COJ (Coastal and Ocean Journal)
e-ISSN: 2549-8223
Journal home page:
https://journal.ipb.ac.id/index.php/coj;
email: journal@pksplipb.or.id

SENYAWA BIOAKTIF, KANDUNGAN FENOL DAN AKTIVITAS ANTIOKSIDAN LAMUN TROPIS *Halodule pinifolia*

BIOACTIVE COMPOUNDS, PHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF TROPICAL SEAGRASS Halodule pinifolia

Joko Santoso[1*], Yulista Noveliyana[1], Sri Purwaningsih[1], Wahyu Ramadhan[1,2]

[1] Department of Aquatic Products Technology, Faculty of Fisheries and Marine Sciences, IPB University, Campus IPB Dramaga Bogor 16680, Indonesia
[2] Center of Coastal and Marine Resources Studies (PKSPL), IPB University, Bogor, 16153, Indonesia

*Corresponding author: jsantoso@apps.ipb.ac.id

ABSTRAK

Lamun yang hidup di daerah pasang surut terpapar cahaya matahari yang kuat, suhu dan kekeringan dalam hidupnya yang dapat menyebabkan meningkatnya produksi spesies radikal reaktif. Dalam rangka mempertahankan kelangsungan hidupnya, lamun tersebut kemungkinan memproduksi senyawa bioaktif dan kandungan komponen gizi dan non-gizinya dapat berubah. Pada penelitian ini, lamun tropis *Halodule pinifolia* diperoleh dari pantai Pangumbahan Sukabumi Indonesia dievaluasi potensi kandungan zat gizi dan komponen antioksidan alami. Lamun segar *H. pinifolia* mengandung abu, protein dan lemak berturut-turut 14,89; 9,74 dan 2,13 g/100 g basis kering; sedangkan kandungan serat pangan larut dan serat pangan tidak larut masing-masing 12,84 dan 27,23 g/100 g basis kering. Kandungan tertinggi total fenol ditemukan pada ekstrak etil asetat (0,31 mg GAE/g basis kering), diikuti ekstrak metanol dan heksana masing-masing sebesar 0,18 dan 0,12 mg GAE/g basis kering. Ekstrak etil asetat juga mempunyai aktivitas penangkapan radikal DPPH tertinggi yang diukur dengan nilai IC50 yaitu 214,38 ppm dibandingkan dengan ekstrak methanol dan heksana. Kesemua ekstrak mengandung komponen bioaktif steroid, flavonoid dan fenol hidroquinon; sedangkan triterpenoid hanya ditemukan pada ekstrak heksana.

Kata kunci: *Antioksidan*, DPPH, Kandungan *Fenol*, Komponen bioaktif, Lamun, Serat pangan

ABSTRACT

Intertidal seagrasses are exposed to a high level of sunlight, temperature, and desiccation daily, which can lead to increase in reactive radical species producing. In order to survive, they may produce some bioactive compounds and may change the content of nutrient and non-nutrient. In this experiment, tropical seagrass Halodule pinifolia collected from the coastal area of Pangumbahan Sukabumi Indonesa was evaluated their potential nutritional value and natural antioxidant compound. Fresh seagrass H. pinifolia contained ash, protein and fat of 14.89, 9.74 and 2.13 g/100 g dry matter; whereas the amounts of soluble dietary fiber, insoluble dietary fiber were 12.84 and 27.23 g/100 g dry matter, respectively. The highest content of total phenol was found in ethyl acetate extract (0.31 mg GAE/g dry matter), followed by methanol and hexane extracts of 0.18 and 0.12 mg GAE/g dry matter, respectively. The extract of ethyl acetate also had the highest activity on DPPH-scavenging measured by IC50 value of 214.38 ppm in compared to methanol and hexane extracts. All of extracts contained bioactive compounds of steroid, flavonoid and phenol hydroquinone; whereas triterpenoid was only found in the extract of hexane.

Keywords*: Antioxidant, Bioactive compound, Dietary fiber, DPPH, Phenol content, Seagrass*

Article history: 06/07/2023; Received in revised from 04/09/2023; Accepted 03/11/2023

1. INTRODUCTION

Seagrasses are submerged marine angiosperms growing abundantly in tidal and subtidal areas of all seas except in the Polar Regions. Seagrasses have a long history of being used for a variety of remedial purposes, such as fever, skin diseases, muscle pains, wounds, and stomach problems (Kannan *et al*., 2012). Intertidal seagrasses are exposed to high levels of light, temperature, and desiccation on daily that can lead to increase in reactive radical species producing. In order to survive, they may produce some active compounds; therefore, they are estimated to possess a large number of active compounds, including antioxidants.

Antioxidants in biological systems have multiple functions, such as protecting from oxidative damage. The major action of antioxidants in cells is to prevent damage caused by the action of reactive oxygen species (ROS), and reactive nitrogen species (RNS). Included in the ROS groups are superoxide (O_2), hydroperoxyl ($HO_2^{\bullet}$), peroxyl ($RO_2^{\bullet}$) and alkoxyl ($RO^{\bullet}$); while belonging to the RNS group are nitric oxide ($NO^{\bullet}$), peroxynitrite ($ONOO^-$) and peroxynitrous acid (ONOOH). Those compounds cause extensive oxidative damage to cells leading to age-related diseases, cancer, and a wide range of other human diseases (Langseth, 2000; Prakash *et al*., 2007; Chen *et al*., 2008; O'Sulivan *et al*., 2011).

