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Research Article

Antioxidant Potentials of Marine Red and Green Algae Extracts *In-vitro*

Yanti[1*], Fendy Heryanto Koesnoto[1], Andre Sutanto[1], Jae-Kwan Hwang[2]
[1]Faculty of Biotechnology, Atma Jaya Catholic University, Jakarta 12930, Indonesia
[2]Department of Biotechnology, Yonsei University, Yonsei-ro 50, Seodaemun-gu, Seoul 120-749, Korea

***Corresponding author**
Yanti,
Email: yanti@atmajaya.ac.id

Abstract: Reactive oxygen species (ROS) is normally found in balance with antioxidant molecules within all aerobic cells. The unbalance condition between ROS and antioxidant molecules will cause oxidative stress that may lead to any damages on nucleic acid, protein, and fatty acid. In this study, we investigated the antioxidant potentials of Indonesian marine algae extracts, including red algae (*Botryocladia* sp. and *Gracilaria* sp.) and green algae (*Caulerpa sertulaioides*, *C. racemosa*, *Codium* sp., *Enteromorpha* sp., and *Halimeda opuntia*) *in vitro*. Seven marine algae were dried and extracted with ethanol, followed by evaporating and freeze-drying treatments to obtain algae extracts. The antioxidant activity was referred to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Our results demonstrated that both red algae (*Botryoclardia* sp. and *Gracilaria* sp.) and green algae (*C. sertulaioides* and *Codium* sp.) at 100 µg/mL significantly possessed 35-40% antioxidant activity, indicating that these marine algae extracts may exert potential natural antioxidant properties.
Keywords: marine algae, antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl.

INTRODUCTION

Indonesia is a maritime country with collection of 782 marine algae species within marine ecosystem that had not been explored yet [1]. Marine algae are eminent for it antioxidant potential. Their habitats are exposed by ultraviolet light that can enhance the reactive oxygen species (ROS) formation in the water. ROS can make any damage for the marine algae cells and for the protection against it, antioxidant is developed by the marine algae. ROS such as superoxide radical, hydrogen peroxide, singlet oxygen, and hydroxyl radical, are formed from the oxygen that very critical for aerobic organisms, including human. ROS is well known to be cytotoxic and related to many human diseases, including cancer, diabetes, and neurodegenerative diseases [2].

Currently, several marine algae species from *Porphyra* sp., *Laminaria* sp., and *Undaria* sp. have been reported for their antioxidant potentials [3]. Also, marine algae synthetize various antioxidant compounds, including polyphenols, alkaloids, halogenated compounds, and polysaccharides [4]. This study was aimed to screen the antioxidant activity from red and green marine algae extracts *in vitro*.

MATERIALS AND METHODS

Collection and extraction of marine algae

Seven marine algae were collected from Binuangen (Banten province) and Garut (West Java province) on April 2012. Red algae (*Gracilaria* sp. and *Botryocladia* sp.) and green algae (*Enteromorpha* sp., *Halimeda opuntia*, *Caulerpa sertulaioides*, *C. racemosa*, and *Codium* sp.) were identified by Dr. Rory A. Hutagalung (Faculty of Biotechnology, Atma Jaya Catholic University, Jakarta). All samples were extracted using ethanol and incubated overnight. The supernatant were separated and the extraction procedures were repeated. Then, evaporation at 55°C with vacuum oven was used to separate the ethanol from the crude extract. The crude extract powder was stored at room temperature.

Preparation of sample stock and working solutions

Crude extracts powder were diluted in 100% dimethyl sulfoxide (DMSO) for preparation of the stock solutions with final concentrations at 10^5 and 10^4 µg/mL. Then, the stock solutions were diluted again with 25% DMSO until final concentrations at 10^3 and 10^2 µg/mL. The working solutions used for the antioxidant assay were arranged with concentration range of 5-250 µg/mL.

