

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF TROPICAL BROWN ALGAE *Padina australis* FROM PRAMUKA ISLAND, DISTRICT OF SERIBU ISLAND, INDONESIA

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Abstract

The proximate composition, dietary fiber, and total phenol contents as well as antioxidant activity of tropical brown alga *Padina australis* collected from the Pramuka Island, District of Seribu Island, Indonesia during the rainy season of 2011 were determined in order to evaluate their potential nutritional value and activity of natural antioxidant compound. The content of ash, protein, and fat were 22.26, 10.76, and 4.17 g/100 g dry matter, respectively; whereas the amounts of soluble, insoluble, and total dietary fibers were 8.4, 5.4, and 13.8 g/100 g, respectively. Methanol extract of *P. australis* contained the highest total phenol of 246.1 mg GAE/1000 g dry sample. The extract also had the highest activity on DPPH radical scavenging, measured by IC₅₀ of 267.1 ppm. Both the total phenol and IC₅₀ value extracts decreased in the following order: methanol > ethyl acetate > n-hexane.

Keywords: antioxidant, dietary fiber, DPPH-scavenging, *Padina australis*, proximate composition

I. INTRODUCTION

Seaweeds or marine macroalgae are potential renewable resource in the marine environment. Approximately 6000 species of seaweeds in the world have been identified and are grouped into different classes *i.e.* green (Chlorophytes), brown (Phaeophytes) and red (Rhodophytes) algae (Devi *et al.*, 2011). Indonesia as an archipelagic country has a large number of seaweeds. Siboga expedition (1899-1900) succeeded to collect 555 species of seaweeds from Indonesian territorial waters, and among them, around 20-30 have been utilized by local people as foodstuff or traditional medicine (Mubarak *et al.*, 1991).

From foodstuff viewpoint, seaweeds become an important part of the diet of many Asian countries, such as Japan, China, and Korea. Japan particularly, is the most important seaweed

consumer (Arasaki and Arasaki, 1983; Nisizawa *et al.*, 1987; Nisizawa, 2002).

Seaweeds are the richest source of minerals (Ruperez, 2000; Norziah and Ching, 2000; Santoso *et al.*, 2006) and rich in dietary fibers contents (Wong and Cheung 2000, Santoso *et al.*, 2002; Benjama and Masniyom, 2012). Seaweeds also contain fat and protein in low concentration (Wong and Cheung, 2000; SÁnshez-Machado *et al.*, 2004; Denis *et al.*, 2010). Moreover, seaweeds contain a wide range of bioactive compounds mainly polyphenols with potential antioxidant activity.

Several studies have been conducted and revealed that extract of green, brown, and red seaweeds had antioxidant activities, measured by several markers such as peroxide value, chelating ion metal, and scavenging of radical (Santoso *et al.*, 2004^a; 2010; Devi *et al.*,

2011; O'Sullivan *et al.*, 2011; Meenakshi *et al.*, 2012). Polyphenols derived from seaweeds may be more potent than analogous polyphenols derived from terrestrial plant sources due to presence of up to eight interconnected phenol rings (Hemat, 2007). In addition, bioactive compounds identified in seaweeds including alkaloids, terpenes, ascorbic acid, tocopherols, carotenoids and phlorotannins have demonstrated antioxidant activity within in vitro studies (Hu *et al.*, 2008; Heo *et al.*, 2009; Li *et al.*, 2011).

The nutrient compositions and bioactive compounds of seaweeds vary according to the species, maturity, environmental growth condition, and seasonal period (Ito and Hori, 1989; Mabeau and Fluerence, 1993; Ortiz *et al.*, 2006). In addition, the changes in ecological condition have an influence on the synthesis of nutrient and non-nutrient compounds, including antioxidant compound (Stengel *et al.*, 2011; Benjama and Masniyom, 2012).

