

## The Isolation of Metabolite Compounds from Seaweed (*Halimeda gracillis*) in the Waters of Teluk Lampung as a Source of Antibacterial Compounds

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**Abstract** The isolation of secondary metabolites of seaweed *Halimeda gracilis* was conducted. Isolation begins with 2 kg macerated dried powder *Halimeda gracillis* in 8 L of n-hexane solvent followed by ethyl acetate and methanol for 3 days. The solvent was filtered and followed by evaporation. Separation and purification of the isolated compounds were done by using chromatography technique. Antibacterial activity was tested by the disc diffusion method with the bacteria *E. coli*, *S. disenteriae*, and *B. subtilis*. Isolated compounds are in the form of white crystals. Based on the spectroscopy analysis data of UV, IR, 1H-NMR, 13C-NMR, HMQC, HMBC, and COSY, the pure compounds isolated are  $\beta$ -sitosterol from n-hexane extract and oleic acid from ethyl acetate extract. Isolated compounds and n-hexane extracts showed non antibacterial active, while the ethyl acetate extract showed weak antibacterial active on three test bacteria at a concentration of 200,000 ppm with clear zones of 9.47; 11.09; and 8.84 mm.

**Keywords** *Halimeda gracillis*; Antibacterial

## Introduction

Seaweed is one of fishery commodities that have high economic value and become one of the sources of export commodities and a source of income from non-oil sector. Seaweed contains many organic materials such as polysaccharides, hormones, vitamins, minerals and bioactive compounds and is one of the plants producing hydrocolloid materials (Anggadireja, 2006). Besides containing hydrocolloid materials as its primary component, seaweed also contains secondary components that have the potential to be developed in various fields such as pharmaceuticals, cosmetics, and other industrial purposes (Atmadja, 1991).

Currently, seaweed has been used as raw materials for industry. Some of the main ingredients produced by seaweeds are jelly, carrageenan and alginate (Aslan, 1998). Some seaweed products are also widely used as food ingredients, additives or adjuvants in the food industry, pharmaceuticals, cosmetics, textiles, paper, paint, and others. In addition, the waste can also be used as fertilizer and animal or fish feed components. When viewed from the benefits and usefulness, it is true that the seaweed in the future may be one of the commodity trade has bright prospects for development, both to meet the needs of the domestic and export markets (Zatnika, 1985).

One of the potential that can provide economic added value to the seaweed ecosystem is its bioactive compounds (Arifudin, 2001). Some types of seaweed that has the ability to produce bioactive compounds are *Halimeda* sp with *Halimeda* sp and *Halimeda Tetrasetat* compounds (Paul and Fenical, 1983a). These compounds allegedly can be used as an antibacterial, antifungal, antifouling, anticancer, antiviral, and so on (Paul and Fenical, 1983b).

Some types of seaweed such as *Sargassum* sp. and *Turbinaria* sp. has been reported to have potential as bioactive compounds that can be used in pharmacy and medicine fields. These compounds are secondary metabolites that are used in defense systems, in order to survive and avoid interference from other organisms in the environment. In addition to the defense, the compound has the capability of pharmacological activity that has the potential to be used in the pharmaceutical field.

Some specific studies on the crude extract of marine algae as an antibacterial against *Staphylococcus aureus* has been conducted. The study specifically found that the species of macroalgae *Caulerpa taylori*, *Halimeda discoidea*, *Ulva rugida*, *Dictyota* sp, and *Osmundea hybrida* effectively inhibit the growth of bacteria *Staphylococcus aureus* with a diameter of approximately 14 mm. Until now there has been lacking research that exploring protein compound of seaweed as a raw material for medicine on human and animal diseases. The use of proteins as raw material medicine has many advantages such as: protein compounds can be accepted by the body and cause fewer side effects, besides that the compounds can be cloned its gen to be produced on a large scale on an industrial scale through genetic engineering techniques.

Teluk Lampung waters have abundant seaweed, both natural seaweed and seaweed that cultivated by farmers. Ecologically, Teluk Lampung waters have characteristics suitable for seaweed habitat. This can be seen from many seaweed discovered around these waters. In addition, Lampung Bay waters characterized as relatively sheltered from the waves and the wind directly. In It is related with its location that rather inside to the land and has a sloping waters. These conditions are ideal for development of seaweed.

This study was conducted to explore the potential and diversity of seaweed in Teluk Lampung and surrounding waters, which until now has been used for a wide range of utilization by humans. Seaweeds found to be grouped based on zoning in accordance with its utilization for further examination for their bioactive compounds (contents) in relation to its ability to inhibit the growth of bacteria (antibacterial), anticancer and antifungal.

## 1 Research Methods

### 1.1 Preparation of sample

The sample of seaweed *Halimeda gracillis* is taken Teluk Lampung. The sample was then dried and pulverized to form a dry powder.

### 1.2 Extraction of seaweed

The dry powder of seaweed *Halimeda gracillis* of 2 kg each macerated using a solvent of n-hexane 8 L then the residue is dried and maceration followed by ethyl acetate and methanol solvent. Maceration is done with three repetitions. The filtrate obtained was concentrated using a *rotary evaporator*. Then each extract be TLC to determine which extracts potentially contain secondary metabolites. Selected extracts is then proceed to the separation and purification.

### 1.3 Separation and purification

#### 1.3.1 Separation and purification of n-hexane *H.gracillis* Extract

N-hexane *H.gracillis* extract of 5 g separated by gravity column chromatography using silica gel G stationary phase 60 F254 (230-400 mesh) and motion phase of n-hexane, n-hexane: ethyl acetate (9:1 - 6:4), and ending with methanol. The eluate collected with vial bottle and analyzed by TLC. Fractions which provide the same stain pattern merged into one, thereby it obtained 7 fractions namely F1 – F7. In Fraction F6 there were crystal after analyzed with KLT, the stain pattern shows a little dirt that then washed with n-hexane.

