THE EFFECT OF RED SEAWEED EXTRACT ON QUALITY OF WHITELEG SHRIMP (*LITOPENAEUS VANNAMEI*) DURING ICED STORAGE

ẢNH HƯỞNG CỦA DỊCH CHIẾT RONG ĐỎ ĐẾN CHẤT LƯỢNG TÔM THẺ CHÂN TRẮNG (*LITOPENAEUS VANNAMEI*) BẢO QUẢN LẠNH

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ABSTRACT

Tyrosinase plays an important role in the formation of melanin. Thus, the process of melanin formation can be reduced through the inhibition of tyrosinase. The objective of this study is to investigate the tyrosinase inhibitory activity of different red seaweeds harvested in the coast of Khanh Hoa Province. All the tested extracts showed inhibitory effect on tyrosinase activity. The *Gelidiella acerosa* extract showed the most tyrosinase inhibitory activity with the IC_{50} value of 3.04mg/mL. The effects of extraction conditions on tyrosinase inhibitory activity of *G. acerosa* were investigated. Water was identified as the most effective solvent for the extraction. The suitable extraction conditions were found to be the solid to liquid ratio (g/mL) of 1/40, the extraction time of 60 min and the extraction temperature of 60°C. The melanosis formation was significantly inhibited in whiteleg shrimp (*Penaeus vannamei*) treated with the *G. Acerosa* extract during storage in ice, compared with the control. Therefore, the red seaweed *G. Acerosa* could be used as a potential melanosis inhibitor in shrimp during refrigerated storage.

Keywords: *Red seaweed, Gelidiella acerosa, tyrosinase inhibitor, melanosis, Litopenaeus vannamei*

TÓM TẮT

Tyrosinase đóng vai trò quan trọng trong quá trình tổng hợp melanin. Do đó, quá trình tạo thành melanin có thể được kiểm soát thông qua ức chế hoạt động của tyrosinase. Mục đích của nghiên cứu này là đánh giá hoạt tính ức chế tyrosinase của dịch chiết một số loài rong đỏ thu mẫu tại vùng biển Khánh Hòa. Kết quả nghiên cứu cho thấy, tất cả dịch chiết rong biển đều có hoạt tính ức chế tyrosinase. Dịch chiết rong *Gelidiella acerosa* có hoạt tính ức chế cao nhất, với giá trị IC_{50} là 3,04mg/mL. Ảnh hưởng của điều kiện chiết đến hoạt tính ức chế tyrosinase của rong *G. acerosa* được nghiên cứu. Điều kiện chiết thích hợp được xác định như sau: Dung môi chiết là nước, tỷ lệ nguyên liệu/dung môi chiết (g/mL) là 1/40, thời gian chiết là 60 phút, nhiệt độ chiết là 60°C. Hiện tượng tạo thành điểm đốm đen (melanosis) của tôm thẻ chân trắng (*Litopenaeus vannamei*) bảo quản lạnh xử lý bằng dịch chiết rong *G. acerosa*, được kiểm soát một cách đáng kể so với mẫu đối chứng. Như vậy, rong đỏ *G. acerosa* là nguyên liệu tiềm năng sử dụng để hạn chế sự tạo thành điểm đốm đen trên tôm trong quá trình bảo quản lạnh.

Từ khóa: *Rong đỏ, Gelidiella acerosa, chất ức chế tyrosinase, điểm đốm đen, Litopenaeus vannamei.*

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Received: 26/5/2021
Revised: 26/6/2021
Accepted: 30/6/2021

1. INTRODUCTION

Melanin are pigments produced in a well-known process called melanogenesis (melanin biosynthesis), in the melanosomes by melanocytes, which are distributed in the basal layer of the epidermis [1, 2]. The color expressed in skin, eye, and hair are the result of melanin, it serves an important protective function against ultraviolet (UV) radiation [3, 4]. More than 80% of the world's population constitutes people with white or yellow skin. Pheomelanin, which is contained in these kinds of skins is much less able to block UV energy and in fact may synergize with UV photons to promote free radical formation, carcinogenesis in the skin [5], thereby resulting in aging.