Seaweeds grown in the tropical climate such as Indonesia are exposed to high level of light, temperature and desiccation that can lead to increase in producing reactive radical species. In order to survive, seaweeds may produce some bioactive compounds and may change the content of nutrient and non-nutrient. In this study, tropical brown alga *P. australis* were collected from the coast of Pramuka Island, Seribu Islands District, Indonesia, during a rainy season.

The objectives of the study were to measure the proximate composition and dietary fiber profile, to determine the phenolic content of tropical brown alga *P. australis* extracted in different solvent, and to evaluate the function of seaweed components as an antioxidant source through analysis of radical-scavenging activity.

II. METHODS

2.1. Location and Sampling

Preparation

Fresh tropical brown alga *P. australis* samples were collected in February 2011 from the intertidal region of Pramuka Island, Seribu Island District, Indonesia (Figure 1). Immediately the samples were placed in plastic bags containing sea water in order to prevent evaporation and transported to the laboratory under refrigerated condition. Then, the plant was washed thoroughly with tap water to remove all sand particles, epiphytes and other impurities. The samples were divided into two groups, specifically fresh and dried samples for the analysis of the proximate composition and the content of dietary fibers, and for extraction of antioxidant compound, respectively. Dried sample forms were obtained after being dried using sun rays for two days and grounded in an electric mixer. The powder sample was then stored in refrigerator for further use.

2.2. *P. australis* Extracts

The procedure of extraction was conducted according to the previous research conducted by Santoso *et al.* (2010) with slightly modification. The powder of *P. australis* was macerated in each solvent *i.e.*, methanol, ethyl acetate, or n-hexane at a ratio of 1:16 (w/v) for 48 h under dark condition. Then the extraction was filtered through glass funnel and Whatman no. 42 filter paper. Each filtrate was concentrated to dryness under reduced pressure at temperature of 40 °C using a rotary flash evaporator until become paste. Each crude extract in paste form was filled up by nitrogen gas to prevent decomposition of active compound inside, then was kept at -20 °C until analysis.

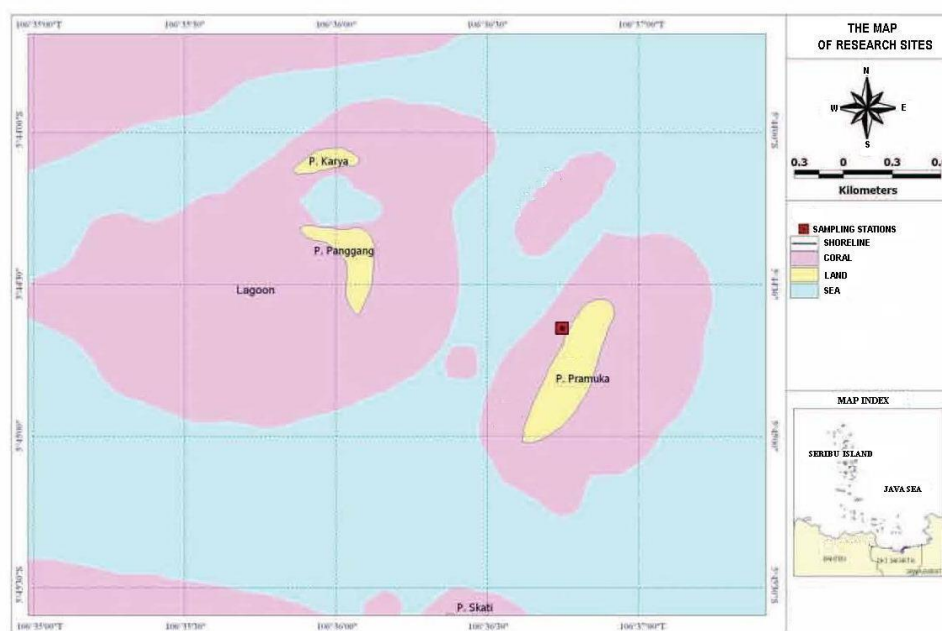


Figure 1. The map of research site in Pramuka Island, Seribu Island District

2.3. Proximate Analysis

The proximate analysis consisted of moisture, ash, fat and protein were carried out according to the procedures of the Association of Official Analytical Chemists (AOAC, 1995). The content of carbohydrate was calculated by subtracting the weights of moisture, ash, fat and protein from 100 g sample.

