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Antioxidant activity of three microalgae *Dunaliella salina, Tetraselmis chuii* and *Isochrysis galbana* clone Tahiti

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Abstract. Natural alternatives antioxidant source has become a trending topic in the past decades to replace synthetic antioxidant. Microalgae have been mentioned to show interesting bioactive properties and one of them is its antioxidant activity. This study aims to evaluate the potential of three microalgae *Dunaliella salina, Tetraselmis chuii* and *Isochrysis galbanaas* new source of natural antioxidant. Proximate analysis and total phenolic content of *D. salina*, *T. chuii* and *I. galbanas* were determined. Antioxidant activity of methanolic extracts of these three species prepared in different concentration (50, 100, 250, 500, and 1000 ppm) was performed through DPPH assay. *I. galbana* clone Tahiti demonstrated a highest antioxidant potential with 61.64 of inhibition at 50 ppm followed by *D. salina* with 58.45 % of inhibition and *T. chuii* with 52.58 % of inhibition. *I. galbana* clone Tahiti was the best antioxidant with total phenol content of 17.798 mg GAE g^{-1}extract at 50 ppm; followed by *T. chuii* 16.868 mg GAE g^{-1}extract and the lowest was *D. salina* with 4.672 mg GAE g^{-1}extract. Results suggest that these microalgae posses antioxidant potential which could be considered for future applications in medicine, dietary supplements, cosmetics or food industries.

Keywords: antioxidant activity, DPPH, microalgae, total phenol.

1. Introduction

Indonesia, as an archipelagic state surrounded by ocean, presents a potential as a microalgae source because of its huge natural biodiversity. Microalgae are distributed everywhere: in salt, brackish or freshwater, in tropical to cold regions and sometimes as symbionts with other organisms, and some species are used and cultivated by farmers.

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Microalgae, also known as phytoplankton, are common name of all the aquatic autotrophic organisms living in suspension in the water column. This name refers to several phyla, mostly eukaryotes but including the photosynthetic prokaryotes known as cyanobacteria. As photosynthetic organisms, this group plays an important role in the productivity of oceans and constitutes the basis of the marine food chain. Moreover, phytoplankton is responsible for half of the oxygen released in the atmosphere.

In the last decades, an increased attention has been paid to the commercial and industrial potential of microalgae. Several species are currently being studied for their ability to synthesize valuable secondary metabolites (pigments, lipids, caretonoids, etc) for biofuel production, pharmaceutical industry or aquaculture applications [1]. Other fields of investigation include nanotechnologies, environmental survey, forensic sciences and paleontology. Similarly, some micro-algae contain and/or excrete pharmacologically active compounds. For example, the dinoflagellates *Gymnodinium* sp. and *Gonyaulax* sp. produce an alkylguanidine compound that affects the central nervous system. Brominated bi-indoles of *Rivularia firma* show pharmacological activity.

Among different compounds with functional properties, antioxidants are the most widely studied since the interest in finding new, safe and powerful antioxidant from natural sources is growing nowadays. Moreover, consumers have demonstrated the important role of antioxidants in human health thus increasing the interest in such products and their demand. Most of commercially available natural antioxidants are extracted from terrestrial plants, such as cocoa, tea grape and rosemary [2]. It appears that unicellular microalgae shows to have promising potential as alternative source of antioxidants [3-5]. Microalgae have been considered as important source of bioactive compounds since they are sustainable resources easily to culture that do not require an arable land unlike the terrestrial plant. Some studies have reported the interesting and remarkable antioxidant potential of microalgae [6-8]. Additionally, microalgae species like *Dunaliella*, *Chlorella*, *Isochrysis*, *Nannochloropsis*, *Nannochloris*, *Chlamydomonas*, *Haematococcus* and *Spirulina* are available to grow in mass production [1].

In regard of these biotechnological challenges, there is a constant effort actually provided for both finding and exploiting new microalgal resources and developing their putative commercial outcomes or industrial valorizations. Thus, this research aims to characterize microalgae of interest for aquaculture for their potential biological activities, especially in antioxidant properties.

2. Material and Methods

2.1.Culture of microalgae

Three species of microalgae, *Dunaliella salina*, *Tetraselmis chuii*, and *Isochrysis galbana* clone Tahiti, were cultured in a batch culture with ratio of algae and seawater was 1:3 (volume). Walne medium was used as nutritional compound for the culture, with ratio of 1:1 (mL L^{-1}); with luminous intensity of 1500–3000 lux and temperature of 23-25^{0}C [9].

The density of the microalgae culture was measured daily with haemocytometer by using binocular microscope. The microalgae were homogenized before the measurement. The cell density was calculated by following formula:

$$\boldsymbol{N} = \frac{(N_1 + N_2)}{2} \times \frac{1}{1.02\ mm^2 \times 0.1\ mm} \times \frac{1\ mm^2}{10^{-3} ml}$$

Where N = cell density (cell.mL^{-1}), N_1 = total cell in 80 small square (replica 1), N_2 = Total cell in 80 small square (replica 2), 0.2 mm = wide of haemocytometer in 80 square, and 0.1 mm = the depth liquid on haemocytometer.

