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Study on Whitening, Anti-glycation, Anti-oxidation and Anti-photoaging(UV) properties of Boysenberry UV-G-OX CLEARER™ Compound Formula

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Abstract: *This study focused on the cosmetic properties of Boysenberry UV-G-OX CLEARER™, including its whitening, anti-glycation, anti-oxidation, and anti-photoaging effects. Using a zebrafish model, we comprehensively evaluated the performance of these functions at various concentrations of the formula. The experimental data clearly demonstrated that the Boysenberry UV-G-OX CLEARER™ compound formula yielded superior results in all test areas, thus providing a solid scientific foundation for practical application in the oral beauty and functional food industry.*

Keywords: *Boysenberry UV-G-OX CLEARER™; Whitening; Anti-glycation; Anti-oxidation, Anti-photoaging(UV)*

1. Study on Whitening efficacy of test item 1

1.1 Testing materials

The sample used in this study was the Boysenberry UV-G-OX CLEARER™ compound formula, which includes Boysenberry, White Tomato, White Onion, Pomegranate, Amla, Olive Fruit, and Red Grape Yeast. The solvent used was standard diluted water. The positive control was arbutin, a white powder with lot number C12020957, provided by Shanghai Maclin Biochemical Technology Co., LTD. It was stored refrigerated, and standard diluted water was used as the solvent. Zebrafish were selected as the experimental animals and were kept in fish culture water at 28℃. The water quality was maintained by adding 200 mg of instant sea salt to every 1 L of reverse osmosis water; the conductivity was 450-550 μS/cm, pH was 6.5-8.5, and hardness was 50-100 mg/L CaCO3. These zebrafish were provided by Hunter Biotechnology, Inc. The experimental animal use license number is SYXK (Zhejiang) 2022-0004, and the feeding and management comply with international AAALAC certification (certification number: 001458). The IACUC Ethics Review number is IACUC-2024-8764-01. The experimental instruments included an anatomical microscope (SZX7, OLYMPUS, Japan), a CCD camera (VertA1, Shanghai Toussen Vision Technology Co., LTD., China), a precision electronic balance (CP214, OHAUS, USA), and a 6-well plate (Zhejiang Berambe Biotechnology Co., LTD., China). The reagents used in the experiment included methyl cellulose (lot number C2004046, Shanghai Aladdin Biochemical Technology Co., LTD., China).

1.2 Detection Methods

Using zebrafish as a biological model, the whitening potential of Boysenberry UV-G-OX CLEARER™ was investigated. The skin structure and function of zebrafish are highly similar to those of humans, and the mechanism of pigment production, especially involving key genes such as tyr, mitf, sox10, and dct, maintains a high degree of consistency with humans [1]. In the experiment, wild-type AB zebrafish at 6 hours post-fertilization (hpf) were selected and given different concentrations of the sample solution and the positive control, arbutin (3000μg/mL), to observe the effect on melanin synthesis. In the experimental design, the transparent state of zebrafish at the early stage of development allows the process of melanin production to be intuitively tracked. On the second day after sample processing, 10 zebrafish were randomly selected from each group, photographed with a high-resolution anatomical microscope, and the melanin signal intensity of the zebrafish head was accurately measured using NIS-

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Elements D 3.20 advanced image analysis software. The weaker the signal strength, the better the whitening effect. All data were presented as mean ± standard error. SPSS 26.0 was used for statistical analysis, and a P-value less than 0.05 was considered significant [2].

1.3 Test Results

In this experiment, the effect of Boysenberry UV-G-OX CLEARER™ was as follows: At doses ranging from 500 to 2000μg/mL, the sample successfully reduced the melanin signal intensity in the zebrafish head, clearly demonstrating its excellent skin-brightening properties with a maximum improvement rate of 78%, significantly better than arbutin. Boysenberry UV-G-OX CLEARER™ at 2000μg/mL proves its usefulness in beauty products.

