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Research article

# Anti-aging potential of cookies from sea grapes in mice fed on cholesterol- and fat-enriched diet: *in vitro* with *in vivo* study

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ABSTRACT

This study determines the effect of cookies made from sea grapes (*Caulerpa racemosa*) on PGC-1α, total cholesterol, and blood glucose levels on mice fed with a Cholesterol- and Fat-Enriched Diet (CFED). The antioxidant activity, tyrosinase inhibition, α-glucosidase, and α-amylase inhibition is also analyzed in order to assess the *in vitro* anti-aging potential of sea grapes cookies. Forty male *Mus muscullus* albino mice weighing 20 g–30 g were used and randomly distributed into four groups of ten animals each. Group A served as a normal control (given a standard dry pellet diet), Group B was given CFED only, and mice in Groups C and D were given CFED with 100 mg and 200 mg/20 g body weight of sea grapes cookies, respectively for 4 weeks. *In vitro* study shows that the percentage of inhibition activity of antioxidant, L-Tyrosine, L-Dopa, α-glucosidase, and α-amylase inhibition were 45.65 ± 1.50, 8.95 ± 0.06, 21.31 ± 0.98, 77.12 ± 4.67 and 70.94 ± 0.98, respectively. This study found that group D had better activity in lowering blood glucose than group C ($p < 0.0001$). In addition, although there was not found significant difference between groups C and D in blood cholesterol reduction and PGC-1α ($p = 0.1482$), both groups experienced the same effect in total cholesterol reduction and PGC-1α in mice (significantly, $p <$ 0001). Thus, we conclude that sea grapes cookies are proven to improve PGC-1α, total cholesterol, and blood glucose levels in mice fed with CFED. Hence, sea grapes cookies is a potential anti-aging novel-functional food.

## 1. Introduction

Marine macroalgae contains not only nutrients, but also functional polyphenol, antioxidant, pigment, and lipid [1]. One of such macroalgae is Sea Grapes (*Caulerpa racemosa*) [1]. This species has been on FAO database as one of two main macroalgae that can be cultivated commercially and is mostly used as food for human consumption in countries such as Philippines, Malaysia and Indonesia [2]. Sea grapes have many health benefits as it is rich in bioactive peptides, fibers, minerals, vitamins, polyunsaturated fatty acids (PUFAs), and bioactive antioxidants and it can potentially be developed into various food products [3].

Sea grapes contain typical compounds, such as caulerpin and caulerpenin [3]. Both compounds are of bioactive antioxidant [4, 5]. In

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addition, sea grapes contain secondary metabolites such as phenols, saponins, tannins, flavonoids, xanthoproteins, sesquiterpenoids, diterpenoids, sitosterol, caulerpicin, gallochincatechin, epicatechin, and catechin gallate [6, 7, 8]. Caulerpin (CLP) as a typical alkaloid sourced from algae of the genus Caulerpa, has shown potent anti-inflammatory activity [9]. Some studies have exposed that the diet of sea grapes has the potential in the prevention and treatment of neurodegenerative, chronic diseases, and aging-related diseases [2, 3, 10].

Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α) is a transcriptional co-activator known for its role in regulating mitochondrial synthesis, glucose-lipid metabolism, and conversion of skeletal muscle fiber types [11]. Recent research proves that PGC-1α can lower Reactive Oxygen Species (ROS)-oxidative stress in diabetic cardiomyopathy patients [12]. Decreased oxidative ROS, blood glucose, and total cholesterol are associated with elevated serum levels of PGC-1α [12]. This suggests that, by improving serum PGC-1α, blood glucose and total cholesterol may be alternatives to the prevention and treatment of neurodegenerative, chronic diseases and aging-related diseases [11, 12, 13].

Aging is the process by which structural and functional changes accumulate in the body due to multiple factors, including oxidative damage caused by reactive oxygen species (ROS) [27]. ROS is a reactive molecule consists of oxygen from the reduction in oxygen molecule within cell [12]. A balance between ROS and detoxification leads to longevity [28, 29]. High concentration of ROS can induce cell damage that plays a vital role in neurodegenerative, cancer, host defense interference, innate immune response related diseases and also metabolic dysfunction due to DNA, RNA, carbohydrate, protein and lipid oxidation that causes cell death [28, 29]. Recently, antioxidant rich functional food has rose into popularity due to its effect on ROS and the emergence of chronic and aging related diseases [30]. ROS can accelerate skin aging process through oxidative stress from UV light, while consumption of antioxidant rich functional food can inhibit the process [28, 29, 30]. Antioxidants, including phenolic acids and flavonoid (phenolic derivatives), that contains hydroxyl group can act as a hydrogen donor that plays a vital role in stabilizing free radicals, thus inhibiting the production of new radicals [31].

Early aging is a result of overproduction of melanin or hyperpigmentation and skin damage that is caused by intense UV light exposure which causes skin to turn dark (depigmentation) [32]. Tyrosinase inhibitor is one of the most effective mechanisms to prevent depigmentation [33]. Tyrosinase converts tyrosine into DOPA (3, 4-dihydroxyphenylalanine), and then further converted into dopaquinone, and melanin as an end product [34]. The (Ultra-Violet) UV, pollutants, smoking, unhealthy diet, and lifestyle, as external factors contribute to the production of free radicals and ROS [35]. This condition stimulates skin inflammation that triggers series of biochemical reaction on the skin which causes collage dermis tissue damage and early skin aging [34, 35].

Referring to the mechanism discussed on the previous paragraphs, sea grapes that contain various bioactive compounds could be developed into several antioxidant rich food products. The study was intended to invent cookies based on the antioxidants of sea grapes and analyzed their effects on blood glucose levels, total cholesterol, and serum PGC-1α on mice *(Mus musculus)* or preclinical (*in vivo*) studies, and also to know the benefits of sea grapes cookies as a potential functional food product for anti-neurodegenerative diseases, chronic diseases, and aging-related diseases. Antioxidant activity, tyrosinase, α-glucosidase, and α-amylase inhibitory activity are also analyzed in order to assess the *in vitro* anti-aging potential of the sea grapes cookies as a functional food product.

## 2. Materials and methods

*In vivo* experimental or preclinical studies were conducted at the Faculty of Mathematics and Natural Sciences Pharmacological Laboratory Center at Sam Ratulangi University, Indonesia.