Research activities on the bioactive compounds of seagrasses had much attention in recent years. Dumay *et al.* (2004) conducted research on variation in the concentration of the phenolic compound of seagrass *Posidonia oceanica*, while Sureda *et al*. (2008) evaluated the antioxidant response of seagrass *P. oceanica* and its secondary metabolites was investigated by Heglmeir and Zidorn (2010). Other seagrasses species *Halophila johnsonii* and *H. decipiens* were evaluated for their antioxidant capacity of flavonoid form (Gavin and Durako, 2011); and Kannan *et al.* (2010) investigated antioxidant activity of ethanol extract from *Enhalus acaroids*. Methanol extracts of seagrasses *Syringodium isoetifolium* and *Cymodocea serrulata* possessed the maximum antimicrofouling and antimacrofouling activities (Iyapparaj *et al*., 2014). The chemical composition and antibacterial activity of Indian seagrasses were studied by Kannan *et al*. (2012) and Kannan *et al*. (2013). In the case of Indonesian seagrasses, we evaluated the phenol contents and antioxidant activities of *Thalassia hemprichii*, *Syringodium isoetifolium*, *Cymodocea rotundata*, and *Enhalus acoroides*, collected from the Pramuka Island, Kepulauan Seribu Jakarta in the rainy season of 2011 (Santoso *et al*., 2012).

From the foodstuff viewpoint, seagrasses may contain beneficial compounds for human beings since certain local people who live in coastal area already have utilized them. Seagrasses, particularly as a rich source of protein, fiber and lipid and for their beneficial effects that have been attributed to diseases, such as obesity and diabetes (Rengasamy *et al*., 2013). The seeds of the tropical seagrass *E. acaroids* have been traditionally eaten in the Philippines (Montano *et al*., 1999). Research on fatty acid composition of *E. acaroids* shows that palmitic, linoleic, and linolenic acids were found as major fatty acid from seagrasses *H. ovalis* and *H. ovata* (Gillan *et al*., 1984), whereas for the seagrass *P. oceanica* the major fatty acids in the leaves are 16:0, 18:2n6 and 18:3n3, and in the rhizomes 16:0, 18:2n6 and 18:1n9 (Viso *et al*., 1993). Furthermore, Iyapparaj *et al.* (2014) reported that

fatty acids (C_{16} to C_{24}) were the major components of seagrasses *S. isoetifolium* and *C. serrulata*. Santoso *et al*. (2012) evaluated the dietary fiber composition of tropical seagrasses from Seribu Island, Jakarta Indonesia.

The nutrient composition and bioactive compounds of seaweeds vary depending on the species, maturity, environmental growth condition and seasonal period (Mabeau and Fluerence, 1993; Ortiz *et al*., 2006; Benjama and Masniyom, 2012, Ramadhan *et al.,* 2022a, Ramadhan *et al.,* 2022b). Therefore, in this study, we used different tropical seagrass, namely *Halodule pinifolia*. This seagrass grown wildly in coast area of National Conservation for Turtle Pangumbahan Sukabumi, West Java Province – Indonesia. The research was carried out to study the chemical composition (proximate composition and dietary fiber profile) of seagrass *H. pinifolia*; to evaluate total phenol content, phytochemical compounds, and antioxidant activities of *H. pinifolia* extract in different solution *i.e.,* methanol, ethyl acetate and n-hexane.

2. METHODS

Chemicals and Reagents

Chemicals and reagents were used in this experiment are methanol, ethyl acetate, n-hexane, pancreatin enzyme, phosphate buffer, gallic acid, butylated hydroxytoluene (BHT), ethanol, acetone, sodium carbonate, 2,2-diphenyl-1-picryldrazyl (DPPH), Follin-Ciocalteu reagents. All the chemicals were analytical grade and obtained from Merck Darmstadt Germany; Sigma Chemical Corp. St Louis, MO USA; Aldrich Steinheim Germany; and Wako Pure Chemical Industries Ltd. Osaka Japan.

Plant Material

Fresh leaves, roots, and rhizomes of tropical seagrass H. pinifolia were collected from the intertidal region of the Conservation Area for Turtle Pangumbahan Sukabumi, West Java Province – Indonesia. Immediately the sample was placed in a plastic bag containing sea water in order to prevent evaporation and transported to the laboratory under refrigeration condition. Then the plant was washed thoroughly with tap water to remove all sand particles and epiphytes. The sample was divided into two groups, specifically fresh and dried samples. Fresh samples were required to be analyzed the proximate composition and content of dietary fiber; whereas the dried samples were used for extraction of antioxidant compound. Dried sample forms were obtained after being dried using sun rays for two days and grounded in an electric mixer. The powder samples were then stored in refrigerator for further use.

Proximate Analysis

The chemical composition of moisture, ash, fat and protein (nitrogen converting factor = 6.25), of fresh seagrass H. pinifolia was analyzed according to the method of AOAC (1995).