#### 1.3.2 Separation and purification of *H.gracillis* ethyl acetate extract

*H.gracillis* ethyl acetate extract 2.5 g were separated by gravity column chromatography using silica gel G stationary phase 60 F254 (230-400 mesh) and eluted n-hexane, n-hexane: ethyl acetate (9:1-1:9), ethyl acetate and ending with methanol. The eluate collected with vial bottle and analyzed by TLC. Fractions which provide the same stain pattern merged into one, thereby it obtained 6 Fractions namely F1 - F6. In Fraction F1 there is a potential compound, so it continued its separation and purification.

F1 fraction separated back by gravity column chromatography silica gel G 60 (70-230 mesh) using the eluent n-hexane and n-hexane: ethyl acetate (9:1). The eluates are contained in vials and the vial on TLC. TLC results obtained 3 fractions namely F1.1, F1.2, and F1.3. The F1.2 is obtained pure compound.

## 1.4 Antibacterial activity test

### 1.4.1 Making tntibacterial test media

*Nutrient agar* as much as 23 g put in erlenmeyer, then dissolved in 1000 ml of distilled water. Then heated on a hot plate and homogenized using a magnetic stirrer until boiling. In the same way, put as much as 13 g of *nutrient Broth* into erlenmeyer, then dissolved in 1000 ml of distilled water, heated on a hot plate and homogenized using magnetic stirrer until boiling. All of the media sterilized by autoclaving at 121 C, a pressure of 15 lbs for 15 minutes.

### 1.4.2 Rejuvenation of test bacteria test

Ose needle sterilized over a Bunsen flame and left for some time to cooling down. Take 1 ose of pure cultures of bacteria test, then inoculated into NA medium slanted aseptically. After that incubated for 24 hours at a temperature of 37 °C.

### 1.4.3 Preparation of test bacteria suspension

The making of suspension of cultures of bacteria is carried by taking 1-2 ose bacteria, which has been rejuvenated aseptically and then inserted into 9 ml of NB medium and shaker until homogeneous.

### 1.4.4 The test of antibacterial activity against *E. coli*, *S. dysenteriae*, and *B. subtilis*

Antibacterial activity test performed by the jelly diffusion method using paper disc with 6 mm diameter. Antibacterial activity test performed with three repetitions. Paper discs dipped into the sample with a concentration of 0% (negative control), 2000 ppm, 1000 ppm, 500 ppm, and 250 ppm. Then put on NA medium that has been inoculated with the test bacteria. Incubation was performed at 37 °C for 2x24 hours. Observations were made on the formation of a clear zone around the paper disc.

## 2 Result and Discussion

### 2.1 Extraction of *H. gracillis*

Maceration results of grams of *H. gracillis* powder obtained 3 g n-hexane extract, 2 g ethyl acetate and 5 g methanol. Furthermore, each extract was analyzed by TLC plate. TLC results showed extract n-hexane and ethyl acetate potentially contain secondary metabolites with the invisibility of the dominant stain pattern fluroscended under UV light, the test is then performed on extracts of n-hexane and ethyl acetate (Figure 1).

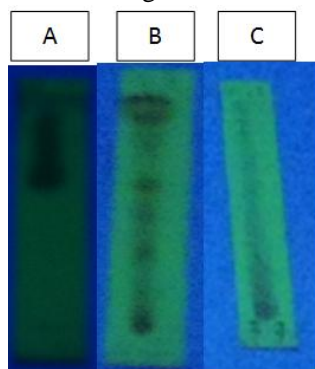


Figure 1 A, KLT of crude extract n-heksan eluen n-heksan: EtOac (8:2); B, etil asetat eluen n-heksan: etil asetat (6:4); and C, metanol with eluen EtOac: MeOH (9:1)

### 2.2 Isolation of secondary metabolites compounds of n-hexane *H.gracillis*

Crude extract of n-hexane *H.gracillis* fraction as much as 3 grams of the separation and purification using gravity column chromatography. The vial results were analyzed using TLC plate and obtained 4 column fractions. F1 0.36; F2 0.11; F3 1.73; and F4 0.51 g. Fraction F3 shows a white crystal and the results of TLC analysis indicates that the compound is still a little impurity. F3 fraction was purified again using a gravitational field, the results obtained 3 column fractions F1.1, F1.2, and F1.3. Fractions were analyzed by TLC F1.2. The compound was pure as just a single stain as much as 1.25 g (Figure 2).

### 2.3 Isolation of secondary metabolites compounds of *H.gracillis* ethyl acetate

The crude extract of ethyl acetate as much as 2 g after gravity column chromatography vial vial-column results were analyzed using TLC. TLC analysis of the results obtained 6 column fractions F1-F6. F1 0.94; F2 0.23; F3 0.11; F4 0.16; F5 0.20; and F6 0.09 g. Fraction F1 suggest the potential of secondary metabolites in the presence of a white powder, TLC analysis results indicate that the compound is not pure then carried back to the field of gravity separation obtained 3 fractions F1.1, F1.2, and F1.3. F1.2 fraction as much as 0.75 g after analyzed by TLC using cerium sulfate indicates that the compound was pure (Figure 3).