Tyrosinase, ubiquitously present in plants and animals, is the key enzyme in the synthesis of melanin that catalyzes hydroxylation of L-tyrosine into L-Dopa (monophenolase activity) and its oxidation to dopaquinone [6, 7]. In addition to the formation of melanin pigment, tyrosinase plays a key role in browning of fruits, vegetables and sea foods [8]**.** The overexpression of tyrosinase may lead to dysregulation in melanogenesis which can trigger hyperpigmentation effects such as melasma, freckles, age-related and chemical-spots etc. [9]. In addition to this, tyrosinase has also been implicated in playing a significant role in neurodegenerative disorders like Parkinson's disease [10, 11]. In food, the quinones (dark, red, brown pigments) formed by tyrosinase action cause

enzymatic browning in plant-based foods and seafood, leading to deterioration during storage and commercial or domestic processing [10, 12]. Melanogenesis and enzymatic browning can be controlled in several ways, including through the inhibition of tyrosinase gene expression and inactivation of the related enzymes [13]. Many synthetic compounds have been demonstrated to show inhibitory effect against tyrosinase enzyme and melanocyte on melanogenesis, such as kojic acid, mercury, hydroquinone, and arbutin. However, they have been associated with dangerous side effects in long-term use [7]. Further research is therefore required to identify natural tyrosinase inhibitors that can be used as whitening agents and antibrowning agents in the cosmetic and food industries respectively.

Marine seaweeds have been a great concern to researchers; their multiple fascinating properties have inspired a lot of studies on various species. Species from the genus Laurencia (Rhodomelaceae, Ceramiales) belonging to red algae, taxonomically classified as Rhodophyta, have been studied and established to possess important biological activities such as antibacterial [14], antifungal [15] anti-predatory [16], anticancer properties [17] and good source of natural antioxidant compounds, with defense systems potent enough to tolerate a wide range of stress-inducing factors [18, 19]. Red algae are primarily being considered in this study because of their tyrosinase inhibitory properties and their ability to retard oxidation that causes aging in humans and enzymatic browning that causes discoloration and aging in fruits and vegetables [20].

The tropical oceans of the world are enriched with the genus *Laurencia*, a significant producer of diterpenes, halogenated sesquiterpenes, and acetogenins, making it the world's most chemically complex seaweed genus [21]. Vietnam has a coastline of about 3260 km, with varying climatic conditions from the northern to southern parts of the country [22]. These physical and climatological characteristics are suitable for the cultivation of seaweed. The abundant algae floral estimated at nearly 1000 species with about 827 species already identified [23], puts Vietnam at an advantage. Seaweeds have long been cultivated in Vietnam. Their biochemical properties have earned them a place in cuisines, traditional medicines, cosmetics, pharmaceutical and more recently, ingredients for bio-industries. The objective of this study is to investigate the tyrosinase inhibitory activity of different red seaweeds harvested in the coast of Khanh Hoa Province. The effect of seaweed extract on the melanosis formation of whiteleg shrimp during cold storage was also investigated.

2. MATERIALS AND METHODS

2.1. Materials

Five red seaweed species (*Gelidiella acerosa*, *Gracilaria salicornia*, *Acanthophora spicifera*, *Hypnea pannosa*, *Kappaphycus alvarezii*) were harvested during May to July, 2018 at the coast of Nha Trang city, Khanh Hoa province. The samples were authenticated in place by a seaweed expert (MSc. Do Anh Duy, Research Institute for Marine Fisheries, Hai Phong city, Vietnam). Seaweed samples were individually rinsed to remove impurities and air-dried in the shade. Dried seaweed samples were cut into small pieces, vacuum packaged in PA bags and stored at 4°C until analysis. whiteleg shrimps (*Penaeus vannamei*) with the size of 80 - 90 shrimp/kg were purchased from a local market in Nha Trang city. These live shrimp were transported to the laboratory in plastic bags with aerated sea water within 30 min. l-3,4-Dihydroxyphenylalanine (l-DOPA) và tyrosinase từ nấm (EC 1.14.18.1) were obtained from Sigma Aldrich (St. Louis, MO, USA). All other reagents and solvents were of analytical grade.

2.2. Seaweed extraction

To screen the tyrosinase inhibitory activitities, 10g of each seaweed sample was extracted with 200mL 80% aqueous methanol for 60 min at 60°C. The mixture was filtered using Whatman No. 1 to obtain the crude extract. The solvent was removed using a rotary evaporator (IKA RV 10 control, Staufen, Germany) under vacuum pressure at 40°C. The obtained extracts were used to evaluate tyrosinase inhibitory activities.

The seaweed *G. acerosa* which showed potential tyrosinase inhibitory activity was selected to investigate the effect of solvent concentration on activities. Different solvent extractions (methanol, ethanol and water) were used to extract under the same procedure as mentioned above. The tyrosinase inhibitory activities were evaluated to find out the suitable extraction solvent concentration. Then, seaweed was extracted using different conditions (temperature, time and seaweed/solvent ratio).