Determination of Dietary Fiber

Soluble and insoluble dietary fibers were determined according to an enzymatic-gravimetric method (Porsky et al., 1988) which has been approved as the legal or recommended procedure for food analysis. However, this method was modified here by using pancreatin (Suzuki et al., 1996; Plaami et al., 1989; Santoso, 2003) because almost

all seagrasses contain little protein and no starch. The procedure consists of following steps: (1) Boiling 2 g of wet sample with 30 mL of water for 5 min. (2) Incubation with 20 mL of 2% pancreatin and 30 mL of phosphate buffer at pH 6.8 in the presence of NaCl (10 mM) for 24 h at 37oC. (3) Waacetone ande dietary fiber was filtered off by a glass fiber filter (GA-100, Adventec Toyo Inc., Tokyo, Japan), washed three times with 20 mL of 78% ethanol, twice with 20 mL of 95% ethanol and once with 10 mL of acetone, and dried at 105 oC. Water soluble dietary fiber was precipitated from the filtrate using 4 volumes of ethanol (at 60 oC) and recovered by filtration in the same way as for insoluble fiber. (5) All samples analyzed were assayed in duplicates and one of the duplicates was used to determine protein content, while the other was used to determine ash content in the fiber precipitate. (6) The final corrected values or the amounts of dietary fiber were calculated by subtracting the weights of ash and protein from the dietary fiber precipitate. The ash and protein contents were analyzed according to the method of AOAC (2005) by furnace and Kjeldahl, respectively.

Preparation of Seagrass Extracts

Three types of solvents with different polarity were used in this experiment i.e., methanol, ethyl acetate, and n-hexane. The dried powder of sample consists of leaves, root, and rhizome (20 g) were extracted for 48 hours in 160 mL of each solvent using automatic shaking for the maceration process at room temperature 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 oC using a rotary flash evaporator until it became paste. Each crude extract in paste form was filled up by nitrogen gas to prevent the decomposition of an active compound inside, then kept at -20 oC until analysis.

Determination of Total Phenol Contents

Total phenol contents of each crude extract from seagrasses were determined by spectrophotometry using Follin-Ciocalteu reagents (Santoso et al., 2004a; Yangthong et al., 2009). Methanol, ethyl acetate, n-hexane extract of each seagrass (0.5 mg) was weighed and diluted with 2 mL of 95% ethanol. Then the solution was added 5 mL of distillated water and 0.5 mL of Follin-Ciocalteu reagents (previously diluted with water 1:1 v/v). The mixture was allowed to stand for 5 min then added 1 mL of sodium carbonate (5% w/v). The homogenized mixture was then incubated in a dark room for one hour. The resulting absorbance was measured by a spectrophotometer (UV-1200 UV-VIS Spectrophotometer, Shimadzu, Kyoto, Japan) at 725 nm. Total phenol 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 100 gram of gallic acid equivalent (GAE).

Qualitative Test of Phytochemical Compounds

A qualitative test of phytochemical was performed to determine the presence or absence of bioactive components contained in each crude extract of seagrass in three types of solvent. The phytochemical test consisted of tests of alkaloids (Dragendorf, Meyer, Wagner), steroids, triterpenoids, flavonoids, saponins, phenols hydroquinone and

saponin. The tests were carried out according to the method described by Harborne (1987).

DPPH Radical-Scavenging Activity

Antioxidant activity assay was measured through the ability of the sample to reduce the stable free radical DPPH according to the method described by Aranda et al. (2009) with slight modifications. One milligram of each crude extract and butylated hydroxytoluene (BHT) as a positive control were weighed and then added to ethanol with a ratio of 1:1000. Furthermore, 1.3 mg of DPPH was diluted with 25 mL of ethanol. One milliliter of ethanol was loaded into the micro-well plate which has been prepared. After that, extracts with several concentrations and the addition of DPPH solution were loaded. The mixture was homogenized and incubated at 37 ° C for 30 minutes. The resulting absorbance was measured by an ELISA reader (Microplate Absorbance Reader 168-1130, Bio-Rad, California USA) at a wavelength of 517 nm. The percentage inhibition of free radical activity obtained from absorbance values of samples. Regression equation obtained from the relationship between sample concentration and percentage inhibition of free radical activity. The inhibitory concentration value of free radical activity by 50%, namely IC50, was calculated using the regression equation as follows: $y = a + b \ln (x)$, where y is the percentage of inhibition, a is an intercept, b is a slope, and x is a concentration of sample (mg/L).

Statistical Analysis

Results are expressed as mean value ± standard deviation. Comparison of means using a significant level of $p<0.05$ was performed by analysis of variance and means separated by F-test and Tukey-test. All computations were done by employing the statistical software (SPSS, version 16).

3. Result and Discussions

Proximate Composition and Dietary Fiber Contents

Table 1 shows the proximate composition and dietary fiber contents of the seagrass *H. pinifolia.* The *H. pinifolia* sample had moisture, ash, protein, fat, insoluble dietary fiber (IDF), soluble dietary fiber (SDF) and total dietary fiber (TDF) contents of 81.42 g/100 g fresh weight, 4.63 g/100 g fresh weight, 1.81 g/100 g fresh weight, 0.40 g/100 g fresh weight, 5.06 g/100 g fresh weight, 2.39 g/100 g fresh weight, and 8.77 g/100 g fresh weight, respectively. After converting them to 100 g dry matter samples, the contents of ash, protein, fat, IDF, SDF and TDF were high. In dried form, TDF was found in the highest number, followed by ash content; whereas the lowest content was found in fat.

Since there is no available data about the proximate composition of seagrass, especially tropical seagrass, therefore we compared it to another marine plant *i.e*., seaweed. From the foodstuff standpoint, seaweeds have a long history and even today, they are an important part of the diet of many Asian countries, particularly Japan, China, and Korea. Among these three, Japan, where seaweeds have a considerable market value, is the most important seaweed consumer (Nisizawa *et al*., 1987; Nisizawa, 2002). In Indonesia, local people living in the coastal area already utilized them in various form: raw as salads, boiled as vegetable, and mixed with various species; however, they consumed in limited number (Santoso, 2003). In our previous work, eight species of Indonesian green, brown and red seaweeds were determined the proximate composition. The range contents of ash, fat and protein in dry matter were 16.5-32.5, 1.2-13.1 and 4.2-17.6 g/100 g,

respectively (Santoso *et al*., 2006). The proximate composition between Indonesian seaweeds and seagrass *H. pinifolia* are almost same.