### 2.1. Cookies sample preparation

Fresh sea grapes was dried at room temperature for about 5 h to reduce moisture. Fresh sea grapes (*Caulerpa racemosa*) are collected from the shallow (510 m above sea level) seawater of Mantehage in North Sulawesi, Indonesia. Plant identification has been confirmed at Sam Ratulangi University (Pharmacology Laboratory) in Indonesia. Rinse the sea grapes thoroughly with water, dry them in an oven at 40 °C with room temperature air, and then smooth them with an electric mill. Following the production of the extract, the coarse powder is accelerated in 95% ethanol for 72 h and each extraction is performed 3 times, resulting in a yield of 34%. The extract was roughly filtered through Whatman 41 filter paper. To produce a viscous extract, the entire filtrate is compressed and evaporated in a reduced pressure (100 mbar) RV 8 IKA rotary evaporator at 40 °C for 90 min and then in an oven at 40 °C. A refrigerator was used to store the extract powder with 10 °C.

Mix the margarine, refined sugar, and eggs. Beat using a mixer until evenly distributed. Put ingredients (wheat flour, cornstarch, milk flour, and banana flour) one by one while sifting using a fine sieve, stirring with a spatula until evenly distributed. Next, add fish bone meal and sea grapes powder, stirring well with a spatula. Preheat the oven. Prepare a baking sheet, apply thinly with margarine. Take 1 tsp of dough and put it on a baking sheet. Flatten using a fork or fingertip. Do it until all the dough is gone. Bake the cake in the oven at 150 °C for 30 min until cooked through. Lift. Refrigerate, store in a jar or airtight aluminum foil packaging.

The cookies formulation above (Table 1) refers to the cookie processing quality standard (Indonesian National Standard, SNI 01-2973-1992) conducted by *Dr. Siti Chairiyah Batubara, S.T.P., M.Si* (Food Technology Expert-Certified, Sahid University Jakarta).

### 2.2. In vitro antioxidant activity test with DPPH

The stock of 100 mg sea grapes cookie sample dissolved in 50 ml methanol is made into several concentrations (100 mg cookies/50 ml in methanol). A total of 0.2 ml of the sea grapes-cookie solution is added with 1 ml 2,2-diphenyl-1-picrylhydrazyl (DPPH) and added with 0.8 mL absolute methanol until the volume is 2 mL. After incubated for 30 min in a dark room, the mixture is measured for its absorbance at 517 nm against the methanol [41].

### 2.3. In vitro tyrosinase inhibitory activity test

Measurements of tyrosinase enzymes (L-tyrosine and L-DOPA) inhibitory activity are associated with Permatasari, 2021 [7]. *In Vitro* tyrosinase enzyme inhibition test, L-tyrosine (Sigma-Aldrich) and L-DOPA (Sigma-Aldrich) as substrates, and $C_6H_6O_4$ (kojic acid; Sigma-Aldrich) was used as a positive control. The sample was dissolved using dimethyl sulfoxide (DMSO, $(CH_3)_2SO$; Sigma-Aldrich) as a stock solution. The change in concentration was made by dissolving collagen using phosphate buffer at pH 6.5. Next, 70 μL of solution was pipetted

**Table 1.** Cookies formulation for one kilogram of dough.

| Ingredients | Tools |
|---|---|
| 600 g (60%) Sea grapes Extract Powder | Oven |
| 50 g Banana Flour *(Musa paradisiaca)* | Mixer |
| 50 g Margarine | Blender |
| 50 g Corn Starch | Container |
| 50 g Refined Sugar | Molder |
| 15 g Skimmed Milk Powder | |
| 50 g Tuna Bone Meal *(Thunnus sp)* | |
| 185 g Egg Yolk | |
| 50 g Wheat Flour | |

into a 96-well plate, then 30 μL of tyrosinase enzyme (Sigma-Aldrich, phosphate buffer of 333 units mL-1) was added and mixed. Then incubate for 5 min. Next, 110 μL of L-tyrosine substrate (2 mM) was added and the solution was incubated (37 °C; 30 min). Absorbance of microplate reader was measured at 492 nm using a spectrophotometer (UV-VIS AMV11).

### 2.4. In vitro α-glucosidase inhibitory activity test

The inhibitory activity of α-glucosidase was determined using the method presented by Permatasari [7]. The α-glucosidase solution (SigmaAldrich; 1.52 IU/ml) was mixed with 1 mg of powder (76 IU) and 50 ml of phosphate buffer (pH 6.9) received. The solution was then stored at 20 °C. Sea grape cookies with a gradient of 0.1 ml (3 mg/ml) were then mixed with 0.35 ml of sucrose (65 mm) and maltose solution (65 mm). After heating (37 °C, 5 min), 0.2 ml of an α-glucosidase solution was added to the preheated system and reacted at 37 °C for 15 min. The reaction was carried out by heating the system in a water bath at 100 °C for 2 min. In this experiment, acarbose was used as a positive control. The control treatment (acarbose) was identical to the treatment of sea grape cookies. The activity of α-glucosidase was expressed as the level of glucose production in the experiment. 0.2 ml of the test solution was added to the solution obtained in the α-glucosidase inhibition test, and then 3 ml of the colorant was added to the reaction system. The system was then heated to 37 °C. The optical density of the solution was measured at a wavelength of 505 nm in a spectrophotometer (UVVIS AMV11) for 5 min.

### 2.5. In vitro α-amylase activity test

500 μl of the diluted sample, 500 μl of 0.02 M sodium phosphate buffer (pH 6.9, 0.006 M NaCl) and 0.5 mg/ml porcine pancreatic amylase (effective concentration 3.2.1.1) were incubated at 25 °C for 10 min. Then, 500 μl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9; 0.006 M NaCl) was added to each mixture. The mixture was incubated for 10 min (25 °C) and quenched with 1.0 ml of staining reagent 3,5-dinitrosalicylic acid (C7H4N2O7). The mixture was incubated in a water bath at 100 °C. for 5 min and left at room temperature. Then, 10 ml of distilled water was added to dilute the mixture, and the optical density was measured at a wavelength of 540 nm with a spectrophotometer (UV-VIS AMV11). The reference sample contains all reagents and enzymes except the sample itself. We then calculated the percent enzyme inhibitory activity of the sea grape biscuit solution according to a previous study. Acarbose (SigmaAldrich) was used as a positive control.

### 2.6. Animal handling and ethical approval

The research protocol or the usage of experimental animals is based on The Council for International Organizations of Medical Sciences (CIOMS) with the Declaration of Helsinki, this protocol has been registered at https://preclinicaltrials.eu (International Register of Preclinical Trials Protocols) with No. PCTE0000257 and has been approved by the Ethics Committee of the General Hospital of Education Prof. Dr. RD. Kandou 084/EC/KEPK-KANDOU/VI/2021. All of the mice in the study had standardized free access to food and water. The Laboratory Animals Farming Makassar, Indonesia, provided forty (40) male Swiss albino mice (*Mus musculus*) (3–5 weeks) weighing between 20 g - 30 g. The animals were separated into groups and placed in cages, where they were housed in conventional laboratory conditions (at 27 ± 2 °C) with light-dark cycles (12 : 12 h). Mice were acclimated to typical laboratory conditions (10 days) before the intervention began. Intake of food and water is affected by ambient temperature; therefore, the environment is strictly maintained at room temperature or standard animal laboratory temperatures.