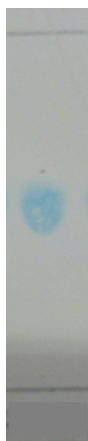


Figure 2 Results TLC pure compound *H.gracillis* fraction of n-hexane at cerium sulfate with the eluent n-hexane: EtOAc (6:4)



Figure 3 Results TLC pure compound *H.gracillis* fraction of n-hexane at cerium sulfate with the eluent n-hexane: EtOAc (8:2)

### 2.4 Identification of isolated compounds results

#### 2.4.1 Identification with <sup>1</sup>H-NMR spectrum

<sup>1</sup>H-NMR spectrum of the compound showed signals typical isolation for a group of steroid/triterpenoid compounds. This can be seen from the signal that accumulated in the area  $\delta$ H 0 - 3 ppm. <sup>1</sup>H-NMR spectrum showed characteristic absorption for the proton attached to the carbon sp<sup>2</sup> (proton vinylic) in the area  $\delta$ H 5.34 ppm (1H, t, J = 5.0 Hz), suspected of protons in this area is methin proton (CH) which cleaved by two neighboring protons. <sup>1</sup>H-NMR spectrum also showed uptake of protons bound to heteroatoms in the  $\delta$ H 3.51 ppm (1H, m) which is suspected as methin proton divided by four neighboring protons. These protons on the steroid/triterpenoids compound usually bound to third carbon atom (C3).

Fragment of <sup>1</sup>H-NMR spectrum showed signals for two methylene protons that each is in the area  $\delta$ H 2.23 ppm –  $\delta$ H 2.34 and  $\delta$ H 1.94 ppm ppm -  $\delta$ H 2.02 ppm (2H, m). Signal in the area  $\delta$ H 1.79 ppm -  $\delta$ H 1.85 ppm (3H, m) is suspected as one of the methin protons and two methylene protons. Proton signals with the integration of the three was also seen in the area  $\delta$ H 1.67 ppm (3H, m) and the signal is thought to also represent the methyl protons and two methylene protons. <sup>1</sup>H-NMR spectrum shows that the proton in the area  $\delta$ H 1.46 ppm –  $\delta$ H 1.50 ppm (6H, m) allegedly representing three methylene protons, then the signal that appears in the  $\delta$ H 1.33 ppm (5H, m) is suspected as four methylene protons and the protons methin. Methylene proton signals were also seen in the above areas  $\delta$ H 1.01 ppm with four integration protons alleged methylene protons. <sup>1</sup>H-NMR spectrum also showed the presence of 4 pieces of signals for methyl protons. Fourth proton signals appear in the  $\delta$ H 1.00 ppm (3H, s);  $\delta$ H 0.82 ppm –  $\delta$ H 0.84 ppm (6H, m);  $\delta$ H 0.79 –  $\delta$ H 0.81 ppm (6H, d) and in the area  $\delta$ H 0.67 ppm (3H, s).

#### 2.4.2 Identification with <sup>13</sup>C-NMR spectrum

<sup>13</sup>C-NMR spectrum of isolated compound showed 29 carbon signals, two signals in the form of C sp<sup>2</sup> namely in the area  $\delta$ c 140.9 ppm and 121.9 ppm  $\delta$ c area and 27 signal in the form of C sp<sup>3</sup>. Signal sp<sup>3</sup> carbon C located in area, respectively:  $\delta$ c 71.9; 56.9; 56.2; 50.3; 46.2; 42.5; 42.4; 39.9; 37.4; 36.7; 36.4; 34.1; 32.1; 32.0; 31.8; 29.1; 28.4; 26.5; 24.5; 23.2; 21.3; 19.8; 19.6; 19.2; 19.0; 12.5, and 12.0 ppm.

DEPT spectrum showed 29 signals that appear in the  $^{13}\text{C}$ -NMR spectrum only looks 25 DEPT spectrum signal at 135. This indicates the presence of 3 pieces of carbon in the form of C kwartener. DEPT 135 spectrum widening seemingly metin 9 carbon atoms, 11 carbon atoms of methylene and methyl 6 carbon atoms. Methyl carbon signal for each - each appear  $\delta\text{c}$  121.9; 71.9; 56.9; 56.2; 50.3; 46.2; 36.4; 32.1; and 29.1 ppm. While the methylene carbon signals for each - each appearing on  $\delta\text{c}$  area 42.3; 39.9; 37.4; 34.1; 31.8; 28.4; 26.5; 24.5; 23.2 and 21.3 ppm. Methyl carbon signals each - each appearing on  $\delta\text{c}$  19.8; 19.6; 19.2; 19.0; 12.5 and 12.0 ppm.

#### 2.4.3 Identification with NMR 2D spectrum

In the HMQC spectrum it seen that the proton at area  $\delta\text{H}$  5.34 ppm bound to the carbon in the area  $\delta\text{c}$  121.9 ppm, then the proton at area  $\delta\text{H}$  3.51 ppm is directly bonded to the carbon at 71.9 ppm  $\delta\text{c}$  area. HMBC spectrum (Figure 4) shows that the proton at  $\delta\text{H}$  5.30 ppm region correlated with the carbon in the area  $\delta\text{c}$  32.2; 36.7; 42.4 ppm. Fragment HMQC spectrum also showed that the proton at  $\delta\text{H}$  2.34 area is attached directly to the carbon at 42.5 ppm  $\delta\text{c}$  area. HMBC spectrum of this proton showed a correlation with the carbon in the area  $\delta\text{c}$  71.9; 121.9 and 140.9 ppm (Figure 5).