Table 1 The proximate composition and dietary fiber contents of the tropical seagrass H. pinifolia.

Composition	Values (g/100 g)	
	Fresh weight	Dry matter
Moisture	81.42 ± 0.23	-
Ash	4.63 ± 0.06	24.89 ± 0.03
Protein	1.81 ± 0.10	9.74 ± 0.41
Fat	0.40 ± 0.01	2.13 ± 0.01
Insoluble Dietary Fiber (IDF)	5.06 ± 0.11	27.24 ± 0.28
Soluble Dietary Fiber (SDF)	2.39 ± 0.07	12.87 ± 0.53
Total Dietary Fiber (TDF)	8.77 ± 1.91	47.15 ± 9.70

For dietary fiber comparison, Santoso *et al.* (2012) analyzed the dietary fiber contents of four Indonesian seagrasses. The contents of total dietary fiber in fresh weight of *T. hemprichii*, *S. isoetifolium*, *C. rotundata*, and *E. acoroides* were 15.39, 14.34, 14.68 and 14.32 g/100 g, respectively. The total dietary fiber content of seagrass *H. pinifolia* (8.77 g/100 g) was lower compared to our previous report. Beside the species, the main factor which may effect on the chemical composition including dietary fiber content is habitat, especially the physical and chemical characteristics of environment or environmental growth condition (Mabeau and Fluerence, 1993; Ortiz *et al*., 2006; Benjama and Masniyom, 2012).

If we compare it to seaweed, the content of dietary fiber in seaweeds is much higher than seagrasses, since seaweed contains sulphuric group in part of soluble dietary fiber as sulphated galactans (Elleuch *et al*., 2011; Benjama and Masniyom, 2012). The contents of dietary fiber in seaweed are 33-75%, particularly rich in the soluble fraction (50-85% of total dietary fiber) (Gomez-Ordonez *et al*., 2011; Jimenez-Escrig and Sanchez-Muniz, 2000). In some Indonesian seaweeds the contents of total dietary fiber were 47.0 – 69.3 g/100 g (Santoso *et al.,* 2002); some Spanish seaweeds were 29.31 – 37.14% (Gomez-Ordonez *et al*., 2011), Tunisia seaweed of *Ulva lactuca* was 54.90% (Yaich *et al*., 2011), Thai seaweeds *Gracialaria fisheri* and *G. verrucosa* contained 57.5 – 64.0% (Benjama and Masniyom, 2012), and Indonesian brown seaweed of *Padina australis* contained soluble, insoluble and total dietary fibers of 8.3, 5.4 and 13.8 g/100 g fresh weight, respectively (Santoso *et al*., 2013).

Total Phenolic Contents

The total phenolic contents of crude extract of seagrass *H. pinifolia* in different solvents are depicted in Table 2. Ethyl acetate extract of *H. pinifolia* had the highest content of total phenol (0.31 mg GAE/g sample), followed by methanol and n-hexane extracts which values were 0.18 mg and 0.12 mg GAE/g sample, respectively. Santoso *et al*. (2012) reported that the solubility of phenolic compounds might differ in each type of solvent and the source of materials.

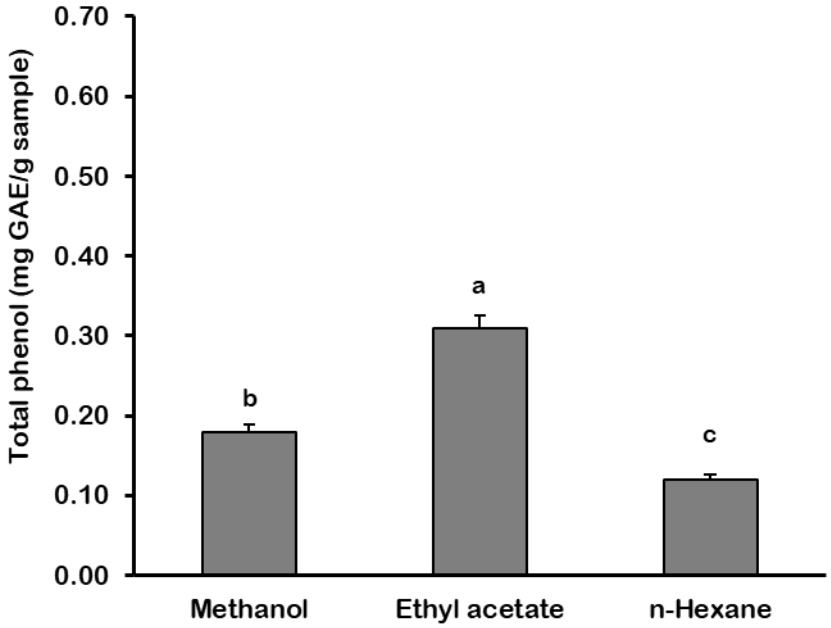

Table 2 Total phenol contents of extract H. pinifolia in different solvent. Letters over each column in the graph not sharing the same are significantly different ($p<0.05$)

Extracted using methanol obtained the highest number of total phenol contents of seagrasses *T. hemprichii* (1022.58 g/100 g fresh weight), *C. rotundata* (335.58 g/100 g fresh weight), and *E. acoroides* (542.56 g/100 g fresh weight). However, in *S. isoetifolium* the phenol compound tended to be soluble in semi-polar solvent (ethyl acetate) with value was 732.66 g/100 g fresh weight (Santoso *et al*., 2012). In the case of n-hexane, obtained the smallest number of total phenols in each seagrass species. This indicated that the phenols contained in seagrasses tended to dissolve in polar and semi-polar solvents (Santoso *et al*., 2012).

