

A Study on the Tyrosinase Inhibitory and Antioxidant Effect of Microalgae Extracts

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Received: December 7, 2020 / Revised: February 16, 2021 / Accepted: February 26, 2021

Reactive oxygen species (ROS) disrupt the cellular redox balance, exert cytotoxic effects, and consequently promote the development of various diseases in humans. Previous studies have reported that antioxidants counteract the adverse effects of ROS. Several studies examine the whitening effects of various agents based on their ability to inhibit tyrosinase activity. Tyrosinase is a critical enzyme involved in the synthesis of melanin, which protects the skin against radiation. Various agents exhibiting antioxidant and tyrosinase inhibitory activities have been synthesized. However, these synthetic drugs are associated with toxicity, decreased safety, and poor skin penetration *in vivo*, which has limited the clinical application of synthetic drugs. This study examined the antioxidant and tyrosinase inhibitory activities of some microalgae. The methanol, dichloromethane, and ethyl acetate extracts of four microalgal species (*Tetraselmis tetraathele*, *Dunaliella tertiolecta*, *Platymonas* sp., and *Chaetoceros simplex*) were prepared. The physiological and whitening effects of microalgal extracts were investigated by measuring the antioxidant and tyrosinase inhibitory activities. The ethyl acetate extract of *D. tertiolecta* exhibited the highest antioxidant and tyrosinase inhibitory activities. Future studies must focus on examining the whitening effects of microalgae on cell lines to facilitate the development of microalga-based therapeutics for skin diseases, functional health foods, and whitening agents. Thus, microalgae have potential applications in the pharmaceutical, food, and cosmetic industries.

Keywords: Antioxidant, cosmeceutical, *Dunaliella*, microalgae, tyrosinase inhibition

Introduction

The pro-oxidant and antioxidant balance in the human body can bust because of physical or environmental factors. The reactive oxygen species (ROS), one kind of direct causative at oxidative stress, has induced oxidation. It rises to cell damage and a pathological disease like cancer [1]. These defense mechanisms against oxidative stress called the anti-oxidation are the mechanism for inhibiting the production or returned to normal levels that the over-produced free radicals at the energy production process *in vivo* [2]. Reactive oxygen species

are produced in the process of stabilizing by taking incomplete electrons generated during the mitochondrial respiratory process from nearby molecules, and types include singlet oxygen (1O_2), superoxide (O_2^-), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2) [2, 3]. These reactive oxygen species are very unstable and highly reactive, easily react with various biological materials, and attack the polymers in the body, causing irreversible damage to cells and tissues, mutations, and cancer. It is also known to cause diabetes, brain disease, and cardiovascular disease. Therefore, for the prevention and treatment of many chronic degenerative diseases in which oxidative stress caused by free radicals acts as a risk factor, many studies have been conducted to find antioxidants from natural products with low toxicity and side effects [3].

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Melanin is essential to protect human skin from radiation, and recent studies have shown that it also plays an important role in regulating skin homeostasis. However, abnormal melanin accumulation causes pigment abnormalities such as blemishes, melanoma, freckles, senile black spots, and damages internal tissues, and causes skin aging due to light and skin quality. It also causes enzymatic browning in fruits and vegetables. Melanin is the final product produced by melanogenesis and is influenced by various factors such as hormonal changes and nutritional status. Besides, melanogenesis can cause damage to cell membranes and DNA by generating free radicals, causing DNA lipid peroxidation, reducing membrane fluidity, and DNA mutations leading to cancer and degenerative diseases [4, 5]. This melanogenesis occurs in melanocytes located in the epidermis basement layer, and tyrosinase acts as the main enzyme in this process. Tyrosinase, also called polyphenol oxidase (PPO), is a monooxygenase containing copper and a major enzyme in melanin synthesis, widely distributed in microorganisms, animals, and plants. Tyrosinase catalyzes two oxidation reactions that hydroxylate L-tyrosine to convert to L-dihydroxyphenylalanine (DOPA) and oxidize L-DOPA to DOPA quinone. Highly reactive DOPA quinone reacts with amino acids and proteins to form high molecular compounds or brown pigments (eumelanin or pheomelanin). It is possible to develop a functional cosmetic or a therapeutic agent to treat pigment disorders as a whitening agent by inhibiting melanin production by inhibiting tyrosinase activity. To date, several tyrosinase inhibitors have been synthesized and isolated to inhibit tyrosinase activity. Still, the use of existing drugs has been limited due to their toxicity, low safety, and insufficient skin penetration and activity. Therefore, efforts have been made to find new tyrosinase inhibitors in natural products to reduce the side effects of synthetic tyrosinase inhibitors [6].

