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Characterization of Non-Polar Organic Compounds from *Padina minor* Macroalgae from Singkawang Waters and Antibacterial Activity Against *Staphylococcus aureus* and*Escherichia coli*

Karakterisasi Senyawa Non-Polar dari Makroalga *Padina minor* Asal Perairan Singkawang dan Aktivitas Antibakteri terhadap *Staphylococcus aureus* dan *Escherichia coli*

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ABSTRACT

Characterization of nonpolar organic compounds of macroalga Padina minor from Singkawang waters and activity against Staphylococcus aureus and Escherichia coli bacteria has been carried out. The stages of this research consisted of maceration, fractionation, phytochemical tests, characterization using FTIR and GC-MS, as well as testing the antibacterial activity with the well-diffusion method. The fractionation process produces n-hexane, dichloromethane, and methanol fractions. The n-hexane fraction was selected for packaging using gradient n-hexane: dichloromethane eluent and yielded 11 combined fractions (F_1RW_1-F_1RW_{11}). The combined fraction F_1RW_2 was separated again using n-hexane: dichloromethane eluent in a gradient manner and 18 combined fractions (F_2RW_1-F_2RW_{18}) were obtained. Then the F_2RW_4 isolate was characterized for GC-MS characterization and the F_2RW_3 isolate was characterized using FTIR. The FTIR interpretation results show the absorption of hydroxyl groups O-H (345.65 cm^{-1}), C=O (1743.65 cm^{-1}), C=C bonds (1633.71 cm^{-1}), C-H (2926.01; 2854.01, 1438.9 and 723.31 cm^{-1}), and C-O-C bonds (1170.79 cm^{-1}). The results of the GC-MS analysis showed that the F_2RW_4 fraction had 2 main compounds, namely methyl decanoate and 11-methyl octadecenoate. The results of the antibacterial activity test showed that the methanol extract and n-hexane fraction of Padina minor were inactive against the inhibiting bacteria Staphylococcus aureus and Escherichia coli.

Keywords: *Padina minor, FTIR, GC-MS, Antibacterial*

ABSTRAK

Telah dilakukan karakterisasi senyawa organik non-polar dari makroalga Padina minor asal kota Singkawang dan aktivitas terhadap bakteri Staphylococcus aureus dan Escherichia coli. Tahap penelitian ini terdiri atas maserasi, fraksinasi, uji fitokimia, karakterisasi menggunakan FTIR dan GC-MS, dan uji aktivitas antibakteri dengan metode difusi sumuran. Proses fraksinasi menghasilkan fraksi n-heksana, diklorometana, dan metanol. Fraksi n-heksana dipilih untuk dilakukan pemisahan menggunakan eluen n-heksana: diklorometana secara bergradien dan menghasilkan 11 fraksi gabungan (F_1RW_1-F_1RW_{11}). Fraksi gabungan F_1RW_2 dipisahkan kembali menggunakan eluen n-heksana: diklorometana secara bergradien dan diperoleh 18 fraksi gabungan (F_2RW_1-F_2RW_{18}). Isolat F_2RW_4 dilanjutkan untuk karakterisasi GC-MS sedangkan Isolat

F_2RW_3 dikarakterisasi menggunakan FTIR. Hasil interpretasi FTIR menunjukkan adanya serapan gugus hidroksil O-H (345.65 cm^{-1}), C=O (1743.65 cm^{-1}), ikatan C=C (1633.71 cm^{-1}), C-H (2926.01; 2854.01, 1438.9 dan 723.31 cm^{-1}), dan C-O-C (1170.79 cm^{-1}). Hasil Analisis GC-MS menunjukkan bahwa fraksi F_2RW_4 memiliki 2 senyawa utama yaitu metil dekanoat dan 11-metil oktadekenoat. Hasil uji aktivitas antibakteri menunjukkan bahwa ekstrak metanol dan fraksi n-heksana Padina minor bersifat tidak aktif dalam menghambat bakteri Sthapylococcus aureus dan Escherichia coli.

***Keyword**: Padina minor, FTIR, GC-MS, Antibakteri*

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1. INTRODUCTION

Macroalgae is a marine biota that contains nutrients such as protein, fat, carbohydrates, and fiber. In addition, seaweed contains secondary metabolites in the form of compounds from alkaloids, flavonoids, phenols, hydroquinones, and tannins (Safia, *et al*, 2020). Macroalgae or seaweed are generally used as food for people living on the coast (Suparmi and Sahri, 2009). Along with the development of technology, seaweed is widely used as a raw material for food, cosmetics and medicines, and others (Alam, 2015).