2.4. Determination of Dietary Fiber Contents

Insoluble and soluble dietary fibers were determined according to an enzymatic-gravimetric method referred to the research conducted by Suzuki *et al.* (1996) and Santoso (2003). Water insoluble dietary fiber was obtained after boiling in water with pancreatin enzyme and phosphate buffer, filtered off by a glass fiber filter with ethanol and acetone. Water soluble dietary fiber was precipitated from the filtrate using ethanol. The final corrected amounts of both dietary fibers were calculated by subtracting the weights of ash and protein from the each fiber precipitate; while the

total dietary fiber was calculated by summing the contents of insoluble and soluble dietary fibers.

2.5. Determination of Total Phenolic Contents

Total phenolic contents of each crude extracts from *P. australis* were determined by spectrophotometry using Follin-Ciocalteu reagents (Yangthong *et al.*, 2009). Each extract of *P. australis* was diluted with ethanol, added with distilled water and Follin-Ciocalteu reagents. The mixture was allowed to stand for 5 min and added with sodium carbonate. Homogenized mixture was then incubated in the dark room for one hour. The resulting absorbance was measured by a spectrophotometer (UV-1200 UV-VIS Spectrophotometer, Shimadzu, Kyoto, Japan) at a wavelength of 725 nm. Phenolic content was expressed in milligram per gram of dry weight samples based on a standard curve of gallic acid (GA), which was expressed as milligrams per 1000 gram of gallic acid equivalent (GAE).

2.6. DPPH Radical-Scavenging Activity

Antioxidant activity assay was conducted through the ability of the sample on reducing the stable free radical DPPH according to the method described by Aranda *et al.* (2009) with minor modifications. Each crude extract was weighed and then was added to ethanol with a ratio of 1:1000. Extracts with several concentrations with the addition of DPPH solution were loaded into the micro-well plate. The mixture was homogenized and incubated at 37 °C for 30 minutes. The resulting absorbance was measured by a microplate reader (Microplate Reader 168-1130, Biorad, California USA) at a wavelength of 517 nm. Regression equation was obtained from the relationship between sample concentration and percentage inhibition of free radical activity.

2.7. Statistical Analysis

All the data were presented as mean \pm standard deviations. Statistical analyses were performed using student's t test and one-way analysis of variance. Multiple comparisons of means were conducted using the least significance difference (LSD) test. All computations were done by employing the statistical software (SPSS version 16).

III. RESULTS AND DISCUSSION

3.1. Proximate Composition and Dietary Fiber Contents

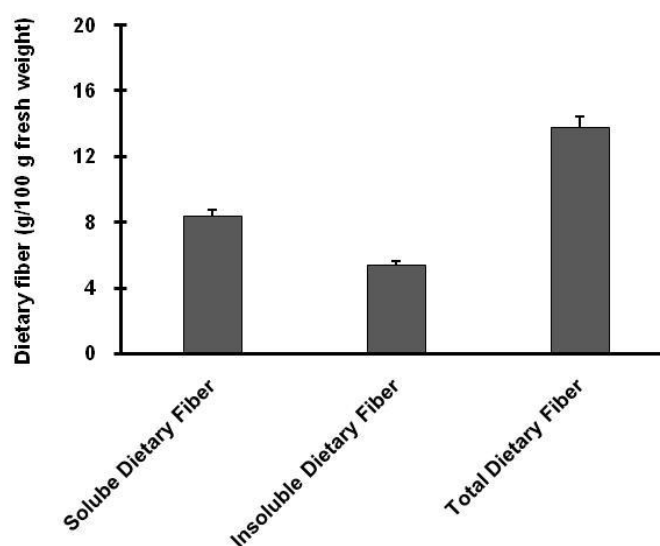
The fresh alga sample contained moisture, ash, protein, fat and carbohydrate of 90.56 g/100 fresh weight, 2.11 g/100 fresh weight, 1.02 g/100 fresh weight, 0.40 g/100 fresh weight and 5.90 g/100 fresh weight, respectively (Table 1). After converting them to 100 g dry matter samples, their content were shown to be

