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# Investigating Anti-Tyrosinase and Anti-Oxidant Potential of the Marine alga *Hormophysa cuneiformis* for Cosmeceutical Application

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## ABSTRACT

Skin hyperpigmentation is a common disorder often associated with various diseases. Melanogenesis is the formation of pigment called melanin in the cells of skin. This process primarily requires the amino acids tyrosine and L-DOPA (3,4-dihydroxyphenylalanine). Consequently, many skin-whitening cosmetics aim to inhibit melanin formation by targeting tyrosinase activity or scavenging L-DOPA. In this study, the brown seaweed *Hormophysa cuneiformis*, collected from Hurghada, Red Sea, Egypt, was investigated for its anti-melanogenic potential through its methanolic extract. The potential of antioxidant in the algae-derived solution was found to be 2.8mg/ g dry weight comparable to ascorbic acid. Phytochemical analysis revealed a total phenol content of 1.87mg/ g dry weight comparable to gallic acid. The flavonoid composition was found to be 0.86mg/ g dry weight comparable to rutin. The anti-melanogenic potential of *Hormophysa cuneiformis* extract was estimated by assessing its cytotoxic impact on the viability of B16F1 melanoma cells via MTT assay using different concentrations compared to kojic acid. Additionally, the inhibition of α-melanocyte stimulating hormone (α-MSH) was analyzed following addition of different doses of the algal extract. The results demonstrated that the algal extract suppressed both extra and intra-cellular activity of tyrosinase in melanoma cells. The intracellular tyrosinase suppression was further confirmed by zymography, which analyzed the expression level of tyrosinase as a protein compound. In conclusion, this study strongly suggests that *Hormophysa cuneiformis* extract possesses significant anti-melanogenic activity, making it a promising candidate for development as a novel natural cosmeceutical compound for skin whitening.

## INTRODUCTION

The aquatic environment is the most valuable source of natural resources. Algae are enriched with many vital compounds including vitamins (A, B, C), omega-3 fatty acids, proteins, polysaccharides minerals, as well as lipids. Numerous therapeutic chemicals with anti-oxidant, antimicrobial, anti-inflammation in addition to cytotoxic compounds have been discovered in algal extracts. The culture environment of marine algae, as evidenced in the diversity of its metabolites, resulted in an infinite number of chemicals with varied uses for treating diseases or disorders that pharmaceutical products failed to

provide. Skin hyperpigmentation is a frequent illness that is associated with a variety of disorders, including 1) POSTINFLAMMATORY HYPERPIGMENTATION, which occurs after trauma or inflammation. 2) MELASMA: During pregnancy or a hormonal imbalance. 3) SOLAR LENTIGINES: following ultraviolet radiation. 4) EPHELIDES: A childhood disease caused by sun exposure in vulnerable persons. 5) CAFÉ AU LAIT MACULES: Patches or lesions in skin of darker color that appear at birth. Hydroquinone (HQ) is considered the most commonly used compound for treating skin pigmentation. General treatment for hyperpigmentation includes topical application of hydroquinone, retinoids, azelaic acid, kojic acid, tranexamic acid, cysteamine, and laser **(Philipp-Dormston, 2024)**.

The standard skin lightening approach is the use of hydroquinone, a tyrosinase inhibitor that suppresses the formation of melanin in skin layers during melanogenesis. Melanin is a dark pigment generated in melanocytes. The synthesied melanin is subsequently packed in melanosomes and delivered to adjacent keratinocytes, resulting in the dark color of skin and hair **(Guo *et al.*, 2023)**. Dopamine, l-DOPA, and l-tyrosine are the three substances essential to melanogenesis. Tyrosinase initiates this process by oxidizing l-tyrosine to dopaquinone. According to this idea, several whitening agents work by blocking tyrosinase or scavenging DOPA to prevent the formation of melatonin **(Karpinski, 2021; García-Mauriño, 2022)**.

Melanogenesis requires dopamine, l-DOPA and l-tyrosine. The mechanism involves the action of tyrosinase to oxidize l-tyrosine to dopaquinone. From this concept, different whitening compounds depend on stopping melatonin formation by inhibiting tyrosinase, or scavenging DOPA **(García-Mauriño, 2022)**.

