ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antioxidant Activity and Identification of Compounds in the Extract of Sea Bamboo's (*Isis Hippuris)* Outer Layer.

Mohammad Sayuti[1]*, Widya Dwi Rukmi Putri[2], and Yunianta[2].

[1] Polytechnic of Marine and Fishery, Sorong, West Papua, Indonesia.
[2]Faculty of Agriculture Technololy, Brawijaya University, Indonesia.

ABSTRACT

Total phenolic test on the results of partial fractionation of the extracted layers of Sea Bamboo (*Isis hippuris)* showed ethyl acetate fraction as much as 8.49±0.15 mg GAE/g followed by the ethanol fraction at 5.26±0.13 mg GAE/g and n-hexane fraction at 3.24±0.16 mg GAE/g. Meanwhile, the result of total flavonoids for ethanol and n-hexane fraction was 6.67%, whilst for ethyl acetate fraction was 5.13%. The test results on antioxidant activities using DPPH showed ethanol fraction as much as 1795.08 ppm, n-hexane fraction 1888.45 ppm and ethyl acetate fraction 2014.54 ppm. The results of FTIR chromatogram showed that there were 9 peaks of ethanol fraction, 10 peaks of ethyl acetate fraction and 12 peaks of n-hexane fraction. GC-MS data analysis on the outer layers of Sea Bamboo (*Isis hippuris)* revealed 53 compounds of ethanol fraction, 40 compounds of ethyl acetate fraction, and 50 compounds of n-hexane fraction. The most dominant compounds were *2-butoxyethanol* (43.68%) of ethanol fraction, *Methyl-D-mannopyranoside* (20.41%) of n-hexane fraction, and *2-Myristynoyl-glycinamide* (19.51%) of ethyl acetate fraction.

Keywords: *Sea bamboo, phenols, flavonoids, Isis hippuris, antioxidant, DPPH, GC-MS, FTIR*

Corresponding author

ISSN: 0975-8585

INTRODUCTION

One of the composers of coral reef is the soft corals (*Octocorallia).* This group is represented by a tribe of *Gorgonacea* which is the group of soft corals that are widely found in Indo-pacific ocean and other oceans especially in tropical. *Gorgonian Isis hippuris* or Sea Bamboo is potential to be explored as it contains huge number of secondary metabolite of steroid compounds which is classified into polyoxygenated, sesquiterpenes, hydrocarbon, phenol and fatty acids. The compounds are believed to play some roles in the cytotoxic activities that can be further enhanced as anticancer (Manuputty, 2002).

Rao and Ramana (1988) extracted the layers of Sea Bamboo (*Isis hippuris)* using ethanol that resulted to 5 new types of *hippuris.* Meanwhile, Sheu et al. (2000) extracted and isolated the *Isis hippuris* using n-hexan and found 5 new sesquiterpen suberosane, in which the five metabolites found in the research showed that there were strong cyctotoxin defenses toward cancer cells P-388, A549, and HT-29. After that, Sheu et al. (2003) continued the extraction and isolation of the Sea Bamboo (*Isis hippuris)* using n-hexan and found five new types of steroids. Different from Sheu, Tanaka et al. (2002) extracted and isolated the Sea Bamboo (*Isis hippuris)* using acetone and found 11 (eleven) new polyoxygenated steroids. Those sterols substances from the extraction of Sea Bamboo (*Isis hippuris)* were also proven to contain various bioactive substances such as anti-cancer substances and anti-inflamation substances (Higa et al., 2001).

The high quantity of Sea Bamboo extractions done by society is closely related to the empirical knowledge that the extraction of the Sea Bamboo contains medication. Scientific research done in laboratory was conducted to prove if there were any primary metabolite and secondary metabolite components in the Sea Bamboo extraction and to find if the compunds had other functions to be used for pharmacy or mediation that would give positive benefits to human. The objectives of this study were to investigate antioxidant activities since there were only few information related to the antioxidant ability of the Sea Bamboo and to identify the content of secondary metabolite of Sea Bamboo (*Isis hippuris)* layers.

MATERIALS AND METHODS

Sample Preparation

Sea Bamboo samples were collected from Biak Sea, Papua. Samples were cleaned and aired for 7 days. Dried samples were separated between the skin and axial(inside) parts. Each part was finely crushed using the machine and sieved by 65 mesh sieve and stored for further tests.

Extraction dan Partition Fractionation

Extraction process employed the ultrasonic method using Branson Ultrasonic tool version 3510. One gram of Sea Bamboo layer powder (*Isis hippuris*) was put into the Erlenmeyer and was added up with methanol dissolver with ration of 1:10 and was then covered with aluminium foil. After that it was extracted using ultrasonic wave in 35^0C for 20 minutes. The extracted substances were then filtered using filter paper and using vacuum filter tool. The filtrate from the filtration was then evaoporated to leave only the solid extraction which was then collected and kept in 0 ^{0}C for the next steps of the test.

The solid extract that was resulted by the process were then fractionated into partitions. The partition extraction was done based on the polarity of the dissolver using liquid extraction inside different funnels to obtain the extracted substances based on the different polarity levels. The dissosiaciton of compounds based on the level of polarity is the basic principle in liquid extraction. Different polarity level of the dissolver avoids the dissolvers to get mixed in order to enable to dissolver to dissolve the compounds based on the similar polarity level to the dissolver liquid which is commonly called *like dissolve like.* Different funnels were prepared, then the extract of the Sea Bamboo (*Isis hippuris*) was weighed and put into the funnels, followed by the n-hexane dissolver and ethanol dissolver with ratio of 2:1 which were then mixed and let it separated itself into two layers. The n-hexane fraction was then evaporated while the ethanol fraction was re-fractionated using ethyl acetate at ratio of 2:1 that resulted 3 fractions which were the n-hexane fraction, ethanol fraction and ethyl acetate fraction which were kept within temperature of 0^{o}C for the next process.

Determining the Total Phenol Substances

Analysis on the total phenol substances of the fractionation results of Sea Bamboo layers (*Isis hippuris*) was tested based on the modificational method, Yangthong et al., (2009); Sharma (2011); and Santoso et al. (2012). The first step was making the curve of gallic acid by creating gallic acid using the concentration serial of 0ppm, 4 ppm, 15 ppm, and 20 ppm which each liquid was added up with 5 ml of aquades, 0.5 m reagent *Folin-Ciocalteau* 50% and then being incubated for 5 minutes to be later added up with 1 ml Na2CO3 at 5% to the liquid. After that, the liquid was homogenized and incubated in a dark place for an hour. The absorbed substances were then measured using spectrophotometer *UV-Vis* at wave length of 725 mm in which absorbance measurement was done three times before being made into standard curve of gallic acid using this equation Y= ax + b, where y= absorbance, and x= concentration of gallic acid liquid. The second step was determining the total phenol by weighing the 5 mg sample of the extract to be dissolved into 2 ml of ethanol PA which was then added up with 5 ml of aquades, 0,5 ml reagent*Folin-Ciocalteau* 50% and was incubated for 5 minutes. After that, 1 ml of 5% Na2CO3 was added up into the liquid which then was homogenized for 5 mnutes and incubated in the dark for an hour. The result of the absorbed substances was measured using spectrophotometer *UV-Vis* at 725 nm wave in three times which was then being calibrated with the standard curve of gallic acid to obtain the total phenol in mm GAE/g. The total phenol was measured in µg mg GAE/g using this following equation.

$$\mathbf{C} = \frac{\mathbf{C_{GAE}\ x\ V}}{\mathbf{G}}$$

Where:

C : Total phenol degree

C_{GAE} : Total phenol degreein the equivalent form of gallic acid (mg/ml)

V : The volume of the extract

G : Mass of the substance (g)