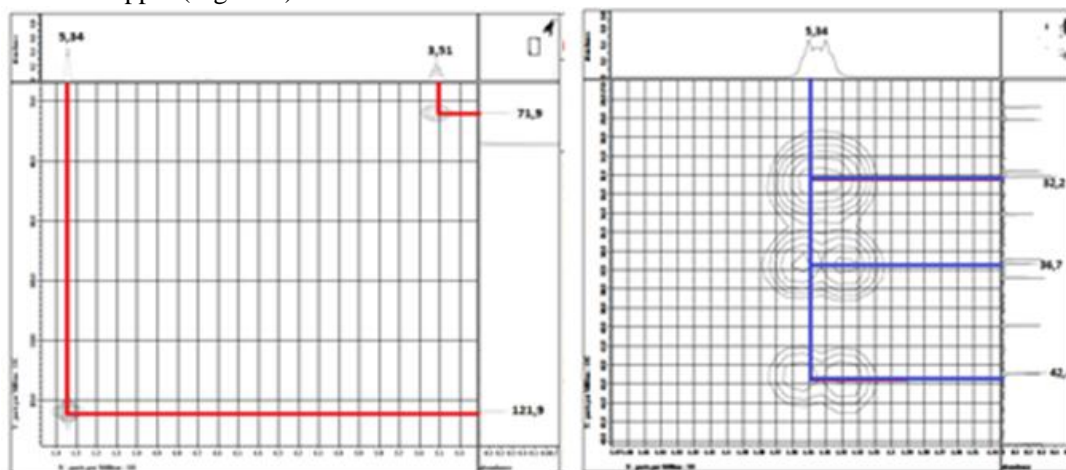


Figure 4 Fragment HMBC spectrum of protons in the area  $\delta\text{H}$  3.51 to 5.34 with the carbon in the  $\delta\text{c}$  71.9 to 121.9 ppm

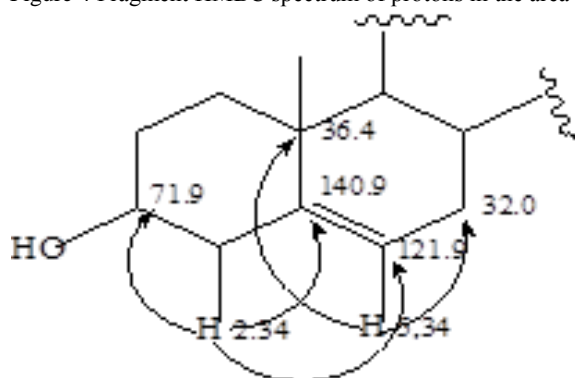


Figure 5 Fragment HMQC spectrum of protons in the area  $\delta\text{H}$  0.60 -  $\delta\text{H}$  1.10 ppm carbon in the area  $\delta\text{c}$  8.0 - 2.70 ppm  $\delta\text{c}$

Spektrum HMBC proton in the area  $\delta\text{H}$  5.34 ppm with carbon  $\delta\text{c}$  27,0 – 46,0 ppm. Protons at  $\delta\text{H}$  0.81 area; 0.83; 0.84; 0.92 ppm respectively - each bonded to carbon in the area  $\delta\text{c}$  19.6; 19.8; 12.0; 19.0. Two other methyl protons appear in the  $\delta\text{H}$  0.67 ppm and 1.01 ppm, respectively - each attached to a carbon at 12.0 ppm and  $\delta\text{c}$  in the area  $\delta\text{c}$  19.2 ppm.

Fragment of HMBC spectrum (Figure 6) shows that the proton at  $\delta\text{H}$  0.81 area; 0.83 and 0.85 ppm correlated with carbon at 46.2 ppm  $\delta\text{c}$  area. Proton  $\delta\text{H}$  0.67 ppm in the area visible in the area correlated dengankarbon  $\delta\text{c}$  39.9; 42.4; 56.2 and 56.9 ppm. Proton  $\delta\text{H}$  0.81 ppm area and protons in the area  $\delta\text{H}$  0.83 ppm correlated with the carbon in the area  $\delta\text{c}$  29.1 ppm. Pieces of the structure in the HMBC correlations presented in Figure 7. Fragment in the HMBC spectrum 8C and 8D  $\delta\text{H}$  0.92 ppm image.



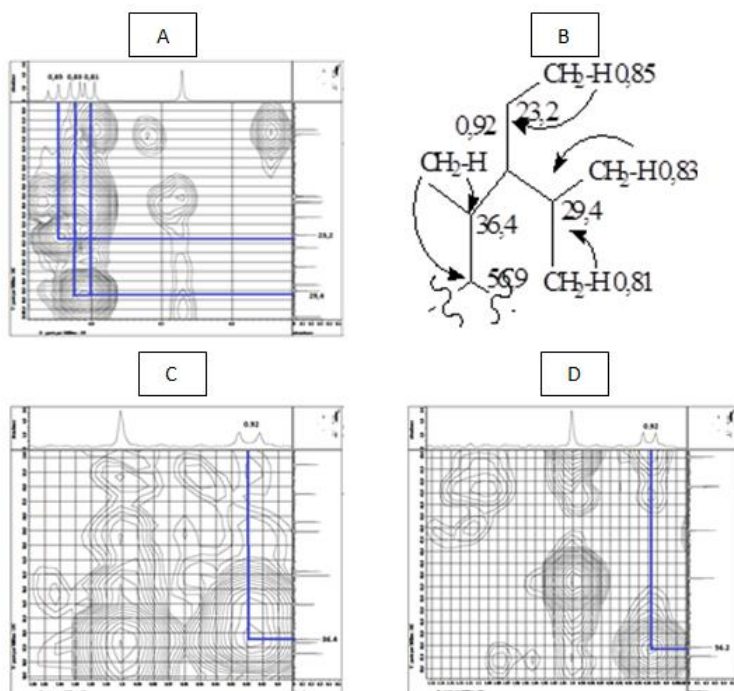


Figure 6 HMBC spectrum fragment area  $\delta H$  0.6 ppm - 0.8 ppm carbon  $\delta H$  in the area  $\delta c$  1.0 ppm - 32.0 ppm  $\delta c$  (A)