Tyrosinase enzyme (EC.1.14.18.1), the triggering compound initiating the formation of melanin, has been in spotlight for the past years, and an astonishing number of agents was marketed to regulate tyrosinase for cosmetic application and skin whitening. Tyrosinase inhibitors have been extracted from many algae, e.g. from *Symphyocladia latiuscula* (Harvey, a red algae) **(Paudel *et al.*, 2019)**, methanolic extracts from the following red macroalgae: *Laurencia papillosa, Laurencia paniculata,* and *Digenea simplex* **(Namjoyan *et al.*, 2019)**, from brown algae such as *Colpomenia bullosa* **(Kurihara & Kujira, 2021)**, from green algae **(Rodrigues *et al.*, 2021)**, from marine microalgae **(Ji *et al.*, 2021; Khan, 2023)**, and from edible red alga *Bangia fusco-purpurea* **(Wang *et al.*, 2023)**.

A study in 2022, investigated different solvents for analyzing tyrosinase activity in the extracts of the macroalgae; *Tetraselmis tetrathele*, *Dunaliella tertiolecta, Platymonas* sp., and *Chaetoceros simplex*, as well as from marine cyanobacteria **(Baba, 2022; He *et al.*, 2022)**. Additionally, plant tyrosinase inhibitors were investigated, including *Ficus deltoidea* (Mas cotek) **(Oh *et al.*, 2011)** and *Asphodelus microcarpus* **(Di Petrillo *et al.*, 2016)**. The tyrosinase enzyme from *Agaricus bisporus* is notable for being both important and cost-effective, sharing significant similarity and homology with human tyrosinase **(Morosanova *et al.*, 2020)**. This enzyme has been widely used as a model for screening

tyrosinase inhibiting agents, investigating melanogenesis, assessing enzymatic-catalyzed reactions, and investigating enzyme-inhibitor relationships **(Zolghadri *et al.*, 2019)**.

The mechanism of tyrosinase action involves the indirect decrease in melanin and melatonin production, as both share pathways involving the amino acid tyrosine. Accordingly, the tyrosinase whitening effect is due to:

A) **Decreasing melanin synthesis**: Tyrosinase catalyzes the hydroxylation of tyrosine to DOPA (dihydroxyphenylalanine). Tyrosinase then oxidizes DOPA to DOPA quinone, which can subsequently lead to melanin production through a series of polymerization reactions. While DOPA is an intermediate in melanin synthesis, it can also be involved in the pathway that leads to melatonin production.

B) **Scavenging DOPA to prevent melatonin formation**: DOPA is decarboxylated to dopamine by the enzyme DOPA decarboxylase. Dopamine can then be further converted to serotonin and eventually to melatonin through a series of enzymatic steps **(Hossain, 2021; Zhang, 2021; Kumar, 2023)**.

The main objective of this present study was to assess the probabilities of *Hormophysa cuneiform* from the Red Sea, Egypt, as natural, cheap, non-toxic treatment for many skin disorders related to hyperpigmentation as well as its use as a cosmetic product for skin whitening. Future prospects will be devoted for culturing *Hormophysa cuneiform* for cosmetic industry.

## MATERIALS AND METHODS

### 1. Algae collection

*Hormophysa cuneiformis*, a brown seaweed, was collected in June 2019 from Hurghada, Egypt. The collection was made at a depth of 1-2 meters during low tide, namely at a latitude of 27.28°N and a longitude of 33.77°E. The harvested algae were thoroughly cleansed using seawater to eliminate any extraneous substances such as epiphytes, sand, pebbles, and shells. Subsequently, the samples were carefully stored in aseptic, pristine plastic bags and conveyed to the laboratory in a refrigerated container. The algae in the laboratory were properly rinsed with tap water to wash out any unwanted particles, salts, and sand. The samples were dried indoors and were grinded using a blender and placed within sterile sealed bags in the refrigerator until the next step. The algal specimens were morphologically identified according to taxonomic descriptions provided by **Jha *et al.* (2009)** and **Dixon and Huisman (2015)** in their respective literature. Specimens were preserved in herbarium sheets and formaldehyded with a final concentration of 2% v/v.

