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Original Research

EVALUATION OF MELANIN INHIBITION EFFICIENCY OF C-PHYCOCYANIN EXTRACTED FROM SPIRULINA ON ZEBRAFISH (DANIO RERIO) MODEL

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ABSTRACT: Melanin overproduction can lead to hyperpigmentation issues such as freckles and melasma, impacting aesthetics and health. This study investigates the inhibitory effects of C-Phycocyanin (C-PC) extracted from Spirulina on melanin synthesis using a zebrafish (Danio rerio) model. C-PC demonstrated significant tyrosinase inhibition and reduced melanin content in zebrafish embryos in a dose-dependent manner. The IC50 value of C-PC for tyrosinase inhibition was determined to be 38.36 ± 19 µg/mL. At the highest tested concentration (10 µg/mL), C-PC reduced melanin content in zebrafish embryos by approximately 48.57%, compared to 71.08% inhibition observed with kojic acid. Importantly, C-PC exhibited low toxicity, with treated embryos maintaining normal morphology and viability. The findings suggest the potential application of C-PC as a natural depigmenting agent in cosmetic formulations.

Keywords: C-Phycocyanin, Spirulina, Melanin Inhibition, Tyrosinase, Zebrafish Model

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1. INTRODUCTION

Melanin is the primary pigment responsible for skin, hair, and eye coloration. Overproduction of melanin leads to hyperpigmentation, which can result from UV exposure, hormonal changes, or aging. Tyrosinase is a key enzyme in melanin biosynthesis, catalyzing the oxidation of L-tyrosine to L-DOPA and subsequent melanin formation [1,2].

C-Phycocyanin (C-PC) is a water-soluble phycobiliprotein extracted from Spirulina, known for its antioxidant, anti-inflammatory, and potential depigmenting properties [3,4]. Previous studies have shown C-PC's ability to inhibit tyrosinase activity in vitro [5]. This study aims to evaluate C-PC's melanin inhibition efficiency using an in vivo zebrafish model, which shares genetic similarities with humans and is a reliable system for pigmentation studies [6,7].

2. MATERIALS AND METHODS

2.1. Materials

C-Phycocyanin (C-PC): Extracted from Arthrospira platensis provided by Institute of Biotechnology. C-PC was extracted by the method described in previous studies [8,9]. Briefly, cell wall was broken by freezing (-20oC, 4 hours) – thawing (4oC, 20 hours) cycles (repeated 3 times), then C-PC was preticipated by 45% ammonium sulfate and dialyzed by dialysis membrane with 14000 daltons molecular weight cuttoff overnight at 4oC in Na bufffer. C-PC with PI of 2.1 was used in the next experiments (cosmetic standard).

Zebrafish (Danio rerio, AB strain): gifted from Biotechnology Center, HCMC, Vietnam.

2.2. Tyrosinase Inhibition Assay

The inhibitory effect of C-PC on tyrosinase was assessed using L-DOPA as the substrate. Reaction mixtures containing tyrosinase, L-DOPA, and various concentrations of C-PC were incubated (table 1), and absorbance was measured at 490 nm (OD490). Kojic acid served as the positive control [10,11].

Table 1. Experimental groups in tyrosinase inhibition assay

Groups	Reaction
Aa	PBS 1X + L-DOPA 300 µg/mL
Ab	PBS 1X + C-PC + L-DOPA 300 µg/mL
Ac	PBS 1X + Tyrosinase 100 IU + L-DOPA 300 µg/mL
Ad	Tyrosinase 100 IU + C-PC + L-DOPA300 µg/mL
Acid Kojic	Tyrosinase 100 IU + Acid kojic 200 µg/mL + L-DOPA 300 µg/mL

Formulation:

% tyrosinase inhibition = [1 – (OD490Ad - OD490Ab)/(OD490Ac - OD490Aa)]x100%

2.3. Zebrafish Melanin Inhibition Assay

The 24 hpf Zebrafish embryos were exposed to different C-PC concentrations (2.5, 5.0, 7.5, 10.0 µg/mL) for 48 hours (50 embryos/group). After 48 hours, the embryos was anesthetized by tricaine, grinded in cold sodium buffer (1% triton X100) to determine absorbance at 420 nm (OD420) and protein concentration (mg). An aliquot of lysate was dissolved in 1M NaOH at 80oC for 2 hours, and then measuring absorbance at 420 nm. The Bradford assay determined protein concentration to normalize melanin content [12].