### 2.7. In vivo studies of cookie sea grapes antioxidant-extract against PGC-1α, total cholesterol, and blood glucose levels

#### 2.7.1. CFED production

The prior study is referenced in the CFED production [7]. CFED was created using conventional mouse feed (dry pellets) supplemented with 1% cholic acid, 2% pure cholesterol powder, 20% fat (animal source), and 2% corn oil. After thoroughly homogenizing all of the ingredients, 1000 mL of distilled water was added and the pellets were shaped into smaller pieces (Pellets). To decrease CFED oxidation by air, the pellets were allowed to dry at ambient temperature under sterile conditions before being kept at 4 °C. Carbohydrates account for 43.57 percent of CFED, whereas protein accounts for 12.38 percent, fiber for 4.73 percent, fat for 3.17 percent, cholesterol for 2%, cholic acid for 1%, animal fat for 20%, maize oil for 2%, total ash for 4%, and moisture for 6.85%.

#### 2.7.2. Cookies sea grapes administration scheme

The mice were randomly divided into 4 groups, each group consisting of 10 mice (10 mice in each treatment group, so there were 10 replications from each group). Group A received a standard diet or standard pellets as a normal control, followed by groups B, C, and D receiving CFED with a 4-week intervention. Group B only CFED diet. Group C were treated with cookies 100 mg/20 g body weight (BW) and group D 200 mg/20 g body weight (BW) (each) daily for 4 weeks. CFED and sea grapes cookies were orally administered.

#### 2.7.3. Biomedical analysis of blood samples [7, 21]

A biochemical analyzer (Roche's COBAS Integra® 400 plus analyzer) was used to check blood glucose and total cholesterol levels. 1X Phosphate Buffered Saline (PBS) with pH 7.4 was used to wash the sample until the laundry liquid is clear. To get pellets and supernatants, a PBS solution containing 1% of the sample is concentrated for 20 min at 3000 rpm. The supernatant is used to test for alpha PGC-1α. Sunlong Biotech Co., Ltd. used the PGC Mouse 1 ELISA Kit to assess the alpha concentration of PGC-1.

### 2.8. Data management and analysis

The data (PGC-1α, Total Cholesterol and PGC-1α measurement data in each group) is statistically analyzed using the MANOVA or Multivariate ANOVA test and the data were presented in the form of Mean ± SEM, via GraphPad Prism for version 9 Software of the MacBook. Antioxidant activity, tyrosinase inhibition, α-glucosidase, and α-amylase inhibition test was carried out in three replicates (Triplo) and the data were presented in the form of Mean ± SEM, using GraphPad Prism for version 9 of the MacBook. Paired T-Test was conducted to determine the significant differences between initial body weight (g) and final body weight (g), in each group. One-Way ANOVA was conducted to determine the significant differences between each parameter (Initial Body Weight (g), Weight Gain (g/day), Food Intake (g), Final Body Weight (g), Water Intake (mL) dan Food Efficiency Ratio (FER, %)) of each group. The data was processed in 95% confidence interval (CI) and presented in Mean ± SEM.

## 3. Results

### 3.1. In Vitro activity

The results of in vitro assays of sea grape Cookies (Caulerpa racemosa) showed by Table 2. Percent inhibitory activity of antioxidants (45.65 ± 1.50), L-Tyrosine (8.95 ± 0.06), L-Dopa (21.31 ± 0.98), α-glucosidase (77.12 ± 4.67), and α-amylase (70.94 ± 0.98). The tyrosine enzyme inhibitory activity using L-Dopa and L-Tyrosine as substrates showed that these sea grape cookies were also better than the positive control (in this case Kojic Acid with an $IC_{50}$ of 8.90 part per million (ppm)). The inhibitory activity of α-glucosidase and α-amylase of sea

**Table 2.** In Vitro study of Cookies from Sea grapes.

| | Antioxidant Activity (%) | *Tyrosinase Inhibition (%) | | Cookies α-Glucosidase Inhibition (%) | Acarbose α-Glucosidase Inhibition (%) | Cookies α-Amylase Inhibition (%) | Acarbose α-Amylase Inhibition (%) |
|---|---|---|---|---|---|---|---|
| | | L-Tyrosine | L-Dopa | | | | |
| 1 | 45.65 | 9.02 | 20.50 | 71.73 | 98.7 | 71.94 | 85.73 |
| 2 | 44.65 | 8.93 | 22.40 | 80.02 | 96.8 | 70.90 | 86.90 |
| 3 | 47.15 | 8.90 | 21.03 | 79.63 | 97.91 | 69.98 | 86.45 |
| Average | 45.65 ± 1.50 | 8.95 ± 0.06 | 21.31 ± 0.98 | 77.12 ± 4.67 | 97.80 ± 0.95 | 70.94 ± 0.98 | 86.36 ± 0.59 |

* Tyrosinase at 1000 ppm, Kojic Acid $^{IC}50$: 8.90 ppm.

grape cookies is close to the inhibitory activity of α-glucosidase and α-amylase of acarbose (antidiabetic drug that is used as positive control) which can inhibit up to 77% and 86% (Table 2).

### 3.2. Body weight characteristic, feed and water intake, and feed efficiency ratio of sample mice

Body weight, weight gain, feed and water intake, and feed efficiency ratio (FER) of the mice was presented in Table 3. On Group B (received only CFED diet), has a final weight of 30.17 ± 0.20 g and daily weight increase of 0.31 ± 0.07 g/day. The increase was significantly higher compared to control group, group C and D. The consumption of both doses of sea grape cookies also affected Final Body Weight (g) and Weight Gain (g/day) within the mice that were given CFED diet. Nonetheless, when Independent T-test on Final Body Weight (g) and Weight Gain (g/day) was conducted on group A and C, the difference was not significant. The result showed that there was not found significant difference on Final Body Weight (g) and Weight Gain (g/day) between group A and C ($p = 0.6962$) (Figure 1). This result showed that the increase of body weight in group C was considered normal. FER was significantly higher in CFED group (6.51 ± 1.59%) compared to the other groups ($p = 0.000$). Feed and water intake between groups were not significantly different $p = 0.599$ and $p = 0.973$, respectively.

Based on the results of the normality test, the data from all three variables (Blood Sugar, Total Cholesterol, and PGC-1α) show $p > 0.05$, meaning that the data distribution is normal (supplementary Table 1; supplementary Table 2 and supplementary Table 3). The data is then analyzed statistically using the MANOVA/Multivariate ANOVA test (supplementary Table 4; supplementary Table 5 and supplementary Table 6).

