Total phenolic content, antioxidant activity and tyrosinase inhibitor from marine red algae extract collected from Kupang, East Nusa Tenggara

M Nursid1*, T S Khatulistiani1, D Noviendri1, F Hapsari2 and T Hardiyati2

1)Research Center for Marine and Fisheries Product Processing and Biotechnology, Ministry of Marine and Fisheries Affair, Jl. KS. Tubun Petamburan VI Jakarta 10260, Indonesia
2)Faculty of Biology, University of Jenderal Soedirman, Jl. Dr. Suparno Purwokerto 53123 Indonesia

*Corresponding author: muhammad.nursid@kkp.go.id

Abstract. Many commercialized synthetic antioxidants are used under strict regulations in certain countries because of their potential health hazards. Thus, the search for alternative antioxidants from natural products Indonesia is needed, and one of the potential material is from seaweed extract. Red seaweed is very potential to be developed as a raw material for medicines and cosmetics because it has antioxidant activity, which from its phenolic compound that can remove the free radicals and can inhibit tyrosinase enzyme activity. The purpose of this research is to know the species of red seaweed in the East Nusa Tenggara sea, its total phenolic content, antioxidant activity, and also inhibitory activity of tyrosinase in red seaweed. Parameters that measured are the number of total phenolic compounds, IC50 that obtain from the DPPH test, and inhibition percentage of Tyrosinase. The result shows that there are ten species of red algae in Kupang, East Nusa Tenggara, and it consists of 4 genera. Some of that red seaweed species are potential as antioxidant and tyrosinase inhibitors source. The result from total phenolic content analysis show the species that have highest total phenolic content is Laurencia sp. (24.97 mg GAE.g-1), the antioxidant activity test show the species that have highest antioxidant activity is Gelidium latifolium (46.68%), and the species that have the highest percentage inhibition of tyrosinase enzyme is Gracillaria foliifera (25.21%). Moreover, the research also shows a strong correlation between the total phenolic content of the seaweed extract with antioxidant activity.

1. Introduction

Marine algae (seaweed) have long been used as food and medicine in Asian countries such as Japan, China, and Korea. Furthermore, Greeks and Romans applied seaweed as medicinal remedies in cosmetics. In folk medicine, marine algae have been used for a wide range of remedial purposes such as treatment of gallstone, vermifuges, stomach ailments, eczema, cancer, and renal disorders [1,2]. Edible marine algae have antioxidants and tyrosinase inhibitory activity [3,4].

Indonesia has the second-longest coastline that is suitable for marine algae growth and development. Marine algae had the potential to be developed as the source of functional compound
Samples rich with antioxidants always related to anti-tyrosinase. It is due to their role in preventing free radical-related skin damage [7]. Compounds such as polyphenols, catechin, flavonols, flavonol glycosides, and phlorotannins have been discovered from methanol extract of red and brown algae. The uniqueness of their molecular structures has contributed to the strong antioxidant activity. Polyphenols, for an instant, use their phenol rings as electron traps for free radicals [8]. Antioxidant compounds play an important role in various fields such as the medical field (to treat cancers, cardiovascular disorders, and chronic inflammations), cosmetics (anti-aging process), and food industries (food preservative) [9]. Over the years, the search for new antioxidant compounds from natural products has mounted. Natural antioxidants gain a positive response from consumer preferences for natural products [10]. Thus, the search for alternative antioxidants from natural products derived aquatic plants such as seaweeds has been increased.

Tyrosinase is a copper-containing enzyme widely distributed in microorganisms, plants, and animals [11]. It catalyzes the oxidation of phenolic substrates to o-quinones [12]. In addition to undesirable color and flavor, the quinine compounds produced in the browning reaction may irreversibly react with amino and sulfhydryl groups of the protein. This reaction decreases the digestibility of protein and bioavailability of essential amino acids, including lysine and cysteine [13]. Therefore, tyrosinase inhibitors have become increasingly important in cosmetic and medicinal products, primarily to control hyperpigmentation [14]. Several synthetic tyrosinase inhibitors exhibited a lack of efficiency or adverse side effects [15].

The study aimed to investigate the antioxidant activity, tyrosinase inhibitory activity, and total phenolic content of the red algae collected from Kupang, East Nusa Tenggara.