Recently, marine resources are attracting attention as raw materials for bioactive compounds to develop new drugs and healthy foods. Among them, marine microalgae are creatures in which more than 50,000 species are distributed in various environments, and they provide various biochemical substances with vast biodiversity. Microalgae are rich in essential trace elements such as vitamins, minerals, iodine, zinc, and various functional polysaccharides and polyphenols, and secondary carot-

enoids such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids and long-chain polyunsaturated fatty acids (PUFA). It is known that metabolites are also produced. Also, research is being conducted to use as a renewable biofuel using microalgae. Microalgae are single-celled organisms with vast biodiversity and are capable of rapidly growing under a large-scale system that is easy to produce large amounts of biomass and can continuously supply desired molecules. Besides, bulk microalgal biomass or fractions thereof can be used for nutritional applications [7, 8]. Therefore, in this study, microalgae, which is an eco-friendly material, was selected as a sample for removing free radicals and inhibiting tyrosinase.

Therefore, this study aimed to investigate the antioxidant activity capable of removing free radical species and the tyrosinase inhibitory activity that can inhibit melanin production by using the microalgae extract, which is a natural product. Through this, it finds microalgae extracts that have both antioxidant and tyrosinase inhibitory activities and provides useful information for researching treatments for skin diseases related to chronic degenerative diseases and pigment disorders caused by oxidative stress and developing health supplements. As such, it is considered that it can be used as a raw material for functional cosmetics.

Materials and Methods

Microalgae

Microalgae were provided and used by the South Sea Fisheries Research Institute of NIFS (National Institute of Fisheries Science). Microalgae were studied by selecting species whose antioxidant activity and whitening activity was not verified. Three green algae species (*Tetraselmis tetrathele*, *Dunaliella tertiolecta*, *Platymonas* sp.) and one diatom species (*Chaetoceros simplex*) were used. All algal cells were cultured in Conwy medium at 25°C for 1–2 weeks under continuous lighting conditions.

Microalgal extracts preparation

For obtained the active substance from microalgae, we performed the extraction using an organic solvent. The microalgal culture was centrifuged at 8,000 rpm for 30 min to obtain 10 g of microalgal cells. 50 ml of methanol was added to the microalgal cells and performed

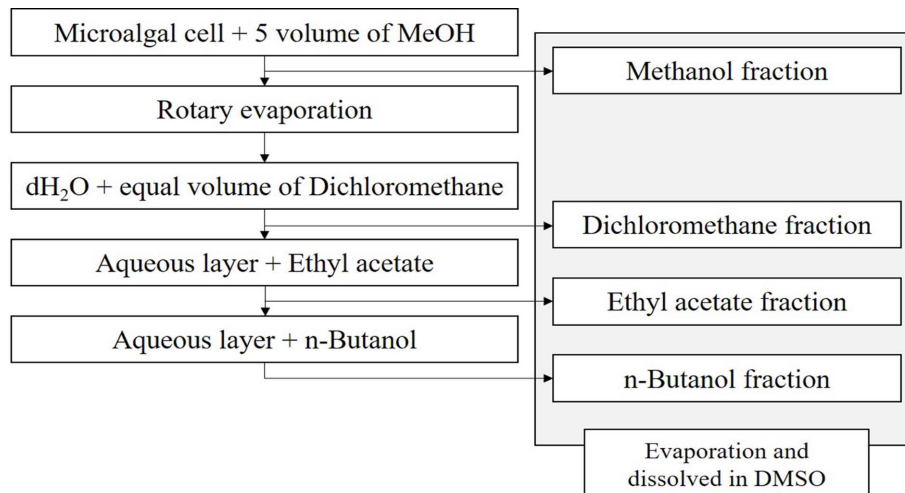


Fig. 1. The extraction process using organic solvent.

extraction at 25°C, 150 rpm for 48 h. Then extracts were concentrated on a rotary evaporator. 100 ml of sterile distilled water and 100 ml of dichloromethane were added in methanol extract and performed extraction at 25°C, 150 rpm for 48 h. In the same way, ethyl acetate fraction was obtained. The butanol fraction was obtained using butanol in the same way. Each obtained solvent fraction layer was concentrated on a rotary evaporator, and then dissolved in DMSO reach at 10 µg/ml. Remain the aqueous layer was used as H₂O extract after evaporation of the solvent (Fig. 1).