Seaweed is classified according to the pigments it contains with attractive colors. Seaweed can be grouped into three, namely green seaweed (*Chlorophyta*), red seaweed (*Rhodophyta*), and brown seaweed (*Phaeophyta*). One type of seaweed that is used is brown seaweed (*Phaeophyta)*. Brown seaweed (*Phaeophyta*) is a type of seaweed that can be found in areas with minimal sunlight (Firdaus, 2019).

Padina minor is a type of brown seaweed (*Phaeophyta*). Padina minor has secondary metabolite compounds in the form of alkaloids, saponins, steroids, and triterpenoids (Diachanty, *et al*, 2017). Research conducted by Triastinurmiatiningsih dan Haryani (2008) stated that methanol extract from Padina minor did not show any antibacterial activity against *Escherichia coli*. The methanol extract from *Padina minor* showed antibacterial activity against *Aeromonas hydrophila* and *Vibrio harveyi* (Natrah, *et al*, 2015). The *Padina australis* and *Padina L. nidifica* showed that there was antibacterial activity from Padina australis and *Padina L. nidifica* extracts which could inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* (Yulheriwarni, *et al*, 2016).

The potential antibacterial activity possessed by brown seaweed (*Phaeophyta*) can be developed as a source of antibacterial raw materials. According to Menurut Radji (2011), many Indonesian people suffer from infectious diseases caused by bacteria, including *Escherichia coli* and *Staphylococcus aureus* bacteria. *Escherichia coli* bacteria are present in the digestive tract which can cause diarrhea and urinary tract infections (Rahayu, *et al*, 2018). *Staphylococcus aureus* bacteria can cause skin problems in children and adults, for example, infections of hair follicles, boils, and infections of wounds (Arfani, 2021). Both of these bacteria are normal flora that can be found in the human body, but if the presence of bacteria outside of their original habitat develops excessively can cause disease in humans (Jawetz, *et al*, 2001).

P. minor macroalgae can be found in Singkawang City. In addition, Padina minor is underused by the community and is mostly found on the beach, where some of the water is carried by the waves. In addition, research on the components of organic compounds and antibacterial activity of macroalgae Padina

minor from Singkawang City has not been found.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study were *Padina minor* macroalgae, agar, aquades (H_2O), hydrochloric acid (HCl), *S. aureus* and *E. coli* bacteria, barium (II) chloride ($BaCl_2$), technical dichloromethane, technical methanol, technical *n*-hexane, sodium chloride (NaCl), peptone, ferrous (III) chloride reagent, Dragendorff reagent, Liberman-Burchard reagent, Mayer reagent, cerium sulfate reagent, Wagner reagent, magnesium powder, tetracycline, and yeast extract. The tools used in this study were aluminum foil, aerator, autoclave, stir bar, spray bottle, vial, petri dish, glass funnel, separating funnel, Erlenmeyer, measuring cup, beaker glass, hot plate, Fourier Transform Infra-red instrument. Shimadzu, Gas Chromatography and Mass Spectroscopy instrument GCMS-QP2010S Shimadzu, caliper, loop needle, cotton, column, laminar flow, micropipette, drop pipette, volume pipette, TLC plate, rotary evaporator DLAB RE100-pro, silica gel 60 (70 -230 mesh), spatula, test tube, maceration container, and wrapping.

2.2. Methods

Sample Preparation and Extraction

Samples of *P. minor* seaweed were washed clean and dried, then the samples were mashed. Samples that have been mashed extracted by maceration method of as much as 800 grams. Then immersed in methanol solvent for 6 x 24 hours. The macerate obtained is filtered using filter paper and evaporated. Then the methanol extract was weighed and the yield was determined (Marzuki, *et al* ,2020).

$$^0/_0\, Yield = \frac{Extract\ Weight}{Dry\ Sampel\ Weight} \times 100\%$$

Fractionation

Fractionation was carried out by partitioning using methanol, dichloromethane, and *n*-hexane. The methanol extract was dissolved and put into a separatory funnel, and *n*-hexane solvent was added, then shaken. The mixture was allowed to stand until two layers were formed, namely the methanol fraction and the n-hexane fraction. Furthermore, the *n*-hexane fraction which is located at the top is separated and accommodated into the container. Then the fractionation was continued with dichloromethane solvent into a separatory funnel and shaken. The obtained methanol, *n*-hexane, and dichloromethane fractions were concentrated using a rotary evaporator. The yield of the fraction obtained is calculated using the following equation (Rudiansyah, *et al*,2018).

$$^0/_0\,\text{Yield} = \frac{Fraction\ Weight}{Methanol\ Extract\ Weight} \times 100\%$$

Phytochemical Test

Phytochemical tests were carried out to identify groups of alkaloids, flavonoids, phenolic compounds, saponins, terpenoids, and steroids in samples with changes in color and precipitate. Identification of secondary metabolite content was carried out on the following compounds (Harbone,1987).