2. Material and Methods

2.1. Sampling Macroalgae

The macroalgae were collected from Kupang Waters, East Nusa Tenggara. Determination of macroalgae was done in the Biotechnology laboratory of Research Center for Marine and Fisheries Product Processing and Biotechnology, Jakarta. The samples obtained were washed using water until they cleaned. Each sample (100 g) was macerated three times using 250 mL ethanol p.a. The extract was filtered using Whatman filter paper and evaporated in a vacuum rotary evaporator to remove ethanol from the extract. The remaining water contained in the extract was then dried with a vacuum concentrator.

2.2. DPPH Assay

DPPH reagent (Merck) was prepared by diluting 3 mg in 5 mL MeOH (p.a). An ethanol diluted seaweed dry extract of 160 µL was poured into 96-well micro plate then a 40 µL of DPPH reagent was added (A). Extract control (containing 160 µL of seaweed extract and 40 µL of MeOH) (B), negative control (160 µL MeOH and 40 µL of DPPH reagent) (C) and a blank (200 µL MeOH) (D) were also use in this evaluation. The absorbance of each well was measured using a microplate reader (Thermo) at 517 nm [16].

2.3. Total Phenolic Content (TPC) Analysis

Seaweed ethanol extract of 1 mg/ml, 10 % Folin Ciocalteu reagent and 7.5% (w/v) Na2CO3, were diluted in dH2O. One milliliter seaweed extract was poured into the glass tube, then 5.0 ml of Folin Ciocalteu and 4.0 ml of Na2CO3 were added. The mixture was incubated at 27-28 °C for 60 min. Absorbance was measured using Microplate UV-Vis Spectrometer (Thermo) at 750 nm. Results were expressed as milligram gallic acid equivalents (GAE)/g extract. Polyphenol concentration in the extract was determined from the gallic acid standard curve for a concentration range of 10 - 50 µg/mL[17].

2.4. Inhibitor Tyrosinase Test
The sample was dissolved with DMSO as a stock solution. Extract concentration was prepared by dissolving concentrated extract using phosphate buffer (pH 6.5). A total of 70 μL of extract solution and 30 μL tyrosinase enzyme (Sigma, 333 units of mL⁻¹ in phosphate buffer solution) were put into 96-well plate and incubated for 5 min. The mixture was then added with 110 μL of a substrate (L-DOPA 12 mM) and incubated at 37ºC for 30 min. The absorbance was measured at a wavelength of 492 nm using Microplate Reader (Thermo) [18].

3. Result And Discussion

3.1. Identification of red algae

Based on the identification result, the red seaweed found in Kupang, East Nusa Tenggara are Gracillaria tikvahiae, Gelidium latifolium, Laurencia sp., Gracillaria foliifera, Laurencia sp., Euchema denticulatum, Euchema cotonii, and Gracillaria Salicornia (Figure 1).

![Figure 1. Red macroalgae that used in this research.](image)

This research started with the extraction of all the red seaweeds. Extraction was carried out by maceration using ethanol p.a. as a solvent which is known to extract useful compounds from red seaweed, according to Sari et al. [19], liquid extraction is intended so that the compound can dissolve in one phase. The use of ethanol in liquid extraction is because ethanol is a polar solvent and not toxic compared to methanol. Then the extract is dried using a vacuum rotary evaporator to separate the extract from the remaining salt content of the seaweed. After that, the seaweed is dried again using vacuum concentrate to get the thick extract. All extraction activities are carried out in the condition of the room that is always dark to keep the extracted active compounds from being lost or damaged during the extraction process.

3.2. Total phenolic content of red algae

Phenolic is a compound that found in almost all types of seaweed and has the potential as an antioxidant. The red algae extract that tested is dissolved using methanol. The phenolic content will...
increase in the extract as the polarity of the solvent increases, which is using methanol [20]. After testing the total phenolic content of red algae extract the results shown in Figure 2, the red algae extract which has a high phenolic content including K2 (24.38 mg GAE.g⁻¹), K3 (24.97 mg GAE.g⁻¹), K6 (24.05 mg GAE.g⁻¹), and K10 (23.37 mg GAE.g⁻¹). The content of polyphenols in seaweed varies by following the season, harvest time, geographical location, and species of seaweed [21].

