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

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

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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 Indonesia 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

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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$_2^•$), peroxyl (RO$_2^•$) and alkoxy (RO$^•$); while belonging to the RNS group are nitric oxide (NO$^•$), 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’Sullivan 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 Hegelmeir 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 acaroides. 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. acaroides have been traditionally eaten in the Philippines (Montano et al., 1999). Research on fatty acid composition of E. acaroides 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 (C₁₆ to C₂₄) 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) Waetone and 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 distilled 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*.

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

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 Fluence, 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.
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, phenylethene derivatives, phenylethane derivatives, phenylpropane derivatives and their esters, chalcones, flavonols, 5a-cholestanes, and cholest-5-enes (Heglmeier and
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).

**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).

![Graph of DPPH-scavenging](image)

![Graph of IC₅₀](image)

**Table 3** The regression model of each extract H. pinifolia in different solvent on ability of DPPH radical scavenging (a) and IC₅₀ 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\textsubscript{50} of each extract. IC\textsubscript{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\textsubscript{50} values, respectively. Ethyl acetate extract of \textit{H. pinifolia} had stronger ability to scavenge DPPH radical in compared to others, stated by IC\textsubscript{50} values. The IC\textsubscript{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 \textit{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 \textit{S. isoetifolium} with values was 96.34 ppm, followed by methanol extracts of \textit{E. acoroides; C. rotundata} and \textit{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 IC\textsubscript{50} 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 ran 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 \textit{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 \textit{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 \textit{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

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th>Type of crude extracts</th>
<th>Description of positive reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>Alkaloid:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Dragendorf</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(b) Meyer</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(c) Wagner</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>hydroquinone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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 taxifolia (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.

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