### 3.3. Effects of Cookies on lowering blood sugar levels

Figure 2 showed that blood glucose improved significantly compared to the control group when given a CFED diet ($p < 0.05$). Blood glucose decreased significantly in both the control group and the treatment group when given the CFED + sea grapes cookie treatment of 100 mg/20 g body weight (BW) and the CFED + sea grapes cookie treatment of 200 mg/20 g body weight (BW) ($p < 0.05$). The effect of administering sea grapes cookies with the dose of 100 mg/20 g body weight (BW) is more effective than the administration of sea grapes cookies with the dose of 200 mg/20 g body weight (BW) in lowering blood glucose mice significantly ($p < 0.05$).

### 3.4. Effect of cookies on total cholesterol levels

Figure 3 showed that total cholesterol increased significantly compared to the control group when given a CFED diet ($p = 0.0001$). Total cholesterol decreased significantly in both the control group and the treatment group when given the CFED + sea grapes cookie treatment of 100 mg/20 g body weight (BW) and the CFED + sea grapes cookie treatment of 200 mg/20 g body weight (BW) ($p < 0.05$). The effect of administering sea grapes cookies with the dose of 100 mg/20 g body weight (BW) was more effective than the administration of sea grapes cookies with the dose of 200 mg/20 g body weight (BW) in lowering total cholesterol in mice, but not significantly ($p > 0.05$).

### 3.5. Effect of cookies on improving PGC-1α

Figure 4 showed that PGC-1α decreased significantly compared to the control group when given a CFED diet ($p = 0.0001$). PGC-1α increased significantly in both the control group and the treatment group when given the CFED + sea grapes cookie treatment of 100 mg/20 g body weight (BW) and the CFED + sea grapes cookie treatment of 200 mg/20 g body weight (BW) ($p < 0.05$). The effect of administering sea grapes cookies with the dose of 100 mg/20 g body weight (BW) was more effective than the cookie of sea grapes with the dose of 200 mg/20 g body weight (BW) in increasing serum PGC-1α on mice, but not significantly ($p > 0.05$).

## 4. Discussion

Sea grapes (*Caulerpa racemosa*) is a natural marine material that can be processed and developed into various forms of food products, such as cookies in this study. Cookies based on this formulation, namely with the addition of the basic ingredients of sea grapes extract powder 60% have 45.65 ± 1.50% antioxidant activity *in vitro* against 2,2-diphenyl-1-picryl-hydrazyl (DPPH) (Table 2). Sea grapes have a lot of health-beneficial

**Table 3.** Body weight characteristic, food and water intake, and feed efficiency ratio of sample mice.

| Groups | A | B | C | D | p-value** |
|---|---|---|---|---|---|
| Initial Body Weight (g) | 22.21 ± 0.40 | 21.49 ± 0.51 | 22.3 ± 0.41 | 22.46 ± 0.47 | 0.456 |
| Final Body Weight (g) | 23.53 ± 0.42 | 30.17 ± 0.20 | 23.7 ± 0.48 | 25.16 ± 0.61 | 0.000 |
| ***p-value**** | ***0.000*** | ***0.000*** | ***0.004*** | ***0.001*** | |
| Weight Gain (g/day) | 0.05 ± 0.01 | 0.31 ± 0.07 | 0.05 ± 0.04 | 0.09 ± 0.03 | 0.000 |
| Food Intake (g) | 4.38 ± 0.63 | 4.78 ± 0.77 | 4.54 ± 0.58 | 4.56 ± 0.62 | 0.599 |
| Water Intake (mL) | 5.15 ± 0.34 | 5.20 ± 0.29 | 5.24 ± 0.56 | 5.20 ± 0.54 | 0.973 |
| FER (%) [1] | 1.11 ± 0.46 | 6.51 ± 1.59 | 1.03 ± 0.74 | 2.13 ± 0.77 | 0.000 |

* Paired T-Test was conducted to determine the significant difference between initial body weight (g) and Final Body Weight (g) of each group. ** One-Way ANOVA was conducted to determine the significant difference of each parameter (Initial Body Weight (g), Final Body Weight (g), Weight Gain (g/day), Food Intake (g), Water Intake (mL) dan Food efficiency Ratio (FER, %) of each group [1]. Food Efficiency Ratio (FER, %) = (Body weight gain (g/day)/food intake (g/day))×100.

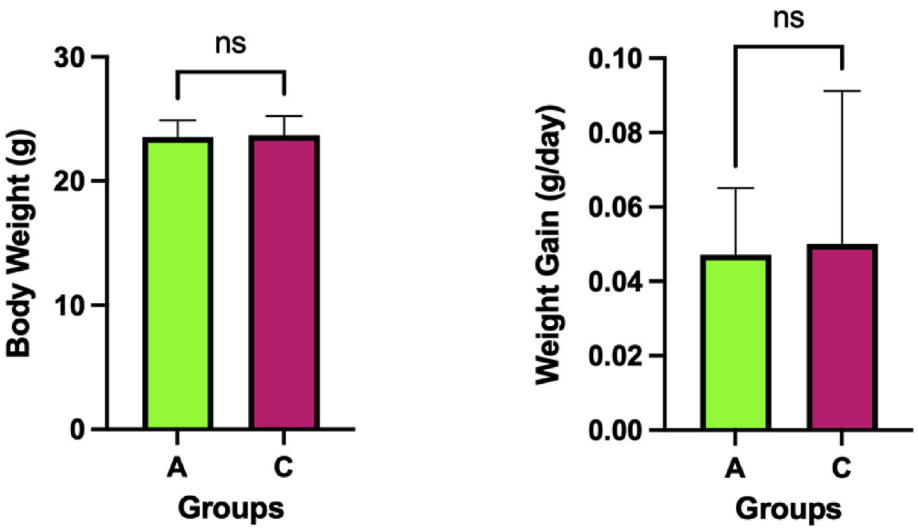


**Figure 1.** Independet-T test for Final Body Weight (g) and Weight Gain (g/day) in group A and C. (ns) = Not Significant p = 0.6962 (p > 0.05) at CI 95%.

potentials, De Gaillande et al. [3] wrote a review study in 2017 that described the profile of sea grapes that is rich in protein, fiber, minerals, vitamins, polyunsaturated fatty acids, and bioactive antioxidants. Some of these bioactive compounds with potential antioxidative activity include phenols, saponins, tannins, flavonoids, xanthoproteins, sesquiterpenoids, diterpenoids, sitosterol, caulerpicin, caulerpin, caulerpenin, gallocatechin, epicatechin, and catechin gallate [6, 7, 8]. Consumption of antioxidants is important in the prevention of degenerative diseases or non-communicable diseases, Systematic studies review has shown that adequate intake of dietary sources of antioxidants is associated with a lower risk of chronic disease and aging [15]. Evidence for the protective effects of antioxidants has been presented in experimental, clinical, and epidemiological studies, which have shown that antioxidants may help treat diabetes and its complications [16, 17, 18]. Therefore, the need for exploration and innovation of functional food sources of antioxidants as an effort to minimize the events of non-communicable diseases such as diabetes and their complications.

