1. Introduction

In the dynamic landscape of skincare, antioxidation stands as a fundamental pillar for maintaining skin vitality and combating aging [1,2]. The skin, our body's largest organ, is continuously exposed to environmental aggressors—ultraviolet (UV) radiation, air pollution, cigarette smoke, and even internal metabolic processes—all of which trigger the production of reactive oxygen species (ROS), such as free radicals. These highly unstable molecules initiate a chain reaction known as oxidative stress, which disrupts cellular equilibrium and inflicts cumulative damage on skin structures [3,4].

Oxidative stress accelerates skin aging through multiple mechanisms. It damages collagen and elastin fibers, the proteins responsible for skin firmness and elasticity, leading to the formation of wrinkles and sagging. Free radicals also compromise the lipid bilayer of cell membranes, weakening the skin barrier and causing moisture loss, dryness, and increased sensitivity. Additionally, they can induce DNA mutations and promote inflammation, contributing to pigmentation disorders, dull complexion, and a compromised ability to repair itself. Over time, these effects diminish the skin's resilience, making antioxidation not just a cosmetic concern but a critical aspect of skin health maintenance [2,3].

Among the vast array of antioxidants, 2R,3R-dihydroquercetin (DHQ) emerges as a standout due to its exceptional potency, unique natural origin, and multifunctional benefits [5]. DHQ is generally originated from Larch and considered as one of the best and most scarce powerful antioxidants [6]. DHQ stands out due to its molecular makeup. It contains five phenolic hydroxyl groups in its molecular structure. This specific arrangement endows DHQ with remarkable antioxidant capabilities, positioning it as one of the most potent natural antioxidants globally [7]. The presence of these hydroxyl groups allows DHQ to efficiently neutralize free radicals, making it a highly sought-after compound in various industries, from health supplements to skincare products.

DHQ demonstrates strong antioxidant activity through several mechanisms [8-10]. Firstly, it can directly scavenge free radicals. Its molecular structure allows it to donate a hydrogen atom to free radicals, thereby stabilizing them and preventing them from reacting with other important biomolecules like DNA, proteins, and lipids. This action helps to protect cells from oxidative damage and maintain their normal function. Secondly, DHQ can modulate the activity of antioxidant enzymes in the body. It upregulates the expression of certain enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). These enzymes are part of the body's natural defense system against oxidative stress. By enhancing the activity of these enzymes, DHQ further boosts the body's ability to neutralize free radicals and maintain a healthy balance. In addition to its direct and enzyme-modulating antioxidant mechanisms, DHQ also exhibits antioxidant activity by chelating metal ions. Transition metal ions, such as iron and copper, can catalyze the formation of highly reactive free radicals through the Fenton reaction. DHQ's molecular structure enables it to bind to these metal ions, preventing them from participating in free radical generating reactions. This chelating property further reduces the levels of harmful free radicals in the body, adding another layer to its antioxidant prowess.

In this study, the antioxidant activity of DHQ was measured and compared with the commonly used antioxidants Vitamin C (VC), Vitamin E (VE), and ergothioneine (EGT). Then, this study placed a particular emphasis on the long-term antioxidant capacity of DHQ under in the

context of environmental stressors like UV exposure and high-temperature incubation. Finally, it's demonstrated that DHQ has the unique ability to remove radicals in a cyclic manner.

2. Materials and Methods

2.1 DPPH radical scavenging rate assay of DHQ, VC, VE and EGT

To assess the DPPH radical scavenging capabilities of DHQ, VC, VE and EGT, the following experimental protocol was implemented. First, 20 μL of sample solutions, each containing 300 μM of DHQ, VC, VE, or EGT respectively, were separately combined with 180 μL of DPPH solution. These mixtures were then left to incubate at room temperature for a duration of 30 minutes. Post-incubation, a spectrophotometer was utilized to measure the absorbance of each mixture at a wavelength of 517 nm, and the values were denoted as T1 for each corresponding sample.

For reference purposes, for each of the antioxidants, the DPPH solution was substituted with ethanol. Then, 20 μL of the sample solution of the relevant antioxidant (DHQ, VC, VE, or EGT) was added to the ethanol. The same incubation and measurement steps were carried out, and the absorbance values were recorded as T0 for each case.

