Preface

PREFACE

On behalf of the Organizing Committee, I would like to extend our warmest regards to all participants of the International Conference on Tropical and Coastal Region Eco-Development (ICTCRED) 2019. This annual conference is the fifth event at Semarang, Central Java, Indonesia that is organized by the Faculty of Fisheries, Universitas Diponegoro. This year we brought an essential global topic the ***Integrated Coastal Zone Management for Sustainable Development.*** The conference aims to provide a forum to exchange ideas and their current achievements for researchers, academicians, professionals, and industries to expose and exchange innovative ideas, methods, and experiences in the areas related to tropical life sciences and coastal development.

We have accepted 156 abstracts for oral and poster presentations coming from different universities and research centers from many countries, which were consisted of 13 big interests. Besides, we have cordially invited five highly respected researchers as keynote speakers with different fields to share their knowledge and expertise. I am grateful for each one of them for setting aside their valuable time to participate in this conference.

The committee extent very kind thank all participants for the success of the conference. They were Rector of Universitas Diponegoro, Dean of Faculty of Fisheries and Marine Science, the keynote speakers. I also would like to acknowledge the Institute of Physics (IOP) for the collaboration in publishing the conference proceedings, our sponsors the Bionesia**,** Faculty of Law, Universitas Diponegoro, COREM Undip, and Deltares.

Finally, we proudly present some selected papers in IOP Conference Series: Earth and Environmental Science. I do hope that the 5th ICTCRED 2019 event brings a fruitful knowledge and be a memorable event not only from the scientific perspective but also in the joy of meeting with other scientists for mutual collaboration.

Guest Editor

Agus Trianto
Chair of Scientific Committee

Scientific Committee/Editor

ORGANIZING COMMITTEE

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In vitro antibacterial study and spectral analysis of brown seaweed *Sargassum crassifolium* extract from Karimunjawa Islands, Jepara

In vitro antibacterial study and spectral analysis of brown seaweed *Sargassum crassifolium* extract from Karimunjawa Islands, Jepara

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Abstract. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are opportunistic pathogenic bacteria that are the main causes of nosocomial infections. These bacteria can infect almost every tissue of the body and there were 15% cases of infection in hospitals. Urinary tract infections, even sepsis, where the death rate reaches 50%. Inappropriate use of antibiotics raises resistance. About 10% of bacterial isolates are generally expressed as *S. aureus* and *P. aeruginosa* Multidrug Resistant (MDR). Sargassum brown seaweed has many potential antimicrobial compounds. This research aims to screen the antibacterial active compounds of *Sargassum crassifolium* seaweed against *S. aureus* and *P. aeruginosa* MDR bacteria. *S. crassifolium* collected from waters of Karimunjawa Islands, Jepara. The research was conducted by laboratory experimental methods. Sample was extracted with diethyl ether, methanol, ethanol and chloroform. The MIC value is done by measuring the diameter of the inhibitory zone in the antibacterial activity test of the agar diffusion method. Furthermore, extracts at MIC concentrations were tested for the antibacterial activity of the diluted method by measuring bacterial OD by spectrophotometric methods. The extract with the best antibacterial activity was performed spectral analysis by GC-MS method. The results showed that the different extracts had different MIC values ($p < 0.05$). Extracts with high antibacterial activity are extracts from diethyl ether solvent. The extract has a MICP value of *P. aeruginosa* 12.7 mg/ml and *S. aureus* 8.4 mg/ml. *P. aeruginosa* has exponential growth at 12 hours and death at 44 hours. While exponential *S. aureus* was at 16 hours and death at 36 hours. Spectral analysis of *S. crassifolium* extract of diethyl ether solvent showed the composition of the presence of eicosane compounds (16.22%), dotriacontane (11.27%), nanocosane (11.09%), dicosane (9.85%), 10.13-octadeadienoic acid (9.52%). 2-butyloctanol (6.33%), pentatriacontane (5.4%), tritriacontane (5.07%), tricosane (1.6%)

1. Introduction

Antibiotics are a group of drugs used to treat and prevent bacterial infections. Infectious diseases can be treated by the use of antibiotics that are rational, appropriate, and safe. But lately, the high rate of infection is caused by bacteria that have been resistant to antibiotics. Antibiotic resistant bacterial infections will endanger the lives of patients because the infection becomes difficult to treat. Bacteria that are often found resistant at the hospital level include *S. aureus* and *P. aeruginosa* Multidrug Resistant (MDR) [1].