Note: Piece structure with HMBC correlation. Fragment HMBC spectrum in the area  $\delta H$  0.92 with carbon in the area  $\delta c$  36.4 and 56.2 ppm (6C and 6D)

Fragment HMQC spectrum (Figure 7) shows that the proton that appears in the  $\delta H$  2.01 ppm and  $\delta H$  1.94 ppm (2H, m) in the HMQC spectrum seen attached to a carbon at 39.9 ppm  $\delta c$  area and 32.1 ppm. Proton in the ppm and 1.85 ppm  $\delta H$  1.83 seen attached to a carbon in the carbon  $\delta c$  31.8 ppm and 37.4 ppm in the area. Proton in the area  $\delta H$  1.51 ppm carbon bonded to the area  $\delta c$  21.3 ppm and the proton HMBC spectrum is seen to correlate with the carbon in the area  $\delta H$  50.3 ppm (Figure 8; Figure 9).

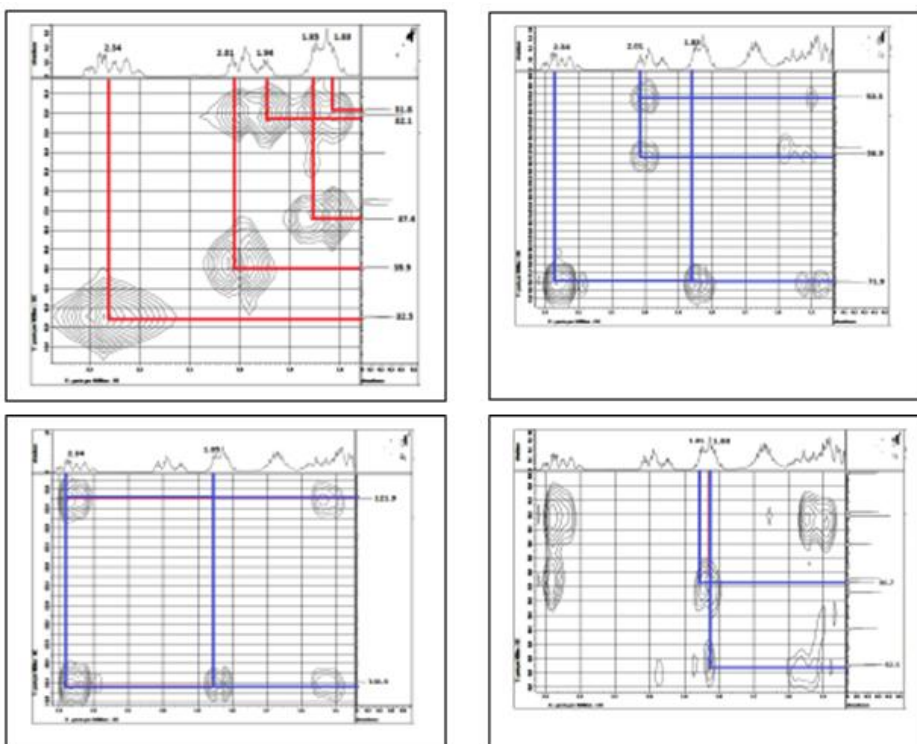


Figure 7 HMBC and HMQC correlation of isolated compounds on the area  $\delta H$  1.94 – 2.34 ppm

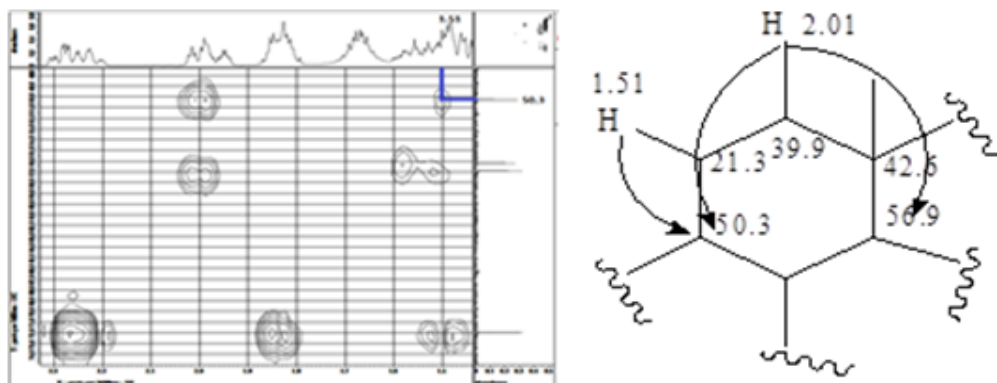


Figure 8 Fragment structures in areas isolated compounds 1.51 and 2.01 ppm in correlation HMBC

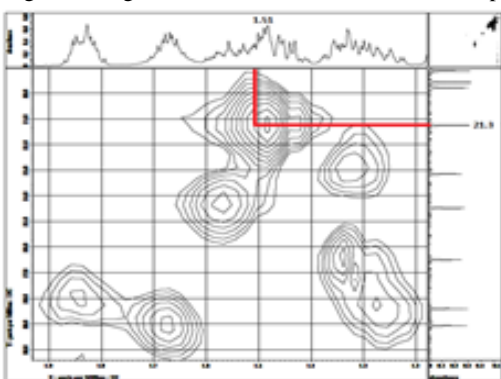


Figure 9 Fragment HMQC spectrum of protons in the region in the area of carbon  $\delta$ H dengan  $\delta$ C 21.3 ppm

Analysis HMBC spectrum (Figure 7) shows that the proton in the area  $\delta$ H 1.85 correlated with the carbon in the area  $\delta$ C 141.9 ppm,  $\delta$ H 71.9 ppm and  $\delta$ H 36.7 ppm, while the protons at 1.83 ppm area correlated with carbon in the area  $\delta$ C 42.5. Fragment isolated structure with HMBC correlation given in Figure 10.