Microalgae were harvested on the stationary phase of culture. The biomass of microalgae was obtained with centrifugation at 5.000 rpm for 10 min and then left to dry for a few days to obtain the dry biomass [10].

2.2. Extraction
Dry biomass was extracted by using methanol solvent with sonication at 50 Hertz for 15 min. The solvents were evaporated by using rotary evaporation [11].

2.3. Antioxidant activity
Antioxidant activity study was divided into 2 steps: first was to identify and drying the specimen. Second step was the extraction of dry specimen and bioactive compound analysis including alkaloid, triterpenoid/steroid, saponin, phenol, flavonoid, tannin [12]. The total phenol test was performed according to [13] and antioxidant activity test with DPPH method [14].

2.4. DPPH antioxidant activity
DPPH antioxidant activity of methanol extract from *D. salina, T. chuii,* and *I. galbana* clone Tahiti were determined according to [15]. The assay was performed at 50, 100, 250, 500, and 1000 ppm. Extract solution (1 mL) (in triplicates) was mixed with 3 mL of 60 µM methanolic solution of DPPH radicals. The mixture was put in the dark for 30 min before the absorbance was taken at 517 nm. The percent inhibition was calculated using the formula:

$$\%\,\boldsymbol{of\ inhibition} = \left\{\left(\frac{\boldsymbol{A-B}}{\boldsymbol{A}}\right) \times \mathbf{100}\right\}$$

Where, A = absorbance of the control (DPPH) and B = absorbance of test sample.

2.5. Determination of total phenol content
Total phenol content of methanol extract from *D. salina, T. chuii,* and *I. galbana* clone Tahiti were determined according to the Folin-Ciocalteu method [16]. 300 µL of extract was dispensed into test tube (in triplicates) and it was added with 1.5 mL of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and a 1.2 mL of Na_2CO_3 solution (7.5 % w/v). The solution was mixed the put 30 min at room temperature before the absorbance at 765 nm. Total Plate Counted was measured in mg g^{-1} Gallic Acid Equivalent (GAE). The calibration equation for Gallic Acid was Y = 0.0645x - 0.0034.

2.6. Phytochemical content
Methanol extracts prepared from all the three different microalgae were used to screen various phytochemicals such as tannins, flavonoids, steroids, saponins, and alkaloids [17].

2.6.1. Flavonoids
The algal extract was diluted and taken some of the water, and put into a test tube. Mg was then added and three drops of HCl into a test tube. Amyl alcohol then added and beat until separated. The reaction was positive if it forms a reddish yellow color to red.

2.6.2. Quinones
Five mL of extract obtained from the experiments to extract flavonoids identification was inserted into a test tube, a few drops of a solution of NaOH 1 N then added. The formation of the red color indicates the class of quinone compound.

2.6.3. Tannins
Algal extracts added to 2-3 drops of 1% $FeCl_3$ solution. Extracts will be positive if it contains tannins greenish-black or dark blue.

2.6.4. Alkaloids
Crude extract was added with 0.5 HCL 2% and was divided into two tubes. The first tube coupled with 2-3 drops of reagent Dragendorff and the second tube was added with 2-3 drops of reagent Mayer. When

an orange precipitation was formed in the first tube and yellowish precipitation was shown by second tube, it indicated the presence of alkaloid.

2.6.5. Saponins
Extract was introduced into a test tube and added with distilled water that has been warmed. It was shaken and allowed to stand for 10 min. Samples showed positive results when the foam was formed and did not disappear until 15 min after the drops of HCl.

2.6.6. Terpenoids and Steroids
The extract was added with three drops acetic anhydride and one drop of sulfuric acid (H_2SO_4). The red color indicates terpenoids, while blue color indicates steroid. Samples showed positive results when the foam was formed and did not disappear until 15 min after the addition of HCl.

3. Results and Discussion

The activity of antioxidant of methanol extracts of three microalgae *D. salina*, *T. chuii*, and *I. galbana* clone Tahiti were studied in this research at different concentrations (1000, 500, 250, 100, 50 ppm). Results showed that *I. galbana* clone Tahiti at 250 ppm showed the best result than other concentration with 67.93 % inhibition. *D. salina* gave the best result of inhibition of 62.19 % at 500 ppm. While *T. chuii* showed the best result with 71.36 % of inhibition at 1000 ppm (Table 1).

Table 1. DPPH Radical Scavenging Assay from methanolic extracts of three microalgae *D. salina*, *T. chuii*, and *I. galbana* clone Tahiti at different concentrations (1000, 500, 250, 100, 50 ppm).