S. aureus and *P. aeruginosa* MDR infections are difficult to eradicate because these bacteria have high intrinsic resistance and are resistant to several different antibiotics. The prevalence of *S. aureus* and *P. aeruginosa* resistance to various types of antibiotics continues to increase. The results showed that *S. aureus* isolates were

resistant to tetracycline antibiotics (64.8%), erythromycin (53.7%), and cloxacillin (40.7%). *P. aeruginosa* is reported to have resistance to several types of antibiotics, such as imipenem (20.8%), cephalosporins such as cefotaxime (90%) and ceftriaxone (85%), aminoglycosides such as tobramisin (70.07%) and gentamicin (71.89%), fluoroquinolones such as ciprofloxacin (35%) and levofloxacin (32%) [2][3].
Resistance to various antibiotic agents has a significant negative impact of an increase in therapy cost to the risk of complications. Resistant bacterial infection has been classified as a very high cause of death in hospitals [4]. This research aims to look for compounds that have antibacterial activity. One source of natural active compounds is from *S. crassifolium* brown seaweed [5][6]. Karimunjawa Islands, Jepara coast have a high abundance of *S. crassifolium* and have the potential to be explored in the field of marine pharmacy. This research is expected to get extracts with MDR antibacterial active compounds.

2. Methodology

2.1. Seaweed sampling.

Sampling was conducted in Karimunjawa Islands, Jepara coast together with local fishermen using a boat and collected by snorkeling. Samples were taken and put in a cool box. Then the seaweed is brought to the laboratory for identification and extraction.

2.2. Seaweed extraction.

Sample preparation was done by washed the seaweed with the fresh-water flow, cut, dried and blended [6]. 500 grams of *S. crassifolium* crude powder soaked in 1.5 liters of solvent for 24 hours in darkroom conditions [7]. Seaweed maceration used 4 different solvents: diethyl ether, methanol, ethanol, and chloroform. The filtration was done by using the Whatman paper filter. The filtrate of each solvent was evaporated using a vacuum rotary evaporator at a temperature of 40°C and a pressure of 500 mbHg. The extract then dried with Freeze Drying and stored in a freezer at -4°C [8].

2.3. Antibacterial Activity Test.

Zobell media 2216e agar is used for purification of *P. aeruginosa* and *S. aureus*. Test of bacterial culture that the OD (Optical Density) has been measured between 0.6-0.8 [9] at a wavelength of 600 nm, 0.1 ml was pipetted and inoculated to the surface of the petri media by spread technique. Afterward, it was incubated for 30 minutes to get diffused. Antibacterial activity test used the extract solutions with concentrations of 100, 75, 50 and 25 ppm. Each extract solution was dropped onto a paper disk of 20 µl [8]. Hereafter, it is stored in an incubator at 37ºC for 24-48 hours.

2.4. MIC and MBC measurement.

Measurement of MIC and MBC were done by the same methods as antibacterial activity tests. The difference is in the extract concentration being tested. There are 3 stages of concentration, stage 1 at concentrations 24, 23, 22, 21, 20, 19, 18, 17 and 16 ppm. Stage 2 at concentrations of 15, 14, 13, 12, 11, 10, 9, 8, 7 and 6 ppm. Stage 3 at concentrations of 5, 4, 3, 2 and 1 ppm. Furthermore, each extract solution was dropped onto a paper disk of 20 µl [8]. Furthermore, it is stored in an incubator at 37ºC for 24-48 hours.

2.5. Analysis of growth kinetics.

Growth measurement aims to determine the growth character of pathogenic bacterial isolated in conditions without exposure and exposed to *S. crassifolium* extract of the solvent diethyl ether at ½ MIC, 1 MIC and 2 times MIC. The culture was using a 2-liter scale fermenter with volume of 1 liter. The conditions of the fermenter are Zobell 2216

E broth media, 1% inoculum concentration with OD 0.01 at A600, pH 8, temperature 35°C, and agitation speed of 150 rpm. Observations were made on the bacterial optical density (OD) values at incubation times of 0, 2, 4, 6, 12, 18, 24, 30, 36, 42, and 48 hours [10].

2.6. GC-MS analysis.

S. *crassifolium* extract of the diethyl ether solvent with the best antibacterial activity then performed by the GC-MS analysis. GC-MS consists of two main component blocks: gas chromatography and mass spectrometer. The GC-MS process is carried out with an active fraction of KCV results, using the GC-MS tool Shimadzu QP2010S type with the method of [11]. This analysis will obtain information about the fraction constituent compounds that are volatile [12].