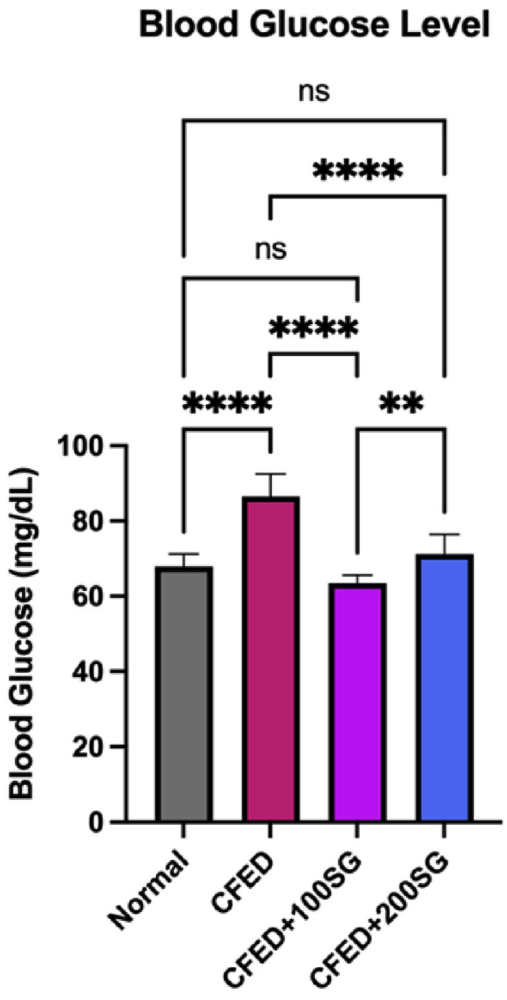


**Figure 2.** Low Doses of Sea grapes Cookies Significantly Lower Blood Glucose. (****)p < 0.0001; (**)p < 0.0021; (ns)p > 0.05.

The difference of average feed and water intake of mice was not significant (Table 3). This range follows the normal conditions (not under stress) which refer to or are in accordance with Heldrich et al, (2004) [14].

In line with the antioxidant levels in these sea grapes-based cookies, the results of *in vivo* studies conducted on mice given cholesterol- and fat-enriched diet showed that the administration of cookies made from sea grapes can significantly reduce blood sugar levels (Figure 2 and Figure 5). Furthermore, Figure 5 shows that a dose of 100 mg/20 g body weight (BW) is more effective than the administration of sea grapes cookies with the dose of 200 mg/20 g in lowering blood glucose significantly (p = 0.0021). These results support other similar studies utilizing sea grapes as a basic ingredient, namely the administration of kombucha tea from sea grapes has the activity of lowering blood glucose levels in mice fed a diet high in cholesterol and fat [7]. In line with this, antioxidants contained in a food ingredient can contribute to the reduction of oxidative stress, cell damage, and protein caused by the incidence of chronic hyperglycemia [19, 20].

The decrease in blood glucose levels in mice in this study also correlated positively with a decrease in total cholesterol. Total cholesterol decreased significantly both in the group when given the 100 mg/20 g body weight (BW) sea grapes cookie treatment and the 200 mg/20 g body weight (BW) sea grapes cookie (p < 0.05) (Figure 3). This is also supported by other studies that show that the administration of sea grapes extract can significantly reduce total cholesterol [21, 22]. As well as research on the utilization of sea grapes into fermented products by Permatasari et al (2021), this sea grapes-based product has a significant total cholesterol reduction activity [7]. The results of the total cholesterol reduction in this study are also in accordance with other studies, providing the evidence that sea grapes has antidiabetic and anti-hyperglycemic activities that are beneficial to health, in particular

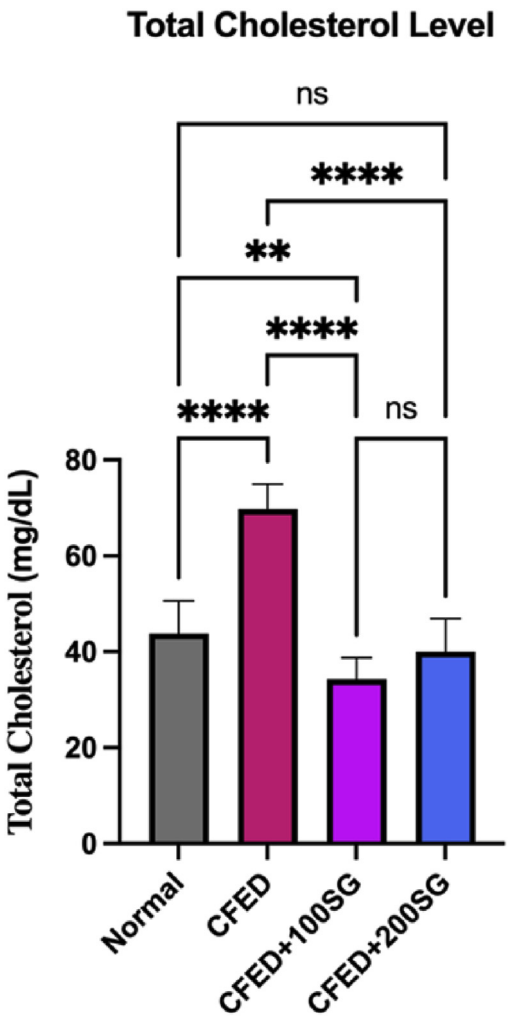


**Figure 3.** Both Doses of Sea grapes Cookies Significantly Lowered Total Blood Cholesterol. (****)$p < 0.0001$; (**)$p < 0.0048$; (ns)$p = 0.4846$.