Total Flavonoid Analysis

The analysis on total flavonoid as the result of the Sea Bamboo layer (*Isis hippuris*) extraction was then tested using the modificational method, Chang et al. (2002); Hassan et al. (2013); and Nugroho et al. (2013). The first step was determining the standard quercetin curve wby making quercetin liquid stock of 1000 ppm, and diluting the liquid stock to obtain the standard liquid with concentration series of 50 ppm, 100 ppm, 150 ppm and 200 ppm. After that, the liquid was added up with 1.5 ml of methano, 0.1 ml of 10% $AlCl_3$, 1 ml of CH_3COOK 1 M, 2.8 ml of aquades. The liquid was then homogenized (*vortex*) and was incubated for 30 munites. The absorbance level of the liquid was measured using spectrophotometer *UV-Vis* at wave length of 415 nm three times. Next, the standard quercetin curve was made using equation: Y= ax +b, where y= absorbance and x= liquid concentration. The regression equation and the $R^2$of the standard quercetin were then measured. The second step was determining the total flavonoid using 0.5 of the fractionation result with 1000 ppm concentration which was put into the reactional tube and was added up with 1.5 ml of ethanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of CH_3COOK 1 M and 2.8 ml of aquades. The liquid was then homogenized (*vortex*) and was then incubated for 30 minutes. The absorbance of the liquid was then measured using spectrophotometer *UV-Vs* at wave length of 415 nm for three times. The next step was calibrating the result with the standard quercetin curve to obtain the total flavonoid which stated in a unit of mg quercetin equivalent /g extract (mg QE/g extract) or in percentage using this following equation:

$$\mathbf{C} = \frac{\mathbf{C_{QE}\ x\ V\ x\ Fp}}{\mathbf{M}}$$

$$\mathbf{C} = \frac{\mathbf{C_{QE}\ x\ V\ x\ Fp}}{\mathbf{M}}\ \mathbf{x\ 100\%}$$

Where:

C : Total flavonoid (mg QE/g extract)

C_{QE} : Quercetin concentration (mg/ml)

V : Volume of the extract (l)

M : weigth of the extract (g)

Fp: dissolving factros

Antioxidant Activity Analysis using DPPH Method (Molyneux 2004).

Making the DPPH 0.0mM ethanol liquid in ethanol pro analyst. Creating the sample of liquid stock in the concentration of 1000 oom ethanol pro analyst which was then diluted to obtain the sample of the substances in concentration series of 50 oom, 100 ppm, 250 ppm, 500 ppm and 1000 ppm. 4 ml of each of the series was taken was the sample which was reacted with 1 ml of 0.2 mM DPPH liquid. Its absorbance was then measured at 517 nm wave length. The same process was also conducted to the blank liquid without the sample of 4 ml of pro ethanol analys with 1 ml of DPPH. The percentage of the inhibition was then measured (% inhibition):

$$Inhibition(\%) = \frac{\text{Abs. Blank liquid} - \text{Abs. Sampel}}{\text{Abs. Blank liquid}} \text{x } 100\ \%$$

The regression between the % inhibition and liquid concentration resulted this equation:

$$Y = a\ (x) + b$$

Notes:

- Y stated the measured IC (inhibitor concentration) at 50.
- X stated the value of IC50. IC50 value stated the concentration of the sample liquid that was needed to reduct the the DPPH at 50%.

Analysis of Functional Cluster with Fourier Transform Infrared Spectrophotometer (FTIR)

The sample of the tested substances in this study was in the form of liquid. The sampling preparation was done by putting the gasket teflon into a holder. The window 1 was then put into the holder, followed by sapacer, window 2, and the range before the holder was closed using a sealer. The sample was injected to the full capacity of the holder and then the injection hole was closed using sealer. Each of the sample was scanned using FTIR. The FTIR system in the Fourier Transfrom Infrared Spectrophotometer was the model name: FTS 1000 Schimitar made by: Agilent Technologies which calibration date was 19 Octeober 2015 and expiry date: 10 Octover 2016, caliberated by: Adista S.N, distributor: Eltra Element Analyzers, Hitachi High-Tech, Scaler, Ina Distibutor: Unitama Analitika Perkasa. The specification of the handpress tool to create pellet was the brand of Pike Technologies with capacity of 9 tons, and pressing duration of 1-3 minutes.

GCMS Analysis

The characteristics of the GC-MS used in this study was the Shimadzu brand type QP2010S with injector temperature at 280^{o}C, mode split injector, and sampling duration of 1 minute, 40 to 270 ^{o}C of column temperature, and initial temperature of 40^{o}C which was restrained for 10 minutes until reaching the temperature of 270 ^{o}C (23 ^{o}C per minute) for 60 minutes. Thus, the total duration for the process was 88 minutes, with detector temperature of 280 ^{o}C, interval temperature of 250 ^{o}C, He gas carrier, main pressure of 500 – 900, flow control mode pressure, pressure of 10.9 Kpa, total flow of 58.8 ml/m, column flow of 0.55 ml/m, linier acceleration of 26.0 cm/dt, cleansing acceleration of 3.0 ml.m, split ratio of 99.8, type of column Rtx-5MS, length of the column 30.00 m, thickness of colum 0.25µm, diameter of 0.5 mm, and ionizer EI (Electron Impact) 70 eV.

RESULTS

Yield Results of the Fractionation

In this research, there were three different types of dissolvers used for fractionation process which were the ethanol (polar), ethyl acetate (semipolar) and n hexane (non polar). The use of those three dissolvers was meant to extract either the polar and non polar chemical components and to see the amoung of yield and the antioxidant propertis of the Sea Bamboo (*Isis hippuris*) extract of each dissolver. The percentage value of the yield partition result from every dissolver can be seen in Figure 1.

ISSN: 0975-8585

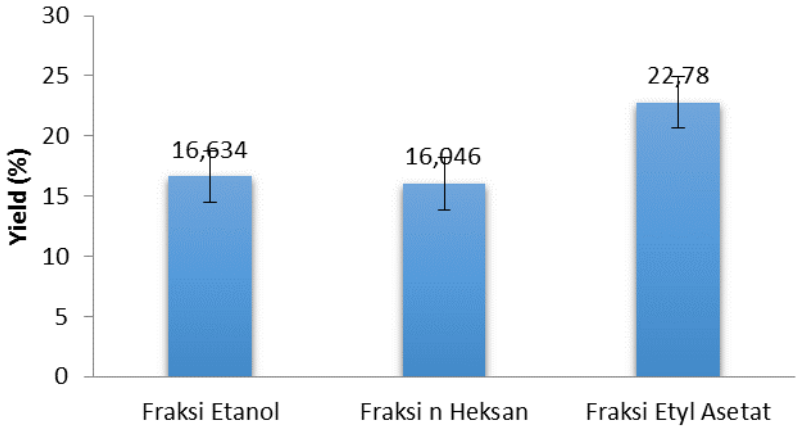

Figure 1. Yield Fractionation Result of Sea Bamboo Extract (*Isis hippuris*)

The highest yield result was found in ethyl acetate fraction at 22.78%. Various result shows that different types of dissolvers used in the fractionation of Sea Bamboo layer extract did not give significant effect (α = 0,05) to the resulted yield of the fractionation. Another test using Turkey 95% method to the yield showed that the result of ethanol fraction, n hexane fraction, and ethyl acetate fraction are not different from each other. Wikanta et al., (2010) reported the result of fractionation of green macroalgae *Ulva fasciata* to the hexane fraction which resulted 20% yield, ethy acetate 37% and water-methanol 15%. The use of different dissolvers with different polarity influenced the compunds that were being extracted. The result of the partition on the *Mahkota Dewa (Phaleria macrocarpa)* to the ethyl acetate fraction contained various compounds such as fatty acids and phitosterol, while the n-butanol fraction contained glycosides flavonoids from benzophenon while the water extract contained carbohydrate compounds (glucose and sucrose) (Soeksmanto et al., 2007).