Table 1. Experimental results of whitening efficacy of the sample (n = 10)

*Compared with the normal control group, **p < 0.01, ***p < 0.001*

tranches	Concentration (μg/mL)	Head melanin signal intensity (pixel, mean ± SE)	Whitening efficacy (%)
normal control group	-	97789 ± 8115	-
Arbutin	3000	68745 ± 4204**	30
Boysenberry UV-G-OX CLEARER™ compound	500	59095 ± 3308	40
	1000	37218 ± 3800**	62
	2000	21348 ± 1387***	78

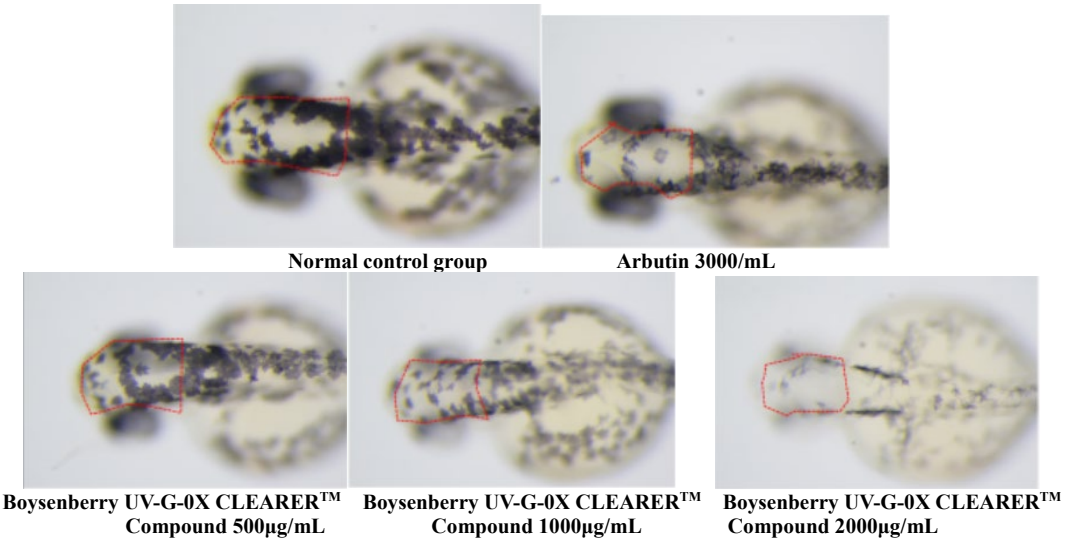

Figure 1. Typical figure of melanin signal intensity of zebrafish head after sample treatment

Note: The red dotted box is the analysis area

2. Study on Anti-glycation efficacy of item 2

2.1 Test materials

This study used Boysenberry UV-G-OX CLEARER™ compound formulation as a powder sample, provided by TRACEL HEALTH (NZ) LIMITED. The samples were stored in cold, dry, and light-free conditions. The experimental animals were selected and kept in fish culture water at 28℃ (the water quality was maintained by adding 200 mg of instant sea salt to every 1 L of reverse osmosis water; the conductivity was 450-550 μS/cm, pH was 6.5-8.5, and hardness was 50-100 mg/L CaCO3). These zebrafish were provided by Hunter Biotechnology, Inc. The experimental animal use license number is SYXK (Zhejiang) 2022-0004, and the feeding management meets the requirements of AAALAC certification.

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Experimental instruments and consumables included an anatomical microscope (SZX7, OLYMPUS, Japan), a CCD camera (VertA1, Shanghai Tousen Vision Technology Co., LTD., China), a precision electronic balance (CP214, OHAUS, USA), a 6-well plate (Zhejiang Berambe Biotechnology Co., LTD., China), a multi-function enzyme meter (SPARK, TECAN, Austria), a high-speed refrigerated centrifuge (Heraeus Fresco17, ThermoFisher, Germany), an automatic sample rapid grinding meter (JXFSTPRP-24L, Science and Technology Department of Shanghai Jinxin Experimental Equipment, China), a thermostatic oscillator (HZ-9211K, Taicang Hualida Experimental Equipment Co., LTD., Taicang, China), and a black 96-well plate (Nest Biotech, China).

Reagents used in the experiment included anhydrous glucose (lot number C15778026, Shanghai McLean Biochemical Technology Co., LTD., China) and POLA B.A anti-glycation skin pill (black pill, POLA, stored in a cool and dry place).