Phenolic compounds are commonly found in plants and have been reported to have several biological activities, including potential antioxidants and free radical scavengers apart from their primary defense role (Soobratte *et al*., 2005). Seagrasses are particularly rich in proanthocyanidins (condensed tannins) (Athiperumalsamy *et al*., 2008). The presence of phytoconstituents, such as phenols, flavonoids and tannin in seaweeds and seagrasses may be responsible for antioxidant activity preventing a number of diseases through free radical scavenging activity (Athiperumalsamy *et al*., 2010).

Eight of the fifteen flavone compounds identified in *H. johnsonii* possess molecular structures indicative of high antioxidant activity due to 3-4-ortho-di-hydroxyl or 3-4-5-ortho-tri-hydroxyl configurations on the B-ring (Meng *et al*., 2008). Flavone compounds *in H. johnsonii* form two distinct groups, in terms of solubility: hydrophilic flavone glycosides and hydrophobic flavones (Meng *et al*., 2008). Butanol extract of a Mediterranean seagrass *Halophila stipulacea* contained malonylated flavone glucoside, genkwanin-4′-O-(6′′malonyl-glucopyranoside) and flavones glucosides 4-9 (Bitam *et al.,* 2010). A total of 51 natural products was reported from *P. oceanica*, including phenols, phenylmethane derivatives, phenylethane derivatives, phenylpropane derivatives and their esters, chalkones, flavonols, 5a-cholestanes, and cholest-5-enes (Heglmeier and

Zidorn, 2010). Several phenolic compounds have been identified in seaweed extracts. Catechin and its derivates *i.e* epicatechin, gallocatechin, epigallocatechin and catechin gallate were found in extracts of Indonesian and Japanese seaweeds, as well as catechol (Yoshie *et al.,* 2002; Yoshie-Stark *et al*., 2003; Santoso *et al*., 2004[b]).

DPPH Radical Scavenging Activity

The effects of antioxidants on DPPH radical scavenging are thought to be due to hydrogen donating ability. When a DPPH solution is mixed with a substrate as a 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).

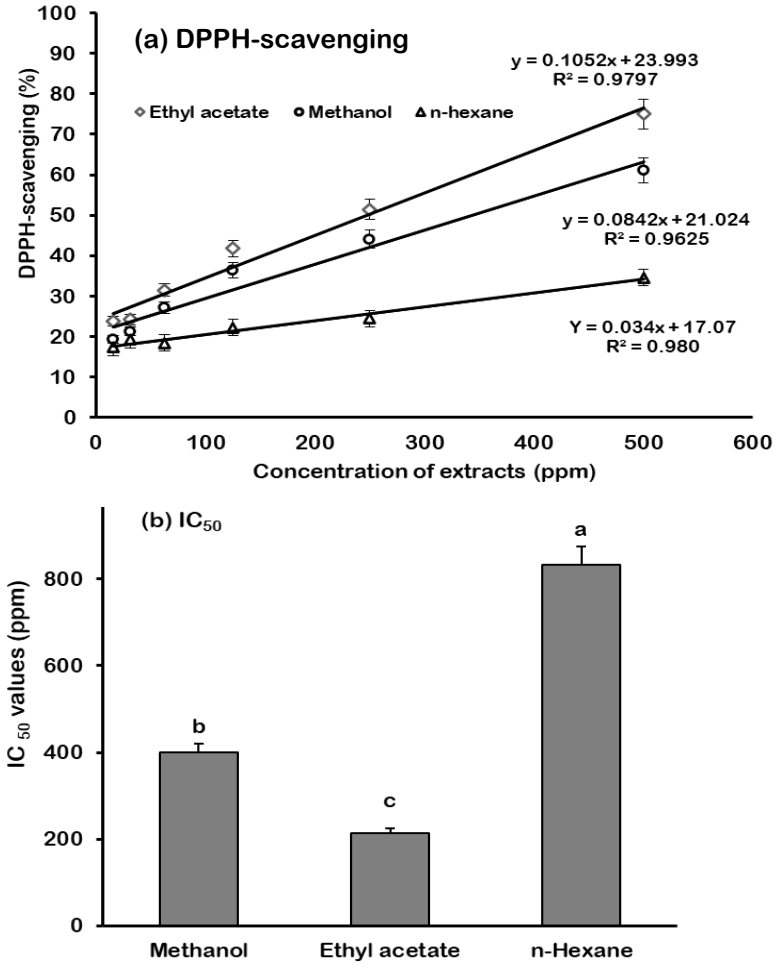

Table 3 The regression model of each extract H. pinifolia in different solvent on ability of DPPH radical scavenging (a) and IC_{50} values of each extract (b). Letters over each column in the graph not sharing the same are significantly different ($p<0.05$)

In order to compare the ability of each extract on DPPH radical scavenging, we determined the IC_{50} of each extract. IC_{50} value is defined as the concentration of substrate that can reduce 50% activity of DPPH radical. Fig. 2(a) and 2(b) show the regression model of each extract on ability of DPPH radical scavenging and IC_{50} values, respectively. Ethyl acetate extract of *H. pinifolia* had stronger ability to scavenge DPPH radical in compared to others, stated by IC_{50} values. The IC_{50} value results decreased in the following order: ethyl acetate > methanol > n-hexane; with values were 214, 400 and 834 ppm, respectively.

