Journal of Coastal Develpopment
Volume 15, Number 2 ,February 2012 : 189-196

ISSN : 1410-5217
Acrredited : 83/Kep/Dikti/2009

Original Paper

PHENOL CONTENT, ANTIOXIDANT ACTIVITY AND FIBERS PROFILE OF FOUR TROPICAL SEAGRASSES FROM INDONESIA

Joko Santoso[1], Siti Anwariyah[1], Ria Octavia Rumiantin[1], Aristi Pramadita Putri[1], Nabila Ukhty[1], and Yumiko Yoshie-Stark[2]

[1]Department of Aquatic Products Technology, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Campus IPB Darmaga Bogor 16680, Indonesia
[2] Department of Food Life Science, Faculty of Life Science, Toyo University, Itakura-machi Ora-gun, Gunma 374-0193, Japan.

Received : January, 5, 2012 ; Accepted : February, 5, 2012

ABSTRACT

Extracts of methanol, ethyl acetate, and n-hexane of four species of Indonesian seagrasses were used to determine the total phenol contents and their antioxidant activities. Total phenol contents of each extract was determined by spectrophotometer using Follin-Ciocalteu reagent, and the antioxidant activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH). The profile of fibers of each fresh seagrass was performed according to an enzymatic-gravimetric method. All of methanol extracts contained high number of total phenol, except Syringodium isoetifolium the highest content was found in ethyl acetate extract; so that methanol extracts of Thalassia hemprichii, Cymodocea rotundata, Enhalus acoroides and ethyl acetate extract of Syringodium isoetifolium had the highest activities on scavenging DPPH radical. The phenol content in tropical seagrasses was tending to soluble in polar and semi-polar solvents. There was no significant different on the content of total fibers, with values ranges was from 14.32 g/100 g to 15.39 g/100 g. However, the highest content of soluble fiber was found in Enhalus acoroides (8.93 g/100 g) and significantly differed to others.

Keywords: Antioxidant; DPPH; fiber content; phenol content; seagrass

Corresponding : Phone : +62 251 8622915; fax. +62 251 862916; e-mail address: joko2209@yahoo.com

INTRODUCTION

Antioxidants in biological systems have multiple functions such as to protect from oxidative damage. The major action of antioxidant in cells is to prevent damage caused by the action of reactive oxygen species and reactive nitrogen species. Those compounds cause extensive oxidative damage to cells leading to age related diseases, cancer and wide range of other human diseases (Food and Nutrition Board, Institute of Medicine, 2000; Prakash, *et al*., 2007).

To prevent free radical damage, living organism, including humans have immune systems which can produce antioxidant naturally from the body namely endogenous antioxidant. In addition, antioxidants can also be obtained from dietary intake namely exogenous antioxidant. Antioxidants produced by the body in the form of enzymes such as catalase, superoxide dismutase, and glutathione peroxidase (Food and Nutrition Board, Institute of Medicine, 2000); whereas antioxidants obtained from food intake can be classified into nutrients antioxidant (vitamins A, C and E) and non-nutrient antioxidant (phenolic compounds) (Papas, 1999). There were also commonly used synthetic antioxidants in foods such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and propylgallate (PG). However, their use is now restricted due to their side effects. Therefore, searching and finding of the natural antioxidant sources namely dietary antioxidant which have been deemed safer more have been carried out and developed in recent years.

From marine plants, there are two groups that can be studied as a candidate for antioxidant sources from the class of seaweed and seagrass. Since seaweeds have become part of the diet in some countries and seagrass has

ISSN : 1410-5217
Acrredited : 83/Kep/Dikti/2009

not yet been claimed as a dietary stuff for human, so that the study of antioxidants on seaweed much done in compared to the seagrass. Several studies have been conducted (*e.g*. Santoso, *et al*., 2004; Santoso, *et al*., 2010[a]; Devi, *et al*., 2011; and O'Sullivan, *et al*., 2011). Although research on the antioxidant activity of seagrass has not been much conducted in compared to seaweed; however, in recent years research activities have been started. Sureda, *et al*. (2008); Heglmeir and Zidorn (2010) evaluated antioxidant response of seagrass *Posidonia oceanica* and its secondary metabolites, respectively. Another seagrasses species *Halophila johnsonii* and *H. decipiens* were evaluated their antioxidant capacity of flavanoid by Gavin and Durako (2011); and Kannan, *et al.,* (2010) investigated antioxidant activity of ethanol extract from *Enhalus acaroids*.