**Fig. 1.** Morphological features of the collected *Hormophysa cuneiformis* used in present study

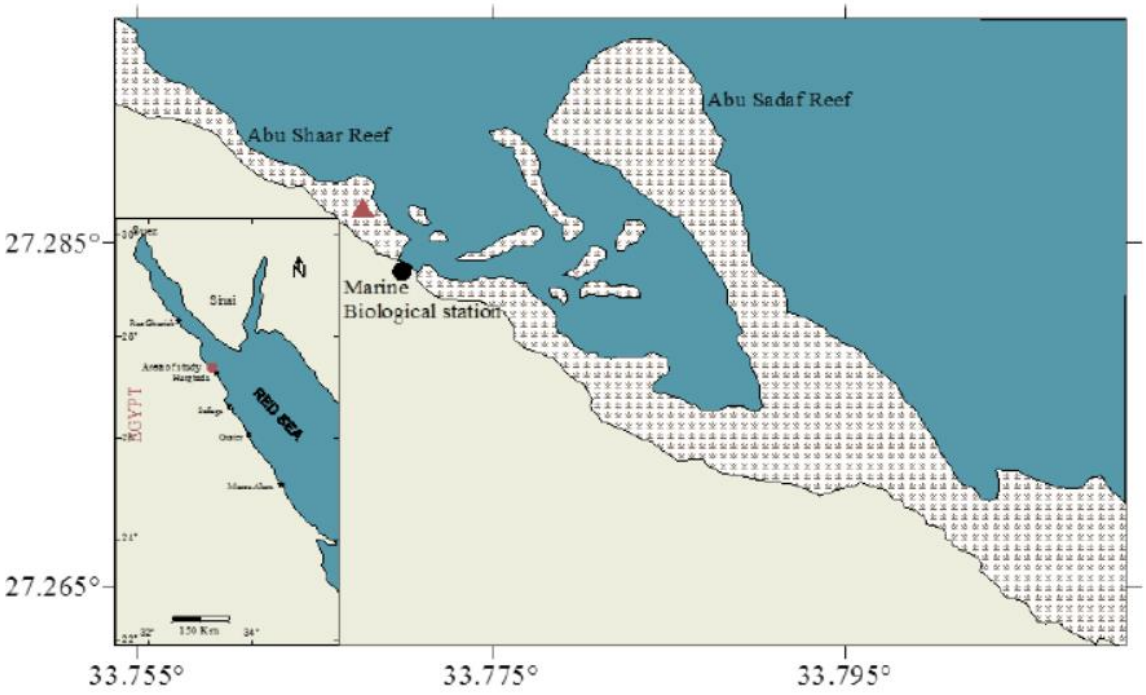


**Fig. 2.** Samples collection area; Red Sea, Hurghada, Egypt

**2. *Hormophysa cuneiformis* extraction**

The extraction procedure adhered to the methodology outlined by **Arokiyaraj *et al.* (2009)**. At first, 15 grams of the algae powder were immersed in 60 milliliters of hexane for a duration of 48 hours. The mixture was then passed through a silk cloth, and finally through filter paper. The resulting liquid was gathered in a sterile container. The same procedure was carried out using other solvents including hexane, ethyl acetate, acetone, and methanol. All extracts were tested for its anti-oxidant capacity, and the one with highest value was chosen for further study.

**3. Total antioxidant capacity (TAC) of *Hormophysa cuneiformis* extract**

The total capacity of antioxidants (TAC) was investigated following the methodology given by **Prieto *et al.* (1999)**. The TAC contained sulphuric acid (0.6 M), sodium phosphate (28 mM), and ammonium molybdate (4 mM). The algal extract was then mixed with 3ml of TAC reagent and incubated at 95°C for 90 minutes. The absorbance was detected at 695nm using ascorbic acid as a standard.

**4. Total polyphenols and flavonoids content of *Hormophysa cuneiformis* extract**

Total phenols were estimated according to **Siddhuraju and Becker (2007)** using gallic acid as the standard. A 20µL aliquot was added to 1ml of a 10% solution of Folin-Ciocalteu reagent. After a 5-minute period of allowing the substance to develop, 700 microliters of 10% sodium carbonate ($Na_2CO_3$) were introduced, and incubated for 2 hours before detecting absorbance at 765nm. A standard curve was developed using different concentrations of gallic acid (20-200mg/ L).