As a control experiment, 20 μL of water was blended with 180 μL of DPPH solution and incubated at room temperature for 30 minutes. The absorbance at 517 nm was measured and noted as C1. For the control reference, the DPPH solution was replaced with ethanol, and 20 μL of water was added. The identical procedure was executed, and the absorbance was recorded as C0.

The DPPH radical scavenging rate for DHQ, VC, VE, and EGT was computed using the formula: [(C1-C0)-(T1-T0)]/(C1-C0)*100%. This formula enabled a straightforward comparison of the antioxidant capacities of DHQ, VC, VE, and EGT based on their proficiency in scavenging DPPH radicals.

2.2 ABTS radical scavenging rate assay of DHQ, VC, VE and EGT

The ABTS radical scavenging rate assay was carried out in accordance with the protocol of the Total Antioxidant Capacity Detection Kit. In detail, 10 μL of sample solutions were each combined with 200 μL of the ABTS working solution. These mixtures were then incubated at room temperature for 6 minutes. After the incubation period, the absorbance of each mixture was measured at a wavelength of 734 nm using a spectrophotometer and was recorded as T1.

For the reference samples, the ABTS working solution was substituted with PBS. Then, 10 μL of sample solutions were mixed with this PBS. The identical steps of incubation and absorbance measurement were followed, and the resulting absorbance was noted as T0.

In the control setup, 10 μL of water was blended with 200 μL of the ABTS working solution and incubated at room temperature for 6 minutes. The absorbance at the same wavelength of 734 nm was measured and recorded as C1. For the control reference, the ABTS working solution was replaced with PBS, and 10 μL of water was added. The same process was carried out, and the absorbance was registered as C0.

The ABTS radical scavenging rate was computed using the formula: [(C1-C0)-(T1-T0)]/(C1-C0)*100%.

2.3 Hydroxyl radical scavenging rate assay of DHQ, VC, VE and EGT

The hydroxyl radical scavenging rate assay was conducted using the Hydroxyl Radical Assay Kit. 0.2 mL of sample solutions were each combined with 0.2 mL of the substrate application solution and 0.4 mL of reagent III application solution. Subsequently, the resulting mixtures

were incubated at a temperature of 37°C for 1 minute. After that, 2 mL of the chromogenic agent was added to halt the reaction. The absorbance of each mixture was then measured at a wavelength of 550 nm and was noted down as T1.
For the blank control group, water was used in place of the samples. The absorbance of this blank control was measured under the same conditions and was recorded as C1. The hydroxyl radical scavenging rate was calculated by applying the formula: (C1-T1)/C1*100%.

2.4 Superoxide anion radical scavenging rate assay of DHQ, VC, VE and EGT

The superoxide anion radical scavenging rate assay was carried out following the protocol of the Superoxide Anion Radical Assay Kit. Initially, 0.05 mL of sample solutions were separately combined with 1 mL of reagent I, 0.1 mL of reagent II, 0.1 mL of reagent III, and 0.1 mL of reagent IV. These mixtures were then placed in a 37°C water bath and incubated for 40 minutes. Subsequently, 2 mL of the chromogenic agent was added, and the resulting solutions were allowed to reach room temperature. After 10 minutes at room temperature, the absorbance of each mixture was measured at a wavelength of 550 nm and was recorded as T1.
For the blank control, water was used instead of the sample solution. The absorbance of this blank control was measured under identical conditions and was noted as C1. The superoxide anion radical scavenging rate was calculated using the formula: (C1 - T1)/C1*100%.