2.3. Mushroom tyrosinase inhibitory assay

The inhibitory activity of mushroom tyrosinase was carried out using a spectrophotometric method with slight modifications of Chang et al. [24]. In brief, 0.1mL of extract together with 2.2mL of phosphate buffer (pH 6.5), 0.1mL of substrate solution (2mM). The mixture was inculated at 37°C for 5 min. Then, 0.1mL of tyrosinase solution (100U/mL) was added and incubated at 37°C for 30 min. Absorbance was measured at 475nm after incubation.

2.4. Preparation of shrimp treated with seaweed extract

The extract of *G. acerosa* with the highest tyrosinase inhibitory activity was used for treatment of shrimp. Whole shrimps were immersed in solution containing seaweed extract at different concentrations (0; 0.78 and 1.59mg/mL, w/v) at 4°C for 30 min. Treated shrimps were drained on a screen for 5 min at 4°C. Shrimps without seaweed extract treatment *were used as the control*. The samples (25 shrimps) were placed on a polystyrene tray, covered with plastic wrap and stored in ice at ≤ 4°C. Samples were analyzed for melanosis and sensory every 3 days up to 12 days. Melanosis of shrimps was evaluated through visual inspection by ten trained panelists using 10-point scoring test. Panelists were

asked to give the melanosis score (0 - 10), where 0 = absent; 2 = slight (up to 20% of shrimps' surface affected); 4 = moderate (20 - 40% of shrimps' surface affected); 6 = notable (40 - 60% of shrimps' surface affected); 8 = severe (60 - 80% of shrimps' surface affected); 10 = extremely heavy (80 -100% of shrimps' surface affected) [25].

2.5. Statistical analyses

All analyses were performed in triplicate and all data are expressed as the mean ± standard deviation (SD). The data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by the Duncan's multiple range test. Statistical analysis was performed using a SPSS package (SPSS 16.0 for windows, SPSS Inc., Chicago, IL, USA).

3. RESULT AND DISCUSSION

3.1. Tyrosinase inhibitory activity of red seaweed extracts

The inhibitory effects of the five different species of red seaweed using mushroom tyrosinase were determined experimentally. Table 1 shows the tyrosinase inhibitory activity at different extract concentrations and the overall IC_{50} values which indicate 50% of the seaweeds' potential in inhibiting tyrosinase activity. The IC_{50} values were different for the five species, varying from 3.04 to 6.53mg/mL. The tyrosinase inhibitory activity of the species decreased in the following order *G. acerosa* > *H. pannosa* > *G. salicornia* > *K. alvarezii* > *A. spicifera.* The differences in extract ability in inhibiting tyrosinase enzyme were majorly caused by differences in bioactive compounds contained in extracts originating from different species. The data demonstrate that *G. acerosa* had the highest tyrosinase inhibitory activity with IC_{50} value of 3.04mg/mL, it was therefore chosen for further investigation. Several studies investigated the phytoconstituents of *G. acerosa,* including flavonoids, alkaloids, tannins, proteins, sulfated polysaccharides, sulfono glycolipid, sesquiterpenes, monoterpenes, phenols and various pharmacological activities [26]. This is however, the first study investigating the tyrosinase inhibitory activity of the species.

Table 1. Tyrosinase inhibitory activity of different red seaweeds

Seaweeds	Tyrosinase inhibitory activity (%)					IC_{50} value (mg/mL)
	2.0 mg/mL	2.5 mg/mL	3.0 mg/mL	3.5 mg/mL	4.0 mg/mL	
G. acerosa	33.01 ±1.22	44.66 ±2.11	50.74 ±1.54	63.94 ±2.09	60.03 ±0.44	3.04 ±0.01^{a}
G. salicornia	16.07 ±2.31	25.87 ±1.72	33.22 ±1.32	38.65 ±2.11	41.09 ±0.65	4.53 ±0.24^{b}
A. spicifera	25.58 ±3.19	31.50 ±1.99	34.08 ±1.51	35.83 ±4.91	36.98 ±1.03	6.53 ±0.47^{c}
H. pannosa	25.29 ±2.59	34.98 ±0.95	43.09 ±4.10	49.67 ±2.47	49.46 ±1.79	4.10 ±0.28^{b}
K. alvarezii	28.59 ±1.68	33.10 ±3.01	34.96 ±1.51	42.32 ±2.53	40.32 ±2.51	5.27 ±1.10^{bc}