Ethyl acetate extract of *H. pinifolia* had the highest activity on DPPH scavenging since the ethyl extract was rich in total phenol content (Fig. 1.). This result was same to the previous research conducted by Santoso *et al.* (2012). They reported that the strongest activity on DPPH scavenging was found in ethyl acetate extract of *S. isoetifolium* with values was 96.34 ppm, followed by methanol extracts of *E. acoroides*; *C. rotundata* and *T. hemrichii* with values were 115.79, 123.72, and 214.68 ppm, respectively. All of seagrasses had the lowest activities when extracted using n-hexane. Those conditions indicated that active compounds inside tended to be soluble in polar and semi-polar solvents.

Based on IC50 value, the antioxidant compound is classified as follows: very powerful antioxidant when the IC 50 values less than 0.05 mg/mL, strong antioxidant if the value of IC 50 between 0.005 to 0.10 mg/mL, intermediate and weak when the IC 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, extract *H. pinifolia* in ethyl acetate belongs to weak activity. This was crude extract, still contained other compounds, and may interfere on antioxidant activity. Since the content of mineral in *H. pinifolia* was the highest (Table 1) and remained in the extract, therefore it could disturb the DPPH radical scavenging activity.

Phytochemical Compounds

The phytochemical test aims to determine the bioactive components present in each crude extract of seagrass. Among the seven tests on the activity of bioactive compounds, it appears that *H. pinifolia* crude extracts contained steroid, flavonoid, and phenol hydroquinone (Table 2). However, there was not detected on alkaloid, triterpenoid, tannin and saponin; except n-hexane crude extract had activity on triterpenoid. In crude extract of n-hexane, we found four bioactive compounds inside, whereas both in methanol and ethyl acetate crude extracts were found only three bioactive compounds.

Table 4 The results of phytochemical test of crude extracts of seagrass H. pinifolia

Phytochemical test	Type of crude extracts			Description of positive reaction
	Methanol	Ethyl acetate	n-Hexane	
Alkaloid:				
(a) Dragendorf	-	-	-	Red or orange precipitate formed
(b) Meyer	-	-	-	Yellowish-white precipitate formed
(c) Wagner	-	-	-	Brown precipitate formed
Steroid	+	+	+	Changed from red color to blue-green
Triterpenoid	-	-	+	Changed from red color to violate
Flavonoid	+	+	+	Amyl alcohol layer formed (red/yellow/ green)
Phenol hydroquinone	+	+	+	Green or blue green color formed
Tannin	-	-	-	Changed from green color to blue
Saponin	-	-	-	Foam formed

Notes: - not detected; + detected

Yu-Tang et al. (2009) stated that methanol can extract low molecular weight compounds and the medium degree of polarity, because methanol has wide solubility properties. Research conducted by Kannan et al. (2010) proved that the tannins content of ethanol extracts of E. acaroides in form of proanthocyanidins was higher than total phenol. Proanthocyanidin is condensed tannin. It is also reported that the presence of condensed tannins in seagrass may act as deterrents against herbivore feeding as well as against fungal and bacterial invasion (McMillan, 1984). However, there was no tannin in the extract of H. pinifolia.

The amount and concentration of the phytochemical components are also influenced by the presence of competitors. Dumay et al. (2004) reported when the seagrass P. oceania is in interaction with Caulerpa taxilofila (green seaweed), it accelerates its production of secondary metabolites. Moreover, the production of bioactive compounds in seagrass is due to increased levels of oxidative stress and antioxidant defenses (Sureda et al., 2008). The chemical composition of Indian seagrass consisted of 24 compounds such as 3,7,11,15-tetramethyl-2-hexadecan-1-ol, 4H-Pyran-4-one-2,3-dihydro-3,5-dihydroxy-6-methyl-, D-allo-se and 5-caranol; determined by GC–MS were reported by Kanan et al. (2012).

4. CONCLUSIONS

From our results it could be concluded that seagrass H. pinifolia contained highly nutritional and non-nutritional compounds namely mineral and dietary fiber. Besides that, H. pinifolia could be a source of natural antioxidant compounds. However, research activities related to the fractionation, purification and identification of components present in it needs to be performed.

REFERENCES

[AOAC] Association of Official Analytical Chemist. 2005. Official Method of Analysis of the Association of Official Analytical of Chemist. The Association of Official Analytical Chemist, Inc., Washington, D.C. 1899 pp.

Aranda, R.S., L.A.P. Lopez, J.L. Arroyo, B.A.A. Garza, and N.W. Torres. 2009. Antimicrobial and antioxidant activities of plants from Northeast of Mexico. Evidence-Based Complement Alternative Medicine, 20(11): 1-6.

Athiperumalsamy, T., V. Kumar, and L. Jessudas. 2008. Survey and phytochemical analysis on seagrasses in the Gulf of Mannar, southeast coast of India. Botanica Marina, 51: 269-277.

Athiperumalsamy, T., D. Rajeswari, S.H. Poorna S, V. Kumar, and L. Jessudas. 2010. Antioxidant activity of seagrasses and seaweeds. Botanica Marina, 53: 251-257.

Benjama, O., and P. Masniyom. 2012. Biochemical composition and physicochemical properties of two red seaweeds (Gracilaria fisheri and G. tenuistipitata) from the Pattani Bay in Southern Thailand. Songklanarin J. of Science and Technology, 34(2): 223-230.