![Figure 2](image-url). The total phenolic content result on red algae sample

Based on the research the species that have highest total phenolic content is Laurencia sp. (24.97 mg GAE.g⁻¹), the result is according to the research by Rosni et al. [22], that the total phenolic content of several red algae collected from Semporna, Sabah, Malaysia which are Laurencia sp. (20.31 mg PGE.g⁻¹), Gracilaria verrucosa (11.27 mg PGE.g⁻¹), Kappaphycus striatum var. sacol (10.90 mg PGE.g⁻¹), Kappaphycus alvarezii (var. green tambalang) (10.39 mg PGE.g⁻¹), Kappaphycus alvarezii (var. aring-aring) (10.09 mg PGE.g⁻¹), Kappaphycus striatum var. sacol (9.90 mg PGE.g⁻¹), Kappaphycus striatum var. sacol (8.94 mg PGE.g⁻¹) and Eucheuma denticulatum (8.59 mg PGE.g⁻¹). Laurencia sp. was found to have the highest total phenolic content compared to the other species that tested.

3.3. Antioxidant activity of red seaweed

This antioxidant test aims to determine the potential of antioxidant activity with the principle of capturing hydrogen atoms from antioxidants possessed by compounds contained in red algae extract by DPPH free radicals. Based on the test, the average percentage of DPPH free radical inhibition at a concentration of 1000 ppm is attached in Figure 3.
Figure 3. DPPH test result on Red algae sample

Based on the data obtained, there are several samples of red algae that have the potential as antioxidants including K2, K3, and K6. These samples were then tested with a dose series to obtain an IC\textsubscript{50} value. Based on the result obtained the antioxidant activity or red algae from Kupang are weak. According to Badarinath [23], antioxidant criteria is very strong (<50), strong (50-100), moderate (100-150), and weak (>200).

The species that have the highest antioxidant activity is \textit{G. latifolium}, but \textit{G. latifolium} has a relatively low IC\textsubscript{50} value. The results show that \textit{G. latifolium} and \textit{S. tomentosum} have lower antioxidant activity compared to some algae tested with IC\textsubscript{50} values of 300 g.mL\textsuperscript{-1}. The highest activity came from \textit{Cystoseira crinite} brown algae with an IC\textsubscript{50} value of 300 g.mL\textsuperscript{-1}. The low antioxidant activity caused by various factors, including the extraction method, was not sufficient for extracting the antioxidant compounds.

3.4. Inhibitor tyrosinase analysis of red algae

The red algae extract which has high inhibitor tyrosinase activity was shown in Figure 4. Tyrosinase enzyme reactions and substrate produce a brown color. Reducing the intensity of brown to yellow shows the inhibitory activity of tyrosinase measured at a wavelength of 475 nm. The test used an L-DOPA substrate with a concentration of 2 mM, and tyrosinase inhibitor activity testing was carried out on all red seaweed extract at a concentration of 1000 ppm. According to Zamani et al. [24], compounds that act as ROS catchers will be more effective as tyrosinase inhibitors. Antioxidant compounds can inhibit the action of the tyrosinase enzyme in oxidizing the L-tyrosine substrate to L-DOPA or L-DOPA to dopaquinon.

Figure 4. Inhibitor tyrosinase result in Red algae sample
Based on the research, the species that have the highest tyrosinase inhibitor activity is *G. foliifera* (25.21%). Tyrosinase inhibitor of several red algae that collected from Jeju Island, Korea, the percentage of inhibition of red algae extract of *Gracillaria* sp. is 28.18%, and it is still relatively low compared to the other species such as *Gellidium* sp. that has 73.87% inhibition of tyrosinase or *Schizymenia* sp. that has highest tyrosinase inhibition percentage (90.75 %) [25]. This result might be due to differences in the environmental condition of Jeju Island and Kupang.

3.5. *Correlation of phenolic content, antioxidant, and tyrosinase inhibitors of red algae extract.*

The result of total phenolic content and antioxidant activity have shown that the species that have high total phenolic content also have high antioxidant activity.

![Figure 5. Correlation curve between Total phenolic content with antioxidant activity and tyrosinase inhibitor](image)

The antioxidant activity had a significant positive correlation to total phenolic content (R=0.91, P < 0.05) (Figure 5). There was a linear correlation between total phenolic compound concentration and antioxidant activity using DPPH method [26]. It showed that phenolic compounds in the extract contributed to antioxidant activity. Phenolic compounds have antioxidant properties because of their ability to act as a reducing agent, hydrogen ion donor, and anti-radical agent. Phenolic compounds also act as a metal chelator that protects metal catalytic function in the radical initiation process [27].