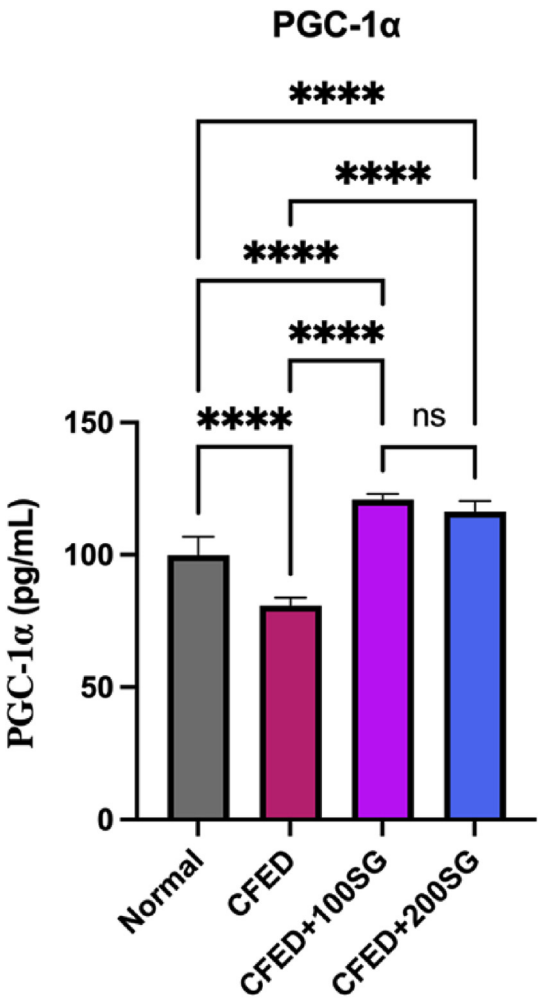


**Figure 4.** Both Doses of Sea grapes Cookies Significantly Increase Serum PGC-1α. (****)$p < 0.0001$; (ns)$p = 0.1294$.

minimizing aging and chronic diseases [6, 19]. The effect of administering sea grapes cookies with the dose of 100 mg/20 g body weight (BW) is more effective than the administration of sea grapes cookies with the dose of 200 mg/20 g body weight (BW) in lowering total cholesterol in mice, but not significantly ($p > 0.05$) (Figure 5).

In addition to the reduction in blood sugar and total cholesterol, the study showed that administering sea grapes-based cookies also significantly increased serum levels of *Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha* (PGC-1α) ($p < 0.0001$) (Figure 4 and Figure 5). This proves that cookies from these sea grapes can have beneficial effects on health because the increase in PGC-1α correlates with increased antioxidant activity, which inhibits oxidative stress induced by reactive oxygen species (ROS) [12]. Polyphenols, antioxidants, and flavonoids in sea grapes may also contribute to these results [23, 24]. Such conditions can improve metabolism and aging, by looking at the function of PGC-1α as a regulator of biogenesis and skeletal muscle angiogenesis [25, 26].

Furthermore, sea grape cookie treatment will reduce glucose and cholesterol levels while increasing PGC-1α levels, along with increasing α-glucosidase inhibitor activity. α-glucosidase inhibitors slow down the digestion of carbohydrates and minimize the uptake of glucose into the circulation [36], hence lowering glucose levels. This sea grape cookie treatment also showed α-glucosidase and α-amylase inhibitory activities of $77.12 \pm 4.67\%$ and $70.94 \pm 0.98\%$ compared to acarbose, an inhibitor of α-glucosidase and α-amylase obtained with inhibitory activity of $97.80 \pm 0.95\%$ and $86.36 \pm 0.59\%$. However, the results of the α-glucosidase inhibitory activity of the sea grape cookie treatment in this study were higher than the α-glucosidase inhibitory activity in other study conducted by Kim et al (2010) using samples from red algae [37]. These results are in line with other studies showing that natural products sourced from the sea have potential α-amylase inhibitory and antidiabetic activities [38].

High melanin production or hyperpigmentation due to excessive UV exposure can darken the skin and cause depigmentation [39]. This case stimulates skin inflammation, causing a series of biochemical reactions in the skin, causing damage to the collagenous tissue of the skin and premature aging of the skin [39]. Therefore, by reducing excess melanin production, the destruction of collagenous tissue in the skin and premature skin aging can be prevented and minimized [40]. Excessive melanin production can be prevented by inhibiting tyrosinase [40]. Table 2 shows the inhibitory activity of the tyrosinase enzyme at a concentration of 1000 ppm. Inhibitory activity of tyrosinase enzyme using L-Tyrosine and LDOPA as substrates indicates that sea grape cookies have inhibitory activity against tyrosine enzyme.

Many additional variables must be observed to be able to claim as an anti-aging and anti-obesity or anti-noncommunicable diseases functional food, such as HDL-C, LDL-C, Cholesterol/HDL-C ratio. Also please be informed due to the limitations of laboratory tools for ophthalmologic-metabolite tests that are contained in this cookie formulation, such as metabolomic tests. Also, this research did not conduct assays regarding aspartate transaminase (AST), alanine aminotransferase (ALT), and blood urea nitrogen (BUN), since this food product is commonly consumed, widely available, and it also does not cause toxicity. The