2.7. Data analysis.

Data on inhibition zone diameter and bacteriocidal zone are presented using histogram graphs, meanwhile the antibacterial activity data, MIC, MBC, OD bacterial cell growth and GC-MS analysis are presented using data tabulation. Data on antibacterial activity, MIC, MBC, and OD bacterial cell growth were further tested for homogeneity, normality and additivity with a sig value of 0.050. If the data is homogeneous, normal and additive, then the data will performed one way ANOVA test with a sig value of 0.050 using the SPSS program version 16.0. If there is an influence between the treatment of the response then the Tukey test is then performed with a sig value of 0.050. OD data on bacterial cell growth was carried out polynomial analysis to determine growth trendsbacteria on the conditions of *Sargassum crassifolium* extract at levels of ½ MIC, 1 MIC and 2 times MIC.

3. Results

3.1. Antibacterial activity test.

Sargassum crassifolium seaweed obtained from Karimunjawa Jepara was extracted with the solvents of diethyl ether, methanol, ethanol and chloroform. Each extract was tested for antibacterial activity against pathogenic bacteria *P. aeruginosa* and *S. aureus*. The antibacterial activity test using a concentration difference treatment (100, 75, 50 and 25 µg/disk). The results of the antibacterial activity test are presented in Table 1.

Table 1. Antibacterial activity of the extract against MDR pathogenic bacteria.

Solvents	Concent-ration (µg/disk)	*P. aeruginosa*	*S. aureus*
Diethyl ether	100	18.32 ± 0.40^{b}	21.70 ± 1.77^{b}
	75	18.86 ± 1.15^{ab}	21.91 ± 1.75^{ab}
	50	17.55 ± 1.21^{ab}	20.54 ± 1.15^{ab}
	25	15.61 ± 1.25^{b}	19.75 ± 1.03^{b}
Methanol	100	16.79 ± 0.83^{b}	20.28 ± 0.49^{b}
	75	15.46 ± 1.01^{ab}	19.46 ± 0.77^{ab}
	50	15.33 ± 0.34^{ab}	17.32 ± 0.29^{a}
	25	14.38 ± 0.64^{a}	17.61 ± 0.55^{a}
Ethanol	100	8.41 ± 0.09^{b}	20.79 ± 0.82^{b}
	75	8.72 ± 0.15^{ab}	18.03 ± 1.02^{ab}
	50	7.14 ± 0.16^{a}	15.45 ± 0.35^{a}
	25	7.41 ± 0.08^{ab}	12.71 ± 0.45^{a}

Chloro-form	100	9.62 ± 0.18^{b}	16.62 ± 0.52^{b}
	75	9.74 ± 0.25^{ab}	13.35 ± 0.22^{ab}
	50	8.11 ± 0.06^{a}	12.03 ± 0.04^{ab}
	25	7.39 ± 0.09^{a}	10.44 ± 0.23^{a}

Note: the value is the average ± standard deviation, the super script letters behind different numbers in one column show significantly different from each other ($p < 0.050$), the super script letters from a to z indicate having a greater average value.

Based on the results of the research, Table 1 shows that the different treatment of extract solvents and extract concentrations gave significantly different inhibitory zone sizes ($p < 0.05$). Besides the pathogenic bacteria *P. aeruginosa* and *S. aureus* showed different responses to the extract. In general, Table 1 shows that higher concentration has higher diameter of the inhibition zone.

3.2. MIC and MBC values.

Based on the results of the antibacterial activity test of the different solvent extracts in Table 1, the research carried out further tests to determine the MIC and MBC values. Further tests were carried out at a lower concentration level. As for the results of the MIC and MBC determination test on pathogenic *P. aeruginosa* bacteria are presented in Table 2.

Inhibition zone diameters presented in Table 2 are zones with bacteriostatic activity. Meanwhile the bacteriocidal zone is a clear zone du to the mortality of bacteria. Table 2 show that the treatment of different extracts gave significantly different values of inhibition zone and bacteriocidal zones to *P. aeruginosa* bacteria.