Analysis on the Total Phenol and Total Flavonoids

Total Phenol Analysis

The total phenol content in every fraction of the Sea Bamboo layer extract has been confirmed equivalent to the gallic acid or Gallic Acid Equivalent (GAE). GAE is the general guide for measurement of phenolic compounds in a chemical substance. From the result of the measurement, the ethyl acetate fraction had the highest total phenol at 8.49±0,15 mg GAE/g. It indicates that in each gram of the n hexane fraction contains 8,49±0,15 mg gallic acid. The total phenol o the fractionation process of Sea Bamboo layer extract (*Isis hippuris)* is presented in Figure 2.

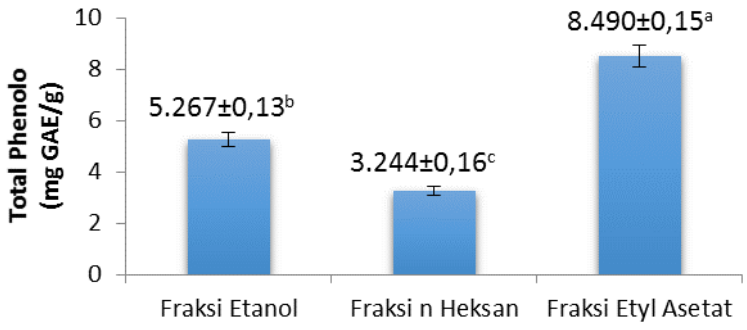

Figure 2. Total Phenol Resulted by Fractionation of Sea Bamboo (*Isis hippuris*) layer extract

Based on the result of obsercation on the amount of total phenol from the fractionation of Sea Bamboo layer extract for the ethyl acetate fraction was at 8.49±0.15 mg GAE/g, followed by the ethanol fraction at 5.26±0.13 mg GAE/g and the n hexane fraction 3.24±0.16 mg GAE/g. It shows that different dissolvers for

different fractionation resuts of the Sea Bamboo extract had significant effect (α = 0,05) toward the amount of the total phenol of the substance. The further result of test using Turkey 95% method to the total phenol of either ethanol fraction, n hexane fraction or ethyl acetate fraction showed a significant difference among each other which was shown by the different notations. The ascending order of the total phenol amont in each fraction of the Sea Bamboo extract is the ethyl acetate fraction, ethanol fraction, and n hexane fraction.

The dissolvers used in an experiment determines the amount of the diluted phenolic. Different dilution characteristics and broad spectrums are owned by polyphenol components (Nur and Astawan, 2011). Thus, extracting phenolic compounds in plants is a difficult for it has quite complicated extraction procedure (Naczk and Shahidi, 2004). Poliphenol fraction that share the same molecule weight as ethyl acetate dissolver such as tannin and flavonol is assumed to cause the high amount of total polyphenol in ethyl acetate dissolver of the Sea Bamboo layer (Nur and Astawan, 2011). Rohman and Riyanto (2006) stated that to extract phenolic compounds in *mengkudu*or (*Morindra citrifolia L)* noni fruit, ethyl acetate is the most suitable dissolver. Besides, the content of total phenol that is obtained from the extraction using ethyl acetate of Indian Plum plants (*Flacourtia jangomas L)* is higher than the one that is extracted using methanol and chlorofoam as the dissolvers (Rahman et al., 2012).

Test on the activity of total phenol is the basic procedure of antioxidant activity tests since the phenolic has been confirmed to play a significant role in preventing oxidation. Besides, most of antioxidant substances found in the original plants are the polyphenol. Total phenol can be obtained from a number of simple molecules of phenolic compounds to the complex molecules such as tannin that can be diluted in organic dissolver (Stevi et al., 2012).

Total Flavonoid Analysis

High amount of total flavonoid found in fractions that are resulted from the fractionation process to the axial and the layer of Sea Bamboo (*Isis hippuris*) is the main objective of total flavonoid analysis. The method to determine the total flavonoid was based on the standard of quercetin mg/mL or %. The total flavonoid result from each Sea Bamboo (*Isis hippuris*) layer extract is presented in Figure 3.

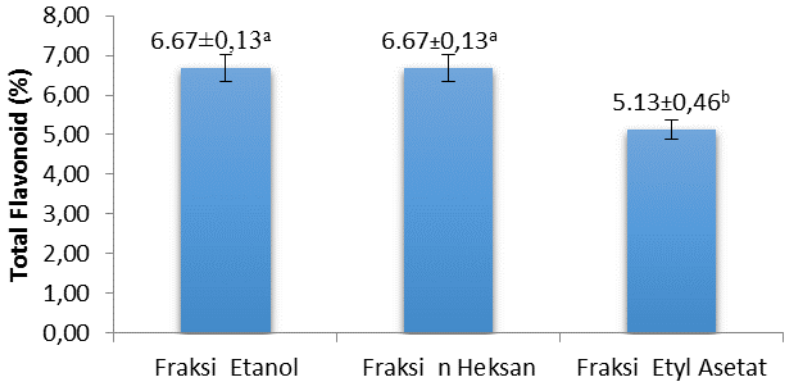

Figure 3. Total Flavonoid from the Extracted Sea Bamboo (*Isis hippuris*) Layer

Figure 3 shows that the highest total flavonoid amount was found in ethanol and n hexane fractions at 6.67%. On the other hand the lowest total flavonoid amount was found in ethyl acetae fraction at 5.13%. However, the overall total flavonoid amount in Sea Bamboo fractions is considered as minor constituent. The various results shoe that the different pearut result of the extracted Sea Bamboo layer fractionation had a significant effect (α = 0,05) toward the total flavonoid in the substances. The result of the total flavonoid using Turkey 95% method showed that the ethanol fraction and n hexane fraction were not different since they had the same notations, while the ethyl acetate fraction had a real difference. The different flavonoid amount of those Sea Bamboo extract fractions reflected the constituent chemical similarity of the plants as the result of different habitat. Temperature, sunlight, ultraviolet, nutrition, water supply, and CO2 amount in the atmosphere are some environmental factors that affect plant metabolism (Summanen, 1999).

ISSN: 0975-8585

Analysis of Antioxidant Activities

Sea Bamboo (*Isis hippuiris*) that live in coast around the depth of 2 to 10 meter generally receive enough sun light. Sun light can trigger biomolecule oxidation to the cell tissue that damages the tissue and the death of living cells due to the ultraviolet that is spread by the sun light. The ultraviolet creates pollutant especially the reactive oxygen species (ROS) (Nursid et al., 2013). Yet, not all of the the Sea Bamboo (*Isis hippuiris*) that are exposed to hazardous ROS attain oxidative damage. It shows that there was a specific protection system that prevents the cells of the Sea Bamboo from getting oxidative stress damage (Nursid et a, 2013). The adaptation ability of the Sea Bamboo toward the sun light is the protection system in which the plant produces antioxidant compunds that prevents itself from pollutant (Budhiyanti et al., 2012). The existence of the pollutant or ROS can be neutralized if the antioxidant compunds produce electrons toward the pollutant (Halliwell, 1999; Mallick and Mohn, 2000; Kelman et al., 2012). The result of the antioxidant activity analysis of Sea Bamboo (*Isis hippuiris*) extract of the ethanol fraction, n hexane fraction, and ethyl acetate fractioncan be seen in Figure 4.