2.5 PTIO radical scavenging rate assay of DHQ, VC, VE and EGT

The experiment for determining the PTIO radical scavenging rate was conducted as follows. First, 20 μL of sample solutions were each mixed with 180 μL of PTIO solution. These mixtures were then incubated at room temperature for 30 minutes. After the incubation period, a spectrophotometer was used to measure the absorbance of each mixture at a wavelength of 557 nm, and the values were recorded as T1.
For the reference samples, the PTIO solution was substituted with ethanol. Then, 20 μL of samples were added to the ethanol. The same incubation and absorbance-measurement procedures were carried out, and the resulting absorbance values were noted as T0.
In the control group, 20 μL of water was blended with 180 μL of PTIO solution and incubated at room temperature for 30 minutes. The absorbance at the same wavelength of 557 nm was measured and recorded as C1. For the control reference, the PTIO solution was replaced with ethanol, and 20 μL of water was added. The identical steps were performed, and the absorbance was recorded as C0.
The PTIO radical scavenging rate was calculated using the formula: [(C1 - C0)-(T1 - T0)]/(C1 - C0)*100%.

2.6 Assessing the long-term antioxidant capabilities of DHQ, VC, VE and EGT

In our daily lives, the skin is constantly bombarded by a plethora of harsh environmental factors, with UV stress and thermal attack being particularly prominent. Therefore, the stability of DHQ under these challenging conditions is crucial for its practical effectiveness in skincare. To assess the stability of DHQ, it was exposed to either a 60°C thermal environment or UV light. Subsequently, the ABTS radical scavenging rates of DHQ were measured at regular intervals. For comparison purposes, the stabilities of VC, VE EGT were measured concurrently.

2.7 Assessing the continuous anti-oxidation capabilities of DHQ, VC, VE and EGT

In our daily lives, free radicals are produced incessantly. On one hand, they are generated through internal cellular respiration processes within our bodies. On the other hand, external

factors such as environmental pollution and UV radiation also contribute to their formation. As a result, the presence of antioxidants that can provide sustained defense against these free radicals is of utmost importance. To comparatively assess the continuous antioxidant efficacy of DHQ, VC, VE and EGT, ABTS radicals were continuously injected into the systems containing DHQ, VC, VE and EGT and the scavenging rate were measured. In detail, besides adding ABTS to the reaction systems initially at 0 h, additional ABTS was introduced at 2-hour intervals, specifically at 2 h, 4 h, 6 h, and 8 h. Subsequently, the ABTS radical scavenging rates were measured and noted at 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, and 24 h.

3. Results

3.1 Anti-oxidation capacity comparison of DHQ with VC, VE and EGT

VC, VE and EGT are well-acknowledged antioxidants celebrated for their robust antioxidative properties. In the present study, radical scavenging rate assay was utilized to compare the antioxidative capacity of DHQ with that of VC, VE, and EGT. As depicted in Figure 1A, 30 μM of DHQ was able to scavenge 89.2% of DPPH radicals. This scavenging ability was 1.89-fold higher than that of VC, 6.66-fold higher than VE, and 2.38-fold higher than EGT. Beyond DPPH radicals, ABTS radicals, hydroxyl radicals, PTIO radicals, and superoxide anion radicals were also employed to assess the outstanding antioxidative property of DHQ and make comparisons with those of VC, VE, and EGT. Figure 1B-E shows that 30 μM of DHQ had scavenging rates of 81.3% for ABTS radicals, 81.5% for hydroxyl radicals, 91.5% for PTIO radicals, and 75.9% for superoxide anion radicals. The radical scavenging rates of DHQ were substantially higher than those of VC, VE, and EGT, with the fold-differences ranging from 1.86-fold to 6.38-fold. These findings clearly demonstrate the remarkable antioxidative capacity of DHQ. They underscore the potential of DHQ to surpass some of the frequently used antioxidants in scavenging various types of radicals, thereby providing enhanced protection against oxidative stress.