Total polyphenol contents

For the measurement of total polyphenol contents according to extraction solvents, 10 µl of sample and 200 µl of 2% Na₂CO₃ were added in 96 well microplates. Next, a reaction for 3 min at room temperature, and then, a reaction for 30 min adding 10 µl of 50% Folin solution. Done to reaction, optical density was measured at 700 nm using a microplate reader and calculates of total polyphenol contents in the following equation.

$$\text{Total polyphenol contents (\%)} = \frac{\text{control OD} - \text{sample OD}}{\text{control OD}} \times 100$$

DPPH radical scavenging activity

To measure the DPPH radical scavenging activity according to extraction solvents, 25 µl of sample and 75 µl of 0.2 mM DPPH solution were added in 96 well

microplates and reaction for 30 min at dark condition. After the reaction, optical density was measured at 517 nm by a microplate reader. Ascorbic acid (1 mg/ml) was used to positive control and calculate the following equation.

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{control OD} - \text{sample OD}}{\text{control OD}} \times 100$$

ABTS radical scavenging activity

The ABTS radical scavenging activity was measured. In a 96 well microplate, 50 µl of sample and 50 µl of ABTS solution were added and reacted for 30 min at dark conditions. After the reaction, measuring the absorbance at 734 nm in the microplate reader. The ABTS solution was used after reaction at 24 h made of one by one mixture as 7.4 mM ABTS and 2.6 mM potassium persulfate. Ascorbic acid (1 mg/ml) was used to positive control and calculate the following equation.

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{control OD} - \text{sample OD}}{\text{control OD}} \times 100$$

The measure of tyrosinase inhibition activity

The 110 µl of 0.1 M sodium phosphate buffer (pH 6.5) and 10 µl of mushroom tyrosinase (500 U/ml) were mixed in order loaded into 96 well microplates. The 20 µl of tyrosine was adding to that mixture and reaction for 15 min at 37°C. The reaction was finished in ice for 5 min, and then absorbance was measured at 475 nm

using a microplate reader. The positive control was used of ascorbic acid (1 mg/ml). The calculate the following equation.

$$\text{Tyrosinase inhibition activity (\%)} = \frac{(\text{control OD} - \text{sample OD})}{\text{control OD}} \times 100$$

In each item's calculation formula, the control OD was calculated by adding the same amount of water instead of the extract. Also, the resulting figure was converted into a relative value for the result of 1 mg/ml ascorbic acid used as a positive control group and is schematically illustrated.

Statistics processing

All experiments were repeated three times. Statistical processing was calculated as mean \pm S.D for each sample, and the significance was verified by calculating the p-value.

Results and Discussion

It is known that there are more than 100,000 kinds of microalgae worldwide, and some of them have been confirmed to function. Active research is being conducted in fields such as energy, industrial material production, and greenhouse gas reduction. In particular, major companies in the world invest in new and renewable energy and are making efforts to secure technology. Among the

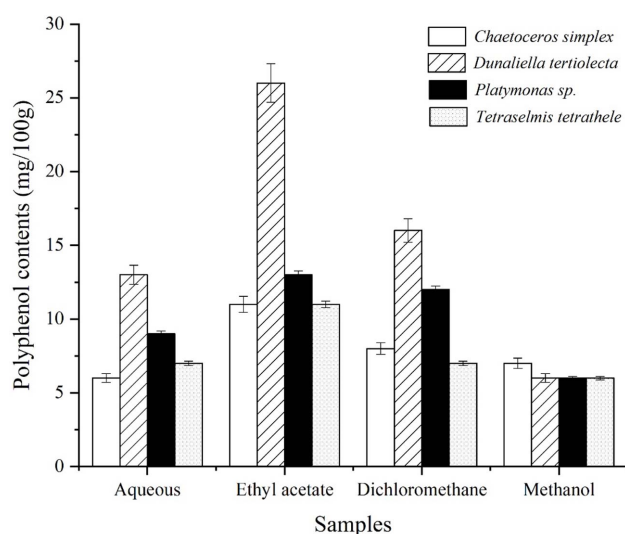


Fig. 2. Total polyphenol contents of microalgal extracts.

fields where the use of microalgae is likely to be expanded is the chemical field. Currently, the food sector industry using *Chlorella* and *Spirulina* is the most active [9], but it is expected to expand into fields such as pharmaceuticals, cosmetic raw materials, and bioplastics [10].