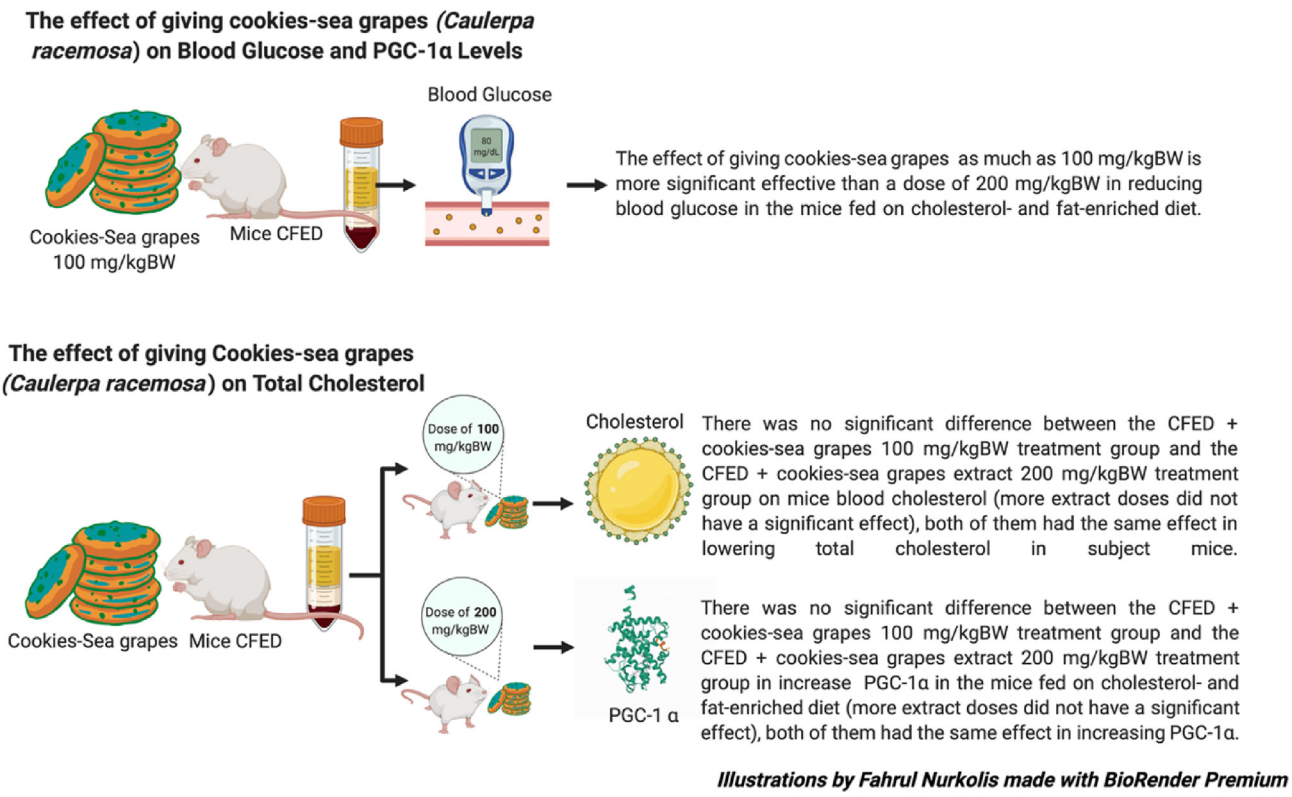


**Figure 5.** Effects of Cookies from Sea grapes *(Caulerpa racemosa)* on Subject Mice.

research done by Aroyehun et al (2020), stated that sea grapes (*Caulerpa racemosa*) can lower glucose concentration, AST, ALT and possess a hepatoprotective activity [38]. However, this study is a recent study aimed at innovating cookies made from sea grapes extracts and observing at their effects on blood glucose levels, total cholesterol, and serum PGC-1α on mice *(Mus musculus)* or preclinical studies.

This study is an *in vivo* or preclinical with *in vitro* study that does not necessarily represent results in humans. However, the formulation and dose of cookies derived from this study can be used as an adjuvant for human clinical trials in future studies. Of course, also seeing the potential of developing these cookies to be produced commercially, food manufacturers, especially cookie products and the government can consider the results of this study to become an instant functional food alternative that can be directly consumed when traveling long distances and for the handling of obesity, especially in Indonesia and Asia.

## 5. Conclusions

This preclinical or *in vivo* in combination with *in vitro* study shows that the utilization of sea grapes as a cookie ingredient can improve blood sugar levels, total cholesterol, and serum PGC-1α, especially at doses of 100 mg/20 g body weight (BW). Aside from that, antioxidant activity, tyrosinase inhibitory activity, α-glucosidase inhibitory activity and α-amylase activity is also observed. These cookies have the potential to become functional food products for anti-chronic diseases and anti-aging.

## Declarations

### *Author contribution statement*

Iskari Ngadiarti, Fahrul Nurkolis: Conceived and designed the experiments; Wrote the paper.

Matthew Nathaniel Handoko, Fachruddin Perdana, Defny Silvia Wewengkang, Siti Chairiyah Batubara: Performed the experiments; Analyzed and interpreted the data.

Happy Kurnia Permatasari, Nurpudji Astuti Taslim, Nelly Mayulu, Sutamara Lasurdi Noor, Melvin Junior Tanner, Nindy Sabrina: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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### *Data availability statement*

Data included in article/supplementary material/referenced in article.

### *Declaration of interests statement*

The authors declare no conflict of interest.

### *Additional information*

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2022.e09348.

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## References


[1] M.K. S R, S.S. FJ B, R.G. M A, Application of seaweeds to develop new food products with enhanced shelf-life, quality and health-related beneficial properties, Food Res. Int. 99 (Pt 3) (2017) 1066–1083.

[2] R.P. Magdugo, N. Terme, M. Lang, et al., An analysis of the nutritional and health values of *Caulerpa racemosa* (forsskål) and *Ulva fasciata* (Delile)—two chlorophyta collected from the Philippines, Mol 25 (12) (2020) 2901, 2020 2901.

[3] C. de Gaillande, C. Payri, G. Remoissenet, M. Zubia, Caulerpa consumption, nutritional value and farming in the Indo-Pacific region, J. Appl. Phycol. 29 (5) (2016) 2249–2266, 2016 295.

[4] R du Preez, M.E. Majzoub, T. Thomas, S.K. Panchal, L. Brown, *Caulerpa lentillifera* (sea Grapes) improves cardiovascular and metabolic health of rats with diet-induced metabolic syndrome, Metabolites 10 (12) (2020) 1–18.

[5] A. Box, A. Sureda, J. Terrados, A. Pons, S. Deudero, Antioxidant response and caulerpenyne production of the alien *Caulerpa taxifolia* (Vahl) epiphytized by the invasive algae *Lophocladia lallemandii* (Montagne), J. Exp. Mar. Biol. Ecol. 364 (1) (2008) 24–28.

[6] A.Q.B. Aroyehun, S.A. Razak, K. Palaniveloo, et al., Bioprospecting cultivated tropical green algae, *Caulerpa racemosa* (forsskal) J. Agardh: a perspective on nutritional properties, antioxidative capacity and anti-diabetic potential, Foods 9 (9) (2020).

[7] H.K. Permatasari, F. Nurkolis, P.S. Augusta, et al., Kombucha tea from seagrapes (*Caulerpa racemosa*) potential as a functional anti-aging food: in vitro and in vivo study, Heliyon 7 (9) (2021), e07944.

[8] P.S. Augusta, F. Nurkolis, S.L. Noor, et al., Probiotic beverage: the potential of anti-diabetes within kombucha tea made from sea grapes (*Ceulerpa racemosa*) containing high antioxidant and polyphenol total, Proc. Nutr. Soc. 80 (OCE3) (2021).

[9] A.M.M. Lucena, C.R.M. Souza, J.T. Jales, et al., The bisindole alkaloid caulerpin, from seaweeds of the genus caulerpa, attenuated colon damage in murine colitis model, Mar. Drugs 16 (9) (2018) 318. Vol 16, Page 318. 2018.

[10] L. Pereira, A. Valado, The seaweed diet in prevention and treatment of the neurodegenerative diseases, Mar. Drugs 19 (3) (2021) 128. Vol 19, Page 128. 2021.

[11] S. Andrzejewski, E. Klimcakova, R.M. Johnson, et al., PGC-1α promotes breast cancer metastasis and confers bioenergetic flexibility against metabolic drugs, Cell Metabol. 26 (5) (2017) 778–787, e5.

[12] N. Zhu, X. Yan, H. Li, H. Wang, Clinical significance of serum PGC-1 alpha levels in diabetes mellitus with myocardial infarction patients and reduced ROS-oxidative stress in diabetes mellitus with myocardial infarction model, Diabetes, Metab. Syndrome Obes. Targets Ther. 13 (2020) 4041.