Different letters within the same column indicate significant difference ($p < 0.05$)

3.2. Effect of extraction conditions tyrosinase inhibitory activity of *G. acerosa*

The tyrosinase inhibitory activity of different solvent (methanol, ethanol and water) extracts of *G. acerosa* was investigated (Figure 1). All three solvents exhibited different tyrosinase inhibitory activity. This indicates that the selection of an appropriate solvent for extraction of bioactive compounds from *G. acerosa* is critical to achieving optimal biological activity, particularly tyrosinase inhibitory activity. The aqueous extract, demonstrated the highest (55.49%) inhibitory activity while the ethanolic extract of the species showed the least inhibitory activity (38.07%). This shows that water is the preferred solvent for optimal *G. acerosa* tyrosinase inhibitory activity. Syad et al. [27] also recorded water as the best extraction solvent of *G. acerosa*. Water was therefore used as the solvent of extraction for subsequent experiments.

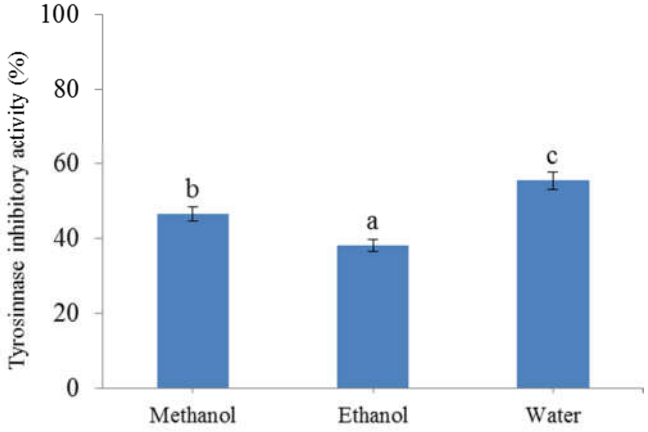

Figure 1. Effect of different solvent extracts on tyrosinase inhibitory activity of *G. acerosa*. Different letters indicate statistically significant difference ($p < 0.05$)

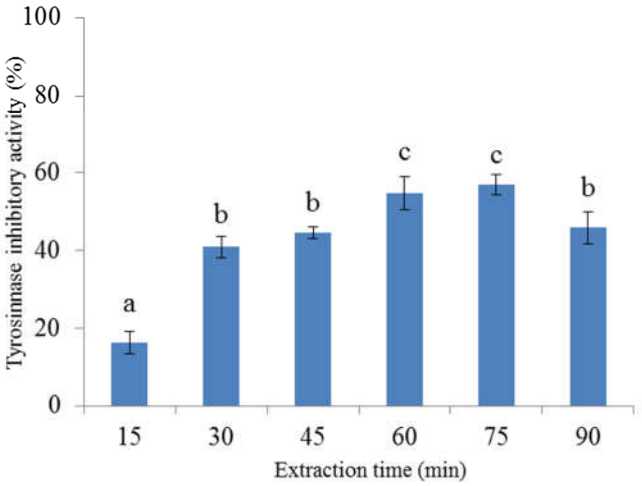

Figure 2. Effect of different extraction time on tyrosinase inhibitory activity of *G. acerosa*. Different letters indicate statistically significant difference ($p < 0.05$)

The effect of six different extraction time on tyrosinase inhibitory activity of aqueous extracts of *G. acerosa* was experimentally determined. Figure 2 shows that there were significant differences among the percentage tyrosinase

inhibitory activity of the extracts at the extraction times investigated (15, 30, 45, 60, 75, and 90 minutes). The tyrosinase inhibitory activity ranged from 16.29% to 56.88%; extraction time of 75mins had the highest activity at 56.88%. The aqueous extracts of *G. acerosa* exhibited a time-dependent increase in tyrosinase inhibitory activity, however, after 75 minutes, increasing the extraction time did not improve the extract inhibitory activity of tyrosinase. Time is essential in optimizing energy requirements and cost during the extraction process. Since there was no significant difference between the inhibitory activity of the extract at 60 and 75 minutes, we can deduce that 60 minutes is the optimal extraction time for maximum tyrosinase inhibitory activity while minimizing energy costs.