The flavonoid content in the methanolic extract of *Hormophysa cuneiformis* was assessed according to **Brighente *et al.* (2007)**. The algal extract and 2% aluminum chloride in methanol were mixed in equal quantities and were put at an ambient temperature for an hour before reading absorbance at 415nm. Different concentrations of rutin (20-200mg/ L) were measured to generate a standard curve and was stated as mg/g of dry weight.

**5. Tyrosinase inhibition assay**

The assay was accomplished according to **Nairn *et al.* (2015)**. The reaction mixture of 1000µL total volume included 685µL of phosphate buffer (0.05 M, pH 6.5), 15µL of mushroom tyrosinase (2500U/ mL), 200µL of algal extract, and 100µL of 5mM L-DOPA (the substrate). The dopachrome formed was detected at 492nm. The algal extract was investigated for its suppression of tyrosinase model from mushroom at dosages ranging from 0 to 0.3mg/ mL in comparison to Kojic acid.

**6. Cytotoxicity of *Hormophysa cuneiformis* extract**

The B16F10 mouse melanoma cells (CRL-6475, ATCC, USA) were propagated and colorimetric MTT assay was utilized to investigate the viability of cells in presence of different doses of algal extract according to the method of **Mosmann (1983)**.

**7. Intra-cellular tyrosinase inhibition**

The methodology outlined by **Pintus *et al.* (2015)** was utilized to assess intracellular tyrosinase activity and melanin concentration. Additionally, the α-MSH-stimulated cells were propagated and were subsequently exposed to the extract at different doses. The protein extracted out of these cells lysates was assayed according to **Bradford (1976)**.

**8. Tyrosinase zymography (L-DOPA staining)**

Zymography of tyrosinase was conducted as stated by **Elias *et al.* (2009)**. The propagated cells were collected, lysed, and electrophoresed on 8% SDS-PAGE, after which the cells were gel stained in buffer supplemented with 10 mM L-DOPA.

## RESULTS

**1. Antioxidant and phytochemical analysis**

*Hormophysa cuneiform* methanolic extract showed the best results compared to other solvents. Antioxidant potential, phenolic, and flavonoid composition is summarized in Table (1).

**2. Cytotoxicity of *Hormophysa cuneiform* extract**

The ability of *Hormophysa cuneiform* extract to impact the survival of melanoma cells was analyzed by adding different concentrations of the extract. The study utilized MTT assay, revealing $IC_{50}$=406.25µg/ ml (Fig. 3).

**3. Mushroom tyrosinase inhibition assay**

The result shown in Fig. (4) obviously demonstrated the remarkable suppression in the activity of mushroom tyrosinase on L-DOPA upon treatment with different doses of *Hormophysa cuneiform* extract comparable to kojic acid.

**4. α-MSH treatment (α-melanocyte stimulating hormone)**

The inhibitory effect of *Hormophysa cuneiform* extract was confirmed via the obvious decrease of the formed melanin in response to treatment of melanoma cells with algal extract (Fig. 5)

**5. Intracellular tyrosinase activity and melanin formation**

Reduced activity of tyrosinase in the melanocytes by the direct inhibition of tyrosinase is shown in Fig. (6), and the melanin formation in cells was remarkably decreased with increasing the concentration of algal extract (Fig. 7).

**Table 1.** Estimation of phenols, flavonoids, and antioxidant capacity in the methanolic extract of *Hormophysa cuneiform*

| | |
|---|---|
| Total phenols (mg gallic acid equivalent (GAE)/g dried weight) | 1.87±0.025 |
| Total flavonoids (mg rutin equivalents (RU)/g dried weight) | 0.86±0.021 |
| Total antioxidant capacity (TAC) equivalent Ascorbic acid /gdw | 2.87±0.075 |

## 6. L-DOPA staining assay (Tyrosinase zymography)

Treated B16F10 cells with *Hormophysa cuneiform* extract were investigated for its protein content by 8% SDS-PAGE (Fig. 8). The effect appeared in the form of melanin bands with intensity that decrease with increasing the concentration of extract upon DOPA staining.