Formulation:

melatin content = OD_{420}/protein (mg)

2.4. Statistical Analysis

Data were analyzed using ANOVA followed by Tukey's test. P-values < 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Tyrosinase Inhibition by C-PC

C-PC inhibited tyrosinase activity in a dose-dependent manner, with significant inhibition observed at concentrations above 50 µg/mL. The IC50 value was determined to be 72.91 µg/mL. Compared to kojic acid, C-PC exhibited notable but slightly lower inhibitory effects [11,13]. **(Table 1)**

Table 1. Tyrosinase Inhibition Rates at Various C-PC Concentrations

C-PC Concentration (μg/mL)	Tyrosinase Inhibition Rate (%)
20	35.4 ± 3.8
50	43.5 ± 6.4
80	65.56 ± 5.0
110	70.2 ± 3.2
140	74.0 ± 3.1
200	83.3 ± 3.1
Kojic Acid (Control)	93.46 ± 1.2

3.2. Melanin Inhibition in Zebrafish

Zebrafish treated with C-PC showed reduced melanin accumulation compared to controls. At 10 μg/mL, C-PC reduced melanin content by approximately 36.8%, compared to 46.7% inhibition by kojic acid. Importantly, C-PC-treated embryos displayed normal morphology and viability, indicating non-toxic effects [14,15]. (Table 2)

Table 2. Melanin Content in Zebrafish Embryos After C-PC Treatment

C-PC Concentration (μg/mL)	Melanin Content (OD420/ pro (mg))	Inhibition Rate (%)
Control (No treatment) (A)	0.483 ± 0.031	
2.5 (B)	0.413 ± 0.017	14.7
5.0 (C)	0.353 ± 0.028	26.9
7.5 (D)	0.313 ± 0.021	35.2
10.0 (E)	0.305 ± 0.025	36.8
Kojic Acid (F)	0.257 ± 0.015	46.7

3.3. Discussion

C-PC's melanin inhibition is attributed to its ability to scavenge free radicals and directly inhibit tyrosinase. Its natural origin and low toxicity profile highlight its potential as a safe alternative to synthetic depigmenting agents like kojic acid [3,16]. The zebrafish model confirmed C-PC's efficacy in vivo, supporting its future application in cosmetic products targeting hyperpigmentation.

Comparing these results with previous studies, Wu et al. demonstrated that C-PC effectively reduced melanin synthesis in melanoma cells by modulating tyrosinase expression [5]. While Wu's study focused on cell cultures, the current zebrafish model provides a more comprehensive in vivo analysis, reinforcing C-PC's depigmenting potential. Additionally, research by Cho et al. highlighted the role of natural antioxidants like feruloylserotonin in inhibiting melanogenesis, similar to the antioxidant properties observed in C-PC [17].

Furthermore, the IC50 value of C-PC in this study (38.36 ± 19 μg/mL) aligns with findings by Patil et al., who reported comparable inhibitory effects on tyrosinase activity [1]. However, C-PC's inhibition

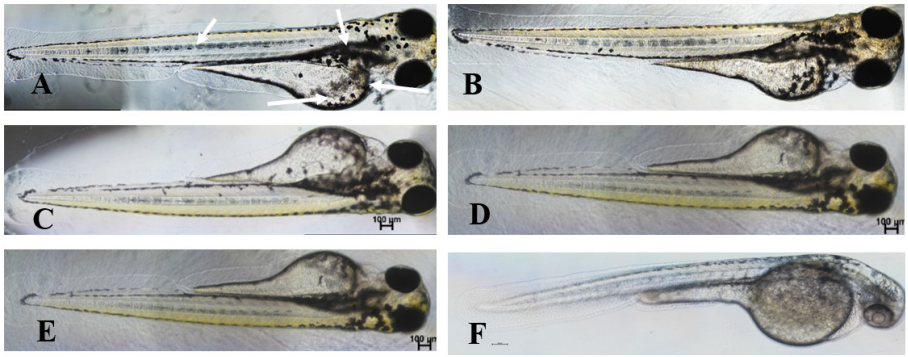

Figure 2. Effects of different concentrations C-PC on zebrafish (the capital letters allow table 2, white arrows indicate melanin location inside)

remains slightly lower than that of kojic acid, a well-established depigmenting agent, though C-PC offers the advantage of lower cytotoxicity [18,19].

The zebrafish model's relevance is further supported by studies like Ferreira et al., which emphasized the utility of zebrafish in pigmentation research due to their genetic similarity to humans [6,20]. The consistency between the current findings and previous literature underscores the robustness of C-PC as a promising natural depigmenting agent [19,22].

4. CONCLUSION

C-Phycocyanin extracted from Spirulina effectively inhibits tyrosinase activity and melanin synthesis in zebrafish models. Its significant depigmenting effects and safety profile underscore its potential as a natural cosmetic ingredient for hyperpigmentation treatment.

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