Flavonoids

As many as 3-5 drops of the extract and fraction solution were dripped onto the drip plate and a little magnesium powder was added, then 3-5 drops of HCl solution were added. The presence of flavonoids was indicated by the formation of orange to red color.

Steroids/Terpenoids

As many as 3-5 drops of extract and fraction solution were dripped into the drop plate and added with Liberman-Buchard reagent qualitatively. The presence of steroids is indicated by the formation of a green or blue color in the solution. While the presence of terpenoids is indicated by the formation of orange-red color.

Alkaloids

As many as 3-5 drops of extract and fraction solution were put into the drip plate, then added Dragendroff reagent, Mayer reagent, and Wagner react. The presence of alkaloids using the Dragendroff reagent is indicated by the formation of an orange to browns color, the Mayer reagent is indicated by a color change with a white precipitate, whereas using the Wagner reagent is characterized by a brown-to-yellow color change.

Saponins

The sample solution and fractions were put into a test tube then added with distilled water and shaken. The presence of saponin compounds was indicated by the formation of stable 1-10 cm high foam in no less than 10 minutes.

Phenolic

A total of 3-5 drops of sample solution and fractions were put into the drip plate and added qualitatively 1% $FeCl_3$ solution. The presence of phenolic compounds was indicated by the formation of dark blue, green to black colors.

Thin Layer Chromatography (TLC)

Extracts and fractions resulting from maceration and partitioning were followed by the identification of secondary metabolite spot patterns using the TLC method. The extract and each fraction were spotted on the TLC plate at a distance of 1 cm from the bottom using a capillary tube. Then eluted using the mobile phase in the form of 100% n-hexane and n-hexane: dichloromethane (9:1, 8:2, and 6:4). The stains formed were then observed under UV light with a wavelength of 254 and 366 nm, then sprayed using cerium sulfate and heated on a hotplate (Forestryana dan Arnida, 2020).

Flash Column Chromatography (FCC)

The packing of the chromatography column uses the wet method, in which silica gel (70-230 mesh) is soaked and occasionally stirred with *n*-hexane for 1 hour, then packed in the column. A total of 1.501 grams of the n-hexane fraction was dissolved and impregnated onto silica gel 60 (70-230 mesh). Then the impreg silica was added to the packed column and eluted using the eluent composition n-hexane: dichloromethane in a gradient manner. The elution results were collected as much as 5 mL in a vial, and TLC was carried out and sprayed with cerium sulfate as a stain remover and heated. Stains that have the same pattern were combined and TLC was repeated. KKT has carried out 2 repetitions (Sayekti, *et al*, 2013).

Activity Test Against Bacteria

Activity test against bacteria was carried out on methanol extract and *n*-hexane fraction. This research refers to research conducted by (Barrow dan Feltham, 1993; Kurniawati, *et al*, 2017) with the following stages.

Concentration Variation Manufacturing

P. minor macroalgae methanol extract and *n*-hexane fraction sample solutions were made with several concentration variations, namely 50 µg/µL, 25 µg/µL, 12.5 µg/µL, and 6.25 µg/µL. The positive controls used were tetracycline 1 µg/µL, methanol, and *n*-hexane as negative controls.

Tool Sterilization

Tool sterilization can be done by washing the equipment that will be used to test the antibacterial activity of the *P. minor* macroalgae extract, then drying it and wrapping it in the paper. Furthermore, it was put into the autoclave at 121˚C.

Rejuvenation of Pure Bacterial Cultures

Rejuvenation of pure bacterial cultures was carried out with nutrient agar (NA) (0.5 g peptone, 0.5 g NaCl, 0.2 g yeast, and 2 g agar) dissolved in 100 mL distilled water and heated. Then sterilized in an autoclave at 121˚C. Pure cultures of *S. aureus* and *E. coli* bacteria were taken as much as 1 ose and streaked on the agar medium. Then incubated at 37˚C for 18-24 hours.

Preparation of Bacterial Test Suspension

Bacterial inoculum using nutrient broth (NB) (0.5 gr peptone, 0.5 gr NaCl, 0.2 gr yeast) then dissolved in 100 mL of distilled water and heated. Then sterilized in an autoclave at 121˚C. Bacterial cultures of *S. aureus* and *E. coli* were taken as much as 1 ose and spread in nutrient broth. Then in the shaker for 24 hours.

Activity Testing Testing

The activity of methanol extract and n-hexane fraction of *P. minor* on the growth of *S. aureus* and *E. coli* bacteria was carried out using the well diffusion method. Nutrient agar (NA) (1 gram of peptone, 1 gram of NaCl, 0.4 grams of yeast, and 4 grams of agar) is dissolved in 200 mL and heated on a hotplate while stirring. Then it was sterilized in an autoclave at 121˚C and poured into a cup of as much as 20 mL and allowed to solidify. Then 200 µL of bacterial suspension was added to NA and wells were made. As much as 20 µL of test solution, positive and negative controls were put into the well. Then incubated at room temperature for 24 hours. The diameter of the inhibition zone formed was measured using a caliper.