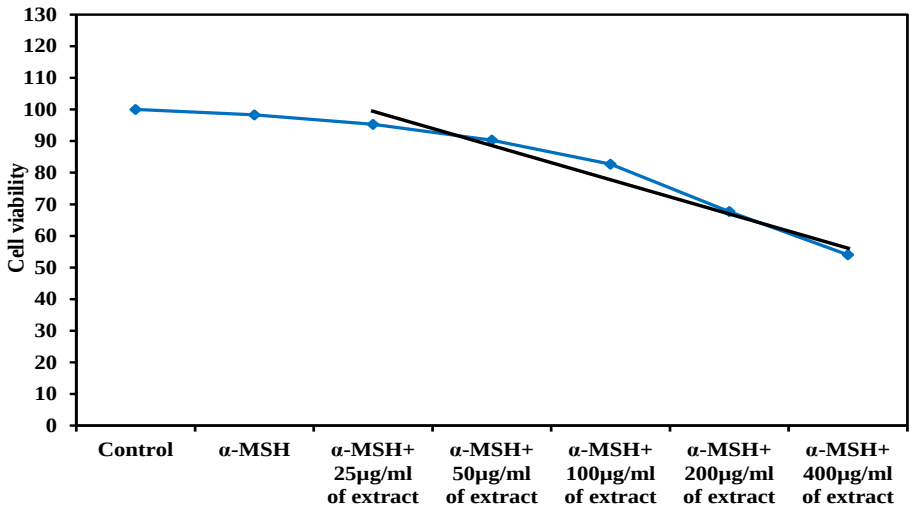


**Fig. 3.** Effect on the viability of B16F10 melanoma cells for serial algae extracts

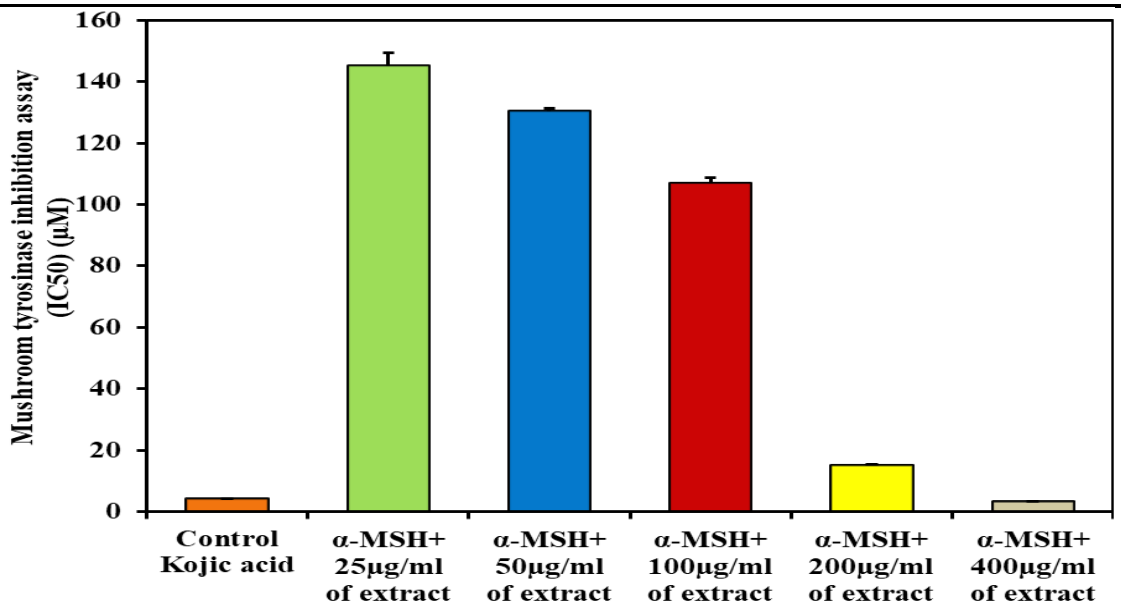


**Fig. 4.** Mushroom tyrosinase inhibition assay on L-DOPA as the substrate. Where 1) Control of kojic acid, 2) L-DOPA+ 25µg/ ml of extract, 3) L-DOPA+50µg/ ml of extract, 4) L-DOPA+100µg/ ml of extract, 5) L-DOPA+200µg/ ml of extract, 6) L-DOPA+400µg/ ml of extract.