2.2 Detection method

Using zebrafish as an experimental model, the potential of substances to inhibit glycosylation can be assessed by observing changes in AGEs levels. In the experiment, 5 dpf wild-type AB zebrafish were selected, and 10 of each batch were placed in 1.5 mL centrifuge tubes. Test samples of different concentrations (including Boysenberry UV-G-OX CLEARER™ compound formula at various concentrations) were administered. A125μg/mL anti-glycation skin pill was used as the positive control, and normal and model control groups were set up. Except for the normal control group, all other experimental groups were given a 0.4 M glucose solution to establish the zebrafish AGEs increase model. The experiment was repeated three times for all groups. After incubation at 60℃ for one day, each group was ground and centrifuged. The supernatant was collected, and the fluorescence values of AGEs were determined using a multifunctional enzyme marker. Data were expressed as mean ± SE, and SPSS 26.0 software was used for statistical analysis. A P-value of less than 0.05 indicated a significant difference [3].

2.3 Test Results

In this experiment, within the concentration range of 500 to 2000μg/mL, the Boysenberry UV-G-OX CLEARER™ compound formulation effectively reduced the fluorescence level of AGEs in zebrafish, demonstrating a significant anti-glycation effect, with a maximum reduction of 34%.

3. Study on Antioxidant efficacy of item 3

3.1 Test material

The sample used in this study was the Boysenberry UV-G-OX CLEARER™ compound formula, with standard diluted water as the solvent. The positive control was N-acetyl-L-cysteine, a white powder with lot number I2016139, provided by Shanghai Aladdin Biochemical Technology Co., LTD. It was stored refrigerated, and ultra-pure water was used as the solvent. Zebrafish were fed in water at 28℃ (water quality: 200 mg of instant sea salt was added to every 1 L of reverse osmosis water; the conductivity was 450-550μS/cm; pH was 6.5-8.5; and hardness was 50-100 mg/L CaCO3). These zebrafish were provided by Hunter Biotechnology, Inc. The experimental animal use license number is SYXK (Zhejiang) 2022-0004, and the feeding and management meet international AAALAC certification standards (certification number: 001458). The IACUC Ethics Review number is IACUC-2024-8764-01.

The instruments used in the experiment included an anatomical microscope (SZX7, OLYMPUS, Japan), a CCD camera (VertA1, Shanghai Toussen Vision Technology Co., LTD., China), a precision electronic balance (CP214, OHAUS, USA), a 6-well plate (Zhejiang Berambe Biotechnology Co., LTD., China), and an electric focusing continuous zoom fluorescence microscope (AZ100, Nikon, Japan). The main reagents used in this experiment included methyl cellulose (lot No. C2004046, Shanghai Aladdin Biochemical Technology Co., LTD., China), Cell ROX Green Reagent (lot No. 2714479, Invitrogen, USA), menadione (lot No. I1817117, Shanghai Aladdin Biochemical Technology Co., LTD., China), and dimethyl sulfoxide (DMSO, lot number I2229063, Shanghai Aladdin Biochemical Technology Co., LTD., China).

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3.2 Detection Methods

This study used zebrafish as a biological model to investigate the antioxidant capacity of the Boysenberry UV-G-OX CLEARER™ compound formulation. The physiological response mechanism of zebrafish is highly consistent with that of mammals, making them an ideal platform for investigating oxidative stress. Zebrafish have a similar antioxidant protection system to humans, including various antioxidant enzymes (such as SOD, CAT, and GSH-Px) and molecules like reduced glutathione. In the experiment, Cell ROX® green dye was used to monitor the fluorescence intensity of the yolk sac of zebrafish to measure the antioxidant performance of the sample. The lower the fluorescence intensity, the lower the ROS level, indicating more significant antioxidant efficacy of the sample [4].

Albino zebrafish at 3 dpf were randomly selected and assigned to 6-well plates, with 30 fish placed in each well. Samples of different concentrations were administered, and normal and model control groups were set up. N-acetyl-L-cysteine (62.5μg/mL) was used as the positive control. In addition to the normal control group, the other experimental groups were given menadione to establish the zebrafish oxidative stress model. After treatment, ROS staining of zebrafish was performed using Cell ROX. After staining, the fluorescence intensity of the yolk sac was observed and recorded by fluorescence microscope. Data were presented as mean ± SE, and statistical analysis was performed using SPSS 26.0 software. A P-value of less than 0.05 was considered statistically significant [5].