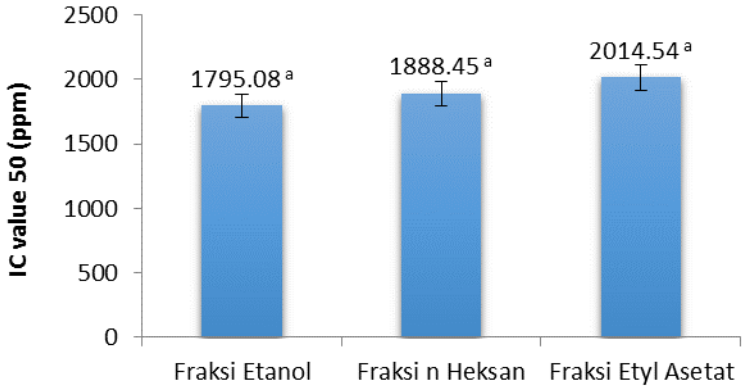

Figure 4. Antioxidant activities of the extracted Sea Bamboo (*Isis hippuiris*) fractionation

The variety analysis showed that different dissolvers used in the fractionation process did not give any significant influence (α=0,05) toward the antioxidant activities of the Sea Bamboo extract. Further analysis using Turkey 95% method showed that the ethanol fraction, n hexane fraction, and ethyl acetate fraction were not different from each other which was proven by the same notation among those three fractions.

Figure 4 showed that almost all of those fractions had IC_5value at above 1000 ppm; ethanol fraction at 1975.08 ppm, n hexane fraction at 1888.45 ppm and ethyl acetate fraction at 2014.54 ppm. It shows that all of the fractions of the extracted Sea Bamboo had low antioxidant activities for their IC_{50} value was above 200 ppm, yet there were still chances for them to become antioxidant substances. Molyneux (2004) stated that a substance has antioxidant characteristics when the IC_{50} value is lower than 200 ppm. If the IC_{50} value is found at around 200 to 1000 ppm, it indicates that the substances is less active. It is assumed that the high IC_{50} value was influenced by the different treatment given to the plant in the sampling preparation, sampling storage, conditional factors, inappropriate duration of extraction process that damaged the extraction process since the antioxidant substances are easily damaged they are put in high temperature of dry atmosphere. The phenolics have aromatic rings with one or more hidroxy (OH-) clusters and other following clusters. Phenol is easily oxydated. Colors of phenol which is left in the open air will be quickly changed due to the results of oxidation process (Kahkonen et al., 1999).

The data related to the low antioxidant activities of the extract of Sea Bamboo (*Isis hippuris*) and its fractions was also supported by the results of phenol tests and total flavonoid tests which generally showed low amount of total phenol and total flavonoid. Phenolic compounds either in the form of phenolic acids or flavonoid are closely relted to antioxidant activities (Kahkonen et al., 1999). The activity of the Sea Bamboo fractions does not rely only on its total phenol and its total flavonoid. Antioxidant activity might be influenced by the existence of secondary metabolites such as fatty acids, karotenoid, and the existence of other vitamins have role up to 33% in influencing antioxidant activity? (Javanmardi et al., 2003).

ISSN: 0975-8585

Compunds Indentification Using GC-MS

The analysis of the fractionantion process results for the layer part and axial part of Sea Bamboo (*Isis hippuris*) extract in each of the ethanol fraction, ethyl acetate fraction and n hexane fraction was then investigated to see the total phenol and the total flavonoids before being analyzed its chemical compounds composition using GC-MS method. The result of the compound were then compared to each of the fractions of either the layer part or axial part. The chromatogram of the GC-MS results and other compounds of the layer part fractions is shown in Figure 5.

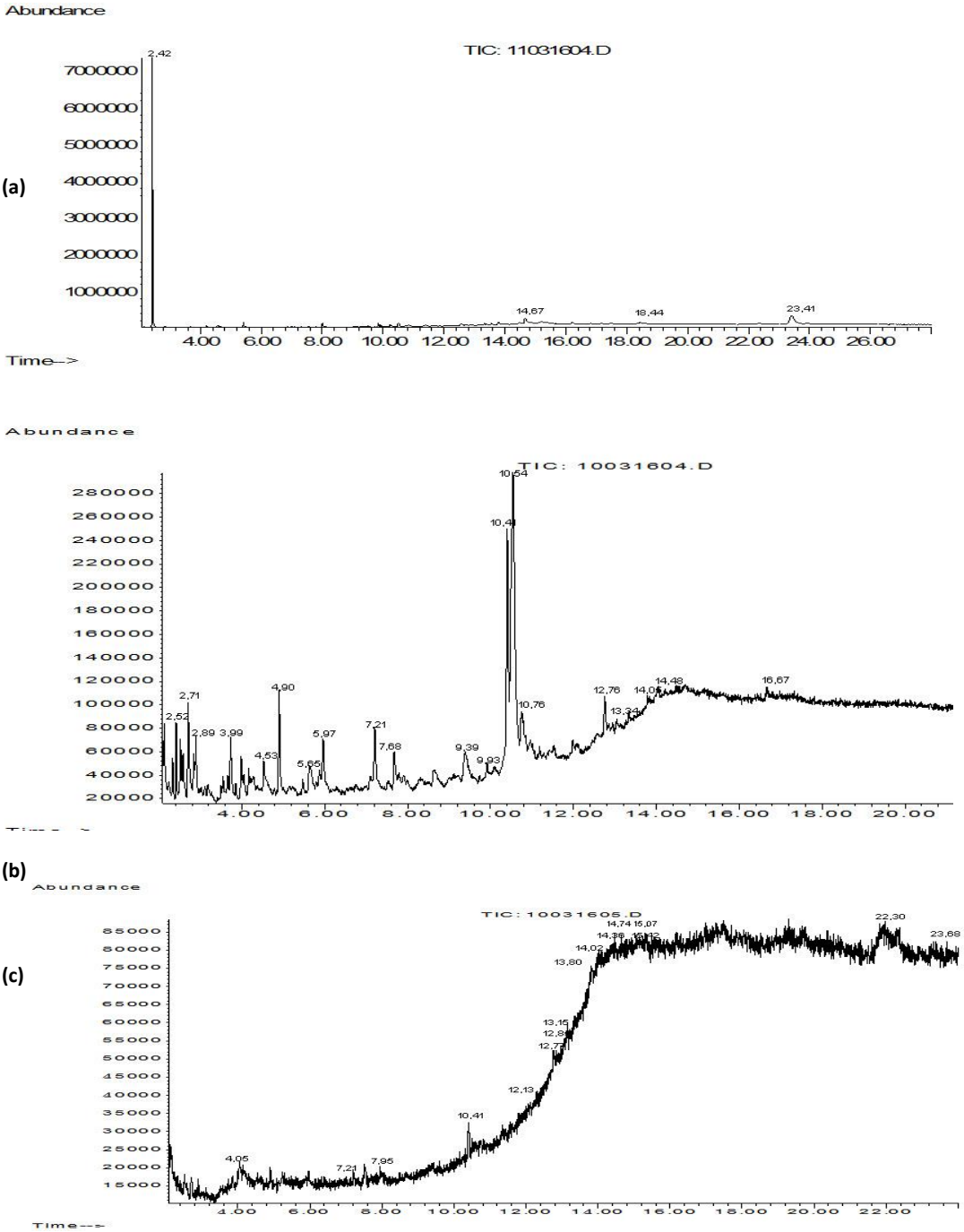

Figure 5. GC-MS Chromatogram of the Fractionation Results of Sea Bamboo (*Isis hippuris*) Extract; (a) Ethanol Fraction; (b) n Hexane Fraction; (c) Ethyl Acetate Fraction

ISSN: 0975-8585

The analyzed data of the GC-MS on the Sea Bamboo (*Isis hippuris*) layer extract fraom ethanol fraction showed that there were 53 compounds found in the extract, while 40 compounds were found in the ethyl acetate fraction and 50 compounds were found in the n Hexane fraction. The chromatogram results of the layer part of the Sea Bamboo extract which is presented in Figure 5 showed that the most dominant compound of the ethanol fraction were the *2-butoxy ethanol* at peak number 2 (43.68%), *Hexanedioic acid*at peak number 52 (12.11%), *n-Hexadecanoic acid*at peak number 44 (5.74%), *1-Naphthalenecarbonitrile* at peak number 45 (2.88 %), *Cyclotetradecane*at peak number 38 dan 43 (2.67%) and*1H-Pyrido[3.4-b]indol-1-one*at peak number 50 (2.6%).