3. RESULTS AND DISCUSSION

3.1. Maceration

Maceration was carried out by soaking for 6 x 24 hours with methanol solvent at room temperature. Methanol viscous extract in the form of a green-black paste with a mass of 15.104 grams with a yield of 1.88%. The resulting yield value indicates that the secondary metabolite content contained is small.

3.2. Fractionation

The methanol extract 9,8088 gr was then partitioned in stages starting from a nonpolar solvent, namely n-hexane, to a semipolar solvent, namely dichloromethane. The results of the brown seaweed fractionation P. minor can be seen in (Table 1).

3.3. Phytochemical Test

The extracts and fractions obtained from P. minor species were subjected to phytochemical tests aimed at identifying the contents of terpenoids, steroids, alkaloids, flavonoids, phenolics, and saponins. The test results for the methanol extract, n-hexane fraction, dichloromethane fraction, and methanol fraction from P. minor can be seen in Table 2 and Figure 1.

Table 1. Fractionation Results of Macroalgae *P. minor*

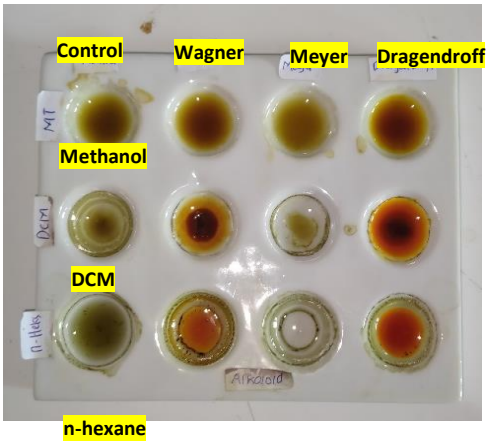
Fraction	Fraction Weight (grams)	Yield (%)
n-hexane	2,209	22,53
Dichloromethane	0,918	9,36

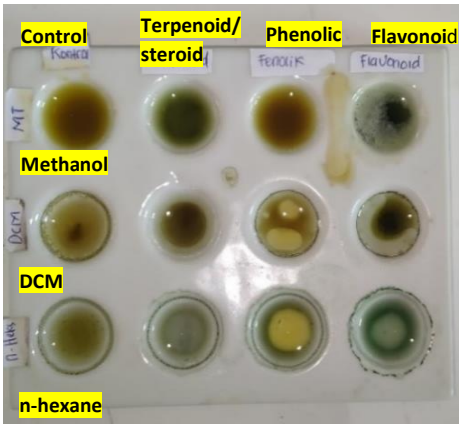
Methanol	3,072	31,32

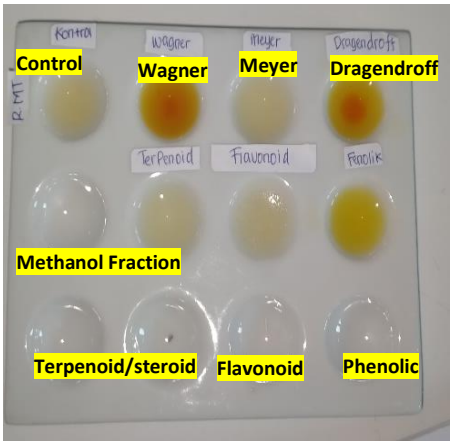
Table 2. Phytochemical Test Result of Macroalgae *P. minor*

Compound	Reagent	Test Result			
		Extract of methanol	Fractions of *n*-hexane	Fractions of dichloromethane	Fractions of methanol
Alkaloids	Dragendroff	++	+++	++	++
	Mayer	-	-	-	-
	Wagner	-	++	++	++
Phenolic	$FeCl_3$	-	-	-	-
Terpenoids/ Steroids	Lieberman-Burchard	+++	++	+++	+
Flavonoids	Mg powder + HCl	+	-	++	-
Saponins	Aquades	+++	+	+++	++