3.3 Test Results

In this trial, within the concentration range of 15.6 to 62.5μg/mL, Boysenberry UV-G-OX CLEARER™ significantly inhibited the fluorescence intensity of zebrafish yolk sacs. This phenomenon indicates that the compound can significantly reduce the level of reactive oxygen species (ROS). Experimental data showed that the fluorescence intensity of the zebrafish yolk sac decreased by about 33% at 62.5μg/mL, indicating that Boysenberry UV-G-OX CLEARER™ has strong antioxidant capacity at this concentration. This superior antioxidant property derives from the active ingredients in the Boysenberry UV-G-OX CLEARER™ compound formula, which protect cells from oxidative damage by removing excess reactive oxygen species, thereby slowing the skin aging process. These results not only demonstrate the antioxidant effectiveness of Boysenberry UV-G-OX CLEARER™, but also provide a solid scientific basis for its use in functional food.

4 Study on Anti-photoaging(UV) efficacy of item 4

4.1 Test material

Boysenberry UV-G-OX CLEARER™ was formulated with standard diluted water as the solvent. The positive control was POLA B.A anti-glycation skin pill, a black pill provided by POLA and stored in a cool, dry place. The solvent used was standard diluted water. The experimental animals were zebrafish, all of which were fed in fish culture water at 28℃ (water quality: 200 mg of instant sea salt was added to every 1 L of reverse osmosis water; the conductivity was 450-550μS/cm; pH was 6.5-8.5; and hardness was 50-100 mg/L CaCO3). These zebrafish were provided by Hunter Biotechnology, Inc. The experimental animal use license number is SYXK (Zhejiang) 2022-0004. The feeding and management meet the requirements of international AAALAC certification (certification number: 001458), and the IACUC ethics review number is IACUC-2024-8764-01.

Instruments and consumables included an anatomical microscope (SZX7, OLYMPUS, Japan), a CCD camera (VertA1, Shanghai Tousen Vision Technology Co., LTD., China), a precision electronic balance (CP214, OHAUS, USA), 6-well plates (Zhejiang Berambe Biotechnology Co., LTD., China), a sunlight simulator (SOL500, Hoenle, Germany), an automatic sample rapid grinding instrument (JXFSTPRP-24L, Science and Technology Department of Shanghai Jingxin Experimental Equipment, China), and methyl cellulose (lot number C2004046, Shanghai Aladdin Biochemical Technology Co., LTD., China).

4.2 Detection Methods

This study used a zebrafish model to investigate the effectiveness of Boysenberry UV-G-OX CLEARER™ against photoaging. Given the similarity of zebrafish skin to human skin, and the fact that ultraviolet (UV) radiation is the primary external cause of skin photoaging [6], attention was paid to the damage to the epidermis, dermis, and subcutaneous tissue of zebrafish skin under UV exposure. This

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damage is manifested by roughness, contraction, and trauma of the tail fin. The effect of the sample was evaluated by measuring changes in the tail fin area of zebrafish, with an increase in tail fin area indicating better anti-photoaging effects [7].

Wild-type AB zebrafish at 3 days post-fertilization (dpf) were selected and given different concentrations of sample solutions. The positive control was a 125μg/mL anti-glycation skin pill, and the normal control and model control groups were maintained with 3 mL per well. In addition to the normal control group, all the experimental groups were exposed to simulated sunlight to create photoaging models of zebrafish. After 1 day of treatment at 28℃, 10 zebrafish were randomly selected for anatomical microscope photography, and the images were recorded. The caudal fin area was analyzed using NIS-Elements D 3.20 advanced image processing software. SPSS 26.0 software was used for statistical analysis. If the P-value was less than 0.05, the difference was considered statistically significant. All data are expressed as mean ± SE.

4.3 Test Results

In this experiment, within the concentration range of 62.5 to 250μg/mL, it was observed that the composite agent significantly increased the surface area of the zebrafish tail, revealing its effective anti-photoaging protection effect, with the maximum protection effect increasing by 8%.

5. Conclusion

This study conducted a comprehensive efficacy analysis of Boysenberry UV-G-OX CLEARER™ compound formulation, revealing its superior properties in whitening, anti-glycation, anti-oxidation, and anti-photoaging (UV). Using a zebrafish experimental model, the data show that the compound formula yields remarkable results in all functional tests: whitening can be increased by up to 78%, anti-glycation efficiency by up to 34%, antioxidant capacity by up to 33%, and anti-photoaging (UV) effect by up to 8%. These findings confirm the multifunctional potential of Boysenberry UV-G-OX CLEARER™ in the oral beauty and functional food industry, establishing a scientific foundation for its practical application in the beauty and skincare industry. Further analysis is needed to explore its mechanism of action, as well as its durability and safety in actual use, to provide consumers with a more comprehensive beauty and health strategy.

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