The use of different extraction temperature has significant effect ($p < 0.05$) on the tyrosinase inhibitory activity of aqueous extract of *G. acerosa* (Figure 3). The result showed that the inhibitory activity of the extract sharply increased when temperature increased from 30°C to 60°C at 13.50% and 56.22%, respectively. However, increasing the temperature from 60°C to 90°C significantly reduced the inhibitory activity at 56.22% to 36.27%. This behavior can be attributed to the loss of biologically active compounds like polyphenols as a result of exposure to high temperatures [28]. It can be deduced from the result that 60°C is the most suitable extraction temperature for optimum inhibitory activity.

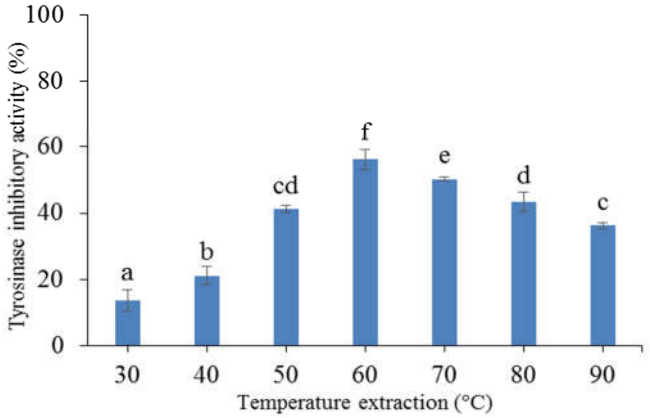

Figure 3. Effect of different extraction temperature on tyrosinase inhibitory activity of *G. acerosa*. Different letters indicate statistically significant difference ($p < 0.05$)

In the data presented, 1g of seaweed sample (*G. acerosa)* was analyzed against varying volume (10 - 50mL) of water to determine the solid/solvent ratio at which the highest tyrosinase inhibitory activity could be obtained. The tyrosinase inhibitory activity increased with the solid-liquid ratio increased until a maximum inhibitory activity reaches at 1/40g/mL (68.92%). The increase of the solid-liquid ratio improves the rate of mass transfer, which results in the increase in the mass transport driving force and the internal diffusion rate [29]. However, the liquid-solid ratio exhibited a slight decrease trend when the ratio was above 40mL/g, which was possible that the contact area was saturated with increasing solvent volume. Similar results on the effect of the solid-liquid ratio on the extraction of bioactive compounds from seaweeds were also reported by Vázquez-Rodríguez et al. [30] and Topuz et al. [31]. It was concluded that the solid-liquid ratio of 1/40g/mL is suitable.

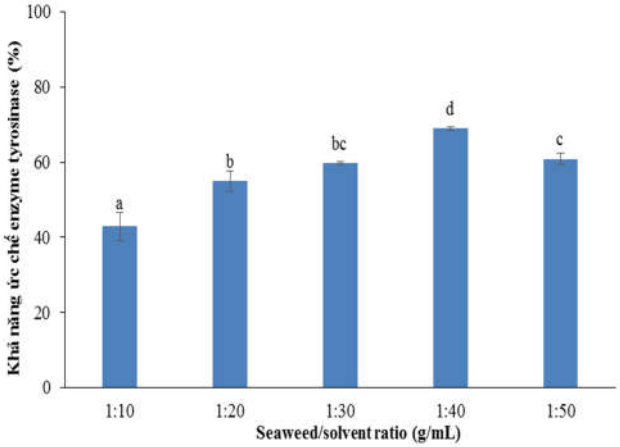

Figure 4. Effect of different extraction ratio (w/v) on tyrosinase inhibitory activity of *G. acerosa*. Different letters indicate statistically significant difference ($p < 0.05$)

3.3. Effect of seaweed extract treatment on melanosis of whiteleg shrimp during refrigerated storage

The inhibition of melanosis in whiteleg shrimp using different concentrations of the *G. acerosa* extract was studied. Melanosis progression in the shrimp is shown in Figure 5. As expected, the melanosis score increased across all the groups with the control group having the highest mean scores (Figure 6). According to the panelists, none of the samples showed melanosis on day 0, melanin started to develop on the third day of storage which then began to increase significantly across the groups. Melanosis score of the shrimp was in descending order: control, 0.78mg/mL and 1.59mg/mL seaweed extract. Photographs of melanosis formation in whiteleg shrimp at different treatments for all the periods of refrigerated storage are shown in Figure 5. Samples in the photograph are representative of the same sample, which were evaluated for melanosis score throughout the refrigerated storage. This study showed that immersing shrimp in seaweed extract inhibited post-harvest melanosis in shrimp; additionally, the development of melanosis decreased as the concentration of the extract increased. Based on the finding, the seaweed extract concentration of 1.59mg/mL was selected for preserving shrimp quality during refrigerated storage. The inhibition of melanosis in shrimp immersed in plant extract has been reported in literature. Sharifian et al. [32] showed that 5% phlorotannins extracted from *S. tenerimum* inhibited melanosis in shrimp. Llanto & Encarnacion [33] observed that the immersion of shrimp in a 1.0% w/v solution of mushroom extract significantly controlled melanosis in the treated shrimp during ice storage and was comparable with the effects of