Seagrasses grown in the tropical climate, like Indonesia are expected to bask in strong ultraviolet radiation. This circumstance can cause to increase levels of reactive radical species. To reduce and/or protect, they may change their metabolism and stimulate to produce some active compounds, therefore tropical seagrasses are estimated possessing a large number of active compounds. The first objective of this research was to determine the phenolic content of four tropical seagrasses extracted in different solvent, estimated the function of seagrasses components as an antioxidant source through evaluating radical-scavenging activity.

Seagrasses may contain useful compound for human being, since certain local people live in coastal area already have utilized them.The seeds of the tropical seagrass *E. acaroids* have been traditionally eaten in the Philippines (Montano, *et al*., 1999). Research on fatty acid composition showed 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 were 16:0, 18:2n6 and 18:3n3, and in the rhizomes were16:0, 18:2n6 and 18:1n9 (Viso, *et al*., 1993). However, there is no information for the fiber composition of seagrasses, although the fiber is the main compound of foodstuff from plants including seagrasess.

Fibers belong to the non-nutritional compounds; however it has been recognized as an important dietary constituent, which possesses a wide range of positive properties (Leontowicz, *et al*., 2001). High fiber diets are an important factor in the low prevalence of colon cancer, ischemic heart disease, diabetes mellitus, gallstones, hemorrhoids and hiatus hernia. Fibers also serve as non-caloric bulking agents, enhance water and oil retention, and improve emulsion and oxidative stability (Elleuch, *et al*., 2011). Research on fiber content of foodstuff from marine water plants mainly focused on seaweeds (*e.g*. Santoso, 2003; Gomez-Ordonez, *et al*., 2011; and Yaich, *et al*., 2011). Therefore it is still fertile area for research activity. Second objective of this experiment was to study the profile of fibers of four species of tropical seagrasses grown in Indonesian water.

MATERIALS AND METHODS

Chemicals and reagents

Chemicals and reagents used in this experiment methanol, ethyl acetate, n-hexane, pancreatin enzyme, phosphate buffer, gallic acid, 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 materials

Fresh leaves, root and rhizome of four species tropical seagrasses namely *Thalassia hemprichii*, *Syringodium isoetifolium*, *Cymodocea rotundata*, and *Enhalus acoroides* were collected on February 2011 from the intertidal region of the Pramuka Island, Jakarta Prefecture. Immediately the samples were placed in plastic bag containing sea water in order to prevent evaporation and transported to the laboratory under refrigerated condition. Then the plants were washed thoroughly with tap water to remove all sand particles and epiphytes. The samples were divided into two groups, specifically fresh and dried samples. Fresh samples were required to be analyzed for fibers

ISSN : 1410-5217
Acrredited : 83/Kep/Dikti/2009

content; whereas the dried samples were used for extraction of antioxidant compound. Dried samples forms were obtained after being dried using sun drying for two days and grounded in an electric mixer. The powder samples were then stored in refrigerator for further use.

Preparation of seagrasses extracts

Three type of solvents with different polarity were used in this experiment *i.e.* methanol, ethyl acetate, and n-hexane. The procedures of extraction were conducted according to the research conducted by Santoso, *et al*., (2010[b]) with minor modification. Dried powder of each sample consist of leaves, root and rhizome (20 g) were extracted for 48 hours in 160 mL of each solvent using automatic shaking for maseration 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 ^{o}C using a rotary evaporator to perform 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 ^{o}C prior to analysis.