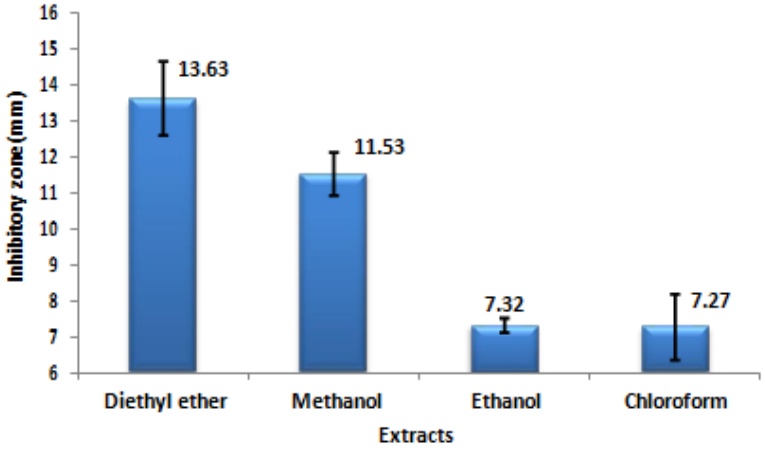

Figure 1. Diameter of inhibitory zone of extract against *P. aeruginosa* bacteria.

The research shows that (Figure 1) *S. crassifolium* extract of the diethyl ether has the largest inhibitory zone diameter against the *P. aeruginosa* bacteria, compared to extract with methanol, ethanol and chloroform. Ethanol and shloroform extracts have inhibitory zone diameters that are not significantly different ($p > 0.05$), but both extracts have different inhibition zones to the methanol and diethyl ether ($p < 0.05$). *S. crassifolium* extract of diethyl ether has the best bacteriostatic activity against *P. aeruginosa* bacteria compared to the other extracts because at small concentrations it is able to inhibit the *P. aeruginosa* bacteria.

Table 2. MIC and MBC extract values for *P. aeruginosa* bacteria.

Solvent	IZ (mm)	MIC (ppm)	BZ (mm)	MBC (ppm)	MIC / MBC
Diethyl ether	13.63 ± 1.03^{c}	4	$13.61 \pm 0{,}98^{b}$	7	0.57
Methanol	11.53 ± 0.60^{b}	8	$10.63 \pm 0{,}71^{ab}$	12	0.67
Ethanol	7.32 ± 0.20^{a}	19	$10.04 \pm 0{,}78^{ab}$	23	0.83
Chloroform	7.27 ± 0.92^{a}	20	$8.94 \pm 1{,}18^{a}$	24	0.83

Note: the value is the average ± standard deviation, the super script letters behind different numbers in one column show significantly different from each other (p <0.050), the super script letters from a to z indicate having a greater average value.

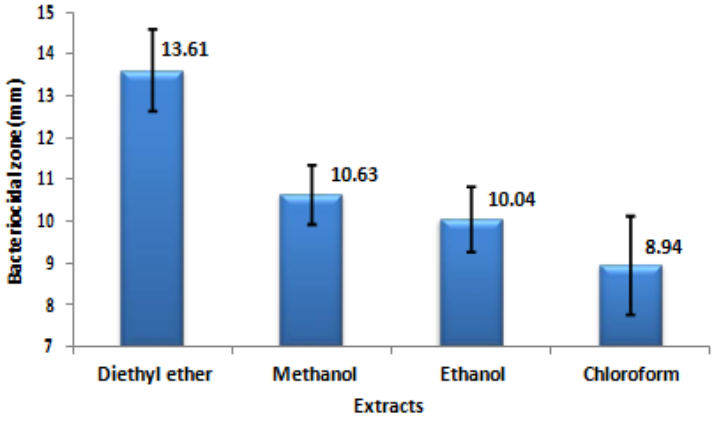

Figure 2. Diameter of bacteriocidal zone of extract against P aeruginosa bacteria.

Table 3 showed that the treatment of different extracts had significantly different inhibitory zone values for the pathogenic *S. aureus* bacteria (p <0.05). In addition, the difference in extracts has a significantly different bacteriocidal zone value for the pathogenic *S. aureus* (p <0.05). Different extracts have different MIC and MBC values, high to low MIC and MBC values are chloroform, ethanol, methanol and diethyl ether extracts.

Table 3. MIC and MBC extract values for *S.aureus* bacteria.

Solvent	IZ (mm)	MIC (ppm)	BZ (mm)	MBC (ppm)	MIC / MBC
Diethyl ether	16.62 ± 0.26^{c}	5	17.39 ± 0.85^{b}	9	0.56
Methanol	14.34 ± 0.65^{b}	8	17.07 ± 1.72^{b}	15	0.53
Ethanol	9.55 ± 0.21^{a}	15	12.74 ± 0.40^{a}	17	0.88
Chloroform	10.12 ± 0.70^{a}	18	13.03 ± 0.73^{a}	22	0.82

Note: the value is the average ± standard deviation, the super script letters behind different numbers in one column show significantly different from each other (p <0.050), the super script letters from a to z indicate having a greater average value.