ascorbic acid and sodium sulfite treatments. Nirmal & Benjakul [34] also reported a reduction in melanosis score in shrimp treated with tea extract. The retardation of melanosis in shrimp by the seaweed extract was most likely due to the high tyrosinase inhibitory activity of *G. acerosa* (Table 1).

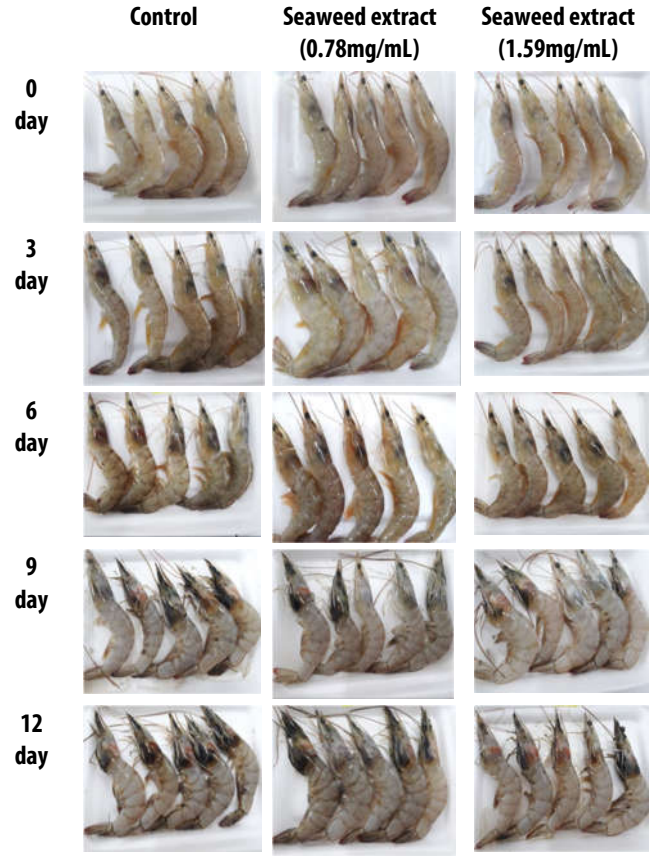

Figure 5. Melanois changes in whiteleg shrimps with and without treatment of seaweed extract at different concentrations

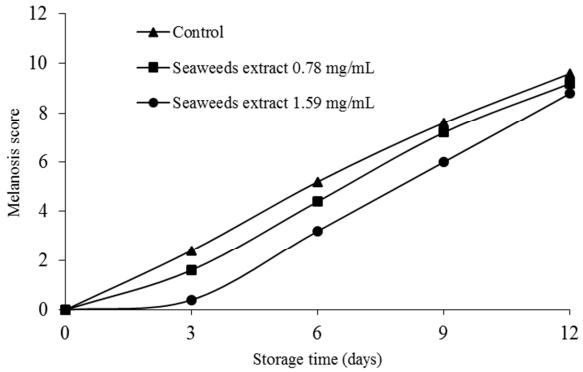

Figure 6. The inhibition of melanosis in whiteleg shrimp using different concentrations of seaweed extract

4. CONCLUSIONS

All red tested seaweed extracts showed inhibition against mushroom tyrosinase inhibitory activity. Among them, the *G. acerosa* extract showed the most tyrosinase inhibitory activity. The suitable extraction conditions for extraction of tyrosinase inhibitors from *G. acerosa* were determined as follows: the solid to liquid ratio (g/mL) of 1/40, the extraction time of 60 min and the extraction temperature of 60°C. The *G. acerosa* extract strongly inhibited tyrosinase and melanoisis formation in whiteleg shrimp during 12 days of refrigerated storage. Thus, the *G. acerosa* extract could be used as a natural tyrosinase inhibitor for controlling melanoisis in shrimp during post storage.

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