Determination of total phenolic contents

Total phenolic contents of each crude extracts from seagrasses were determined by spectrophotometry using Follin-Ciocalteu reagents (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 allowed to stand for 5 min then added 1 mL of sodium carbonate (5% w/v). Homogenized mixure 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 100 gram of gallic acid equivalent (GAE).

DPPH radical-scavenging activity

Antioxidant activity assay was measured through the ability of the sample on reducing the stable free radical DPPH according to the method described by Aranda, *et al*., (2009) with slight modifications. One milligram of each crude extract was weighed and then added to ethanol with a ratio of 1:1000. Furthermore, 1.3 mg of DPPH 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 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.

Determination of fibers contents

Soluble and insoluble fibers were determined according to an enzymatic-gravimetric method referred to the research conducted by Suzuki, *et al*., (1996) and Santoso (2003). 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 37^{o}C. (3) Water insoluble 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 ^{o}C. Water soluble fiber was precipitated from the filtrate using 4 volumes of ethanol (at 60 ^{o}C) and recovered by filtration in the same way as for insoluble fiber. (5) All samples analyzed were assayed in duplicate and one of the duplicate 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 fibers were calculated by subtracting the weights of ash and protein from the fiber precipitate. Both of ash and protein contents were analyzed according to the method of AOAC (2005) by furnace and kjeldahl, respectively.

ISSN : 1410-5217
Acrredited : 83/Kep/Dikti/2009

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 using SPSS, version 16 software.

RESULTS AND DISCUSSION

Total phenolic 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). The total phenolic contents of each extract of seagrass in different solvent are presented in **Table 1.** Methanol extract of *T. hemphrichii* had the highest content of total phenol, whereas the smallest was found in n-hexane extract of *C. rotundata* which values were 1022.58 and 2.63 mg GAE/100 g, respectively. In general, the methanol solvent was able to extract the component phenols in high quantities in all three seagrass species namely *T. hemphrichii*, *C. rotundata*, and *E. acoroides*; whereas in *S.isoetifolium*, ethyl acetate solvent produced the high quantities. The highest content of phenol in each extract was statistically difference to others ($p<0.05$). Extracted using n-hexane obtained the smallest number of total phenol in each seagrass species. This indicates that the phenols contained in seagrasses tended to dissolve in polar and semi-polar solvents.

Table 1. Total phenol content of crude extract of methanol, ethyl acetate and n-hexane of four seagrasses species

Seagrasses species	Solvent for extraction		
	Methanol	Ethyl acetate	n-Hexane
Thalassia hemprichii	$1022.58 \pm 193.28^{a/p}$	$36.19 \pm 10.67^{b/q}$	$5.29 \pm 0.64^{b/q}$
Syringodium isoetifolium	$94.36 \pm 6.71^{b/q}$	$732.66 \pm 61.77^{a/p}$	$10.04 \pm 2.21^{a/q}$
Cymodocea rotundata	$335.58 \pm 14.92^{b/p}$	$37.24 \pm 0.04^{b/q}$	$2.63 \pm 0.15^{b/q}$
Enhalus acoroides	$542.56 \pm 14.90^{ab/p}$	$66.50 \pm 5.38^{b/q}$	$2.90 \pm 0.17^{b/r}$

Notes: All values are mean ± SD mg GAE/100 g
Values within columns followed by differ superscript letters a, b are significantly different ($p<0.05$), and values within rows followed by differ superscript letters p, q, r are significantly different ($p<0.05$)

Harborne (1987) stated that the phenolic compounds tended to be soluble in polar solvents. However, the solubility of phenolic compounds may differ in each type of solvent and the source of material. So that, in *S. isoetifolium* the phenol compound tended to be soluble in semi-polar solvent (ethyl acetate). Research conducted by Kannan, e*t al.,* (2010) showed that using ethanol solvent was able to extract phenolic compound from leaf, root and rhizome of *E. acoroides* with values were 0.323, 0.258, and 0.103 mg TAE/g, respectively.