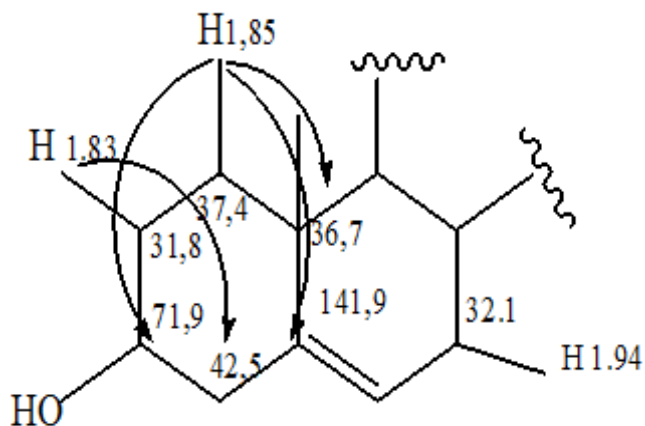


Figure 10 A fragment structure of isolated compounds with correlation HMBC

COSY spectrum analysis of isolated compounds, showing a correlation between the protons in the area  $\delta$ H 3.54 with protons in the area  $\delta$ H 2.24 ppm. Proton in the area  $\delta$ H 3.54 also shows a correlation with the proton in the area  $\delta$ H 1.83 ppm. Proton signals in the area  $\delta$ H 5.34 ppm correlated with protons in the area  $\delta$ H 1.94 ppm. Fragment of proton COSY spectrum - these protons are presented in Figure 11. This portion of the structure with COSY correlations are presented in Figure 12.

Based on the analysis of  $^1$ H-NMR spectrum,  $^{13}$ C-NMR and DEPT 135 then identified isolated compounds are a group of steroid compounds, namely  $\beta$ - sitosterol (Figure 13) with the molecular formula  $C_{29}H_{50}O$  BM 414 g/mol and DEPT of 5 pieces.

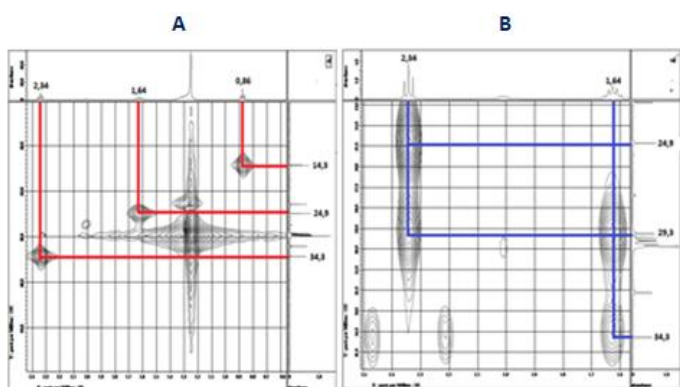


Figure 11 HMQC spectrum (A)  $\delta C$  area of 0.6 ppm and 2.4 ppm - $\delta C$  HMBC spectrum (B) daerah  $\delta H$  1.6 ppm - 2.5 ppm

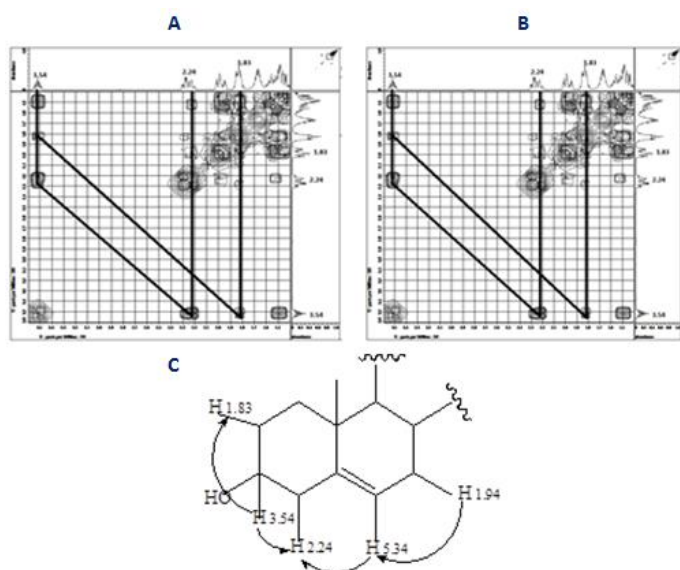


Figure 12 COSY spectrum fragment in the 1.83 to 5.34 ppm (A and B), fragment of structure of the isolated compounds regions  $\delta H$  1.94 -  $\delta H$  5.34 ppm with a correlation COSY (C)

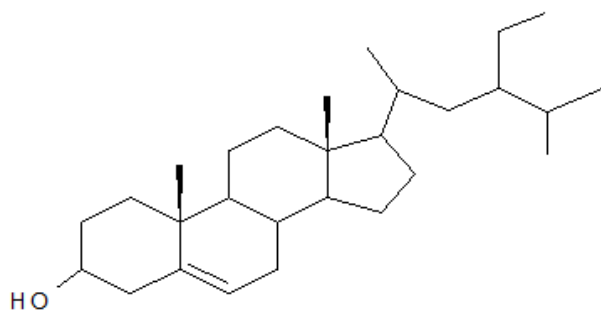


Figure 13 B-sitosterol compounds isolated

## 2.5 Identification of 2 isolated compound result structure

### 2.5.1 Identification with $^1H$ -NMR spectrum

$^1H$ -NMR spectrum showed signals at  $\delta H$  2.34 ppm region (2H, t,  $J = 7.8$  Hz) with the integration of two protons is suspected as a signal for the methylene protons (CH<sub>2</sub>). Methylene signal with multiplet appears in the  $\delta H$  1.64 ppm (2H, m), the proton is thought to be cleaved by more than two neighboring protons. Proton methyl (CH<sub>3</sub>) seemingly appeared in the area  $\delta H$  0.86 ppm (3H, t,  $J = B$ ). Proton in this area appears as a triplet signal and thought to be cleaved by two neighboring protons.