The standard substance in the measurement of total polyphenol content was using tannic acid. The results in measured tannic acid equivalent polyphenol contents, the best one was ethyl acetate extract fraction of *D. tertiolecta*. The content was 26 mg/100 g polyphenol. The next one was the dichloromethane extract fraction of *D. tertiolecta* (16 mg/100 g). Besides, at the aqueous layer of *D. tertiolecta* and the ethyl acetate extract fraction of *Platymonas sp.* were observed 13 mg/100 g of polyphenol contents (Fig. 2). The result was not described in the butanol fraction because no activity was observed in all measurement items. The DPPH radical scavenging occurred only ethyl acetate extract fraction. The scavenging activities were 39.05%, 15.29%, 10.33%, and 8.06% in order *D. tertiolecta*, *C. simplex*, *Platymonas sp.* and *T. tetrahele*, respectively (Fig. 3). The highest value of ABTS radical scavenging activity was 89.77% at ethyl acetate extract fraction of *D. tertiolecta*. In sequentially, ethyl acetate extract fraction and dichloromethane extract fraction of *C. simplex* were showed 62.67% and 58.27%. The dichloromethane extract fraction of *D. tertiolecta*, was showed 57.18% results in the ABTS scavenging activity (Fig. 4). The difference in ABTS and

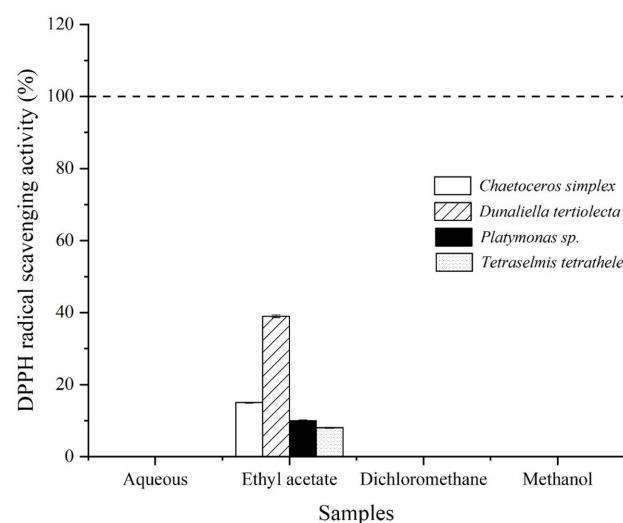


Fig. 3. DPPH radical scavenging activity of macroalgal extracts.

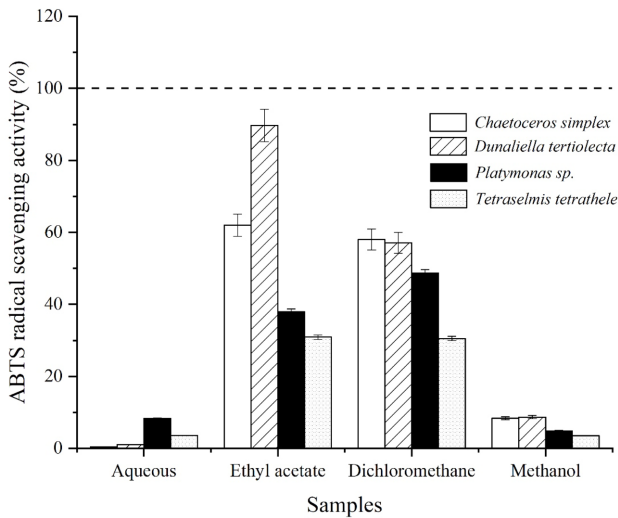


Fig. 4. ABTS radical scavenging activity of macroalgal extracts.

DPPH radical scavenging activity according to the extraction solvent is expected to be due to the difference in the binding capacity of cation radical and free radical. This study investigated the possibility of development as a therapeutic agent for skin diseases, health functional foods, and whitening agents using four types of microalgae and extracted antioxidants using organic solvents to measure antioxidant and tyrosinase inhibitory activities. As a result of the antioxidant activity test, the best activity was found in the ethyl acetate extract of *D. tertiolecta*. The dichloromethane extract of *D. tertiolecta* and the ethyl acetate extract of *C. simplex* also showed relatively good antioxidant activity. Besides, some antioxidant activity was also observed in ethyl acetate extract of *Platymonas sp.* According to a study by Wang *et al.*, it has been reported that total polyphenol compounds are soluble in polar organic solvents such as acetone [11]. Another study reported that ethyl acetate could be a better solvent for polyphenol extraction than water [12]. As a result of this study, when comparing the antioxidant activity according to the organic solvent, ethyl acetate extract and dichloromethane extract showed high antioxidant activity. It is thought that the extraction efficiency was high in the polar organic solvent as the results of the previous researchers.