DPPH radical-scavenging activity

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).

Methanol extracts of seagrasses had stronger ability to scavenge DPPH radical in compared to others, which was expressed by IC 50 values, except extract of *S. isoetifolium*. IC50 value is defined as the concentration of substrate that can reduce 50% activity of DPPH radical. The strongest activity 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

ISSN : 1410-5217
Acrredited : 83/Kep/Dikti/2009

compound inside tended to soluble in polar and semi polar solvents.

Based on IC50 value, the antioxidant compound are 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 0.05 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, the highest value of each seagrass extract belongs to intermediate or weak activity. This crude extract still contained other compounds which were not belong to the antioxidant compound, therefore may interfere on antioxidant activity.

Table 2. IC 50 values of crude extract of methanol, ethyl acetate and n-hexane of four seagrasses species

Seagrasses species	Solvent for extraction		
	Methanol	Ethyl acetate	n-Hexane
Thalassia hemprichii	$123.72 \pm 9.99^{b/q}$	$250.72 \pm 1.52^{ab/q}$	$8134.70 \pm 251.94^{b/p}$
Syringodium isoetifolium	$520.91 \pm 66.36^{a/q}$	$96.34 \pm 31.47^{b/r}$	$3998.65 \pm 51.91^{c/p}$
Cymodocea rotundata	$214.68 \pm 52.55^{b/q}$	$362.56 \pm 81.07^{a/q}$	$8752.80 \pm 201.11^{a/p}$
Enhalus acoroides	$115.79 \pm 3.55^{b/q}$	$153.40 \pm 34.92^{b/q}$	$937.62 \pm 103.73^{d/p}$

Notes: All values are mean ± SD ppm
Values within columns followed by differ superscript letters a, b are significantly different ($p<0.05$), and values within rows followed by differ superscript letters p, q, r are significantly different ($p<0.05$)

There was a positive relationship between total phenol content and antioxidant activity determined by DPPH radical scavenging. The results are in line with the previous research conducted by Kannan, *et al*. (2010) that there was a significant correlation between total phenolic content and total antioxidant activities measuring by DPPH and FRAP assays in ethanol extract of *E. acoroides*. Phenol in form of condensed tannin (proanthocyanidins) was found as the main phenolic compound in *Thalassia testudium* (Arnold *et al*., 2008), and *E. acoroides* (Kannan, *et al*., 2010). Four phenolic acids consisted of gallic, caffeic, p-coumoric, and ferulic acids were present in leaves of eelgrass (*Zostera marina*) (Vergeer and Develi, 1996); whereas in *P. oceania*, 23 compounds were identified belong to phenol group with acetosyringone and ferulic acid found in highest concentration (Agostini, *et al*., 1997). Futhermore, Heglmeir and Zidorn (2010) reported that 51 natural products were found in *P. oceania.*

The contents of fibers

The soluble, insoluble and total fibers contents of four species of Indonesian seagrasses are shown in **Fig. 1.** The highest soluble fiber was found in *E. acoroides* (8.93 g/100 g) and *S. isoetifolium* had the lowest content of soluble fiber (6.21 g/100 g). In opposite, *S. isoetifolium* contained the highest number of insoluble fiber and *E. acoroides* had the lowest insoluble fiber content; with the values were 8.13 and 5.29 g/100 g, respectively. There was an inverse relationship between soluble fiber and insoluble fiber contents of four tropical seagrass species. As a result the total content of fiber was not significantly different, with values were in narrow range from 14.32 g/100 g to 15.39 g/100 g. As for the percent of soluble fiber against total fiber, *E. acoroides* was the highest (62.4%), followed by *C. rotundata* (54.1%), *T. hemprichii* (50.9%), and *S. isoetifolium* (43.3%). This indicated that the proportions between soluble and insoluble fibers contents in seagrasses were almost same.