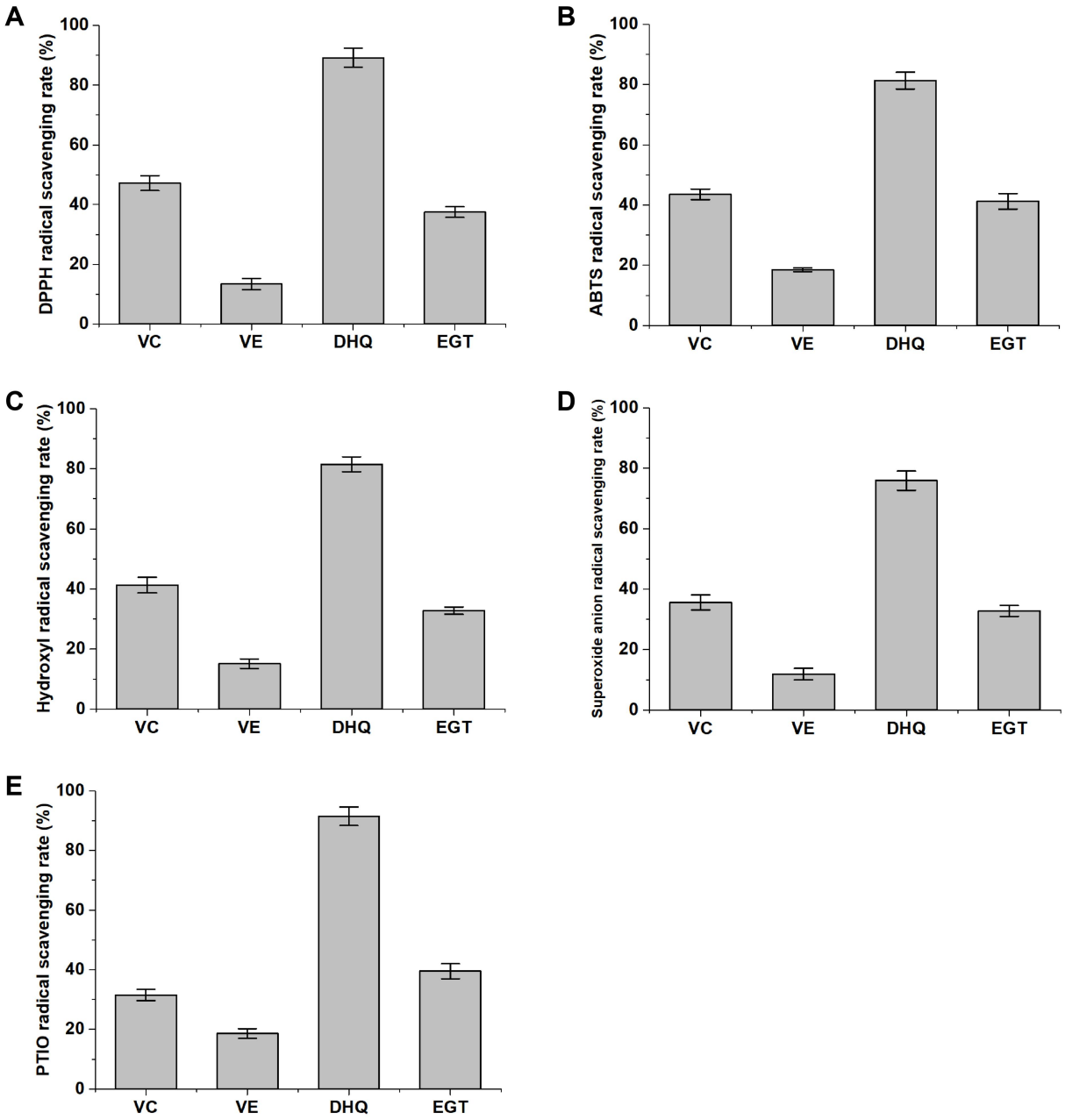

Figure 1. Antioxidation capacity evaluation of DHQ in comparison with VC, VE and EGT. DPPH (A), ABTS (B), hydroxyl (C), superoxide anion (D) and PTIO (E) radical scavenging rate of 30 μM of DHQ, VC, VE and EGT.

3.2 Long-term antioxidant capability evaluation of DHQ

Our skin endures daily challenges from diverse environmental elements, with UV exposure and high-temperature stress being particularly notable. Thus, the stability of DHQ under such conditions holds great importance for its real-world antioxidant capacity. To determine if DHQ could maintain its activity over an extended period under these circumstances, DHQ was subjected to UV light or incubated at 60°C, and its ABTS radical scavenging rate was measured. Figure 2A reveals that after 96-hour incubation at 60°C, the ABTS radical scavenging rate of 30 μM DHQ remained at 81.4%, comparable with the 0 h data. In sharp contrast, under the same conditions, the ABTS radical scavenging rate of 30 μM EGT plummeted to merely 16.5%. Even more remarkably, 30 μM VC completely lost its antioxidation activity after just 40 hours, and 30 μM VE followed suit after 48 hours.