Antioxidant assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used for determining the antioxidant activity from marine algae extracts [5]. DPPH working solution was prepared with dilution in methanol until final concentration at 0.003 mM. Each 125 µL marine algae working solutions was mixed with 50 µL DPPH in the 96-well microtiter plate. Positive control used was 50 µL DPPH mixed with 125 µL DMSO 25% and for the negative control, 175 µL DMSO 25% was used. Ascorbic acid was referred as the antioxidant standard. The reaction between DPPH and samples was for 30 minutes. The absorbance was measured using microtiter plate reader at 515 nm. The antioxidant activity of all samples was determined using the formula of:

$$1- \frac{\text{(OD 515 samples-OD 515 negative control)}}{\text{(OD 515 positive control-OD 515 negative control)}} \text{ x 100\%}$$

Statistical analysis

All experiments were performed in tetraplicate with three repeats. All data collected were analyzed statistically using mean ± standard deviation. Statistical analysis of untreatment and marine algae treatment was performed by analysis of variance (SPSS 11.0 for Windows).

RESULTS AND DISCUSSION

Table 1 showed that *Gracilaria* sp. extract at 100 µg/mL showed the highest antioxidant activity (40%) compared to those of other algae extracts. Also, there were 3 algae extracts exhibited potential antioxidant effects including *Botryocladia* sp., *C. sertulaioides*, and *Codium* sp. within a range of 35-38% antioxidant activity, respectively. All algae extracts represented a dose-independent antioxidant activity (Figure 1). The highest antioxidant effect of each extract was reached at concentration of 100 µg/mL. Meanwhile, ascorbic acid at 10 µg/mL had 43% antioxidant activity.

Table-1:Antioxidant activity of marine algae extracts *in vitro*

Marine algae (µg/mL)	Antioxidant Activity (%)					
	5	**10**	**25**	**50**	**100**	**250**
Red algae						
Botryocladia sp.	24 ± 3	32 ± 2	31 ± 7	26 ± 3	35 ± 2	24 ± 4
Gracilaria sp.	26 ± 1	27 ± 6	30 ± 5	35 ± 3	40 ± 3	33 ± 4
Green algae						
Caulerpa racemosa	18 ± 2	13 ± 1	14 ± 2	17 ± 1	10 ± 5	11 ± 1
C. sertularioides	27 ± 4	25 ± 1	32 ± 1	28 ± 3	35 ± 1	27 ± 3
Codium sp.	16 ± 3	26 ± 4	28 ± 3	34 ± 2	38 ± 1	32 ± 4
Enteromorpha sp.	19 ± 2	20 ± 1	17 ± 1	22 ± 1	27 ± 5	27 ± 3
H. opuntia	15 ± 2	19 ± 1	22 ± 1	24 ± 2	15 ± 3	28 ± 0