Since there are no available data about the fiber content on seagrass, therefore seaweed was chosen for comparison. Its well known that seaweed contain large amounts of polysaccharides, most of which are not digested by humans, whose gastrointestinal tract does not produce the required degradation enzymes; therefore they can be regarded as dietary fibers. The contents of fiber in seaweed are 33-75%, particularly rich in the soluble fraction (50-85% of total fiber) (Gomez-Ordonez, *et al*., 2011; Jimenez-Escrig and Sanchez-Muniz, 2000). Consequently, the total content of fiber from seaweeds was much higher than seagrasses. In some Indonesian seaweeds the contents of total fibers were 47.0 – 69.3 g/100 g (Santoso, 2003);

ISSN : 1410-5217
Acrredited : 83/Kep/Dikti/2009

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).

The contents of soluble fibers in some Spanish seaweeds were 14.67 – 23.63% (Gomez-Ordonez, *et al*., 2011); some Japanese edible seaweeds were 7.2 - 25.6 g/100 g dry weight (Suzuki, *et al*., 1996). However, in case of some Indonesian seaweeds the amount of soluble fiber was very low of 0.9 – 10.7 g/100 g dry weight (Santoso, 2003).

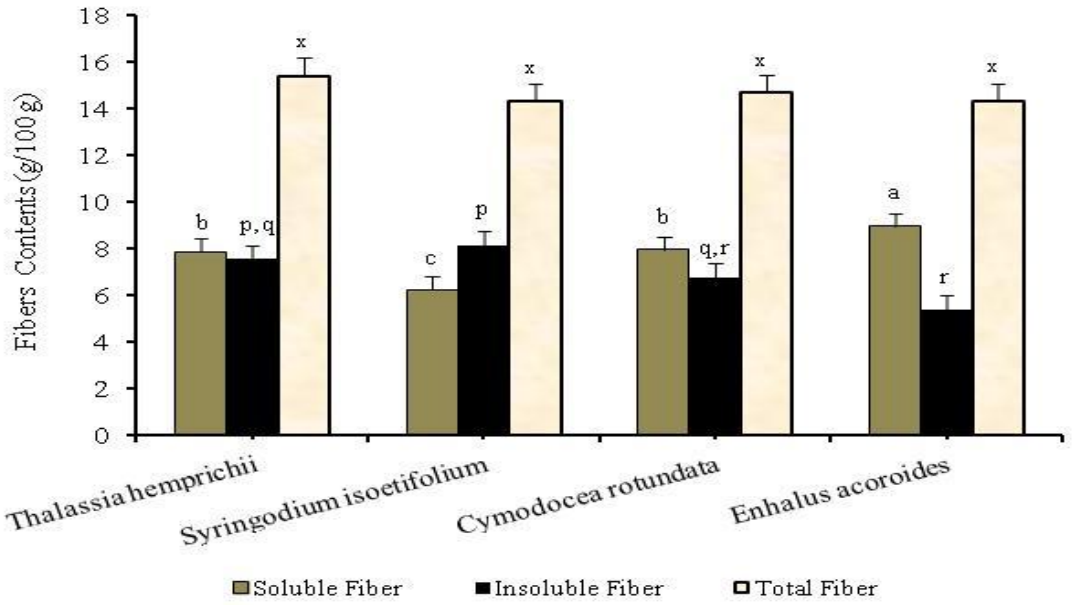

Fig.1. Letter over each column in the graph followed by differ letters are significantly different ($p<0.05$) and same letters are not significantly different ($p>0.05$) in each type of fiber, soluble fiber (a, b); insoluble fiber (p, q, r); and total fiber (x).

CONCLUSIONS

This study revealed that four tropical species of Indonesian seagrasses could be a source of natural antioxidant compounds. Among them, *S. isoetifolium* and *E. acoroides* could be a prime candidate, therefore research activities related to the fractionation, purification and identification of components present in them needs to be done. The content of soluble fiber in *E. acoroides* was the highest; therefore next researches to evaluate the characteristic of fibers are required. We planned to analysis each part of seagrasses (leaf, root and rhizome) in correlation to the phenol compound and antioxidant activity, as well as the fibers content.