high. Among three nutrient compounds, ash (mineral) became the highest content in compared to protein and fat. In our previous research, we determined the proximate composition of fresh *P. australis* collected in dry season. The content of ash, protein and fat were 32.54, 8.97 and 4.73 g/100 g dry matter, respectively (Santoso *et al.*, 2006). The content of ash was higher in dry season, whereas the content of protein was higher in rainy season. The content of fat both in rainy and dry season was almost same. Benjama and Masniyom (2012) reported that the high ash content of *G. fisheri* and *G. tenuisipitata* also was found in summer season, whereas the protein content was found high in rainy season. However, the high fat content of *G. fisheri* and *G. tenuisipitata* were found in summer and rainy seasons, respectively. These levels varied depending on the species, environmental growth condition and seasonal period (Ito and Hori, 1989; Mabeau and Fluerence, 1993; Ortiz *et al.*, 2006).

Dietary fibers belong to the non-nutritional compounds as an important dietary constituent, which possesses a wide range of positive properties (Leontowicz *et al.*, 2001). In this study, the amounts of soluble, insoluble and total dietary fibers of 8.4, 5.4 and 13.8 g/100 g fresh weight, respectively (Figure 2). The content of total dietary fiber of brown algae *P. australis*, *Sargassum polycystum* and *Turbinaria conoides* were 9.6, 10.1 and 9.5 g/100 g fresh weight, respectively (Santoso *et al.*, 2006). However, *G. fisheri* contained total dietary fiber of 57.5 - 64.0 g/100 g dry weight and *G. tenuisipitata* had total dietary fiber of 56.6 - 60.2 g/100 g dry weight (Benjama and Masniyom, 2012).

Table 1. Proximate composition of fresh brown alga *P. australis*

Composition	Values (g/100 g)	
	Fresh weight	Dry matter
Moisture	90.56 ± 0.16	
Ash	2.11 ± 0.17	22.26 ± 1.98
Protein	1.02 ± 0.04	10.76 ± 0.54
Fat	0.40 ± 0.01	4.17 ± 0.04
Carbohydrate (<i>by difference</i>)	5.90 ± 0.37	62.21 ± 3.34

Figure 2. The contents of soluble, insoluble and total dietary fiber of *P. australis*

The ratio of soluble dietary fiber and insoluble dietary fiber of *P. australis* was 1.6. This value was higher in compared to red algae *G. fisheri* (0.39 - 0.42) and *G. tenuisipitata* (0.35 - 0.45) (Benjama and Masniyom 2012), since the genus *Gracilaria* has high content of soluble dietary fiber as sulphated galactants (Wong and Cheung 2000). Different with dietary fiber from terrestrial plants, dietary fibers in seaweeds contain some acidic group such as sulphuric group; therefore they have different characteristics in physicochemical and physiological effects, such as water holding capacity (Suzuki *et al.*, 1996; Wong and Cheung, 2000), oil holding capacity (Wong and Cheung, 2000), swelling capacity (Wong and Cheung, 2000), binding of vitamins

and minerals (Yoshie *et al.*, 2000), binding of bile salts (Wang *et al.*, 2001), and lipid metabolism effect (Wang *et al.*, 2002).

3.2. Total Phenol Contents

Phenolic compounds were commonly found in plants and have been reported to have several biological activities including potential antioxidants and free radical scavengers apart from primary defense role (Soobratte *et al.*, 2005). Methanol extract of *P. australis* contained the highest total phenol of 246.1 mg GAE/1000 g dry sample, followed by extract of ethyl acetate and n-hexane with values were 90.17 mg and 17.3 mg GAE/1000 g dry sample, respectively (Figure 3).

