

## DIVERSITY OF ANTIBACTERIAL COMPOUNDS FROM *EUCHEUMA SERRA*, *HALIMEDA OPUNTIA*, AND *HYDROCLATHRUS CLATHRATUS*

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### Abstract

Fourteen compounds were isolated from acetone extracts of three species of seaweeds (*Eucheuma serra*, a red seaweed, *Halimeda opuntia*, a green seaweed, and *Hydroclathrus clathratus*, a brown seaweed) using bioautographic TLC methods and identified using GC-MS. From *Eucheuma serra* were isolated 8 compounds (3 fatty acids, 3 steroids, and 2 aldehyds). Only two compounds of fatty acid came from *Halimeda opuntia*, whereas *Hydroclathrus clathratus* produced 6 compounds (4 fatty acids, one compound each of steroid and ether). All isolated single compounds were tested for their antibacterial activities by the agar diffusion method against the Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and *Streptococcus faecalis*, and the Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. All 14 compounds showed activity against Gram-positive bacteria, especially *Bacillus subtilis*, and only 2 compounds showed activity against Gram-negative bacteria *Escherichia coli*. Nine compounds showed activity against *Staphylococcus aureus*, and 4 compounds showed activity against *Streptococcus faecalis*. All compounds were not active against *Pseudomonas aeruginosa* and *Salmonella typhimurium* bacteria. This study indicated that there is indeed a diversity both in kinds and in molecular structures of the antibacterial substances.

**Key Words:** seaweed, isolated single compound, antibacterial activity

### 1. INTRODUCTION

Marine organisms including seaweed are important sources of biologically active compounds. Therefore, it is important to examine the pharmacological and antimicrobial potentials of metabolite compounds derived from seaweed<sup>1,2,3)</sup>. Chemical investigations on seaweeds have proven that these organisms produce a wide variety of structurally unique secondary metabolite compounds. Some of them have been shown to possess biological activity of potential medicinal value<sup>1,4,5,6,7,8)</sup>. From

the *Laurencia obtusa*, Kurosawa and his group isolated two new cyclic polyethers with a squalene skeleton, and one of them is teurilene while the other is thyriferyl 23-acetate was remarkably cytotoxic against P<sup>388</sup> cells<sup>9)</sup>. Bhakuni & Silva<sup>10)</sup> and Aubert *et al.*<sup>11)</sup> listed a number of the brominated isolated compounds from *Laurencia* spp. with quite toxic properties, such as laurencin, laureatin, and laurenterol. From numerous Caribbean and Pacific species of *Halimeda* were isolated the highly bioactive diterpenoid

of halimedatrial<sup>12,13</sup>). Acyclic and monocyclic sesquiterpenes and linear diterpenoids were isolated from *Caulerpa* spp., also some sesquiterpenes derived from *Laurencia* spp. of red seaweed was shown a moderate cytotoxic properties<sup>14</sup>).

A total of 18 derivatives of O,O-dialkylthio- and O,O-dialkylthiophosphoric acids were tested for antimicrobial activity using *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Candida albicans*; the activity was found to depend on the length of the alkyl substituent and/or the cation size; all derivatives were ineffective against *E. coli* and *C. albicans*<sup>15</sup>). Some fatty acid compounds isolated from several species of red, green, and brown seaweeds were shown to have antibacterial activity<sup>16,17,18,19,20,21,22</sup>). In a preliminary study, all crude extracts of 21 species of seaweeds collected from Warambadi seashore of Sumba Island were tested against Gram-positive and Gram-negative bacteria. Acetone and methanol extracts of *Eucheuma serra* and acetone extract of *Halimeda opuntia* showed activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. N-hexane extracts of *Halimeda opuntia* were positive against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. Acetone and methanol extracts of *Hydroclathrus clathratus* also indicated activities against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*<sup>23</sup>). In a further study, acetone extracts of *Eucheuma serra*, *Halimeda opuntia*, and *Hydroclathrus clathratus*, were fractionated, then isolated by Column Chromatography and Thin Layer Chromatography (TLC)<sup>24,25,26,27</sup> and the compound isolated were identified by Gas Chromatography-Mass Spectrometry (GC-MS)<sup>28,29</sup>). These compounds belong to fatty acids, steroids, alkaloids, amine, aldehyde, hydrocarbons, and alcohols groups<sup>30</sup>). Since it is of interest to see the bioactivities of these isolated single compounds, they were tested against Gram-positive and Gram-negative bacteria using the bioautographic TLC assay.