The chromatogram result of the layer part extraction shown in Figure 5b showed that the most dominant compunds of the n hexane fraction were *Methyl-D-mannopyranoside*at peak number 32 (20.41%), *2.6-Dimethyl-3-(methoxymethyl)-benzoquinone*peak number 31 (8.16%), *Octadec-9-enoic acid* nomor puncak 44 (6,99%), *2-Furancarboxaldehyde* peak number 21 (4.98 %), *D-Glucopyranoside*peak number (4.03%), *1-Butanone*peak number38 (3.32%), *Heptanoic Acid*peak number29 (3.12%), *2.5-Furandione*peak number 6 (3%), *2(1H)-naphthalenone* nomor peak number 28 and 48 (2.86%), *4H-Pyran-4-one*peak number 17 (2.7%), *2-Methoxy-4-vinylphenol*peak number 23 (2.57%), *Cyclohexane*peak number 25 and 45 (2.26%) dan *6-Octenal* peak number47 (2.16%).

The chromatogram result of the layer part extraction shown in Figure 5c showed that the most dominant compunds of the ethyl acetate fraction were *2-Myristynoyl-glycinamide*peak number 22, 26, 36 and 37 (19.51%), *1H-Indole*peak number 7, 14,15, 21 dan 40 (17.64%), *8.beta.,12-Epoxy-13,14,15,16,17,19-hexanorlabdane*peak number 23 (11.63%), *9-Octadecenoic* acid peak number 25 and 30 (7.31%), *10-Octadecenoic acid*peak number 24 (6.21 %), *Hexahydropyridine*peak number 29, 32 and 33 (5.51%), *Styryltrimethylgermane*peak number 28 (4.81 %), 5-*methyl*-3-*methoxy*-7-*nitroindazole*peak number 17 (3.9%), *Trideuterio Acetyl Carnitine* nomor peak number 31 (3.35%), *Cyclotrisiloxane*peak number 5, 8, 10, 11 and 13 (3,11%), *Propiophenone*peak number 20 (2.98%), *Cyclohexene*peak number 27 (2.35%), *Heptadecene-(8)-Carbonic Acid*peak number 36 and 39 (2,18%), *Propanedinitrile*peak number 34 and 38 (2.16 %) dan *Benzenamine*peak number18 (2.1%).

Based on those identified compounds, there were more compounds with similarity > 90% compared to those in the WILEY275L, NTIST02.L database which is assumed to be the compound that influence the antioxidant activity. The chemical compounds that construct the layer part and the axial part of Sea Bamboo (*Isis hippuris*) can be seen in Table 1.

Table 1. Dominant Compounds from GC-MS of the Fractionation Results of the Sea Bamboo (*Isis hippuris*) Extract (% relative higher than 2%)

No	IUPAC Name	Formula	Molecule weight	% Relative		
				F. Ethanol	F. ethyl Acetate	F. n Hexane
1.	2-butoxyethanol	$C_6H_{14}O_2$	118,17	43.68	-	-
2.	Methyl-D-mannopyranoside	$C_7H_{14}O_6$	194,18	-	-	20.41
3.	2-Myristynoyl-glycinamide	$C_{16}H_{28}N_2O_2$	280,41	-	19.51	-
4.	1H-Indole	C_6H_7N	117,15	-	17.64	-
5.	Hexanedioic acid	$C_6H_{10}O_4$	146,14	12.11	-	-
6.	8.beta.,12-Epoxy-13,14,15,16,17,19-hexanorlabdane	$C_{14}H_{24}O$	208,34	-	11.63	-
7.	1,4-Dihydrophenanthrene	$C_{14}H_{12}$	180,25	-	-	8.16
8.	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282,46	-	7.31	-
9.	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282,46	-	-	6.99
10.	10-Octadecenoic acid	$C_{18}H_{34}O_2$	282,46	-	6.21	-
11.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256,42	5.74	-	-
12.	Hexahydropyridine	$C_5H_{11}N$	85,15	-	5.51	-
13.	2-Furancarboxaldehyde	$C_5H_4O_2$	96,08	-	-	4.98
14.	Styryltrimethylgermane	$C_{11}H_{16}Ge$	220,88	-	4.81	-
15.	D-Glucopyranoside	$C_6H_{12}O_6$	180,16	-	-	4.03
16.	5-methyl-3-methoxy-7-nitroindazole	$C_9H_9N_3O_3$	207,19	-	3.9	-
17.	trideuterio acetyl carnitine	$C_9H_{14}D_3NO_4$	208,14	-	3.35	-

18.	1-Butanone	C_4H_8O	72,11	-	-	3.32
19.	Heptanoic Acid	$C_7H_{14}O_2$	130,18	-	-	3.12
20.	Cyclotrisiloxane	$H_6O_3Si_3$	138,30	-	3.11	-
21.	2,5-Furandione	$C_4H_2O_3$	98,06	-	-	3
22.	Propiophenone	$C_9H_{10}O$	134,18	-	2.98	-
23.	1-Naphthalenecarbonitrile	$C_{11}H_7N$	153,18	2.88	-	-
24.	2(1H)-naphthalenone	$C_{10}H_8O$	144,17	-	-	2.86
25.	4H-Pyran-4-one	$C_5H_4O_2$	96,08	-	-	2.7
26.	Cyclotetradecane	$C_{14}H_{28}$	196,37	2.67	-	-
27.	1H-Pyrido[3,4-b]indol-1-one	$C_{11}H_6N_2O$	182,18	2.6	-	-
28.	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150,17	-	-	2.57
29.	Cyclohexene	C_6H_{10}	82,14	-	2.37	-
30.	Cyclohexane	C_6H_{12}	84,16	-	-	2.26
31.	HEPTADECENE-(8)-CARBONIC ACID	$C_{18}H_{34}O_3$	298,46	-	2.18	-
32.	Propanedinitrile	$C_3H_2N_2$	66,06	-	2.16	-
33.	6-Octenal	$C_8H_{14}O$	126,2	-	-	2.16
34.	Benzenamine	C_6H_7N	93,13	-	2.1	-

Table 1 shows that there were some dominant compounds of the layer part of Sea Bamboo (*Isis hippuris*). The most dominant compounds for the ethanol fraction were *2-butoxyethanol* (43.68%), *hexanedioic acid* (12.11%), for the ethyl acetate fraction were*2-Myristynoyl-glycinamide* (19.51%), *1H-Indole* (17.64%), *8.beta.,12-Epoxy-13,14,15,16,17,19-hexanorlabdane/ 2(1H)-Naphthalenone, octahydro-4a- methyl- 7-(1-methylethyl)* (11.63%),while for the n Hexane fraction, the most dominant compounds were *Methyl-D-mannopyranoside* (20.41%), *2.6-Dimethyl-3-(methoxymethyl)-benzoquinone* (8.16%).

Hexanedioic acid compounds were found in the ethanol fraction of the extracted layers and ethanol fraction as well as n hexane fraction of the exteracted axial of the Sea Bamboo. *Hexanesiodic acid* can be produced by using green chemical concept, a new method that has veen developed in which the process involved the oxidation of cyclohexena, hydrogen peroxide, catalic tungsten and catalic transfer phase (Sato et al., 1998). Based on the results of some research, *hexanedioic acid* functions as antibacterial (Choi and Jiang, 2014) as the biological labeling (Zhang et al., 2009). In addition, *hexanedioic acid* functions as *antieczematic* (prevents skin from irritation), *antieseborrheic* (prevents scalp from inflammation), and *antihypoxic* (prevents from lack of oxygen), sklerosan (curing muscle cramps and varises), *antioxidant,* prevents eye from irriation, antimutagenetic (lowering the risk of cancer) (Way2Drug, 2016).