Information: +++ : Strong ++ : Moderate + : Weak - : None







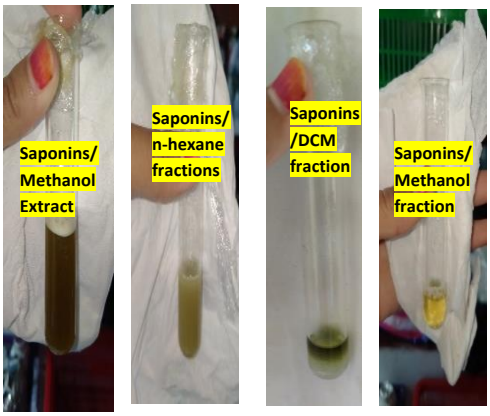


Figure 1. Results of Phytochemical Test

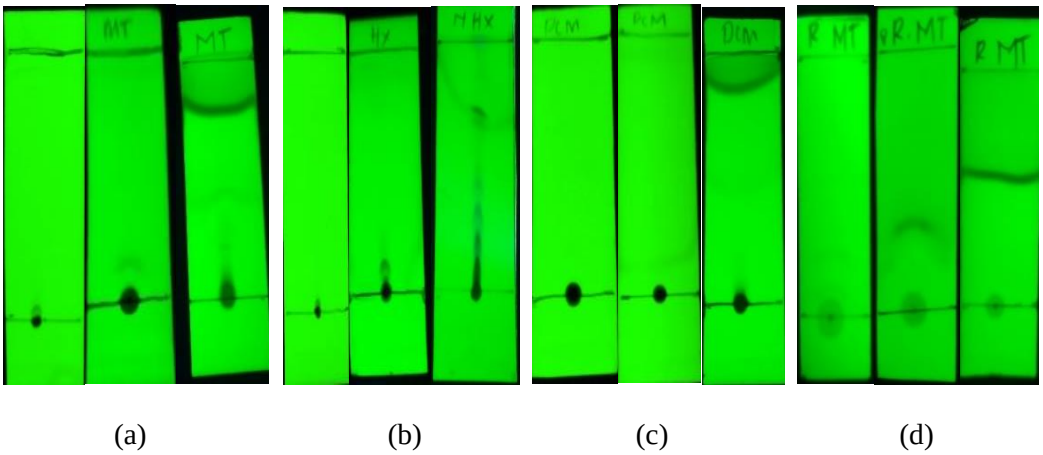
The terpenoid test is orange and the steroid test produces a blue or green color when tested using the Lieberman-Burchard reagent. This is because terpenoid and steroid compounds have the ability to form colors by sulfuric acid in acetic anhydrous solvents, while the color differences produced between terpenoids and steroids are due to differences in groups on C-4 (Marlina and Saleh, 2011). The alkaloid test was identified using Dragendroff reagent to form an orange-to-brown color, tested with Wagner's reagent to form a brown-to-yellow color change, and used Mayer to form a white precipitate. In the alkaloid test, there was a change of ligand, nitrogen which has a lone electron pair (PEB) in the alkaloid formed a covalent coordination bond with K^+ ions originating from potassium tetraiodobismutate to produce a potassium-alkaloid complex which could precipitate (Haryati, *et al*, 2015).

The flavonoid test forms an orange-to-red color when tested using mg powder and concentrated HCl. The mg metal functions to attract the carbonyl groups to bond with each other, while the use of hydrochloric acid aims to reduce the αα-benzopyrene nucleus present in the flavonoid structure and form orange or red flavillum salts (Afriani, *et al*, 2016). The phenolic test will form a color change to dark blue, and green to black when tested using the $FeCl_3$ reagent, this is because FeCl3 reacts with aromatic -OH groups and forms complex compounds with Fe^{3+} ions (Haryati,*et al*,2015). Saponin uses distilled water and is shaken with the marked formation of constant foam because saponins have glycosyl as a polar group and a steroid or triterpenoid group as a nonpolar group so that they are surface active and form micelles when reacting with water (Sangi, *et al*, 2008).

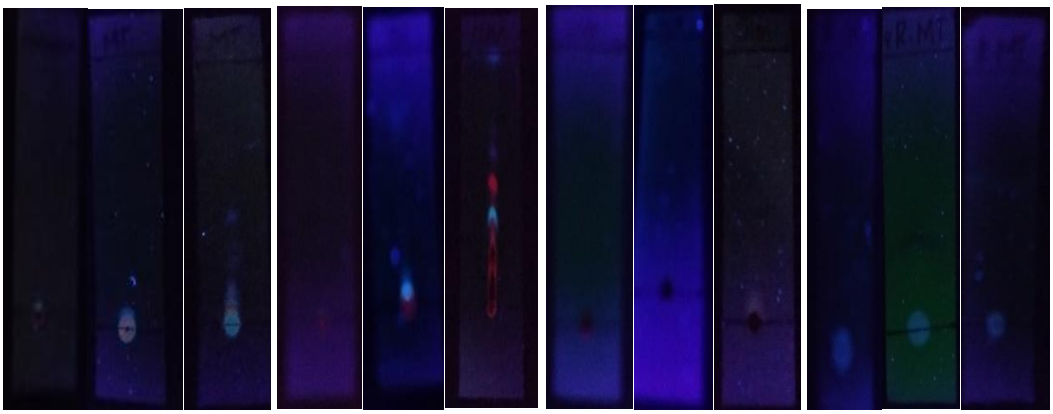
(Table 2.) shows that *P. minor* contains secondary metabolites in the form of alkaloids, terpenoids, steroids, flavonoids, and saponins. This is following the results of research by Diachanty *et al* (2017) that *P. minor* has secondary metabolite compounds in the form of alkaloids, saponins, steroids, and triterpenoids originating from the Seribu Islands Waters. These compounds are dispersed in the extract and its fractions. Methanol extract, *n*-hexane fraction, dichloromethane fraction, and positive methanol fraction contained alkaloids, terpenoids, steroids, flavonoids, and saponins. However, the n-hexane and methanol fractions did not contain flavonoids.