ACKNOWLEDGEMENT

The authors gratefully acknowledge to Mrs. Sri Andajani, Head of Kepulauan Seribu National Park, Indonesian Institute of Science, who have given permission to take samples at Pramuka Island.

REFERENCES

Agostini, S., J.M. Desjobert, and G. Pergent. 1997. Distribution of phenolic compounds in the seagrass *Posidonia oceania. Phytochem.* 48(4): 611-617.

[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., Arlington.

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 Complemen. Alter. Med.* 2011: 1-6.

ISSN : 1410-5217
Acrredited : 83/Kep/Dikti/2009

Arnold, T.M., C.E. Tanner, M. Rothen, and J. Bullington. 2008. Wound-induced accumulations of condensed tannins in turtlegrass, *Thalassia testudinum*. *Aquat. Bot*. 89: 27-33.

Devi, G.K., K. Manivannan, G,M. Thirumaran, F.A.A. Rajathi, and P. Anantharaman. 2011. In vitro antioxidant activities of selected seaweeds from southeast coast of India. *Asian Pacific J. Trop. Med*. 205-211.

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 Chem*. 95: 37-43.

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

Food and Nutrition Board, Institute of Medicine. 2000. Proposed Definition and Plan for Review of Dietary Antioxidants and Related Compounds. National Academy Press, Washington, DC

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

Gillan, F.T., R.W. Hogg, and E.A. Drew. 1984. The sterol and fatty acid composition of seven tropical seagrasses from North Queensland Australia. *Phytochem.* 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 Res. Inter*. 43: 2289-2294.

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

Heglmeier, A., and C. Zidorn. 2010. Secondary metabolites of *Posidonia oceania* (Posidoniaceae). *Biochem. System. Ecol.* 38: 964-970.

Jimenez-Escrig, A. and F. Sanchez-Muniz. 2000. Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutr. Res*. 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. Trop. Med*. 898-901.

Leontowicz, M., S. Gorinstein, E. Bartnikowska, H. Leontowicz, G. Kulasek, and S. Trakhtenberg. 2001. Sugar beet pulp and apple pomace dietary fibers improve lipid metabolism in rats fed cholesterol. *Food Chem*. 72: 73-78.

Molyneux, P. 2004. The use of the stable free radical diphenylpicryl-hydrazil (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol*. 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. *Aquat. Bot.* 65: 321-325.

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 Chem*. 126: 1064-1070.

ISSN : 1410-5217
Acrredited : 83/Kep/Dikti/2009

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 Chem*. 104: 783-790.

Papas, A.M. 1999. Diet and antioxidant status. *Food Chem. Toxicol*. 37: 999-1007.

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

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

Santoso, J., D. Fitriani, and Y. Wardiatno. 2010[a]. Phenol content and antioxidant activity of benthic macroalga *Caulerpa racemosa* (Forsskal) from Hurun Bay, Lampung. *Biota*. 15 (3): 369-378.

Santoso, J., R. Maulida, and S.H. Suseno. 2010[b]. Antioxidant activities of methanol, ethyl acetate and hexane extracts of green seaweed *Caulerpa lentillifera*. *J. Mar. Sci.* 2 (special edition): 310-319.

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. *Mutat. Res*. 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*. *Mar. Environ. Res*. 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. *Fish. Sci*. 62: 454-461.

Verger, L.H.T. and A. Develi. 1996. Phenolic acids in healthy and infected leaves of *Zostera marina* and their growth-limiting properties towards *Labyrinthula zosterae*. *Aquat. Bot*. 58: 65-72.

Viso, A.C., D. Pesando, P. Bernard, and J.C. Marty. 1993. Lipid components of the Mideterranean seagrass *Posidonia oceania*. *Phytocem.* 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 Chem.* 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 Nutr.* 64: 218-223.