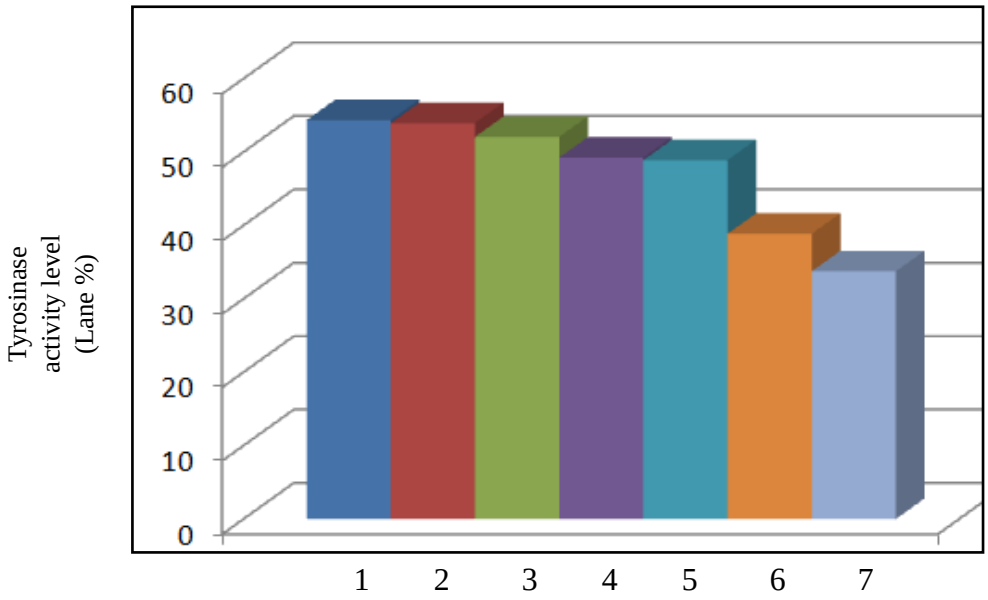


**Fig. 5.** Tyrosinase activity levels (Lane %) on α-MSH-stimulated B16F10 cells response to serial concentration of algae extract. Where: 1) Control, 2) α-MSH, 3) α-MSH+ 25µg/ ml of extract, 4) α-MSH+ 50µg/ml of extract, 5) α-MSH+ 100µg/ ml of extract, 6) α-MSH+ 200µg/ml of extract, 7) α-MSH+ 400µg/ ml of extract