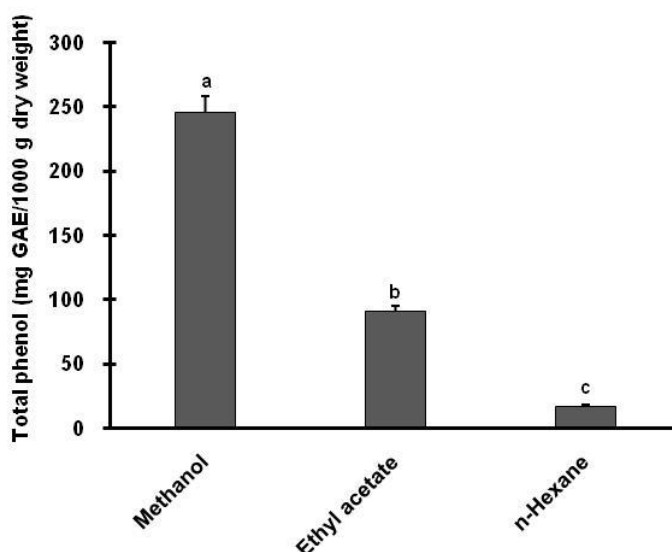


Figure 3. Total phenol content of each extract of *P. australis*. Letters over each column in the graph not sharing the same are significantly different ($p < 0.05$)

Compared to ethanol extract of brown alga *Sargassum pallidum*, the total phenol content was higher than *P. australis* in spite of different standard and extraction method. Fractionated ethanol extract of *S. pallidum* with chloroform, ethyl acetate and n-butanol contained the total phenol of 1.80, 11.54 and 12.12 mg CHA/g extract (CHA = chlorogenic acid) (Ye *et al.*, 2009). The levels of phenols in the methanol extract of brown algae ranged from 1.5 mg GAE/g dry weight in *Laminaria hyperborea* to 4.5 mg GAE/g dry weight in *Ascophyllum nodosum* (O'Sullivan *et al.*, 2011). Ganesan *et al.* (2008) observed methanol extract of red seaweeds *Euclima cottonii* and *Gracilaria edulis* contained total phenol of 1.5 and 4.1 mg GAE/g dry weight, respectively. Chandini *et al.* (2008) on the other hand observed lower levels of phenols in the aqueous fractions of *Sargassum marginatum* and *Turbinaria conoides* which contained of 0.29 and 0.86 mg GAE/g on a dry weight basis, respectively. Furthermore, methanol extract of *T. conoides* contained total phenol of 1.23 mg GAE/g (Devi *et al.*, 2011). Chew *et al.* (2008) reported that

phenol content can vary quite considerably depending on the variety of seaweed.

3.3. DPPH Radical-Scavenging Activity

Methanol extracts of *P. australis* had stronger ability to scavenge DPPH radical in compared to others (Figure 4). IC₅₀ value is defined as the concentration of substrate that can reduce 50% activity of DPPH radical. The IC₅₀ value results decreased in the following order: methanol > ethyl acetate > n-hexane; with values were 267.1, 1160.2 and 1629.5 ppm, respectively.

The effects of antioxidants on DPPH radical scavenging is thought to be due to hydrogen donating ability. When a DPPH solution is mixed with a substrate as hydrogen atom donor, a stable non-radical form of DPPH is obtained with simultaneous change of the violet color to pale yellow (Molyneux, 2004). Hence, DPPH has been used extensively as a free radical to evaluate reducing substances and is a useful reagent for investigating the free radical scavenging activities of compound (Duan *et al.*, 2006).