## 2. MATERIAL AND METHOD

### 2.1. Material

#### 2.1.1. The fraction samples

The fractions used were ES 1.2, ES 1.2.1, ES 1.2.2, ES 1.6, ES 1.7, ES 1.8, and ES 1.11 from *Eucheuma serra*, HO 2.1.2, HO 2.2, HO 2.3.2, and HO 4.1 from *Halimeda opuntia*, and HC 1.4, HC 2.2, HC 3.1, HC 3.2, and HC 3.3 from *Hydroclathrus clathratus*.

#### 2.1.2. Bacteria for antibacterial testing

The bacteria used for antibacterial activities testing were the Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, and *Streptococcus faecalis* ATCC 29212, whereas the Gram-negative bacteria were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella typhimurium* ATCC 14028.

#### 2.1.3. Chemicals and media

The solvents and media used were dichloromethane (Merck), acetone (Merck), methanol (Merck), ethanol (Merck), ether (Merck), nutrient agar (OXOID), Mueller Hinton Agar (OXOID), McFarland suspension, paper discs Ø 6 mm (Schleicher & Schuell), standard Penicillin-G 10 unit (OXOID), Streptomycin 10 µg (OXOID) discs, silica gel plates Si-60 F<sub>265</sub> (Merck No. 0554), and sephadex LH-20 (Sigma).

#### 2.1.4. Apparatus

Vacuum rotary evaporator (Buchii RE-111), laminar airflow, vortex mixer, oven (Memmert-WG), analytical balance (Mettler), micro pipettes (Socorex), GC-MS (GC Hewlett Packard, HP 6890 Series, Mass selective detector MSD 5972), GC-MS (Shimadzu QP 5050A type).

## 2.2. Method

### 2.2.1. Preparation of samples

The TLC was carried out using TLC plate silica gel Si-60 F<sub>265</sub> (Merck 0554). Standard chromatograms were prepared by applying fractions to a silica gel TLC plate and developing it with ether: methanol (75:25; v/v) under saturated conditions<sup>24,25,26,27</sup>. According to the procedures of the bioautographic TLC assay, the developed TLC plates were placed directly on the agar surfaces and incubated at 35°C for 18 hours. After incubation, plates were taken, the spots which affected the bacterial growth were chosen and the Retention factor (Rf) were measured<sup>27,31</sup>. The same fractions which indicated to have effect to the bacterial growth were passed through TLC with the same method. The spots with the same Rf and affected the bacteria were taken off carefully scrapped from the plates and diluted by acetone. Column chromatography was carried out using a Pharmacia column (Ø 3 cm x 50 cm). To purify the compound solution, the column was filled with the adsorbent Sephadex LH-20 and then developed with acetone. The fractions were then collected and dried to be used for the antibacterial testing and GC-MS analysis<sup>27,28,29,32,33</sup>.

### 2.2.2. Preparation of bacterial suspension

All bacteria were inoculated on nutrient agar slants and incubated at 35°C for 18 hours. Five ml of sterilized distilled water was added and the bacterial culture was scrapped with an inoculating needle. The bacterial suspension was poured into a sterile test tube and homogenized using a Vortex mixer. The McFarland standard was used for turbidity testing. The bacterial suspension was diluted to obtain a concentration of (5 - 7) x 10<sup>6</sup> cells per ml.

### 2.2.3. Evaluation of antibacterial activity by agar diffusion

All compounds were tested *in vitro* using paper discs of 6 mm in Ø each containing 50 µg of the extract. The paper discs containing Penicillin-G and Streptomycin (10 µg concentration of each) were used as positive control while the paper discs containing dichloromethane, methanol, and acetone as negative control.

The amount of 0.5 ml of the bacterial suspension was poured onto the Mueller-Hinton Agar surfaces on Petri dishes, and spread out. The paper discs containing each compound were placed on the agar surfaces and incubated at 35°C for 18 hours. After incubation, the diameter of the clear inhibition zones were measured<sup>34,35,36</sup>.