Bitam, F., M.L. Ciavatta, M. Carbone, M. Ernesto, and M.M. Gavagnin. 2010. Chemical analysis of flavonoid constituents of the seagrass Halophila stipulacea: First finding of malonylated derivatives in marine phanerogams. Biochemical Systematics and Ecology, 38: 686-690.

Chen, Y., L. Cai, C. Zhao, H.C. Xu, C.Y. Cao, and Y. Liu. 2008. Spectroscopic, stability and radical scavenging properties of a novel pigment from Gardenia. Food Chemistry, 109: 269-277.

Duan, X.J., W.W. Zhang, X.M. Li, and B.G. Wang. 2006. Evaluation of antioxidant property of extract and fractions obtained from a red alga, Plysiphonia urceolata. Food Chemistry, 95: 37-43.

Dumay, O., J. Costa, J.M. Desjobert, and G. Pergent. 2004. Variation in the concentration of phenolic compounds in the seagrass Posidonia oceania under condition of competition. Phytochemistry, 65: 3211-3220.

Elleuch, M., D. Bedigian, O. Roiseux, S. Besbes, C. Blecker, and H. Attia. 2011. Dietary fibre and fibre-rich by-products of food processing: Characterization, technological functionality and commercial applications: A review. Food Chemistry, 124: 411-421.

Gavin, N.M., and M.J. Durako. 2011. Localization and antioxidant capacity of flavonoids from intertidal and subtidal Halophila johnsonii and Halophila decipiens. Aquatic Botany, 95: 242-247.

Gillan, F.T., R.W. Hogg, and D.E. Drew. 1984. The sterol and fatty acid composition of seven tropical seagrasses from North Quensland Australia. Phytochemistry, 23: 2817-2821.

Gomez-Ordonez, E., A. Jimenez-Escrig, and P. Ruperez. 2011. Dietary fibre and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. Food Research International, 43: 2289-2294.

Harborne, J.B. 1987. Phytochemical Methods 2nd edition. Chapman and Hall, New York. 184 p.

Heglmeier, A., and C. Zidorn. 2010. Secondary metabolites of Posidonia oceania (Posidoniaceae). Biochemical Systematics and Ecology, 38: 964-970.

Iyapparaj, P., P. Revathi, R. Ramasubburayan, S. Prakash, A. Palavesam, G. Immanuel, P. Anantharaman, A. Sautreau, and C. Helli. 2014. Antifouling and toxic properties of the bioactive metabolites from the seagrasses Syringodium isoetifolium and Cymodocea serrulata. Ecotoxicology and Environmental Safety, 103: 54-60.

Jimenez-Escrig, A., and F. Sanchez-Muiz. 2000. Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. Nutrition Research, 20(4): 585-598.

Kannan, R.R.R., R. Arumugam, and P. Anantharaman. 2010. In vitro antioxidant activities of ethanol extract from Enhalus acoroides (L.F.) Royle. Asian Pacific J. of Tropical Medicine, 898-901.

Kannan, R.R.R., R. Arumugam, and P. Anantharaman. 2012. Chemical composition and antibacterial activity of Indian seagrasses against urinary tract pathogens. Food Chemistry, 135: 2470–2473.

Kannan, R.R.R., R. Arumugam, P. Iyapparaj, T. Thangaradjou, and P. Anantharaman. 2013. In vitro antibacterial, cytotoxicity and haemolytic activities and phytochemical analysis of seagrasses from the Gulf of Mannar, South India. Food Chemistry, 136: 1484-1489.

Langseth, L. 2000. Antioxidant and their effect on health. In: Schmidl, M.M., and T.P. Labuza (eds.). Essential of Functional Foods. Aspen Publisher Inc., Gaithersburg, Maryland. pp. 303-317.

Mabeau, S., and J. Fluerence. 1993. Seaweeds in food products: Biochemical and nutritional aspects. Trends in Food Science and Technology, 4:103-107.

McMillan, C. 1984. The contensed tannins (proanthocyanidin) in seagrasses. Aquatic Botany, 20: 351-357.

Meng, Y., A.J. Krzysiak, M.J. Durako, J.I. Kunzelman, L.C. Jeffrey, and J.L.C. Wright. 2008. Flavones and flavone glycosides from sea grass Halophila johnsonii. Phytochemistry, 69: 2603-2608.

Molyneux, P. 2004. The use of the stable free radical diphenylpicryl-hydrazil (DPPH) for estimating antioxidant activity. Songklanarin J. of Science and Technology, 26(2): 211–219.

Montano, N.M.E., R.S. Bonaficao, and R.G.O. Rumbaoa. 1999. Proximate analysis of the flour and starch from Enhalus acaroides (L.f) Royle seeds. Aquatic Botany, 65:321-325.

Nisizawa, K., H. Noda, R. Kikuchi, and T. Watanabe. 1987. The main seaweeds in Japan. Hydrobiology, 151/152: 5-29.

Nisizawa, K. 2002. Seaweeds Kaiso. Bountiful harvest from the seas. Sustenance for Health and Well Being by Preventing Common Life-style Related Diseases. Japan Seaweed Association, Kochi. 105 p.

Ortiz, J., N. Romero, P. Robert, J. Araya, J. López-Hernández, C.E. Bozzo,C.E. Navarrete, A. Osorio, and A. Rios. 2006. Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds Ulva lactuca and Durvillaea antartica. Food Chemistry, 99: 98-104.