Extract differences had significantly different bacteriocidal zone values (p <0.05). *S. crassifolium* extract with chloroform had the lowest bacteriocidal zone but did not differ significantly (p> 0.05) against methanol and ethanol extracts, but the bacteriocidal zone of the chloroform extract was significantly different (p <0.05) from the diethyl ether. *S. crassifolium* extract of diethyl ether has the largest diameter of the bacteriocidal zone (figure 2). The extract has high antibacterial activity because at the lowest concentration, it is able to kill the *P. aeruginosa*

bacteria compared to the other extracts. MIC and MBC determination test was also carried out on *S. aureus* pathogenic bacteria. As for the results of MIC and MBC values in the pathogenic bacteria *S. aureus* are presented in Table 3.

Figure 3 shows that it has inhibitory zone values for different *S. aureus* bacteria ($p < 0.05$). The extract with the lowest inhibition zone was chloroform extract and did not differ significantly ($p > 0.05$) against ethanol. however, the two extracts were significantly different ($p < 0.05$) against the extracts of diethyl ether and methanol.

The results of the study in figure 4 show that the treatment of different extracts gave a significantly different diameter of the bacteriocidal zone of the *S. aureus* bacteria ($p < 0.05$). The diameter of the bacteriocidal zone of ethanol and chloroform extracts did not differ significantly ($p > 0.05$) as well as diethyl ether and methanol extracts had no bactericidal zone that was not significantly different ($p > 0.05$). However, the two groups differed markedly ($p < 0.05$). Based on the bacteriocidal zone values indicate that diethyl ether was the best solvent to extract *S. crassifolium*.

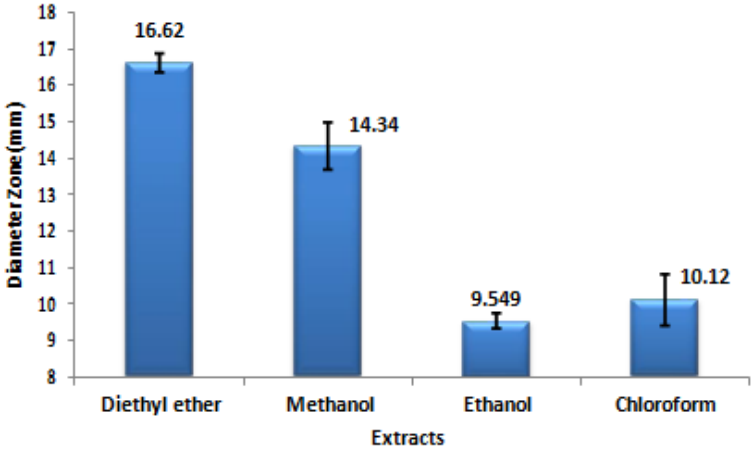

Figure 3. Diameter of inhibitory zone of the extract against S aureus bacteria.

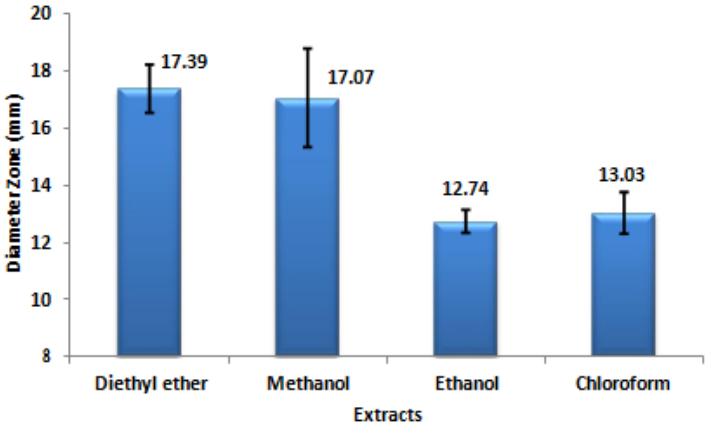

Figure 4. Diameter of bacteriocidal zone of extracts against S aureus bacteria.