## 3. RESULT

The results of bioautographic TLC assay showed that there were 16 spots affected the bacterial growth (Figure 1.). The Rf were (0.07), (0.12), (0.2), (0.25), (0.28), (0.32), (0.38), (0.45), (0.50), (0.55), (0.60), (0.67), (0.73), (0.78). Three spots had the same Rf (0.50). So that there were 14 spots which were processed further.

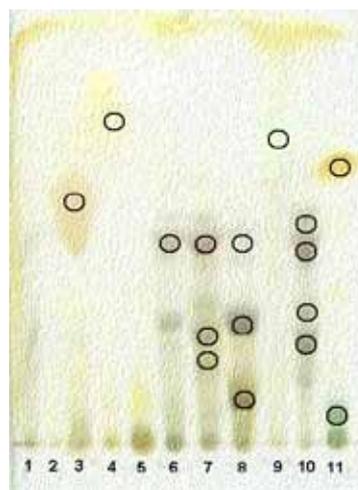


Figure 1. Result of the Bioautographic TLC Assay

The results of the antibacterial testing and compound identification were shown in Table 1., and the list of active compounds per species shown in Table 2. The 14 identified compounds active against bacteria were: **(1)** methyl trans-8-octadecenoate or 8-octadecenoic acid, methyl ester (E); **(2)** 3-hydroxy-nonadeca-5,8,11,14-tetraenoic acid; **(3)** 3-hydroxy-20-methyl heneicosa-5,8,11-trienoic acid; **(4)** dioctyl phthalate or 1,2- benzene dicarboxylic acid, dioctyl ester; **(5)** isodecyl octyl phthalate or 1,2- benzene dicarboxylic acid, isodecyl octyl ester; **(6)** methyl arachidonate or arachidonic acid methyl ester; **(7)** methyl eicosa-5,8,11,14,17-pentaenoate; **(8)** androstan-3-one, 17-(acetyloxy)-, (5 $\alpha$ , 17 $\beta$ ) or dihydrotestosteron acetic acid; **(9)** androstan-11-one, 3-(acetyloxy)-17-iodo-, (17 $\alpha$ ) or 3-acetoxy-17 $\alpha$ -iodo-androstan-11-one; **(10)** pregn-15-en-20-one, 17-methyl-, (5 $\alpha$ -17.xi.); **(11)** cholest-5-en-3 $\beta$ -ol, 24-propylidene; **(12)** 9-[3-(1-hydroxy-butyl)-oxiranyl]-nonanal; **(13)** 11-[3-(1-hydroxy-butyl)-oxiranyl]-undecanal; **(14)** 3,7,11-trimethyl-1-(3-methyl-butoxy)-dodec-2-en (Figure 2. up to 15.).

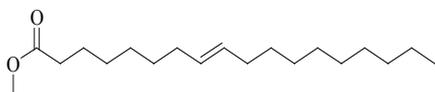


Figure 2. Compound (1), Methyl trans-8-octadecenoate or 8-Octadecenoic acid, methyl ester, (E)

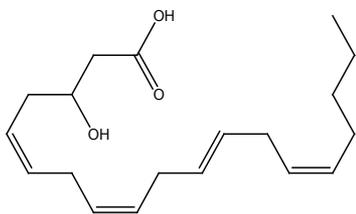


Figure 3. Compound (2), 3-hydroxy-nonadeca-5,8,11,14-tetraenoic acid

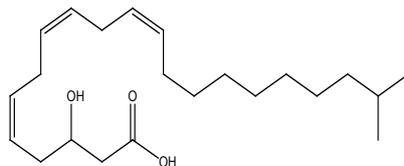


Figure 4. Compound (3), 3-hydroxy-20-methyl heneicosa-5,8,11-trienoic Acid

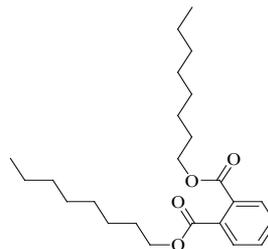


Figure 5. Compound (4), Dioctyl phthalate or 1,2- Benzenedicarboxylic acid, dioctyl ester

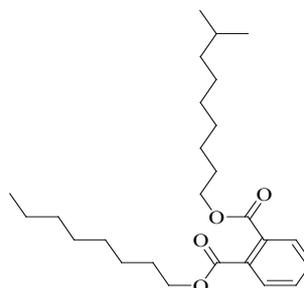


Figure 6. Compound (5), Isodecyl octyl phthalate or 1,2- Benzenedicarboxylic acid, isodecyl octyl ester