The total phenolic content has a weak correlation to tyrosinase inhibitor activity (R= 0.291, P > 0.05 (Figure 5). Contrastingly, Nur et al. [28] found that the presence of phenolic and flavonoid compounds that have been reported, such as flavonols, stilbens, phenolic acids, and quartzite contribute to inhibiting tyrosinase activity thereby preventing skin depigmentation. The result might occur because the extract that used are not fractionated first so the substance that might be acting as tyrosinase inhibitor is affected by the other substance so, the activity of the substance to inhibits the tyrosinase enzyme are not effective.

4. **Conclusion**

Red algae from Kupang, East Nusa Tenggara were *Gracillaria tikvahiae, Gelidium latifolium, Laurencia* sp., *Euchema cotonii, Gracillaria foliifera, Laurencia* sp., *Euchema denticulatum, and Gracillaria Salicornia*. *Laurencia* sp showed the highest total phenolic content. *Gelidium latifolium* had the highest antioxidant activity. The highest tyrosinase inhibitor activity was *Euchema cotonii*.

**Acknowledgment**
This study was financially supported by the Ministry of Marine Affairs and Fisheries 2018

References
[1] Hoppe HA and Levr ing T 1982 Marine Algae in Pharmaceutical Science 2 (Berlin: Walter de Gruyter)
[2] Srivastava R and Kulshreshtha DK 1989 Phytochemistry 28 2877-2883
[3] Kumar KS, Ganesan K, and Rao PVS 2008 Food Chem. 107 289-295
[4] Yoon NY, Eom TK, Kim MM and Kim SK 2009 J Agric Food Chem. 57 4124-4129.
[5] Kelman D, Posner EK, McDermid KJ, Tabandera NK, Wright PR and Wright AD 2012 Mar. Drugs 10 403-416
[6] Firdaus M, Nurdiani R and Prihanto AA 2015 In Marine Algae Extracts: Processes, Products, and Applications (Wiley VCH)
[7] Chang VS and Teo SS 2016 Int. Food Res. J. 23 2370-2373
[8] Zakaria, Aili N, Ibrahim D, Sulaiman SF and Supardy NA 2011 J. chem. pharm. res. 3 182-191
[9] Kohen R and Nyska A 2002 Toxicol. Pathol. 30 620-50
[10] Safer AM and Al-Nughamish AJ 1999 Histol Histopathol 14 391-406
[11] Whitaker, JR. 1995 Polyphenol oxidase (New York: Chapman & Hall) pp 271-307
[12] Friedman M 1996 J Agric Food Chem. 44 631-653
[13] Kim YJ and Uyama H 2005 Cell Mol Life Sci. 62 1707-1723
[14] Parvez S, Kang M, Chung HS and Bae H 2007 Phytother Res. 21 805-816
[15] Hamon VLM and Criton M 2009 Biol Pharm Bull. 32 301-303
[16] Sachindra NM, Sato E, Maeda H, Hosokawa M, Niwano Y, Kohno and Miyashita K 2007 J. Agric Food Chem. 55 8516-8522
[17] Anesini C, Ferraro GE and Filip R 2008 J Agric Food Chem. 56 9225-9229
[18] Batubara I, Julita I, Darusman LK, Muddathir AM and Mitsunaga T 2016 Procedia Chem. 14 216-224
[19] Sari, Bina Lohita, Nurulila Susanti and Sutanto 2015 Int. J. Pharm Sci Rev Res. 2 2407-2354
[20] Ganesan P, Chandini SK and Bhaskar N 2008 Bioreour Technol. 99 2717-2723.
[21] Rajauria G, Foley B and Abu-Ghannam N 2016 Innov. Food Sci. Emerg. 37 261-268.
[22] Rosni MS, Fisal A, Azwan A, Chye F Y and Matanjun P 2015 Int. Food Res. J. 22 1483-1493
[23] Badarinath, AK 2010 Int. J. Pharmtech Res. 2 (2) 276-1285
[24] Anesini NP, Gazali M and Batubara I 2015 Marine Scientific Research & Development 5 1-5
[25] Cha SH, Ko SC, Kim D and Jeon YJ 2011 J. Dermatol 38 354-63
[26] Nursid M, Marraskuranto E, Atmojo KB, Hartono TMP, Meinita MDN and Riyanti 2016 Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology. 11 59-67
[27] Wu X J and Hansen C 2008 J. Food Sci. 73 434-438.
[28] Nur, Syamsu, Rumiyati and Lukitaningsih E 2017 Tradit. Med. Res. 22 63-72