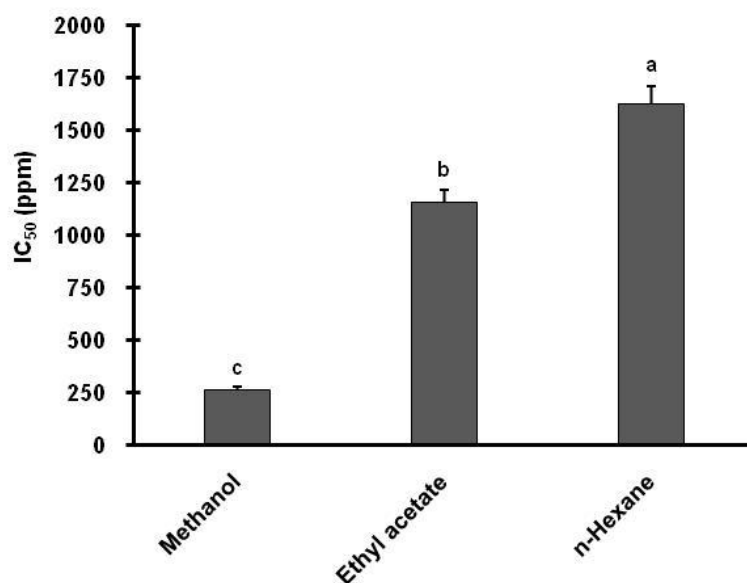


Figure 4. DPPH radical-scavenging activity of each extract of *P. australis* measured by IC₅₀. Letters over each column in the graph not sharing the same are significantly different ($p < 0.05$)

Based on IC₅₀ value, the antioxidant compound are classified as follows: very powerful antioxidant when the IC₅₀ values less than 0.05 mg/mL, strong antioxidant if the value of IC₅₀ between 0.005 to 0.10 mg/mL, intermediate and weak when the IC₅₀ values ranged from 0.10 to 0.15 mg/mL and from 0.15 to 0.20 mg/mL, respectively (Molyneux, 2004). According to the classification, the highest value of methanol extract belongs to weak activity.

There was a positive correlation between total phenol content and antioxidant activity determined by DPPH radical scavenging. Devi *et al.* (2011) reported that methanol extract of *T. conoides* contained the highest number of phenolic compound, exhibited higher radical scavenging activity. Similar result was reported by O'Sullivan *et al.* (2011) that methanol extract of *A. nodosum* had total phenol content of 4.5 mg GAE/g dry weight also had highest activity on DPPH-radical scavenging of 25%. In contrary methanol extract of *Fucus vesiculosus*

only contained total phenol of 2.5 mg GAE/g dry weight; however, it had highest activity on reducing a ferric oxidant to a ferrous complex by electron-transfer with value was 109.8 μ M ascorbic acid.

Bioactive compounds identified in seaweeds including alkaloids, terpenes, ascorbic acid, tocopherols, carotenoids, and phlorotannins (Hu *et al.*, 2008; Heo *et al.*, 2009; Li *et al.*, 2011). The antioxidant activity of polyphenols depends on their nature (*i.e.* phenolic acids, hydroxycinnamic acids, flavonoids, etc.) and chemical structure (mono or dihydroxylation, etc.) (Cuvelier *et al.*, 1992; Pulido *et al.*, 2000). Furthermore, Santoso (2003) and Santoso *et al.* (2004^b) succeeded to identify several active compounds from seaweed namely gallic acid, epigallocatechin, catechin, epicatechin, epigallocatechin gallate, gallic acid gallate, epicatechin gallate, catechin gallate, and catechol.

There are different mechanisms of the antioxidant defense system, *i.e.* (1) scavenging of oxygen and hydroxyl

radicals, (2) reduction of lipid peroxy radical, (3) inhibition of lipid peroxidation, or (4) chelation of metal ions (Pulido *et al.*, 2000). Therefore, the different results are often come out depend on the method which used as a marker.

IV. CONCLUSION

Brown alga *P. australis* contained highly nutritional compound namely mineral. Brown alga *P. australis* also could be a source of natural antioxidant compounds. However, it is remaining to study on activities related to the fractionation, purification and identification of components.

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