3.3. MIC and MBC values.

The results of OD measurements of *P. aeruginosa* bacteria are presented in table 4. Table 4 shows that the treatment of different extract concentration exposures significantly influences the OD value of Pseudomonas aeruginosa bacterial growth ($p < 0.05$). OD values at each observation time between treatments showed significantly different

(p <0.05). Results of the OD spectrophotometer were then carried out by trend analysis on the growth kinetics of the *P. aeruginosa* bacteria shown in Figure 5.

Based on Figure 6 shows that the difference in extract exposure concentration affects the growth kinetics of *S. aureus*. The kinetics of bacterial growth without exposure to extracts (negative control) have normal growth. The control bacterial growth pattern consisted of the lag phase, exponential phase, stationary phase and mortality as well as in plants exposed to half MIC concentration extracts. Whereas in treatment with exposure to extract according to MIC and twice the MIC occurred growth pressured. The growth phase does not look well, due to both treatments inhibit growth and even kill bacteria.

Table 4. Growth density of *P. aeruginosa* bacteria exposed to *S. crassifolium* extract of solvent diethyl ether.

Observation (Dayth)	*Pseudomonas aeruginosa*			
	K	1/2*MIC	MIC	2*MIC
0	0.118 ± 0.0150^{a}	0.112 ± 0.0150^{a}	0.116 ± 0.0150^{a}	0.112 ± 0.0150^{a}
2	0.155 ± 0.0073^{b}	0.148 ± 0.0060^{b}	0.146 ± 0.0060^{b}	0.113 ± 0.0058^{a}
4	0.218 ± 0.0035^{d}	0.181 ± 0.0169^{c}	0.147 ± 0.0065^{b}	0.117 ± 0.0015^{a}
6	0.323 ± 0.0221^{d}	0.193 ± 0.0124^{c}	0.161 ± 0.0085^{b}	0.123 ± 0.0087^{a}
12	0.495 ± 0.0315^{d}	0.230 ± 0.0072^{c}	0.182 ± 0.0106^{b}	0.120 ± 0.0028^{a}
18	0.663 ± 0.0352^{d}	0.386 ± 0.0236^{c}	0.294 ± 0.0185^{b}	0.122 ± 0.0028^{a}
24	1.162 ± 0.0361^{d}	0.667 ± 0.0213^{c}	0.380 ± 0.0150^{b}	0.123 ± 0.0015^{a}
30	1.488 ± 0.0586^{d}	1.029 ± 0.0177^{c}	0.369 ± 0.0133^{b}	0.123 ± 0.0015^{a}
36	1.843 ± 0.0422^{d}	1.007 ± 0.0267^{c}	0.345 ± 0.0075^{b}	0.122 ± 0.0015^{a}
42	1.742 ± 0.0391^{d}	0.966 ± 0.0309^{c}	0.323 ± 0.0041^{b}	0.116 ± 0.0005^{a}
48	1.589 ± 0.0682^{d}	0.859 ± 0.0273^{c}	0.280 ± 0.0110^{b}	0.109 ± 0.0005^{a}

Note: the value is the average ± standard deviation, the super script letters behind different numbers in one column show significantly different from each other (p <0.050), the super script letters from a to z indicate having a greater average value.

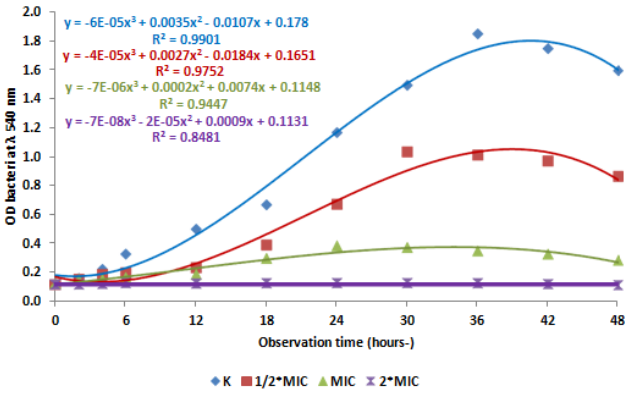

Figure 5. Growth kinetics of *P. aeruginosa* bacteria exposed to *S. crassifolium* extract of solvent diethyl ether.