[13] K. Sharabi, H. Lin, C.D.J. Tavares, et al., Selective chemical inhibition of PGC-1α gluconeogenic activity ameliorates type 2 diabetes, Cell 169 (1) (2017) 148.

[14] H.J. Hedrich, H. Mossmann, W. Nicklas, Housing and Maintenance. Lab Mouse 1, 2004, pp. 395–408. Published online January.

[15] A. Jayedi, A. Rashidy-Pour, M. Parohan, M. Sadat Zargar, S. Shab-Bidar, Dietary antioxidants, circulating antioxidant concentrations, total antioxidant capacity, and risk of all-causemortality: a systematic review and dose-responsemeta-analysis of prospective observational studies, Adv. Nutr. 9 (6) (2018) 701–716.

[16] F.A. Matough, S.B. Budin, Z.A. Hamid, N. Alwahaibi, J. Mohamed, The role of oxidative stress and antioxidants in diabetic complications, Sultan Qaboos Univ. Med. J. 12 (1) (2012) 5.

[17] R. P, N. PP, K. G, M. W, L.P. HJT, The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society, Diab. Metab. Res. Rev. 17 (3) (2001) 189–212.

[18] G.-O. S, H.B. R Y, S B, The effects of combined treatment of antioxidants on the liver injury in STZ diabetic rats, Dig. Dis. Sci. 54 (3) (2009) 538–546.

[19] C. Turpin, A. Catan, A. Guerin-Dubourg, et al., Enhanced oxidative stress and damage in glycated erythrocytes, PLoS One 15 (7) (2020), e0235335.

[20] S.J. Forrester, D.S. Kikuchi, M.S. Hernandes, Q. Xu, K.K. Griendling, Reactive oxygen species in metabolic and inflammatory signaling, Circ. Res. 122 (6) (2018) 877–902.

[21] M. Kuswari, F. Nurkolis, N. Mayulu, et al., Sea grapes extract improves blood glucose, total cholesterol, and PGC-1α in rats fed on cholesterol- and fat-enriched diet, F1000Research 10 (2021) 718.

[22] M. Cao, Y. Li, A.C. Famurewa, O.J. Olatunji, Antidiabetic and nephroprotective effects of polysaccharide extract from the seaweed *Caulerpa racemosa* in high fructose-streptozotocin induced diabetic nephropathy, Diabetes, Metab. Syndrome Obes. Targets Ther. 14 (2021) 2121.

[23] P. Yang, D.Q. Liu, T.J. Liang, et al., Bioactive constituents from the green alga Caulerpa racemosa, Bioorg. Med. Chem. 23 (1) (2015) 38–45.

[24] W.F. Yap, V. Tay, S.H. Tan, Y.Y. Yow, J. Chew, Decoding antioxidant and antibacterial potentials of Malaysian green seaweeds: *Caulerpa racemosa* and *caulerpa lentillifera*, Antibiotics 8 (3) (2019).

[25] J.M. Chambers, R.A. Wingert, PGC-1α in disease: recent renal insights into a versatile metabolic regulator, Cells 9 (10) (2020) 2234, 2020 2234.

[26] M. Khani, P. Motamedi, M.R. Dehkhoda, S. Dabagh Nikukheslat, P. Karimi, Effect of thyme extract supplementation on lipid peroxidation, antioxidant capacity, PGC-1α content and endurance exercise performance in rats, J. Int. Soc. Sport Nutr. 14 (1) (2017) 1–8, 2017 141.

[27] C.T. Madreiter-Sokolowski, C. Thomas, M. Ristow, Interrelation between ROS and Ca2+ in aging and age-related diseases, Redox Biol. (2020) 101678.

[28] T. Finkel, N.J. Holbrook, Oxidants, oxidative stress and the biology of ageing, Nature 408 (6809) (2000) 239–247.

[29] S. Kumari, A.K. Badana, R. Malla, Reactive oxygen species: a key constituent in cancer survival, Biomark. Insights 13 (2018), 1177271918755391.

[30] S. Lordan, R.P. Ross, C. Stanton, Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases, Mar. Drugs 9 (6) (2011) 1056–1100.

[31] F. Shahidi, P. Ambigaipalan, Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects–A review, J. Funct.Foods 18 (2015) 820–897.

[32] N.G. Jablonski, G. Chaplin, Human skin pigmentation as an adaptation to UV radiation, Proc. Natl. Acad. Sci. Unit. States Am. 107 (Supplement 2) (2010) 8962–8968.

[33] Y.J. Kim, H. Uyama, Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future, Cell. Mol. Life Sci. CMLS 62 (15) (2005) 1707–1723.

[34] E.J. Land, P.A. Riley, Spontaneous redox reactions of dopaquinone and the balance between the eumelanic and phaeomelanic pathways, Pigm. Cell Res. 13 (4) (2000) 273–277.

[35] K. Naidoo, M.A. Birch-Machin, Oxidative stress and ageing: the influence of environmental pollution, sunlight and diet on skin, Cosmetics 4 (1) (2017) 4.

[36] E. Thilagam, B. Parimaladevi, C. Kumarappan, S.C. Mandal, α-Glucosidase and α-amylase inhibitory activity of Senna surattensis, J. Acupunc. Merid. stud. 6 (1) (2013) 24–30.

[37] K.Y. Kim, T.H. Nguyen, H. Kurihara, S.M. Kim, α-Glucosidase inhibitory activity of bromophenol purified from the red alga Polyopes lancifolia, J. Food Sci. 75 (5) (2010) H145–H150.

[38] Q.B. Aroyehun, S. Abdul Razak, K. Palaniveloo, T. Nagappan, N. Suraiza Nabila Rahmah, G. Wee Jin, D.K. Chellappan, J. Chellian, A.P. Kunnath, Bioprospecting cultivated tropical green algae, Caulerpa racemosa (Forsskal) J. Agardh: a perspective on nutritional properties, antioxidative capacity and anti-diabetic potential, Foods 9 (9) (2020) 1313.

[39] Nityanand Maddodi, Ashika Jayanthy, Vijayasaradhi Setaluri, Shining light on skin pigmentation: the darker and the brighter side of effects of UV radiation, Photochem. Photobiol. 88 (5) (2012) 1075–1082.

[40] I.A. Ahmed, M.A. Mikail, N. Zamakshshari, A.S.H. Abdullah, Natural anti-aging skincare: role and potential, Biogerontology 21 (3) (2020) 293–310.

[41] R. Rompies, N. Mayulu, F. Nurkolis, F. Faradila, B.J. Kepel, H. Natanael, Antioxidant capacity of snack cookies made from mango and pineapple fermentation, Food Res. 5 (5) (2021 Oct) 145–148.

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