The most dominant compounds found in the extracted layer part of the Sea Bamboo of the ethanol fraction was 2-*butoxyethanol* which is the ether glycols with simple surfactant characteristic (can be used as mutual solvent). Ether glycol has been used since 1930s as dissolver that dilute two water-dilutable substances and hydrophobic substances. Ether glycol consists of two components which are the alcohol and ether. 2-butoxyethanol in industrials is used as paint dissolver and surface coverm and tint-cleaner products (Rebsdat, 2002). 2-butoxyethanol prevents eye irritation, skerosan (prevents muscle cramps and varises), prevents skin irritation, phobia therapy, antiseptic (antibacterial), antiseborrheic (prevents scalp irritation), antioxidant, acaricide (bug and mite killer) (Way2Drug, 2016).

The most dominant compound of the Sea Bamboo layer extract was the 2-*Myristynoyl-glycinamide* which functions as mucocytis medication (mouth cavity inflammation), spasmilitic (stomach spasmodic) (Way2Drug, 2016). 1H-Indole placed the second rank, in which this substance functions as preneoplastic medication, mucomembranous protector, phobia therapy (Way2Drug, 2016). Other subtansces were following such as the 8beta compounds, *12-Epoxy-13,14,15,16,17,19-hexanorlabdane*in the Sea Bamboo layer extract of the ethyl acetate fraction which is related to the Fe(III)EDTA, beta-tetralone, thujopsene, 6-methoxy-2- 6-methoxy-1-methylquinolinium, 1,1,6-trimethyl-1,2-dyhidronaphthalene, N-acetylgalactosa minyl-(1-4)-glucose, tetrahydronoot katone, longipinene, spiniferin-1, tetrahydronootkatone (Pubchem, 2005). 8.beta.,12-Epoxy-13,14,15,16,17,19-hexanorlabdane functions as anriseborrheic (prevents sclap inflammation), antineoplastic (anticancer), mucomembranous protector (Way2Drug, 2016).

In the ethyl acetate fraction of the layer part, *9-Octadecenoic acid*was found which functions as the *antiinflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor antiandrogenic, antiarthritic, anticoronary,*

insectifuge. Whilst, in the axial part, there was found *1.2-benzenedicarboxylic acid*compounds which functions as antimicrobial and antifouling (Kalaivani et al., 2012).

9-Octadecenoic acid functions as aniteczematic (prevents skin irritation), phobia therapy medication, anti mutagenic (lowering risk of cancer), preventing eyes from irritation, *antiseborrheic* (prevents scalp irritation), *antisecretoric* (anti diarrhea), *angiogenesis stimulant* (increasing awareness), eye inflammatory medication, *antihypercholesterolemic* (prevents high cholestrol), sklerosan (prevents muscle cramps and varises), *anti hypoxic* (prevents lack of oxygen), *choleretic* (disgestive juices stimulous), *fibrinolytic* (prevents blood coogulation), antioksidan, *antithrombotic* (prevents hematoma) (Way2Drug, 2016).

Dominant most compound found in the n hexane fraction of the sea bamboo outer layer extract was *alpha.-Methyl-D-mannopyranoside*which is a part of mannose structure. Mannose is the monomer of glucose from the aldohexose carbohydrate series. Mannose is the epimer C-2 glucose. Mannose is important to maintain metabolism system in human, especially for the glicolization of certain protein. Some hereditary abnormality of glicolization is relted to the mutation on the enzimes of the mannose metabolism (Freeze and Sharma, 2010). Generally, mannose has two rings with different sizes and piranose (has six models) of shape and furanose (has five models) of shape. Each end of every ring can have alpha or beta configuration in anomerical position. Chemical substances that has either one of those four shapes are easily isomerized. It has been confirmed that D-mannose powder helps the body in preventing itself from urinary infection (Kranjčec *et al.,* 2013). *Methyl alpha-D-mannopyranoside*functions as osmotic diuretic (increasing urine excression using a certain mechanism based on different osmosa pressure), *Lipotropic* (fat burner), sweetener, antiinfesi, *antineoplastic*, *antihypoxic, radioprotector, antiviral (influenza), dementia treatment, hepatoprotectant, antitoxic, antidiabetic, analeptic, anticarcinogenic, sclerosant, cytostatic, antineoplastic, chemopreventive, vasodilator, peripheral* (Way2Drug, 2016).

Functional Cluster Analysis using Fourier Transform Infra Red (FTIR) Spectrophotometry

The fractionation result of the Sea Bamboo layer extract (*Isis hippuris*) was then analyzed to find its antioxidant activites and its GC-MS. After that, the functional clustering of the substance was analyzed using Fourier Transform Infra Red (FTIR). The result form the analysis of its functional clusters was then compared to the fractionation result of the extracted layer of the Sea Bamboo (*Isis hippuris*) as presented in Figure 6.

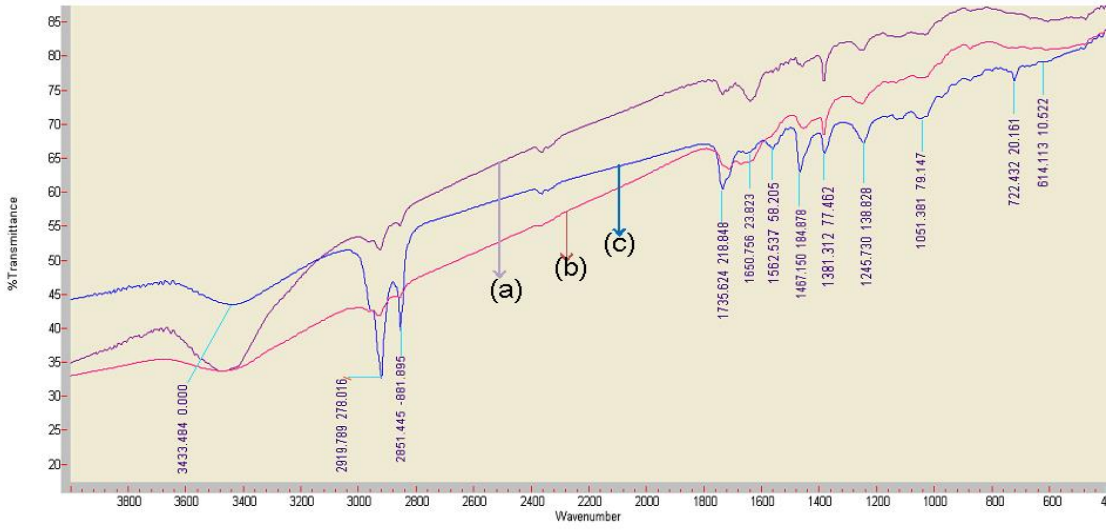

Figure 6. The Infra Red Spectrum of the Fractionation Result of Extracted Sea Bamboo Layer (*Isis hippuris*); (a) Ethanol Fraction; (b) Ethyl Acetate Fraction; (c) n Hexane Fraction.

The chromatography of FTIR in Figure 6 shows that there were 9 peaks found in the ethanol fraction, (a) 10 peaks found in ethyl acetate fraction (b), and 12 peaks of the n hexane fraction (c). Numbers under the peaks shows the peak frequency that shows the types of certain functional clusters as presented in Table 2.

