function, and erythema status in a human model of compression-induced skin hypoxia, providing crucial evidence for its application in anti-hypoxic skin care.

From a mechanistic perspective, the finding that *Hippophae rhamnoides* fruit oil enhances the oxygen content of hypoxic epidermal cells by activating the HIF-1α pathway is of great significance. Although HIF-1α is generally regarded as a “survival switch” for cells under hypoxic conditions [7], and its excessive activation can exacerbate inflammation and cell damage, the results of this study suggest that *Hippophae rhamnoides* fruit oil may precisely regulate the expression level of HIF-1α, promoting the cells towards an adaptive response. This may be related to the ability of palmitoleic acid, which is abundant in *Hippophae rhamnoides* fruit oil, to regulate cell membrane fluidity and enhance the efficiency of oxygen transmembrane transport.

The results of the human efficacy test further validate the potential clinical application of *Hippophae rhamnoides* fruit oil. Thirty minutes after the application of the fruit oil, the transcutaneous oxygen pressure in the corrector-compressed skin area increased significantly by 19% and returned to the normal level. This rapid response indicates that the oil can quickly improve skin microcirculation. This may be attributed to the regulation of vascular endothelial cell function by unsaturated fatty acids in *Hippophae rhamnoides* fruit oil, which promotes the release of nitric oxide (NO), thereby dilating blood vessels and increasing blood perfusion. Meanwhile, the skin moisture content increased, and transepidermal water loss decreased, indicating that *Hippophae rhamnoides* fruit oil effectively repaired the skin barrier damaged by hypoxia. This is consistent with the mechanism reported in the literature, where fatty acids participate in the reconstruction of the lipid bilayer of the stratum corneum and enhance intercellular adhesion [8]. In addition, the decrease in skin hemoglobin content and the alleviation of erythema suggest that *Hippophae rhamnoides* fruit oil has anti-inflammatory and anti-angiogenic effects, which may be related to its antioxidant properties and the inhibition of the release of inflammatory cytokines (such as TNF-α and IL-6) [9].

Compared with existing research, the innovation of this study lies in clarifying the unique role of *Hippophae rhamnoides* fruit oil in the hypoxic skin environment. Previous studies have mostly focused on the application of *Hippophae rhamnoides* fruit oil in wound healing, repair of ultraviolet damage, and other aspects [10]. This study is the first to reveal its direct effect on improving skin oxygenation. However, the sample size of the human trial was relatively small,

and no long-term follow-up was conducted, making it difficult to evaluate the sustained efficacy and safety of *Hippophae rhamnoides* fruit oil. Furthermore, the specific contributions of each active component (such as fatty acids, polyphenols, and carotenoids) in *Hippophae rhamnoides* fruit oil and their synergistic mechanisms remain unclear, and further investigation through component separation and targeted intervention experiments is required.

5. Conclusion

In conclusion, *Hippophae rhamnoides* fruit oil demonstrates significant protective effects against hypoxic skin damage by regulating cellular oxygenation, repairing the skin barrier, and reducing inflammation, providing a natural and safe choice of active ingredients for the development of new functional skin care products.

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