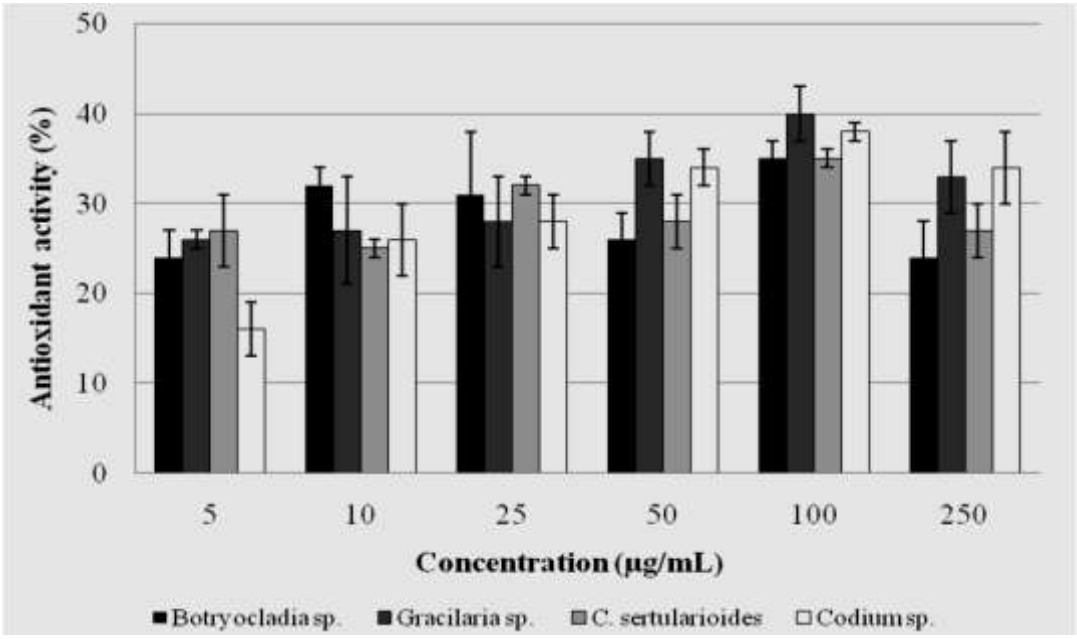

Fig- 1. Antioxidant activity of *Gracilaria* sp., *Botryocladia* sp. , *C. sertulaioides* and *Codium* sp. extracts at various concentrations (5-250 µg/mL). Standard of ascorbic acid at 10 µg/mL had 43% antioxidant activity.

Screening of antioxidant potential from natural products is a fast-growing field of research, and many antioxidant potentials have been investigated using various methods. DPPH assay is based on the ability of DPPH reagent as a stable radical to decolorize in the presence of antioxidants. It offers a quick, reliable, and low-cost method that has been frequently used for antioxidative potential from many natural products [6].

The ethanolic extracts of *Gracilaria* sp, *Botryocladia* sp., *C. sertularioides*, and *Codium* sp. at 100 µg/mL exhibited a dose-independent high antioxidant potential (Figure 1). The range of antioxidant potentials from all algae extracts was 35 - 40%. It has been reported that *Gracilaria* sp. contained phycobiliprotein, a bioactive compound rich in C-phycoerythrin, for prevention of oxidative stress due to its nucleophilic activity to neutralize oxidants [7]. In addition, the polysaccharide fractions in *Gracilaria* sp. were also known for its inhibitory activity on the formation of oxygen radical [4]. These results are in line with *Botryocladia* sp. extract that also contained phycobiliprotein for its action as an antioxidant agent [8].

Samples from Chlorophyta, i.e. *C. sertularioides* and *Codium* sp., also showed antioxidant activity of 35% and 38%, respectively (Table 1). *C. sertularioides* and *Codium* sp. were reported for their high antioxidant potential [9, 10]. Recent studies showed that marine algae from *Caulerpa* genus contain high levels of antioxidant enzymes, such as superoxide dismutase and catalase. These two enzymes have functions to transform oxygen radical become oxygen and water [11]. Chlorophyta class is rich in carotenoid contents in particular β-carotene. This compound is known for its antioxidant capability due to its reaction with peroxyl radical formed β-carotene radical, a compound that will inactivate peroxyl radical [12].

Recent study reported that several marine algae have been identified for also containing vitamin C and α-tocopherol, gold standards for antioxidant, which have synergist effect on stabilizing free radicals [13]. Vitamin C is the main antioxidant with neutralizing activity on free radical and as the recycler of α-tocopherol. Furthermore, α-tocopherol, a derivative from vitamin E, also has scavenging radical activity and functions on protecting lipoprotein and cell membranes [14].

The potential of antioxidant from marine algae has been associated with the antioxidant compounds found in marine algae. Marine algae are well known for its ability to synthesize various compounds including secondary metabolites, for stabilizing free radical [4]. It has been reported that antioxidant action may possess various mechanisms in stabilizing free radicals. In this study, DPPH method was used for measuring the antioxidant activity which donating the hydrogen ion for reducing the DPPH. Other antioxidant assay such the ferric reducing ability of plasma (FRAP) was also employed for measuring the electron donor activity from marine algae extract [15].

The antioxidant activity from all red and green marine algae used in this study could be enhanced with some modified methods, including the optimatization of solvent extraction, the choice of antioxidant assays, and the isolation of antioxidant compounds derived from marine algae. For the extraction procedure, previous study showed that methanolic extract of plant natural products showed higher antioxidant activity compared to those of extracts from ethanol, chloroform, and n-hexane [16]. Crude extract of marine algae may contain several active compounds which responsible for antioxidant properties through various molecular mechanisms.

CONCLUSION

There were four marine algae extracts, i.e. *Gracilaria* sp., *Botryocladia* sp., *C. sertulaioides*, *Codium* sp., showed high antioxidant activities *in vitro*. Further study on the isolation and fractionation of the bioactive compound derived from the marine algae is needed to observe the exact mechanism of specific antioxidant compound from marine algae as an antioxidant agent.

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