<sup>1</sup>H-NMR spectrum also showed the presence of methylene protons with the integration of the twenty-four protons in the area  $\delta$ H 1.29 ppm (24H, m). Proton in this area thought to represent 12 protons methylene (CH<sub>2</sub>). Signals - signals that appear in the area  $\delta$ H 5.34 ppm and in the area  $\delta$ H 2.0 ppm suspected as a signal for carbon impurities. It is seen from the integration of the two pieces of proton signals with very little integration.

### 2.5.2 Identification with <sup>13</sup>C-NMR spectrum

Identification of the isolated compounds with <sup>13</sup>C-NMR spectrum, showed ten carbon signals. One signal sp<sup>2</sup> carbon in the form of C that appears in the  $\delta$ C 180.5 ppm and allegedly as carbonyl signal. Nine other signals in the form of C sp<sup>3</sup> each - each appear in the  $\delta$ C 29.9; 29.8; 29.6; 29.5; 29.4; 29.3; 24.9; 22.9; and 14.3 ppm.

To clarify the types of carbon atoms that appear in the <sup>13</sup>C-NMR spectrum then be identified by DEPT spectrum 135. Identify the DEPT 135 spectrum is clear signal on carbon spectrum whether in the form of metin, methylene, methyl or having carbon kwartener. DEPT spectrum showed the presence of carbon atoms in the form of C kwartener in the area  $\delta$ C 180.5 ppm. Methyl carbon signals seen in the area  $\delta$ C 14.3 ppm and the methylene sinyel to appear in the area  $\delta$ C 34.3; 32.1; 29.8; 29.7; 29.6; 29.5; 29.4; 29.3;  $\Delta$ C 24.9 and in areas 22, 9 ppm. The results of the data analysis of 1D NMR spectrum allegedly isolated compounds are a group of fatty acid compounds.

### 2.5.3 Structure identification with NMR 2D

In the HMQC spectrum (Figure 13) shows that the proton at  $\delta$ H 2.34 area; 1.64 and 0.86 ppm respectively - each bound to the carbon in the area  $\delta$ C 34.3; 1.64; and 14.3 ppm. HMBC spectrum (Figure 13B) shows a correlation between the proton at  $\delta$ H 2.34 ppm region with carbon in the area  $\delta$ C 29.3 and 24.9 ppm. HMBC spectrum also shows that the proton at  $\delta$ H 1.64 ppm region has a correlation with the carbon in the carbon  $\delta$ C 34.3 ppm and 29.3 ppm in the area  $\delta$ c.

Fragment of HMBC spectrum isolated compounds also showed that the proton at  $\delta$ H 2.34 ppm region and protons at  $\delta$ H 1.64 ppm region correlated with the carbon in the area  $\delta$ c 180.5 ppm. HMBC spectrum (Figure 14) shown a correlation between the proton at  $\delta$ H 0.86 ppm region with carbon at 32.1 ppm  $\delta$ c area. HMQC and HMBC total spectrum isolated compounds are given in Appendix 7 and pieces of the structure of HMBC correlations are given in Figure 15.

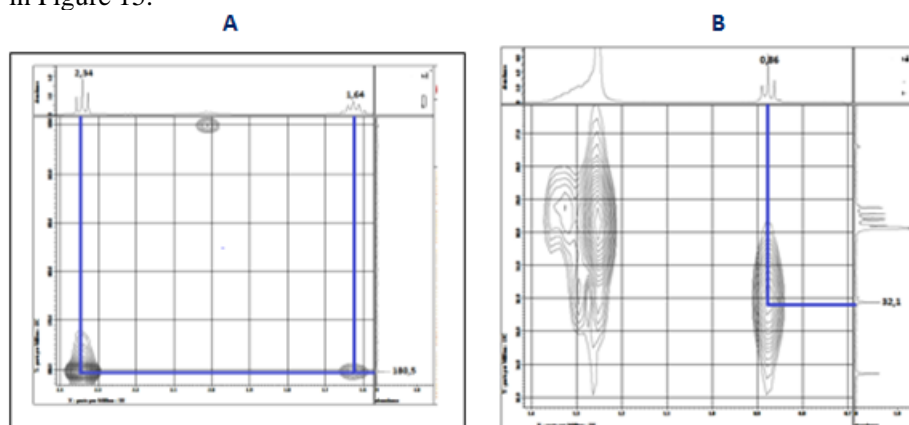


Figure 14 HMBC spectrum fragment (A) area  $\delta$ H 1.6 ppm - 2.4 ppm  $\delta$ H. HMBC spectrum (B) area of 0.7 ppm - 1.4 ppm  $\delta$ H

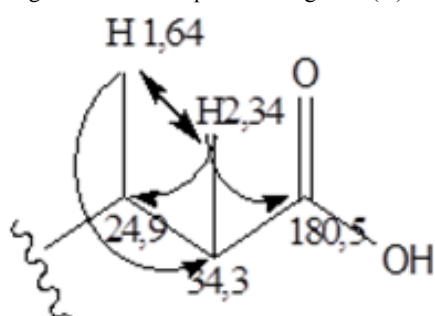


Figure 15 Pieces of the structure of isolated compounds with HMBC and COSY correlations

COSY spectrum (Figure 16) isolated compounds shown that there is a correlation between the protons at  $\delta H$  2.34 ppm region deng protons in the region of 1.64 ppm. This spectrum also shows that the proton at  $\delta H$  1.29 ppm regions correlated with the proton at  $\delta H$  0.86 ppm region.