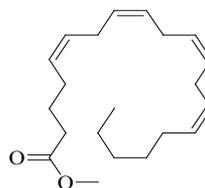


Figure 7. Compound (6), Methyl arachidonate or Arachidonic acid methyl ester

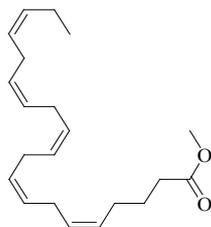


Figure 8. Compound (7), Methyl eicosa-5,8,11,14,17-pentaenoate

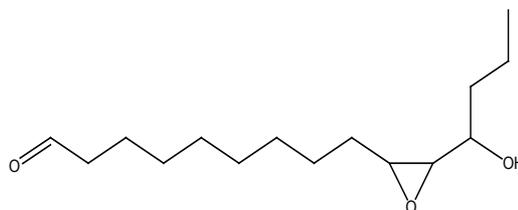


Figure 13. Compound (12), 9-[3-(1-hydroxybutyl)-oxiranyl]-nonanal

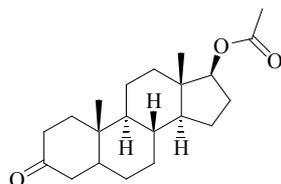


Figure 9. Compound (8), Androstan-3-one, 17-(acetyloxy)-, (5 $\alpha$ , 17 $\beta$ ) or Dihydrotestosteron acetic acid

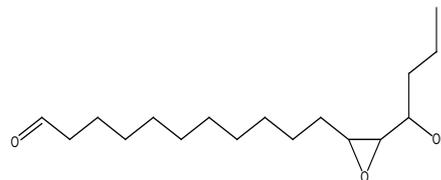


Figure 14. Compound (13), 11-[3-(1-hydroxybutyl)-oxiranyl]-undecanal

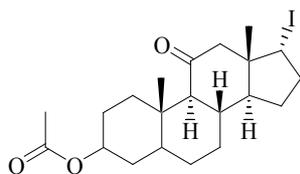


Figure 10. Compound (9), Androstan-11-one, 3-(acetyloxy)-17-iodo-, (17 $\alpha$ ) or 3-Acetoxy-17 $\alpha$ -iodo-androstan-11-one

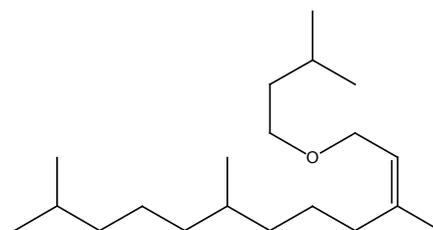


Figure 15. Compound (14), 3,7,11-trimethyl-1-(3-methyl-butoxy)-dodec-2-en

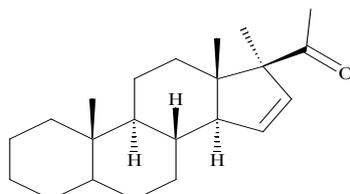


Figure 11. Compound (10), Pregn-15-en-20-one, 17-methyl

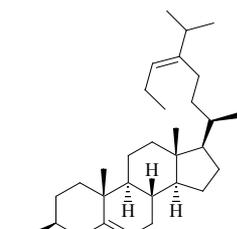


Figure 12. Compound (11), Cholest-5-en-3 $\beta$ -ol, 24-propylidene

The compounds (1) up to (7) are of the fatty acid group, compounds (8) up to (11) are of the steroid group, compounds (12) and (13) are of the aldehyde group, and compound (14) of the ether group. All of the 14 compounds showed activity against Gram-positive bacteria, especially *Bacillus subtilis* (100% of the identified compounds). Only 2 out of 14 compounds (14,3 %) were active against Gram-negative bacteria of *Escherichia coli*. All compounds were not active against *Pseudomonas aeruginosa* and *Salmonella typhimurium*. Nine out of the 14 compounds (64.3 %) were active against *Staphylococcus aureus*, and 4 out of the 14 compounds (28.6 %) were active against *Streptococcus faecalis*.