As presented in Figure 2B, following 120 minutes of continuous UV exposure, the ABTS radical scavenging rate of DHQ still remained at a remarkably high level of 80.6%. Intriguingly, this value showed no discernible decline when compared to its activity at 0 hours, indicating a high degree of stability. Conversely, the situation for VC and EGT was quite different. The ABTS radical scavenging rate of VC plummeted to a mere 21.5%, and that of EGT dropped even lower, to just 13.4%. Additionally, VE completely lost its ability to scavenge ABTS radicals under the same UV exposure conditions.

These comprehensive results unambiguously demonstrate that DHQ exhibits exceptional resilience against both UV exposure and thermal stress. In comparison to VC, VE, and EGT, DHQ clearly outperforms them under these harsh environmental conditions. Its superior stability and sustained antioxidant activity make DHQ a more reliable candidate for applications where protection against environmental stressors is crucial, such as in high-quality skincare products designed to shield the skin from the damaging effects of daily UV exposure and temperature fluctuations.

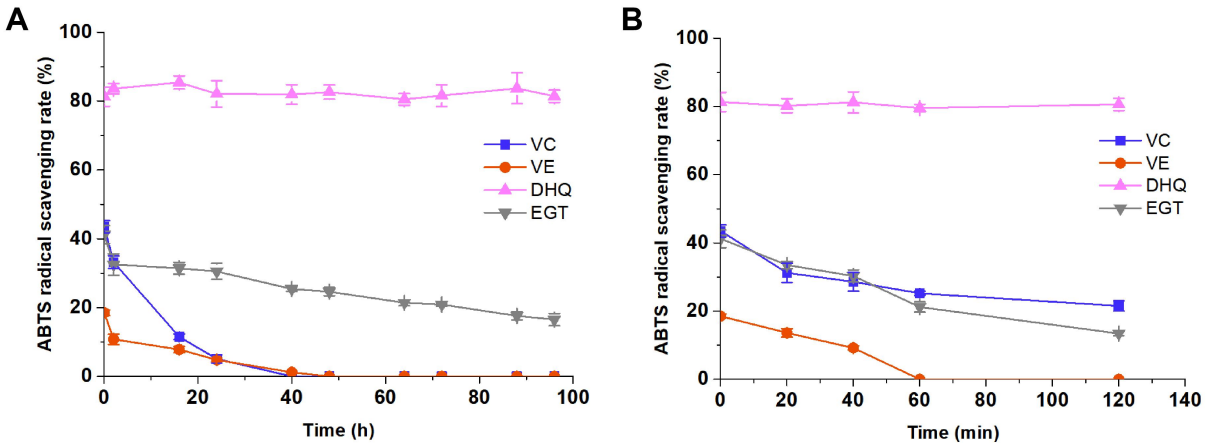

Figure 2. Stability evaluation of DHQ under 60°C incubation (A) or UV exposure (B)

3.3 Continuous anti-oxidation property evaluation of DHQ

Free radicals are generated relentlessly in our bodies. They originate from endogenous processes such as mitochondrial respiration and exogenous factors like UV radiation and air pollution. Since free radicals can initiate lipid peroxidation, protein denaturation, and DNA mutations within milliseconds, a continuous antioxidant effect is crucial to maintain a dynamic equilibrium against oxidative stress. Transient antioxidant interventions are insufficient as they cannot provide real - time neutralization of free radicals, which are constantly being produced, thus failing to halt the cascading cellular damage. The continuous anti-oxidation property of DHQ was evaluated and compared with that of VC, VE, and EGT. As depicted in Figure 3, in the initial 6-hour period, DHQ exhibited an ABTS radical scavenging rate above 60%. In contrast, the rates for VE and EGT dropped to 6.2% and 6.5% respectively, and VC completely lost its activity at 6 hours. By the end of the 24-hour assessment, while VC, VE, and EGT had almost entirely lost their free radical scavenging capacity, DHQ was still able to scavenge ABTS radicals. This significant difference in performance clearly showcases the unique and persistent nature of DHQ's continuous antioxidation capabilities, highlighting the importance of such sustained antioxidant effects in the face of the ceaseless generation of free radicals.