ISSN : 1410-5217
Acrredited : 83/Kep/Dikti/2009

Table 1. Total phenol content of crude extract of methanol, ethyl acetate and n-hexane of four seagrasses species

Seagrasses species	Solvent for extraction		
	Methanol	Ethyl acetate	n-Hexane
Thalassia hemprichii	$1022.58 \pm 193.28^{a/p}$	$36.19 \pm 10.67^{b/q}$	$5.29 \pm 0.64^{b/q}$
Syringodium isoetifolium	$94.36 \pm 6.71^{b/q}$	$732.66 \pm 61.77^{a/p}$	$10.04 \pm 2.21^{a/q}$
Cymodocea rotundata	$335.58 \pm 14.92^{b/p}$	$37.24 \pm 0.04^{b/q}$	$2.63 \pm 0.15^{b/q}$
Enhalus acoroides	$542.56 \pm 14.90^{ab/p}$	$66.50 \pm 5.38^{b/q}$	$2.90 \pm 0.17^{b/r}$

Notes: All values are mean ± SD mg GAE/100 g
Values within columns followed by differ superscript letters a, b are significantly different ($p<0.05$), and values within rows followed by differ superscript letters p, q, r are significantly different ($p<0.05$)

Table 2. IC 50 values of crude extract of methanol, ethyl acetate and n-hexane of four seagrasses species

Seagrasses species	Solvent for extraction		
	Methanol	Ethyl acetate	n-Hexane
Thalassia hemprichii	$123.72 \pm 9.99^{b/q}$	$250.72 \pm 1.52^{ab/q}$	$8134.70 \pm 251.94^{b/p}$
Syringodium isoetifolium	$520.91 \pm 66.36^{a/q}$	$96.34 \pm 31.47^{b/r}$	$3998.65 \pm 51.91^{c/p}$
Cymodocea rotundata	$214.68 \pm 52.55^{b/q}$	$362.56 \pm 81.07^{a/q}$	$8752.80 \pm 201.11^{a/p}$
Enhalus acoroides	$115.79 \pm 3.55^{b/q}$	$153.40 \pm 34.92^{b/q}$	$937.62 \pm 103.73^{d/p}$

Notes: All values are mean ± SD ppm
Values within columns followed by differ superscript letters a, b are significantly different ($p<0.05$), and values within rows followed by differ superscript letters p, q, r are significantly different ($p<0.05$)

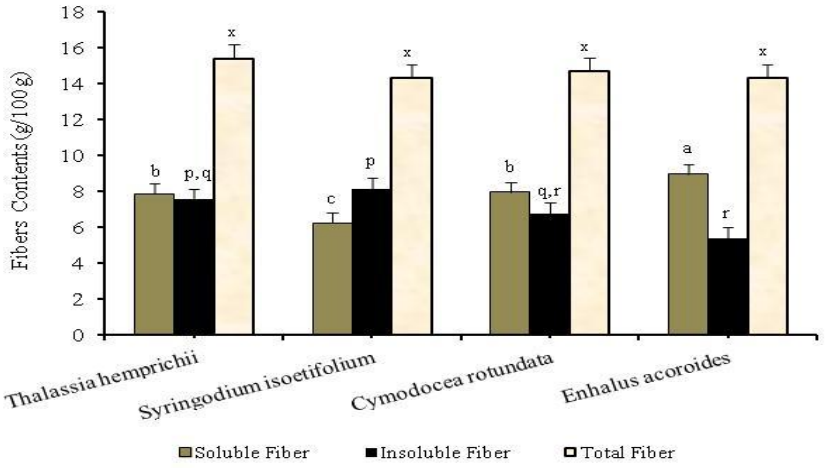

Fig. 1. The content of soluble fiber, insoluble fiber and total fiber of four seagrass species.

Letter over each column in the graph followed by differ letters are significantly different ($p<0.05$) and same letters are not significantly different ($p>0.05$) in each type of fiber, soluble fiber (a, b); insoluble fiber (p, q, r); and total fiber (x).