3.4. Thin Layer Chromatography (TLC)

Extracts and fractions that have been obtained are then carried out by TLC using eluent as the mobile phase and polar silica as the stationary phase. The methanol extract, n-hexane fraction, dichloromethane fraction, and methanol fraction were analyzed by TLC using various eluents namely n-hexane 100% and n-hexane: dichloromethane (9:1, 8:2, and 6:4). The results obtained at the TLC orientation that had the best separation were eluents with a ratio of n-hexane: dichloromethane (6:4) in the n-hexane fraction (Figure 2).



(a) (b) (c) (d)

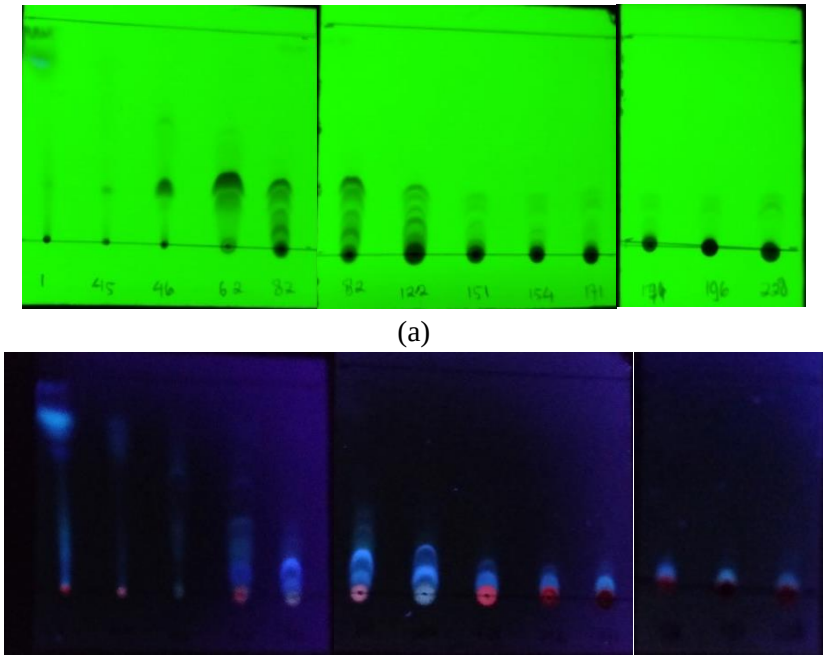


(a) (b) (c) (d)

Figure 2. Eluent Search Chromatogram Profile at UV 254 and 366 nm
(a) Eluent methanol Extract (*n*-hexane 100%, *n*-hexane: dichloromethane (8:2 and 6:4))
(b) Eluent *n*-hexane fraction (*n*-hexane 100%, *n*-hexane: dichloromethane (8:2 and 6:4))
(c) Eluent dichloromethane fraction (*n*-hexane 100%, *n*-hexane: dichloromethane (8:2 and 6:4))
(d) Eluent methanol fraction (*n*-hexane 100%, *n*-hexane: dichloromethane (8:2 and 6:4))

3.5. Flash Column Chromatography (FCC)

The n-hexane fraction was separated on a column using a silica gel 60 stationary phase (70-230 mesh). A total of 1.709 grams of the n-hexane fraction was impregnated, then put into an FCC column which had been packed wetly, where the silica was soaked using n-hexane for 1 hour. Summit was conducted to see the complexity of the compounds in each fraction. This aims to determine the fraction that will proceed to the purification stage. Fractions having the same stain pattern were combined to obtain 11 combined fractions (F_1RW_1-F_1RW_{11}) (Figure 3).