Microalgae	**Concetration (ppm)**				
	1000	**500**	**250**	**100**	**50**
D. salina	53.17	62.19	58.43	59.01	58.45
T. chuii	71.36	66.18	52.38	42.19	52.58
I. galbana clone Tahiti	53.77	55.87	67.93	64.02	61.64

Note: The results in % inhibition DPPH.

Table 2. Total Phenol Content from methanolic extracts of three microalgae *D. salina*, *T. chuii*, and *I. galbana* clone Tahiti at 4 concentrations (1000, 500, 250, 100, 50 ppm).

	Concentration (ppm)				
Microalgae	**1000**	**500**	**250**	**100**	**50**
D. salina	0.539	0.839	1.223	2.336	4.672
T. chuii	0.466	1.015	2.918	8.95	16.868
I. galbana clone Tahiti	0.678	1.408	3.167	8.02	17.798

Note : The results in mg GAE g^{-1} extract

Phytochemical screening using methanol of three marine microalgae strains was evaluated. The phytochemicals present in the microalgae strains were identified as flavonoids, tannin, alkaloids, saponin and steroids; while quinon was absent (Table 3).

Table 3. Screening of phytochemicals of methanol extracts of three microalgae

Microalgae	Flavonoid	Tannin	Alkaloid	Saponin	Steroid	Quinon
D. salina	+	+	+	+	+	-
T. chuii	+	+	+	+	+	-
I. galbana clone Tahiti	+	+	+	+	+	-

The use of methanol as solvent on extract of *D. salina* and *T. chuii* has been studied before [18]. Furthermore, on the screening on antioxidant and phytochemical contents of different solvent extracts (acetone, methanol, ethanol, chloroform) of five different microalgal strains including *Tetraselmis sp*., *Dunaliella sp*; showed that the maximum antioxidant activity were found in acetone extracts, followed by methanol extracts of *Tetraselmis sp*. and *Dunaliella sp*.[18]. The study on screening on nine Moroccan microalgae by using the ethanolic extracts showed that the antioxidant activity of *Dunalliela sp* (IC50 283 ± 0.09 µg mL^{-1})., *Tetraselmis sp* (IC_{50} 247 ± 0.01 µg mL^{-1}) were highest antioxidant potentials among nine microalgae studied. *D. salina* and *T. chuii* were two potentials microalgae on antioxidant activites and DPPH was the simple method to elucidate the activity by using different solvents [4].

The total phenolic content of methanolic extracts of *D. salina*, *T. chuii*, and *I. galbana* clone Tahiti was evaluated using the Folin–Ciocalteu method. The phenolic content varied from 4.672 to 17.798 mg GAE g^{-1}extract at concentration of 50 ppm. Among five concentrations tested (1000, 500, 250 100 and 50 ppm), 50 ppm gave the best result. The highest phenolic content was found on *Isochrysis galbana clone Tahiti* (17.798 mg GAE g^{-1}extract) followed by *Tetraselmis chuii* 16.868 mg. g^{-1} GAE and the lowest was found in *Dunaliella salina* (4.672 mg GAE g^{-1}extract) (Table 2).

The result of this study on *I. galbana* clone Tahiti was higher than those found by [4] by using the ethanolic extracts of *Isochrysis s*p (13.4 ± 0.16 mg GAE g^{-1} extract) and by [2] on extracts of *Isochrysis sp*. (4.6 mg GAE g^{-1} extract). The total phenol content of methanolic extract of *Tetraselmis sp* in this study (16.868 mg. g^{-1} GAE) was higher than showed by [2] (3.8 mg GAE g^{-1} extract); meanwhile the result of this study was lower than those showed by [4] using the ethanolic extracts (25.5±1.5 mg GAE g^{-1} extract). In addition, total phenol content of methanolic extract of *D. salina* of this study (4.672 mg GAE g^{-1} extract) was also lower than those showed by [4] by using the ethanolic extracts (14.0 ± 0.43 mg GAE g^{-1} extract). It is suggested that the different results obtained between the present study and that of by [4] was due to the type of solvent. Indeed, different solvent used will generate different content of bioactive compound. Methanol is commonly used to extract bioactive compound from any organisms as this solvent dissolves various polar compounds and certain non-group of non-polar as well. Phenolic compounds are often extracted using polar solvent yet the most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone and ethyl acetate [19][20]. In general, methanol is favourable for lower molecular weight phenolic compounds [21].

5. Conclusion

The methanolic extracts of three-tested microalgae *I. galbana clone Tahiti*, *T. chuii* and *D. salina* showed high antioxidant activities. The higher antioxidants potentials were obtained in *Dunalliela sp*., *Tetraselmis chuii*. These microalgae posses antioxidant potential, which could be considered for future applications in medicine, dietary supplements, cosmetics or food industries. In the future however it is crucial to perform characterization of phenolic compounds from *D. salina, T. chuii*, *and I. galbana* clone Tahiti.

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