The 8 identified compounds from *Eucheuma serra* which were active against

bacteria are 3 fatty acids, 3 steroids, and 2 aldehydes. Only two compounds, all fatty acids, from *Halimeda opuntia* were active against bacteria. Six compounds from *Hydroclathrus clathratus* which were active against bacteria are 4 fatty acids, one compound a steroid and another one an ether.

#### 4. DISCUSSION

The performance of antibacterial activities as indicated by inhibition zone in this study was substantially lower than the data reported in a previous study which used whole extracts. These seem to indicate that a synergism effect occurred between compounds in the whole extract sample compared to the single compound.

Most of the identified compounds showed an inhibition activity against both Gram-positive as well as Gram-negative bacteria. Almost all identified compounds had a greater effect against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* (Table 1.). It is important to distinguish between the agents which are active against Gram-positive bacteria only and those which are also active against Gram-negative bacteria. Generally, many compounds have antibacterial capability against Gram-positive bacteria, but lesser number was capable of attacking Gram-negative bacteria<sup>37)</sup>. The Gram-negative bacteria have outer membrane which protected bacteria cells from toxic agents<sup>34,38)</sup>. The outer membrane and the quite narrow of porin channels in the membrane have added protection to the Gram-negative bacteria. For this reason, the diffusion of water-soluble antibacterial agents larger than 700 Daltons through the outer membrane of Gram-negative bacteria is severely limited<sup>34,37,38,39)</sup>. Furthermore, the lipid bi-layer region of the Gram-negative bacteria's outer membrane usually has a low permeability toward lipophilic molecules. Consequently, the larger and more lipophilic antibiotics tend to have significant activity

against Gram-positive bacteria only<sup>37,40)</sup>.

There were seven identified compounds of fatty acid group and four compounds of steroid group were active against bacteria, as well as two other compounds of the aldehyde group and one compound of ether also showed active against bacteria (Table 1.). The fatty acids, e.g. oleic acid and arachidonic acid (eicosatetra-5,8,11,14-enoic acid) are also found in cell membranes, but in addition they act as precursors of biologically active metabolites, for example, oleic acid is the biosynthetic precursor of polyacetylenes like ceprenynic acid<sup>41)</sup>. Arachidonic acid is the biosynthetic precursor of a large family of metabolites known as eicosanoids, which include the pharmacologically important prostaglandins and leukotrienes<sup>41)</sup>. The biosynthesis of fatty acids is under the control of enzyme called fatty acid synthases, and the main building block of fatty acid biosynthesis is acetate in the form of acetyl-S-Coenzyme-A or acetyl-S-CoA<sup>42)</sup>. Acetyl-S-Coenzyme A is also the main building block of the terpenoids and steroids. All organisms contain a discrete cell nucleus (eukaryotes) synthesize steroids, or have the capacity to affect a cyclization of squalene to form steroid-like molecules, or have an absolute requirement for them in their diet. Other steroids are metabolized to produce biologically active molecules like the human hormones and vitamins D<sup>41,43)</sup>. Some marine invertebrates share fatty acid profiles which are unique in their process of biosynthesizing high amount of polyunsaturated fatty acids with a variety number and position of unsaturated group. Some of marine fatty acids, such as (5Z,9Z)-14-methyl-5,9-pentadecadienoic acid, are antibacterial against Gram-positive bacteria *Streptococcus* and *Staphylococcus*<sup>44)</sup>.

More active compounds were found in *Eucheuma serra* if compared to *Halimeda opuntia* and *Hydroclathrus clathratus*. Two interesting active compounds found in this study are worth noting. There are methyl *trans*-8-octadecenoate or 8-Octadecenoic

acid, methyl ester (E) (1), a fatty acid in trans formation from *Hydroclathrus clathratus*, and androstan-11-one, 3-(acetyloxy)-17-iodo-, (17 $\alpha$ ) or 3-Acetoxy-17 $\alpha$  -iodo-androstan-11-one (9), a steroid containing iodine from *Eucheuma serra*. Both compounds were active against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus faecalis*) and Gram-negative bacteria (*Escherichia coli*), especially the compound (9) was active against all the Gram-positive bacteria (Table 1.).

Robles *et al.*,<sup>45)</sup> suggested that antibiosis in algae is extremely complex and involves numerous different activities which manifest themselves differently with respect to habitat, season and life history stages. It means that the difference in quantities and qualities of seaweed's active secondary metabolite products were synthesized under different conditions, such as seasons, geographic location, habitat factors and genetics which affect the spectrum of biologically active secondary metabolites produced. The different results indicated the possibility that genera, species, and their physiological state or life stages, affected the capability of the seaweed to synthesize different quantities and qualities of bioactive secondary metabolites .