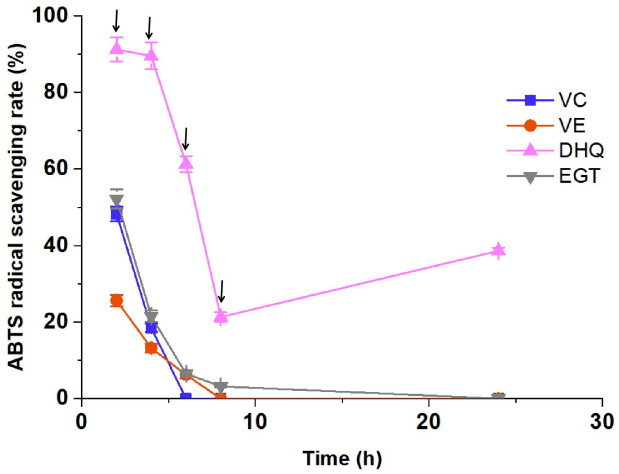

Figure 3. Continuous anti-oxidation property evaluation of DHQ, VC, VE and EGT. The arrow indicates the time of ABTS supplement.

4. Discussion

In this study, the antioxidative properties of DHQ were comprehensively evaluated and compared with well-known antioxidants VC, VE, and EGT. The findings highlight DHQ's superiority in multiple aspects.

DHQ demonstrated an outstanding ability to scavenge various types of radicals, including DPPH, ABTS, hydroxyl, PTIO, and superoxide anion radicals. With scavenging rates significantly higher than VC, VE, and EGT across these radical types, the fold-differences ranging from 1.86-fold to 6.66-fold, DHQ clearly shows a remarkable antioxidative capacity. This broad-spectrum radical-scavenging ability implies that DHQ can potentially neutralize free radicals generated from different sources within the body, whether from endogenous metabolic processes or exogenous environmental factors.

The stability of DHQ under UV exposure and high-temperature stress was also investigated. After 96-hour incubation at 60°C, DHQ maintained an ABTS radical scavenging rate of 81.4%, while VC lost its activity after 40 hours and VE after 48 hours, and EGT dropped to a mere 16.5%. Under 120 minutes of continuous UV exposure, DHQ's ABTS radical scavenging rate remained at 80.6%, with no discernible decline. In contrast, VC and EGT experienced drastic drops, and VE completely lost its scavenging ability. These results emphasize DHQ's exceptional resilience, making it more suitable for applications where exposure to environmental stressors is common, such as in skincare products protecting against daily UV and temperature changes.

Given the relentless generation of free radicals in the body from both endogenous and exogenous sources, a continuous antioxidant effect is essential. DHQ showed a unique and persistent continuous antioxidation property. In the initial 6-hour period, it had an ABTS radical scavenging rate above 60%, and by the end of the 24-hour assessment, it was still able to scavenge ABTS radicals, while VC, VE, and EGT had almost entirely lost their capacity. This indicates that DHQ can provide long-term protection against oxidative stress, which is crucial considering the rapid propagation of free radical induced damage like lipid peroxidation, protein denaturation, and DNA mutations.

Overall, DHQ shows great potential as an antioxidant. Its high antioxidant capacity, long-term protection property against environmental stress, and continuous antioxidation property make it a promising candidate for various applications, especially in areas where protection against oxidative stress is of utmost importance.

5. Conclusion

The data unequivocally prove that DHQ is a highly competitive antioxidant. Its exceptional antioxidant capabilities are further enhanced by its unique characteristics of long-term radical-removal and cyclic-action. These two features, are what truly set DHQ apart from other antioxidants. DHQ shows great promise in revolutionizing the skincare industry by offering a more effective and stable antioxidant solution.

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