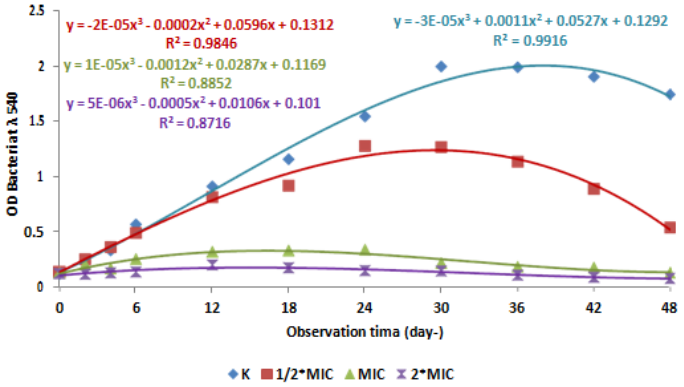

Figure 6. Growth kinetics of *S. aureus* bacteria exposed to *S. crassifolium* extract of solvent diethyl ether.

Table 5. Growth density of *S. aureus* bacteria exposed to *S. crassifolium* extract of solvent diethyl ether.

Observation (Dayth)	Staphylococcus aureus			
	K	1/2*MIC	MIC	2*MIC
0	0.114 ± 0.010^{a}	0.134 ± 0.040^{a}	0.124 ± 0.020^{a}	0.118 ± 0.010^{a}
2	0.226 ± 0.010^{c}	0.249 ± 0.011^{d}	0.200 ± 0.011^{b}	0.113 ± 0.005^{a}
4	0.327 ± 0.010^{c}	0.359 ± 0.024^{d}	0.163 ± 0.011^{b}	0.124 ± 0.003^{a}
6	0.564 ± 0.029^{d}	0.485 ± 0.027^{c}	0.250 ± 0.013^{b}	0.133 ± 0.008^{a}
12	0.908 ± 0.103^{d}	0.811 ± 0.010^{c}	0.315 ± 0.003^{b}	0.196 ± 0.012^{a}
18	1.154 ± 0.047^{d}	0.914 ± 0.011^{c}	0.328 ± 0.007^{b}	0.170 ± 0.010^{a}
24	1.542 ± 0.041^{d}	1.275 ± 0.045^{c}	0.335 ± 0.005^{b}	0.145 ± 0.003^{a}
30	1.994 ± 0.124^{d}	1.265 ± 0.040^{c}	0.214 ± 0.001^{b}	0.141 ± 0.003^{a}
36	1.988 ± 0.086^{d}	1.131 ± 0.019^{c}	0.179 ± 0.007^{b}	0.103 ± 0.002^{a}
42	1.901 ± 0.118^{d}	0.885 ± 0.035^{c}	0.172 ± 0.006^{b}	0.084 ± 0.003^{a}
48	1.742 ± 0.068^{d}	0.536 ± 0.009^{c}	0.126 ± 0.001^{b}	0.074 ± 0.002^{a}

Note: the value is the average ± standard deviation, the super script letters behind different numbers in one column show significantly different from each other ($p < 0.050$), the super script letters from a to z indicate having a greater average value.

Table 5. Growth density of *S. aureus* bacteria exposed to *S. crassifolium* extract of solvent diethyl ether.

Observation (Dayth)	Staphylococcus aureus			
	K	1/2*MIC	MIC	2*MIC
0	0.114 ± 0.010^{a}	0.134 ± 0.040^{a}	0.124 ± 0.020^{a}	0.118 ± 0.010^{a}
2	0.226 ± 0.010^{c}	0.249 ± 0.011^{d}	0.200 ± 0.011^{b}	0.113 ± 0.005^{a}
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24	1.542 ± 0.041^{d}	1.275 ± 0.045^{c}	0.335 ± 0.005^{b}	0.145 ± 0.003^{a}
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Note: the value is the average ± standard deviation, the super script letters behind different numbers in one column show significantly different from each other ($p < 0.050$), the super script letters from a to z indicate having a greater average value.

3.4. GC-MC analysis of S. crassifolium extract.

T. crassifolium extract of the solvent diethyl ether has the best antibacterial activity then performed with GC-MS analysis. GC-MS consists of two main component blocks: gas chromatography and mass spectromater. The GC-MS process is carried out with an active fraction of KCV results, using the GC-MS tool Shimadzu QP2010S type with the method of Khotimah *et al*. (2013). This analysis will obtain information about the fraction constituent compounds which are non-folatil. The results of the GC-MS analysis of *S. crassifolium* extract of the solvent diethyl ether are presented in Table 6.