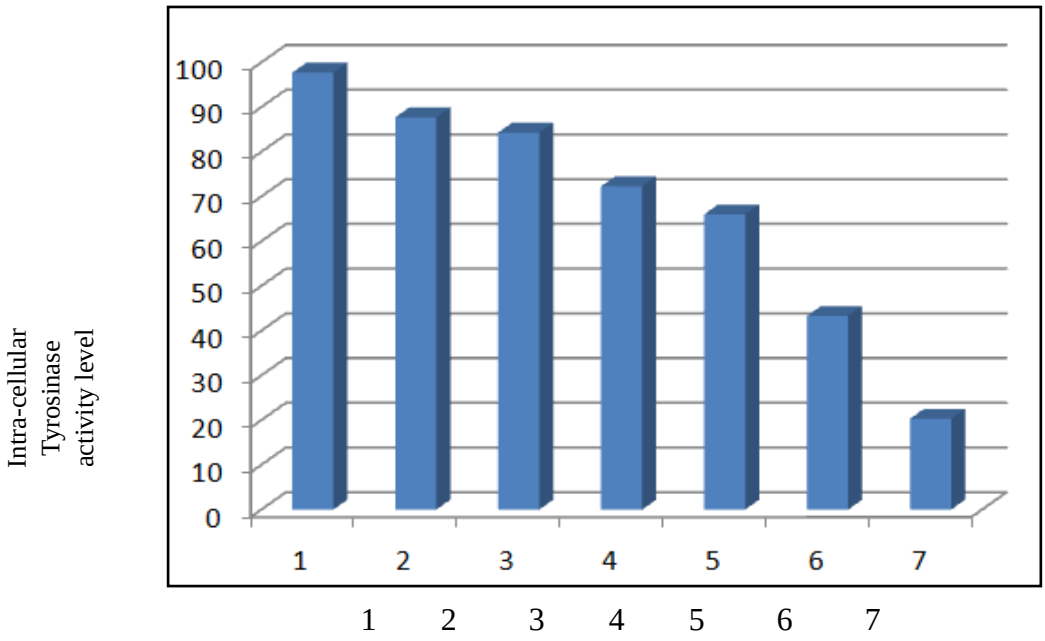


**Fig. 6.** Intracellular tyrosinase activity for serial algae extract. Where: 1) Control, 2) Kojic acid, 3) α-MSH+ 25µg/ ml of extract, 4) α-MSH+ 50µg/ ml of extract, 5) α-MSH+ 100µg/ ml of extract, 6) α-MSH+ 200µg/ ml of extract, and 7) α-MSH+ 400µg/ ml of extract

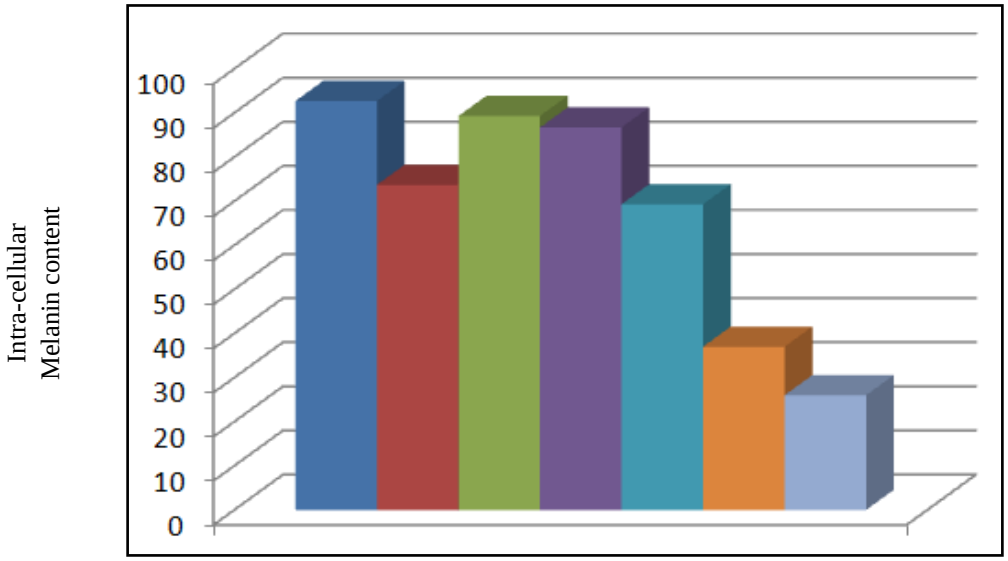


**Fig. 7.** Melanin content for serial algae extract Where: 1) Control, 2) Kojic acid, 3) α-MSH+ 25µg/ ml of extract, 4) α-MSH+ 50µg/ ml of extract, 5) α-MSH+ 100µg/ ml of extract, 6) α-MSH+ 200µg/ ml of extract, and 7) α-MSH+ 400µg/ ml of extract