The active compounds were presented in Table 2., displayed fatty acid, steroid, aldehyde, and ether groups, respectively. The antibacterial compounds of fatty acids are unsaturated groups. Based on the molecular structures of active compound, it could be suggested that the number and position of unsaturated in fatty acids are the key of its antibacterial activity. As in 3-hydroxy-20-methyl heneicosa-5,8,11-trienoic acid (Figure 4.) which has three double bond showed more active than its analog of 3-hydroxy-20 methyl heneicosa-5,8-dienoic acid. Moreover, the number of unsaturated was also supposed as the factor of the activity of methyl eicosa-5,8,11,4,17-pentaenoate (Figure 8.). Active compound was also showed by phthalate derivatives such as dioctyl phthalate (Figure

5.) and isodecyl octyl phthalate (Figure 6.). But the other phthalate derivatives, namely, 1,2-benzendicarboxylic acid, bis (2-ethylexyl) ester was not active, because of the presence of 2 ethyl substituents which caused steric hindrance at carbonyl ester groups.

The active compounds of aldehyde group were also obtained. The activity factor of this group may be caused by the presence of epoxy group as in 9-[3-(1-hydroxy-butyl)-oxiranyl]-nonanal and 11-[3-(1-hydroxy-butyl)-oxiranyl]-undecanal respectively (Figure 13. & 14.). The other active compounds were steroid and ether groups. The activity of steroids could be caused by ketone group in the steroid ring and side chain of acetyloxy group, as well as the presence of halogen (iodine) substituent. Moreover, the ether compound was also active as showed by 3,7,11-trimethyl-1-(3-methyl-butoxy)-dodec-2-en (Figure 15.).

## 5. CONCLUSION

The results of this study indicated that three seaweed species (*Eucheuma serra*, *Halimeda opuntia*, and *Hydroclathrus clathratus*) which were collected from Warambadi seashore of Sumba Island, showed a significant capacity to produce a variety of compounds with antibacterial activities.

The 14 compounds with an active antibacterial activity, consisted of 8 compounds from *Eucheuma serra* (3 fatty acids, 3 steroids, 2 aldehyds), two fatty acids compounds from *Halimeda opuntia*, and six compounds from *Hydroclathrus clathratus* (4 fatty acids, and one compound each of the steroid and ether group). All compounds were active against Gram-positive bacteria especially *Bacillus subtilis* and only 2 compounds showed active against the Gram-negative *Escherichia coli*. All compounds were not active against *Pseudomonas aeruginosa* and *Salmonella typhimurium*.

In term of antibacterial activity, two interested active compounds are methyl *trans*-8-octadecenoate or 8-octadecenoic

acid, methyl ester (E) (a fatty acid in *trans* formation from *Hydroclathrus clathratus*), and androstan-11-one, 3-(acetyloxy)-17-iodo-, (17 $\alpha$ ) or 3-acetoxy-17 $\alpha$  -iodo-androstan-11-one (a steroid contain iodium from *Eucauma serra*), both of compounds were active against Gram-positive and Gram-negative bacteria.

The molecular structures produced showed that the fatty acids active compounds are of the unsaturated group. It is likely that the number and position of unsaturated in fatty acids are important factors of its antibacterial activity. Two of four compounds of aldehydes were active substances, and in this case may be caused by the presence of epoxy group in the molecular structures, whereas, the active compounds of steroids could be caused by the presence of ketone group in the steroid ring and acetyloxy group in the side of chain, as well as the presence of iodine substituent.