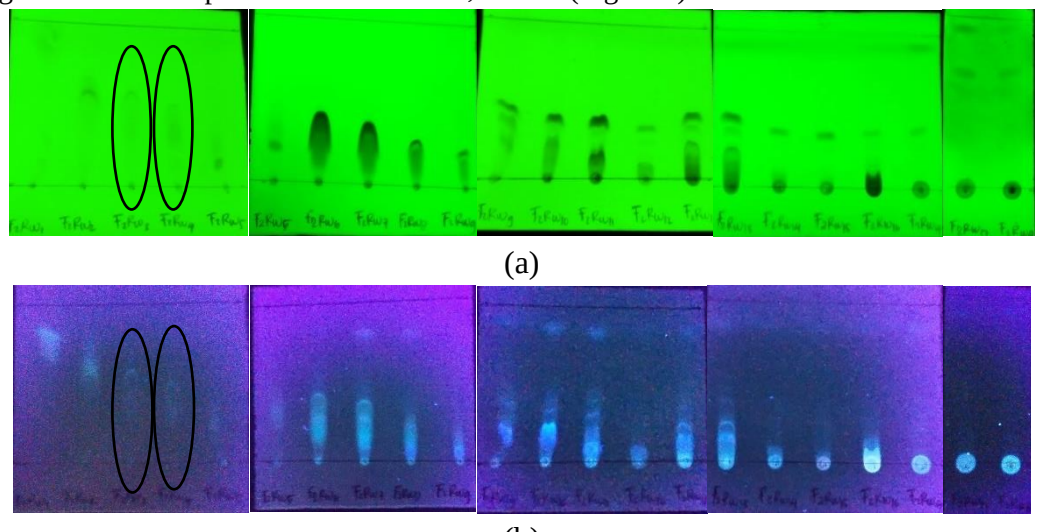


(a)

(b)

Figure 3. First FCC Combined Chromatogram Profile at UV (a) 254 nm (b) 366 nm

Based on the results of the combined TLC, the F_1RW_2-F_1RW_5 were recombined and again separated using a second column of pressure chromatography (FCC). The F_1RW_2-F_1RW_5 fraction has physical characteristics that tend to be the same, namely a yellow color like oil in the fraction. The second FCC fraction having the same stain pattern was combined, resulting in 18 combined fractions (F_2RW_1-F_2RW_{18}), and the combined TLC was performed using n-hexane: dichloromethane as eluent. The combined TLC profile shows a single spot for F_2RW_4 as the target isolate. F_2RW_4 isolates were analyzed by GC-MS and F_2RW_3 isolates were analyzed by FTIR (Figure 4).



(a)

(b)

Figure 4. Second FCC Combined Chromatogram Profile at UV (a) 254 nm (b) 366 nm

3.6. Isolate Analysis

FTIR analysis of Isolate F_2RW_3

Interpretation of the FTIR spectrum (Figure 5) of isolate F_2RW_3 shows the absorption of the hydroxyl functional group -OH at 3450.65 cm^{-1}, carbonyl group C=O at 1743.65 cm^{-1}), C=C bond (1633.71 cm^{-1}), alkane bond C-H (2926.01 cm^{-1}, 2854.01 cm-and 723.31 cm^{-1}), C-OH bonds (1438.9 cm^{-1}), C-O bonds (1026.49 cm^{-1}), C-O-C bonds (1170.79 cm^{-1}). The absorption by functional groups from the F_2RW_3 isolate can be seen in Table 3 and compared with references. Based on the results of this interpretation it indicates the presence of ester compounds in isolate F_2RW_3.