Based on the analysis of NMR data 1D and 2D NMR it is suspected that the isolated compounds are a group of compounds, namely fatty acids oleic acid with the molecular formula  $C_{17}H_{33}COOH$  with a molecular weight of 282 g/mol with DBE as much as 1 piece. The structure of compound identification results are given in Figure 17.

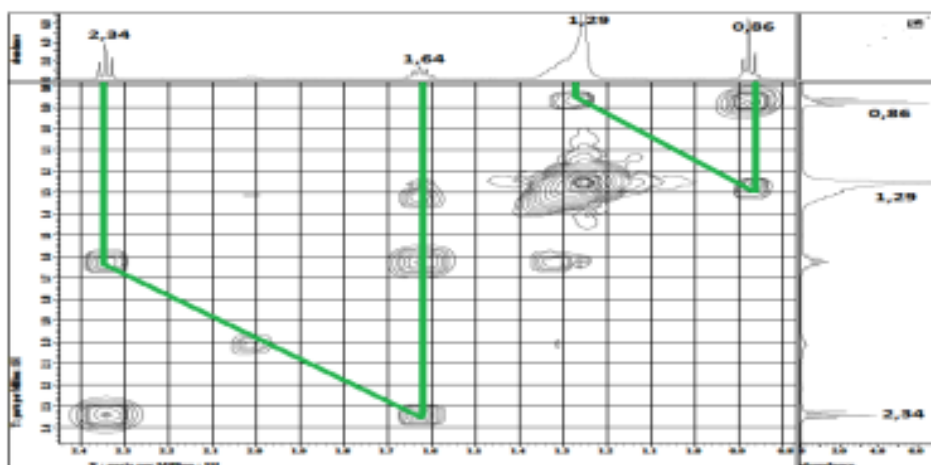


Figure 16 COSY spectrum fragment area  $\delta H$  0.8 ppm - 2.4 ppm  $\delta H$

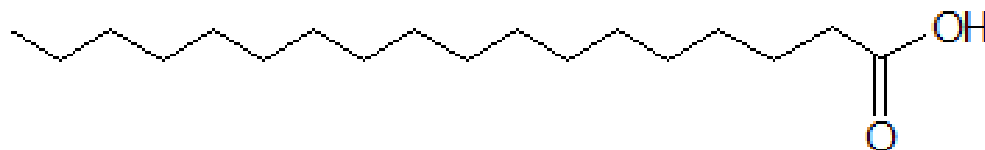


Figure 17 Oleic acid compound identification results

## 2.6 Measurement of antibacterial activity of crude extract

Antibacterial activity test using disc diffusion method using test bacteria *Escherichia coli*, *Shigella dysenteriae*, and *Bacillus subtilis* which extracts tested antibacterial activity with three repetitions. The formation of a clear zone is indicative of antibacterial activity.

The results of antibacterial activity test (Table 1) shows that the crude extract provides activities for the third test bacteria *E. coli*, *S. dysenteriae* and *B. subtilis*. Criteria antibacterial power of inhibition zone diameter 1-9 mm including categories weak inhibition, inhibition zone diameter of 10-14 mm category inhibitory medium and inhibition zone diameter of 15-20 mm strong category (Davis and Stout, 1971). So based on these data it can be concluded that the crude extract n-hexane *H. gracillis* not active antibacterial and ethyl acetate extract weak against bacteria related *B. subtilis* and categories are against *E. coli* and *S. dysenteriae*.

Table 1 Average data of test results crude extract antibacterial activity against *E. coli* bacteria test, *S. dysenteriae*, and *B. Subtillis*

Sampel Ekstrak Kasar	Diameter Zona Bening (mm)		
	<i>E. coli</i>	<i>S. dysenteriae</i>	<i>B. subtilis</i>
n-heksan	0	0	0
Etil asetat	13,40	9,65	8,20
Kontrol (-)	0,0	0,0	0,0

The results of antibacterial activity test (Table 2) showed that the crude extract of *H. gracillis* showed clear zone at a concentration of 200,000 ppm in weak category, while the concentration of 100,000 and 50,000 did not show any antibacterial activity.

Table 2 Data on average test results of activity of varying concentrations crude extract of *H. gracillis* to test bacteria *E. coli*, *S.dysenteriae*, and *B. subtilis*

Sampel uji	Konsentrasi (ppm)	Diameter Zona Bening (mm)		
		<i>E. coli</i>	<i>S. dysenteriae</i>	<i>B.subtilis</i>
Ekstrak Kasar etil asetat	200.000	9,47	11,09	8,84
	100.000	0,0	0,0	0,0
	50.000	0,0	0,0	0
Kontrol (-) Etil asetat	0	0,0	0,0	0,0

### 3 Conclusion

Two of secondary metabolites were isolated from seaweed *Halimeda gracillis* namely  $\beta$ -sitosterol from n-hexane extract and oleic acid from ethyl acetate extract.

The ethyl acetate extract of the active antibacterial is in weak category at a concentration of 200,000 ppm.

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