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Table 1. Antibacterial activity of identified compounds

No	Compound	S.aureus	B.subtilis	S.faecalis	E.coli	P.aeruginosa	S.typhimurium
Fatty Acids							
1	Methyl trans-8-octadecenoate or 8-Octadecenoic acid, methyl ester, (E)	6.8	7.4	-	6.5	-	-
2	3-Hydroxy-nonadeca-5,8,11,14-tetraenoic acid	6.5	7.0	-	-	-	-
3	3-Hydroxy-20-methyl heneicosa-5,8,11-trienoic acid	6.8	6.8	-	-	-	-
4	Diocetyl phthalate or 1,2-Benzenedicarboxylic acid, dioctyl ester	6.7	7.4	-	-	-	-
5	Isodecyl octyl phthalate or 1,2- Benzene dicarboxylic acid, isodecyl octyl ester	7.0	7.5	6.5	-	-	-
6	Methyl arachidonate or Arachidonic acid methyl ester	6.5	6.8	-	-	-	-
7	Methyl eicosa-5,8,11,14,17-pentaenoate	-	6.5	-	-	-	-
Steroids							
8	Androstan-3-one, 17-(acetyloxy)-, (5 $\alpha$ , 17 $\beta$ ) or Dihydrotestosteron acetic acid	-	6.6	-	-	-	-
9	Androstan-11-one, 3-(acetyloxy)-17-iodo-, (17 $\alpha$ ) or 3-Acetoxy-17 $\alpha$ -iodo-androstan-11-one	7.0	7.8	7.0	6.5	-	-
10	Pregn-15-en-20-one, 17-methyl	-	6.8	6.5	-	-	-
11	Cholest-5-en-3 $\beta$ -ol, 24-propylidene	7.0	7.0	-	-	-	-
Aldehydes							
12	9-[3-(1-hydroxy-butyl)-oxiranyl]-nonanal	-	6.7	-	-	-	-
13	11-[3-(1-hydroxy-butyl)-oxiranyl]-undecanal	-	6.7	-	-	-	-
Ether							
14	3,7,11-Trimethyl-1-(3-methyl-butoxy)-dodec-2-en	6.7	7.0	6.5	-	-	-
P-10 = Penicillin-G (Conc. 10 $\mu$ g)		8.8	8.3	8.0	7.8	-	8.0
S-10 = Streptomycin (Conc. 10 $\mu$ g)		18.5	20.5	18.0	2.0	10.5	11.0
C (Acetone) = control		-	-	-	-	-	-

*Gram-positive bacteria* : *Staphylococcus aureus* ATCC 25923; *Bacillus subtilis* ATCC 6633; *Streptococcus faecalis* ATCC 29212; *Gram-negative bacteria* : *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853; *Salmonella typhimurium* ATCC 14028

Table 2. List of the active compounds of *Eucheuma serra*, *Halimeda opuntia*, and *Hydroclathrus clathratus*

No.	Compound
<b><i>Eucheuma serra</i></b>	
	<b>Fatty Acids</b>
1	3-Hydroxy-nonadeca-5,8,11,14-tetraenoic acid
2	3-Hydroxy-20-methyl heneicosa-5,8,11-trienoic acid
3	Diethyl phthalate or 1,2- Benzenedicarboxylic acid, diethyl ester
	<b>Steroids</b>
4	Androstan-3-one, 17-(acetyloxy)-, (5 $\alpha$ , 17 $\beta$ ) or Dihydrotestosteron acetic acid
5	Androstan-11-one, 3-(acetyloxy)-17-iodo-, (17 $\alpha$ ) or 3-Acetoxy-17 $\alpha$ -iodo-androstan-11-one
6	Pregn-15-en-20-one, 17-methyl
	<b>Aldehydes</b>
7	9-[3-(1-hydroxy-butyl)-oxiranyl]-nonanal
8	11-[3-(1-hydroxy-butyl)-oxiranyl]-undecanal
<b><i>Halimeda opuntia</i></b>	
	<b>Fatty acids</b>
1	Diethyl phthalate or 1,2- Benzenedicarboxylic acid, diethyl ester
2	Isodecyl octyl phthalate or 1,2- Benzene dicarboxylic acid, isodecyl octyl ester
<b><i>Hydroclathrus clathratus</i></b>	
	<b>Fatty acids</b>
1	Methyl trans-8-octadecenoate or 8-Octadecenoic acid, methyl ester, (E)
2	Methyl arachidonate or Arachidonic acid methyl ester
3	Methyl eicosa-5,8,11,14,17-pentaenoate
4	Diethyl phthalate or 1,2- Benzenedicarboxylic acid, diethyl ester
	<b>Steroids</b>
5	Cholest-5-en-3 $\beta$ -ol, 24-propylidene
	<b>Ether</b>
6	3,7,11-Trimethyl-1-(3-methyl-butoxy)-dodec-2-en