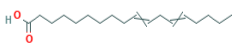
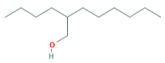
GC-MS analysis shows that the extract has a complex composition of compounds. The composition of the extract consists of the compound Cyclopentylacetic acid; Tonalid; 10,13-Octadecadienoic acid; Tritriacontane; 2-Butyl-1-octanol; Pentacosane; Eicosane; Tetratetracontane; Dotriacontane; Octacosane; Nonacosane; Heneicosane; Eicosane; Docosane; Pentatriacontane; Tetracosane and Eicosane, 2-methyl. Molecular weight of compounds possessed ranged from 186 - 619 g/mol. Extract components have different contents. The lowest percentage of extract component is tonalid, while the highest component is eicosane.

4. Discussion

Extracts used were different types of solvents based on their polarity level. Based on the type of solvent used the extract had a significantly different activity ($p < 0.05$). The results of antimicrobial activity tests on extracts provide information that the overall extract data that has the lowest antimicrobial activity is extracts with ethanol. According to [13][14], the extent or level of extract activity on disc paper depends on the diffusion rate of the extract on agar media and the potential extract. The extract with high potential bioactivity may have physical properties that are difficult to diffuse on the media which is the diameter of inhibition of microbes that formed is small or absent.

The results of this study are in line with research conducted [15][16] that Sargassum seaweed extracted using diethyl ether has the largest inhibitory zone when compared to hexane and methanol solvents. Factors affecting the size of the inhibitory area were culture medium, agar diffusion rate, organismic sensitivity and incubation conditions. The factors that influence the speed of agar diffusion are media composition, microorganism concentration, incubation time and temperature [17][18].

Table 6. GC-MC analysis of *S. crassifolium* extract of solvent diethyl ether.

Structure	Compounds	RT	Molecular formula	BM (g/mol)	Persentage (%)
	Cyclopentylacetic acid	20,751	C7H12O2	128.17	5.11
	Tonalid	27,551	C18H26O	258,4	2.09
	10,13-Octadecadienoic acid	31,815	C18H32O2	280.4	7.51
	Tritriacontane	32,374	C33H68	464.9	4.20
	2-Butyl-1-octanol	33,454	C12H26O	186.33	5.75
	Pentacosane	34,487	C25H52	352.7	3.61
	Eicosane	36,258	C20H42	282.5	12.00
	Tetratetracontane	36,371	C44H90	619.2	3.99
	Dotriacontane	37,223	C32H66	450.88	8.26
	Octacosane	38,198	C28H58	394.8	2.82
	Nonacosane	38,552	C29H60	408.8	9.66
	Heneicosane	39,489	C21H44	296.6	7.65
	Eicosane	39,797	C20H42	282.5	7.88
	Docosane	41,753	C22H46	310.6	7.80
	Pentatriacontane	45,725	C35H72	492.9	4.22
	Tetracosane	46,018	C24H50	338.7	4.77
	Eicosane, 2-methyl	48,534	C21H44	296.57	2.68

The amount of inhibition zone formed by seaweed extract which is extracted using diethyl ether is suspected due to the diethyl ether which has lipophilic and hydrophilic properties [19][20]. This condition causes the antibacterial compound extracted with ethyl acetate to have optimum polarity, which is antimicrobial activity occurs both hydrophilic and lipophilic balance therefore the interaction of antibacterial compounds and tested bacteria is maximized [21].

Generally antibacterial activity test results showed that higher concentration of extract treatment affected in the effect of no zones into bacteriostatic zones then bacteriocidal zones, besides that higher

concentrations obtain greater zones. According to [22][23], bacteriostatic agents work by inhibiting protein synthesis by temporarily binding the ribosome of an organism. The bonds are not very strong as of concentration and stability decrease, antimicrobial agents release ribosomes which bacteria can grow back. This is different from the mechanism of bacteriocidal agents that work by tightly binding to target cells, not released again and microorganism cells will be killed.

5. Conclusions

The results showed that differences in extracts had different MIC values ($p < 0.05$). Extracts with high antibacterial activity are extracts from diethyl ether solvent. The extract has a value of 12.7 mg/ml *P. aeruginosa* mic and 8.4 mg/ml *S. aureus*. *P. aeruginosa* has exponential growth in the 12th and 44th hours. Whereas *S. aureus* is exponential at the 16th hour and 36th mortality. Spectral analysis of *S. crassifolium* diethyl ether extract solvent showed the composition of eikosana (16.22%), dotriacontane (11.27%), nanocosane (11.09%), dicosane (9.85%), 10.13 octadiadienoic acid (9.52%), 2-butyloctanol (6.33%), pentatriacontane (5.4%), tritriacontane (5.07%), tricosane (1.6%).

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