O'Sullivan, A.M., Y.C. O'Callaghan, M.N. O'Grady, B. Queguineur, D. Hanniffy, D.J. Troy, J.P. Kerry, and N.M. O'Brien. 2011. In vitro and cellular antioxidant activities of seaweed extracts prepared from five brown seaweeds harvested in spring from the west coast of Ireland. Food Chemistry, 126: 1064-1070.

Plaami, S., M. Saastamoinen, and J. Kumpulainen. 1989. Effect of variety of environment on dietary fiber content of winter rye in Finland. J. of Cereal Science, 10: 209-215.

Prakash, D., G. Upadhyay, B.N. Singh, and H.B. Singh. 2007. Antioxidant and free radical-scavenging activities of seeds and agri-wastes of some varieties of soybean (Glycine max). Food Chemistry, 104: 783-790.

Prosky, L., N.G. Asp, T.F. Schweizer, J.W. DeVries, and I. Furda. 1988. Determination of insoluble and total dietary fiber in foods and food product: Intercollaborative study. J. of Association Official Analytical Chemist, 71: 1017-1023.

Ramadhan, W., Uju, Hardiningtyas, SD., Pari., Nurhayati, Sevica, D. 2022a. Ultrasonic wave assisted extraction of ulvan polysaccharide from Ulva lactuca seaweed at low temperature. JPHPI, 2(1): 132-142

Ramadhan, W., Khoirunnisa Z., Uju, Tarman K., Oktaviarty V., Meydia. 2022b. Meta-analysis on the effect of ingredients on chemical quality of nori-like product. COJ (Coastal and Ocean Journal). 6(2): 63-72.

Rengasamy, R.R.K., A. Radjassegarin, and A. Perumal. 2013. Seagrasses as potential source of medicinal food ingredients: Nutritional analysis and multivariate approach Biomedicine & Preventive Nutrition, 3(4): 375-380.

Santoso, J., Y. Yoshie, and T. Suzuki. 2002. The distribution and profile of nutrients and catechins of some Indonesian seaweeds. Fisheries Science, 68 (suppl): 1647-1648.

Santoso, J. 2003. Studies on nutritional components and antioxidant activity in several Indonesian seaweeds [dissertation]. Tokyo University of Fisheries, Tokyo. 119 p.

Santoso, J., Y. Yoshie, and T. Suzuki. 2004a. Antioxidant activity of methanol extracts from Indonesian seaweeds in an oil emulsion model. Fisheries Science, 70: 183-188.

Santoso, J., Y. Yoshie, and T. Suzuki. 2004b. Polyphenolic compounds from seaweeds: Distribution and their antioxidative effects. Development in Food, 42: 169-177.

Santoso, S., S. Gunji, Y. Yoshie-Stark, and T. Suzuki. 2006. Mineral content of Indonesian seaweeds and mineral solubility affected by basic cooking. Food Science and Technology Research, 12(1): 59-66.

Santoso, J., S. Anwariyah, R.O. Rumiantin, A.P. Putri, N. Ukhty, and Y. Yoshie-Stark. 2012. Phenol content, antioxidant activity and dietary fiber profile of four tropical seagrasses from Indonesia. J. Coastal Development, 15(2): 189-196.

Santoso, J., F. Podungge, and H. Sumaryanto. 2013. Chemical composition and antioxidant activity of tropical brown alga Padina australis from Pramuka Island – District of Seribu Island, Indonesia. J. Ilmu dan Teknologi Kelautan Tropis, 5(2): 287-297.

Soobrattee, M.A., V.S. Neergheen, A. Luximon-Ramma, O.I. Arouma, and T. Bahorun. 2005. Phenolic as potential antioxidant therapeutic agents: Mechanism and actions. Mutation Research, 579: 200-213.

Sureda, A., A. Box, J. Terrados, S. Duedero, and A. Pons. 2008. Antioxidant responses of the seagrass Posidonia oceania when epiphytized by the invasive macroalgae Lophlocladia lallemandii. Marine Environmental Research, 66: 359-363.

Suzuki, T., Y. Ohsugi, Y. Yoshie, T. Shirai, and T. Hirano. 1996. Dietary fiber content, water-holding capacity and binding capacity of seaweeds. Fisheries Science, 62: 454-461.

Viso, A.C., D. Pesando, P. Bernard, and J.C. Marty. 1993. Lipid components of the Mideterranean seagrass Posidonia oceania. Phytochemistry, 34(2): 381-381.

Yaich, H., H. Garna, S. Besbes, M. Paquot, C. Blecker, and H. Attia. 2011. Chemical composition and functional properties of Ulva lactuca seaweed collected in Tunisia. Food Chemistry, 128: 895-901.

Yangthong, M., H.T. Nongporn, and W. Phromkunthong. 2009. Antioxidant activities of four edible seaweeds from the southern coast of Thailand. Plant Foods Human Nutrition, 64: 218-223.

Yoshie, Y., W. Wang, D. Petillo, and T. Suzuki T. 2002. Distribution catechins in Japanese seaweeds. Fisheries Science, 66: 998-1000.

Yoshie-Stark, Y., Y.P. Hsieh, and T. Suzuki. 2003. Distribution of flavonoids and related compounds from seaweeds in Japan. J. Tokyo University of Fisheries, 89: 1-6.

Yu-Tang, T., C. Jyh-Horng, H. Ch-Chang, P. Hsiang-Chi, C. Ya-Ling, and Y. Suh-Ching. 2009. Protective effect of acacia confuse bark extract and its active compound gallic acid against carbon tetrachloride-induced chronic liver injury in rats. Food Chemistry and Toxicology, 47: 1385-1392.