ISSN: 0975-8585

Table 2. Analysis of Functional Clusters of the Fractionation Results of the Sea Bamboo Layer (*Ethanol Fraction, n Hexane Fraction and Ethyl Acetate Fraction*) Using Fourier Transform Infra Red (FTIR)

No.	Types of Vibration	Wave Length (cm^{-1})		
		Ethanol Fraction	Hexane Fraction	Ethyl Acetate Fraction
1	OH stretching (4000-3200 cm^{-1})	3471,28	3433,48	3468,01
2	CH Stretch dalam C-CH_3/ OH Stretching, hidrogen berikatan asam karboksilat (2970-2830 cm^{-1})	2923,92	2919,79	2928,03
3	CH Stretch dalam alkana (2860-2850 cm^{-1})	2857,14	2851,45	2860,23
4	C=O stretch (1765-1720 cm^{-1})	1735,79	1735,62	-
5	C=O stretch alifatik jenuh (1725-1705 cm^{-1})	-	-	1714,07
6	C-C stretch (1690-1670 cm^{-1})	-	-	1671,63
7	C=C stretching (cis isomer), alkena (1665-1635 cm^{-1})	1638,11	1650,76	-
8	NO_2 stretch alifatik (1570-1550 cm^{-1})	-	1562,54	-
9	NO_2 stretch aromatik, CH_2 bend (1480-1460 cm^{-1})	-	1467,15	-
10	CH_3 bend antisym (1470-1440 cm^{-1})	1456,99	1467,15	1454,71
11	H-C=O bend. Alifatik aldehid (1440-1320 cm^{-1})	1383,96	1381,31	1383,71
12	C-O-C stretch alkil-aril eter (1280-1220 cm^{-1})	1249,14	1245,73	1250,57
13	C-OH stretch (1060-1030 cm^{-1})	1032,52	1051,38	1034,65
14	C-N stretch (920-830 cm^{-1})	-	-	874,89
15	CH bend cis (730-665 cm^{-1})	-	722,43	-
16	C-Br stretch (650-500 cm^{-1})	-	614,11	-

FTIR spectroscopic analysis was administered based on the characteristics of each functional cluster in each of the fraction. The data shown in Table 2 shows that the fractionation result of the Sea Bamboo (*Isis hippuris*) layer extract of the ethanol fraction, n hexane fraction and ethyl acetate fraction contained phenol which was shown by the existence of active OH clusters found in each number of the waves which was 3471,28 cm^{-1}, 3433,48 cm^{-1}, 3468,01 $cm^{-1.}$ The wave numbers indicate the existence of stretch hydroxile vibration (OH) that constructed the phenol (Socrates, 2001). In addition, the OH clusters in the wave numbers shows that loose fatty acids were identified in the clusters. According to Peternelli et al, (2008), the absorbance of the wave numbers between 3600 to 2500 cm^{-1}showed OH clusters that represents the loose fatty acids.

The bounding of OH in carboxylate acids for ethanol fraction, n hexane fraction and ethyl acetate fraction were shown by the wabe number of 2923,92 cm^{-1}, 2919,79 cm^{-1}, 2928,03 $cm^{-1.}$ Besides, those numbers show the strectch virbration of aliphatic C-H that gives clues to the existence of metal clusters (CH_3) and methylenes (CH_2) that indicate the existence of fatty acids. This funding is in line with Ghrek et al, (2008) who stated that the asimetrical CH stretching from CH_3 existed in the peak wave number around 2956 $cm^{-1.}$ In addition, the wave numbers also show the ß karoten spectrum since it is in the wave number of range 2922-2862cm^{-1} (Ammawath and Che, 2009).

The wave number of 2857,14 cm^{-1}, 2851,45 cm^{-1}, 2860,23 cm^{-1}which were the fractionation result of the ethanol fraction, n hexane fraction and ethyl acetate fraction of the layer part of Sea Bamboo (*Isis hippuris*) extract, showing the the stretching vibration of aliphatic C-H in the alkane indicated the existence of groups of metallic or methylene in those fractions (Socrates, 2001).

Meanwhile, the vibration path C=O at 1735,79 cm^{-1}, 1735,62 cm^{-1}, 1714,07 cm^{-1}shoes that there were some compounds from esther groups or carboxylic acids (Socrates, 2001). Besides, the wave numbers indicated the existence of vitain E as stated by Ahmed (2010) that the structure of the vitamin E in FTIR was signified by the existence of cluster C=O stretching (1746 cm^{-1}) CH_3 and CH_2*scissoring* (1377 and 1467 cm^{-1}) and cluster C-O *stretching* (1118,1163 dan 1238 cm^{-1}).

Cluster C=C stretching aromatic was obtained in the wave number 1638,11 cm^{-1}, 1650,76 $cm^{-1}$of ethanol and n hexane fractions of the Sea Bamboo (*Isis hippuris*) layer. It is in line with Cobbniah (2008) who found the C=C aromatic stretching around the wave number of 1613 cm^{-1} and 1510 cm^{-1}. It has been well-known

unsaturated fatty acids with two or more double bonds are easily oxydated. It is being influenced by its ability in donating electrons. Thus, unsaturated fatty acids are preventive antioxidant that can also play role as peroxides (Huang and Wang, 2004). Moreover, the seizure of radical pollutant and antioxidant activites mostly rely on the number and position of hydroxile groups which donate their hydrogen to the aromatic rings of the phenol molecules. The antioxidant activities in phenol also rely on some factors such as the glycolization of glycogene which is included in the donator group H (-NH, -SH) (Cai et al., 2004).

The wave number 1456,99 cm^{-1}, 1467,15 cm^{-1}, 1454,71 cm^{-1}, 1383,96 cm^{-1}, 1381,31 cm^{-1}, 1383,71 cm^{-1}indicated the methylene and metal bending clusters. There were also some other functional cluster which is C-O in the wave number 1249,14 cm^{-1}, 1245,73 cm^{-1}, 1250,57 cm^{-1} indicating the glucose rings. There were also OH which were bounded to the edge chain in the wave number 1032,52 cm^{-1}, 1051,38 cm^{-1}, 1034,65 cm^{-1}which were the OH that were bounded to each og the glucose ring. As stated by El-Batal (2008) in which the bound C-O-C of the hexane ring was detected around the wave number of 1160 $cm^{-1}$and the C-OH was located in the edge of the chain around the wave number of 1078 $cm^{-1.}$

REFERENCES

[1] Ammawath, W., and M.Y. Che. 2009. A Rapid Method for Determination Commercial ß-carotene in RBD Palm Olein by Fourier Transform Infrared Spectroscopy. International Conference on the Role of Universities in Hands-On Education. Rajamanggala University of Technology Lanna. Chiang-May.

[2] Budhiyanti, S.A., Raharjo, Marseno, D.W., and Y.B. Lelana. 2012. Antioxidant activity of brown algae Sargassum species extract from the coastline of Java Island. American Journal of Agricultural and Biological Sciences. 7(3): 337–346.

[3] Cai, Y., Luo, Q., Sun, M., and H. Corke. 2004. Antioxidant Activity and Phenolic Coumpounds of 112 Traditional Chinese Medicinal Plants Associated with Anticancer. Life Science J. 74: 2154-2184.

[4] Chang, C. C., Yang, M. H., Wen, H. M., and Chern, J. C., 2002, Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods, J. Food. Drug Anal, 10 : 178-182

[5] Choi, W.H., and M.H. Jiang. 2014. Evaluation of antibacterial activity of hexanedioic acid isolated from Hermetia illucens larvae. Journal of applied biomedicine 12:179–189. http://dx.doi.org/10.1016/ j.jab.2014.01.003

[6] Cobbinah, E.A. 2008. Antioxidant and Antibacterial Activities of The Chemical Constituents of Terminalia ivorensis chev. Thesis. Kwame Nkrumah of Science of Technology.

[7] El-Batal. 2008. Amelioreting Effect of Yeast Glucan with zinc Bisglycinate on Histological Change in γ–Irradiated Rats. Friends Science Plubisher.

[8] Freeze, H.H., and V. Sharma. 2010. Metabolic manipulation of glycosylation disorders in humans and animal models. Seminars in Cell & Developmental Biology 21 (6):655–662. doi:10.1016/j.semcdb. 2010.03.011. PMC 2917643. PMID 20363348

[9] Halliwell, B. 1999. Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radic. Res. 31: 261–272.

[10] Hassan, I., Konchock, D., Abdul, S., Parvaiz, A. 2013. Sunsreens and antioxidant as Photo-Protective Measure: An Update, Our Dhermatol Online, Vol. 4, No. 3 pp 369-374.