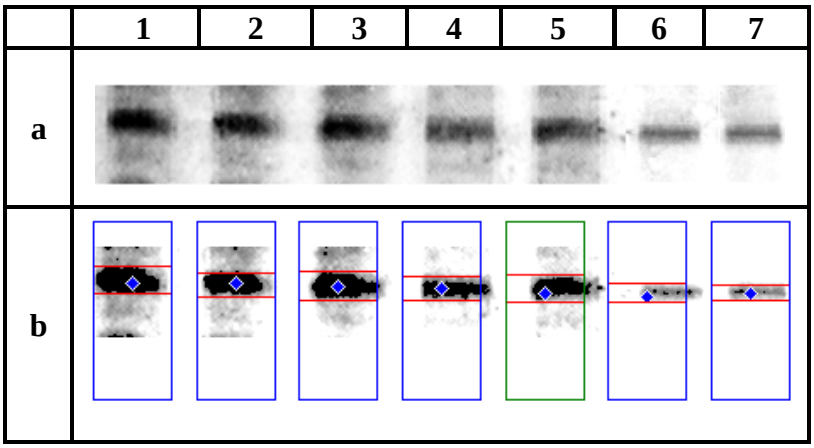


**Fig. 8.** Tyrosinase activity levels for serial algae extract represented as bands of melanin upon L-DOPA staining. Where: 1) Control, 2) α-MSH, 3) α-MSH+ 25µg/ ml of extract, 4) α-MSH+ 50µg/ ml of extract, 5) α-MSH+ 100µg/ ml of extract, 6) α-MSH+ 200µg/ ml of extract, and 7) α-MSH+ 400µg/ ml of extract **(a)**, and computerized detection of tyrosinase activity levels **(b)**

## DISCUSSION

There is significant interest in discovering novel anti-melanogenic compounds from natural sources with antioxidant properties. Given the crucial role of tyrosinase in the melanogenesis pathway, investigating tyrosinase inhibitors has become essential for cosmeceutical products that could serve as potent skin whitening agents to treat various skin disorders.

Marine ecosystems are rich in bioactive secondary metabolites, which promotes the production of entirely novel natural substances with therapeutic applications. Seaweeds establish a huge number of beneficial substances. *Hormophysa cuneiformis* is an integral part of the seaweed species throughout the coast of the Red Sea, particularly in Hurghada and Safaga **(El-Sharouny *et al.*, 2001)**.

Previous studies have discussed the antibacterial and antihyperlipidemic activities of this species **(Shoubaky & Salem, 2014a, b; Abdel-Raouf *et al.*, 2015)**, as well as its antifungal activity **(Mohamed & Saber, 2019)**. This study evaluates the anti-tyrosinase inhibition potential of *Hormophysa cuneiformis* as a novel natural whitening compound for hyperpigmentation disorders associated with various skin diseases and as a cosmetic product.

*Hormophysa cuneiformis'* methanolic extract demonstrated an exceptional antioxidant potential. The investigation of antioxidant activity is based on the reduced

ions of phosphomolybdate upon treatment with an antioxidant, which generates the detectable green complex of phosphate/MoV. The obtained results revealed significant levels of phenolic and flavonoid composition, indicating the substantial relationship within phenolic level and antioxidant capacity in algal and plant extracts **(Prasedya *et al.*, 2021)**. Furthermore, the anti-melanogenic activity of algal extracts could be linked to flavonoids, a kind of polyphenol known for suppressing the formation of reactive oxygen species (ROS) **(Choi *et al.*, 2008; Shan *et al.*, 2009; Ye *et al.*, 2010; Nasr Bouzaiene *et al.*, 2016)**.

Tyrosinase is essential in the first two critical phases of melanogenesis. The present study investigated whether *Hormophysa cuneiformis* extract has a supressing effect on tyrosinase activity. The impact of the methanolic extract on melanin formation was investigated in B16F1 cells of melanoma. B16F1 cells treated with varying quantities of *Hormophysa cuneiformis* extract in the presence of α-MSH showed significantly higher amounts of melanin compared to kojic acid, as a positive control.

Tyrosinase zymography, a method for detecting intracellular tyrosinase, showed that *Hormophysa cuneiformis* extract degraded intracellular tyrosinase to levels similar to the untreated control with α-MSH. Furthermore, the mushroom tyrosinase activity assay revealed that *Hormophysa cuneiformis* extract acts as a tyrosinase inhibitor. The reduction in melanin synthesis is affected by several factors among which is supressing the levels of intracellular tyrosinase and reducing the direct activity of tyrosinase.

In conclusion, *Hormophysa cuneiformis* extract can be utilized for the production of novel natural medications with high levels of phytonutrients and fewer adverse side effects compared to chemical whitening products.

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