The tyrosinase inhibitory activity according to the microalgae extract was highest at 124% in ethyl acetate extract of *Platymonas sp.* Moreover, observed 107% and

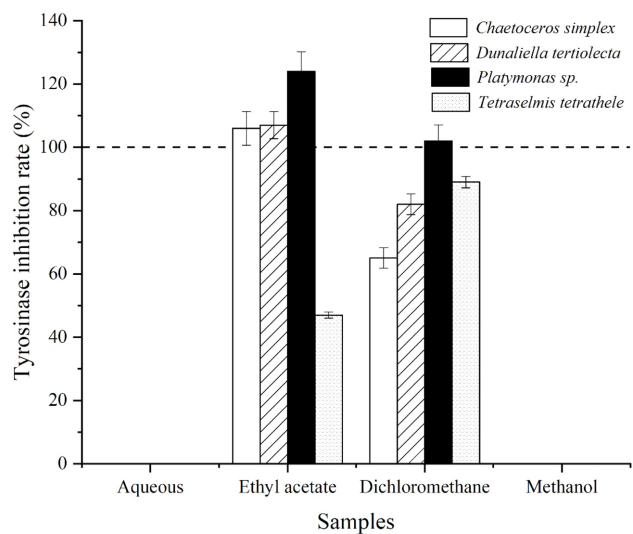


Fig. 5. Tyrosinase inhibition activity of algal extracts.

106% in ethyl acetate extract of *D. tertiolecta* and *C. simplex*. Also, dichloromethane extract of *Platymonas sp.* was 102%. Also, could confirm that the inhibition activity of the 89%, 82%, and 65% in dichloromethane extract on *T. tetrahele*, *D. tertiolecta*, and *C. simplex*, respectively (Fig. 5). As a result of tyrosinase inhibitory activity, ethyl acetate extract of *Platymonas sp.* showed the best activity, and ethyl acetate extract of *D. tertiolecta* and *C. simplex*, and dichloromethane extract of *Platymonas sp.* showed relatively good inhibitory activity. The tyrosinase inhibitory activity was found in ethyl acetate and dichloromethane fraction, but not in the aqueous layer and methanol fraction. Therefore, the active material is expected to have an intermediate polarity. Since they are natural products that have similar ascorbic acid activity and are harmless to the human body, they are considered competitive whitening active substances as their substitutes compared to chemical compounds. When comparing tyrosinase inhibitory activity according to the organic solvent, ethyl acetate extract and dichloromethane extract showed high antioxidant activity.

Among the microalgae used in this study, *Dunaliella* is known to biosynthesize carotenoid pigments such as α -, β -carotene, violaxanthin, neoxanthin, zeaxanthin, and lutein [13]. Also, the use of glycerol biosynthesis, wastewater treatment, and SCP has already been studied [14]. However, *D. tertiolecta* has been studied for

antihypertensive, bronchodilator, antiserotonin, polysynaptic block, analgesic, muscle relaxant, and antioedema activities [15–17], but no results have been reported for antioxidant and whitening activities. A study on the antioxidant activity of *C. simplex* can be found in a report by Karthikeyan *et al.* [18] about 55.69% of DPPH radical scavenging activity was confirmed in acetone extract. As a result of the antioxidant activity measurement of *T. tetrathele*, there is a study report that it has an activity of 2.99 mg GAE/g [19]. As described above, the antioxidant activity of the microalgae used in this study has already been revealed, but this study has a difference as a study that reveals that it has both antioxidant and whitening activity and is further used in product development utilizing the results of this study. It is believed to have provided us with useful information and secured better usability. Abd-El-Baky *et al.* reported that when microalgae were cultured in a stressful environment, the accumulation of antioxidants increased [20]. Also, the *Nannochloropsis oculata* improvement strain, which enhanced the EPA content, obtained by NMU (N-nitroso-N-methylurea) and quinalofop-ethyl treatment [21], and the *Arthrospira platensis* improvement strain, which improved CO₂ fixation through EMS (ethyl methane sulfide) are also reported [22]. To increase the antioxidant and whitening activity of the microalgae used in this study, it is expected that positive and effective utilization in the healthy functional food and cosmetics field is possible if further studies are conducted based on the previous research.

Acknowledgments

This work was supported by the Research Grant of Pukyong National University (year 2019).

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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