[11] Higa, T., J. Tanaka, I.I. Ohtani, M. Musman, M.C. Roy and I. Kuroda, 2001. Bioactive compounds from coral reef Invertrebrates. Pure Apllied Chem. , 73: 589-593.

[12] Huang, H.C., and B.G. Wang. 2004. Antioxidant Capacity and Lipophilic Content of Seaweed Collected From The Qingdao Coastline. Journal Agric. Food Chem. 52: 4993-9997.

[13] Kahkonen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., and M., Heinonen. 1999. Antioxidant activity of extracts containing phenolic compounds, J. Agric. Food Chem. 47:3954-3962.

[14] Kalaivani, C.S., Sathish, S.S., Janakiraman N., and M. Johnson. 2012. GC-MS studies on Andrographis paniculata (Burm.f.)Wall ex. Ness-A Medicinally Important Plant. Int. J. Med. Arom. Plants. 2(1): 69-74

[15] Kelman, D., Posner, E.K., McDermid, K.J., Tabandera, N.K., Wright, P.R., and A.D. Wright. 2012. Antioxidant activity of Hawaiian marine algae. Mar. Drugs. 10: 403–416.

[16] Kranjčec, Bojana, Papeš, Dino, Altarac and Silvio. 2013. d-mannose powder for prophylaxis of recurrent urinary tract infections in women: a randomized clinical trial. World Journal of Urology 32 (1): 79–84. doi:10.1007/s00345-013-1091-6. ISSN 0724-4983

[17] Mallick, N. and F.H. Mohn. 2000. Reactive oxygen species: Response of algal cells. J. Plant Physiol.157:183–193.

[18] Manuputty, A. E. W. 2002. Karang Lunak (Soft Coral) Perairan Indonesia. LIPI, Jakarta

[19] Molyneux, P. 2004. The use of the stable free radicals diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci. Technol26(2): 211-219.
[20] Naczk, M., and F. Shahidi. 2004, Extraction and Analysis of Phenolic in Food. Journal of Chromatography A. 1054:95-111
[21] Nugroho, A.E., Malik, A. and Pramono, S. 2013. Total Phenolic and Flavonoid contents, and in vitro antihypertension activity of purified extract of Indonesian cashew leaves (Anacardium occidentale L.), International Food Research Jounal 20 (1): 299-305
[22] Nur, A.M., dan M. Astawan. 2011. Kapasitas Antioksidan Bawang Dayak (Eleutherine palmifolia) Dalam Bentuk Segar, Simplisia dan Keripik, Pada Pelarut Nonpolar, Semipolar dan Polar, Skripsi, Bogor: Departemen Ilmu dan Teknologi Pangan Institut Pertanian Bogor
[23] Nursid, M., Wikanta, T., Susilowati, R. 2013. Aktivitas antioksidan, sitotoksisitas dan Kandungan fukosantin ekstrak rumput laut coklat Dari pantai Binuangeun, Banten. JPB Kelautan dan Perikanan 8(1): 73–84.
[24] Peternelli, E.F.O., Barbosa, L.C.A., and T.M.C.D. Lucia. 2008. Isolation of Compounds Attractive to the Leaf-cutting ants Atta Sexdens rubropilosa Forrel (Hymenoptera: Formicidae) from Mabea fistulifera Elaisome. Quim Nova, 31 (3): 475-478.
[25] Rahman, M., Habib, R., Hasan, R., Islam, A. M. T., and I.N. Khan. 2012. Comparative Antioxidant Potential Of Different Extracts Of Flacourtia Jangomas Lour Fruits. Asian Journal of pharmaceutical and Clinical Research 5(1):73-75.
[26] Rao, C.B., and K.V. Ramana. 1988. Metabolites of the Gorgonian Isis hippuris from India. Journal of Natural Products 51(5):954-958.
[27] Rebsdat, S. 2000. Dieter Mayer "Ethylene Glycol" in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim,.doi: 10.1002/14356007.a10_101
[28] Rohman, A. & Riyanto, S., 2006, Aktivitas Antiradikal Bebas Ekstrak Kloroform Buah Mengkudu (Morinda citrifolia, L.) dan Fraksi-Fraksinya, Artocarpus, Vol.6.No.1 Maret 2006, 39.
[29] Santoso, J., Anwariyah, S., Rumiantin, R.O., Putri, A.P., Ukhty, N., and Y. Yoshie. 2012. Phenol Content, Antioxidant Activity and Fibers profile of Four Tropical Seagrasses from Indonesia. Journal of Coastal Development 15 (2) : 189-196.
[30] Sato, K., Aoki, M., and R. Noyori. 1998. A "Green" route to adipic acid: direct oxidation of cyclohexenes with 30 percent hydrogen peroxide.Science 281 (5383): 1646–7. doi:10.1126/science
[31] Sharma, G.N. 2011. Phytochemical Screening and Estimation Total Phenolic Content in Aegle marmelos Seeds. International Journal of Pharmaceutical and Clinical Reseach 2(3).27-29.
[32] Sheu, J.H., Chen, S.P., Sung, P.J., Chiang, M.Y. and C.F. Daic. 2000. Hippuristerone A, a novel polyoxygenated steroid from the gorgonian Isis hippuris. Tetrahedron Letters 41:7885–7888
[33] Sheu, J.H., Huang, L.F., et al. 2003. Hippuristerones E-I, New Polyoxygenated Steroids from the Gorgonian Coral Isis hippuris. J. Nat. Prod. 66: 917-921.
[34] Socrates, G. 2001. Infrared and raman Characteristic Group Frequencies: Tables and Charts. Third Edition. Jhon Wiley & Sons, Ltd. Formerly of Brunel. The University of West London, Middlesex. UK
[35] Soeksmanto, A., Hapsari, Y., dan P. Simanjuntak. 2007. Kandungan Antioksidan pada Beberapa Bagian Tanaman Mahkota Dewa, Phaleria macrocarpa (Scheff) Boerl. (Thymelaceae). Biodiversita 8(2): 92-95.
[36] Stevi, G., Dungira., Dewa, G., Katja., Vanda dan S. Kamu. 2012. Aktivitas Antioksidan Ekstrak Fenolik Dari Buah Manggis (Garcinia mongostana L). Jurnal MIPA Online 1 (1):11-15. Unsrat Manado.
[37] Summanen, J. A. 1999. A Chemical and Ethnopharmalogical Study on Phyllantus emblica (Euphorbiaceae). Disertasi. Faculty of Science, University of Helsinki, Helsinki
[38] Tanaka, J., Trianto, A., et al. 2002. New polyoxygenated steroids exhibiting reversal of multidrug resistance from the gorgonian Isis hippuris. Tetrahedron 58:6259–6266.
[39] Way2Drug. 2016. Predictive Services. Understanding Chemical-Biological Interactions. www.pharmaexpert.ru/PASSonline/predict.php. accessed in June 2016.
[40] Wikanta, T., Prabukusuma, A., Ratih, D., dan H.I. Januar. 2010. Bioaktivitas ekstrak kasar aseton, fraksi dan subfraksinya dari Ulva fasciata terhadap sel lestari tumor HeLa. Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan, 5(1): 1-10.
[41] Yangthong, M., Hutadilok-Towatana, M. and Phromkunthong, W. 2009. Antioxidant activities of four edible seaweeds from the Southern Coast of Thailand. Plant Foods Human Nutrition 64: 218-223.
[42] Zhang, Q., Song, K., Zhao, J., Kong, X., Sun, Y., Liu, X., Zhang, Y., Zeng, Q., and H. Zhang. 2009. Hexanedioic acid mediated surface–ligand-exchange process for transferring NaYF4:Yb/Er (or Yb/Tm) up-converting nanoparticles from hydrophobic to hydrophilic. Journal of Colloid