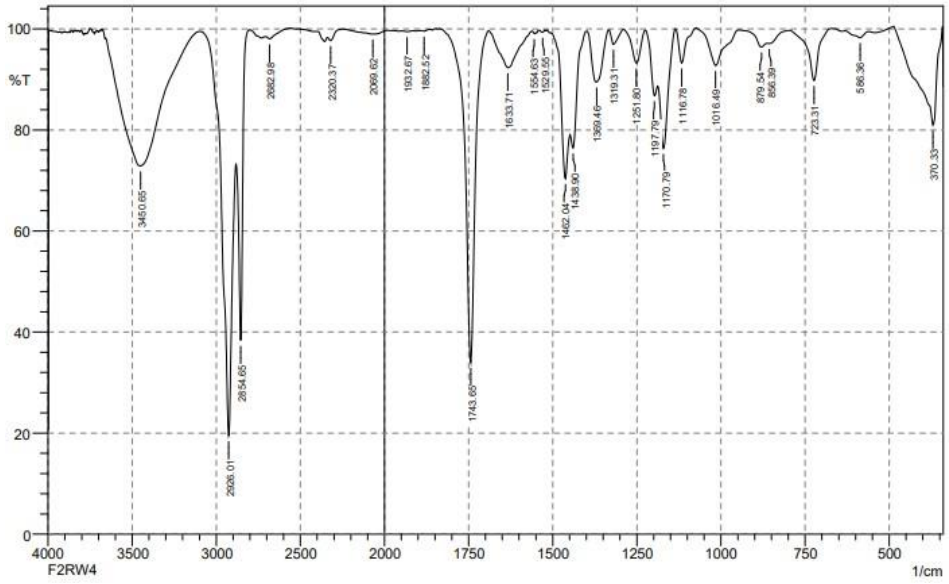


Figure 5. FTIR Spectrum of F_2RW_3 Isolate

Table 3. Interpretation of the FTIR Spectrum Result from $F_2RW_{3\ Isolate}$

Fraction Absorption F_2RW_3	Reference Lutfia, *et al*, 2020	Functional Groups
3450.65	3421	Hydroxyl (-OH)
2926.01; 2854.01	2935	Alkyl (C-H)
1743.65		Carbonyl (C=O)
1633.71	1630	Bond (C=C)
1438.9	1420	Alkyl (C-H)
1170.79		Bond (C-O-C)
1026.49	1036	Bond (C-O)
723.31		Alkyl (C-H)

GC-MS analysis of F_2RW_4 Isolate

The results of the GC-MS analysis (Figure 6) on isolate F_2RW_4 showed that there were 6 components of organic compounds (Table 4) and two of them were the main components of the ester group with a similarity index above 90%. Ester compounds obtained on GC-MS have a correlation with the functional groups produced on the FTIR instrument, namely the presence of ester groups.

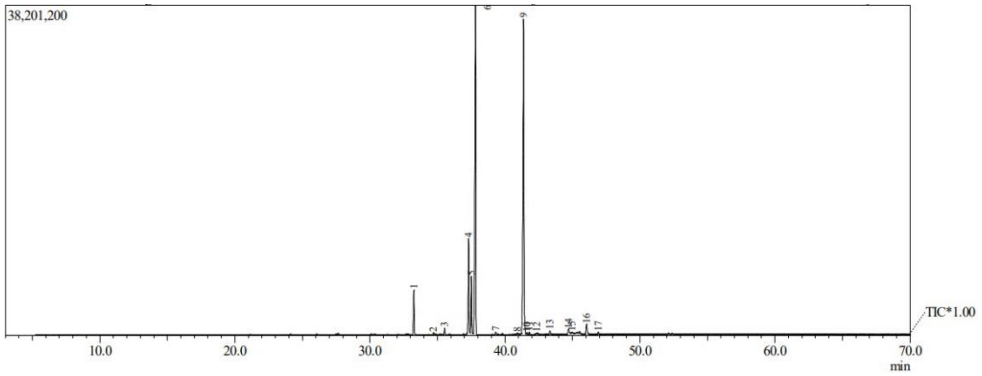


Figure 6. GC Chromatogram of F_2RW_4 Isolate

Table 4. Interpretation of GC-MS Spectrum Result of F_2RW_4 Isolate

No.	Retention Time	Compounds	Molecular Formula	Area (%)
1.	33.256	Metyl 14-pentadecanoate	$C_{17}H_{34}O_2$	4.06
2.	37.317	Methyl 2-undecyl cyclopropane pentanoate	$C_{20}H_{38}O_2$	8.94
3.	37.820	Metyl decanoate	$C_{11}H_{22}O_2$	37.57
4.	41.383	Metyl 11-Oktadecenoate	$C_{19}H_{36}O_2$	38.90
5.	43.330	Decylacetate	$C_{12}H_{24}O_2$	0.37
6.	46.910	1-Hexadecanol acetat	$C_{18}H_{36}O_2$	0.24

The GC-MS results in the table show that there are chemical compounds that make up F_2RW_4 isolate with a similarity level with an index above 90. The main compound that will produce the highest peak is methyl 11-octadecenoate/methyl oleate (38.90%) with an SI of 93%, methyl decanoate/ methyl caprate (37.57%) with an SI of 91%. The brown macroalgae *P. australis* has a high concentration of polyunsaturated fatty acids, namely oleic acid (21.62%) (Kumari, *et al*, 2013). Whereas a study conducted by Caf, *et al* (2015) stated that *P. pavonica* has a high monounsaturate content in the form of palmitic acid of (42.89%), eicosapentaenoic acid with a PUFA/SPA ratio of 0.68%. Based on this, Padina macroalgae can be used as a food supplement.

Figure 7 is the mass spectra of the 11-octadecenoate methyl compound ·+$C_{19}H_{36}O_2$ with m/z = 296. This compound can be fragmented to form +$C_{18}H_{33}O$ (265 m/z) by releasing CO (31 m/z), then the +$C_{18}H_{33}O$ fragment (265 m/z) is fragmented by releasing C_2H_2O (42 m/z) to form +$C_{16}H_{31}$ (223 m/z). Fragment +$C_{16}H_{31}$ (223 m/z) forms +C_9H_{17} (125 m/z) by releasing C_7H_{17} (98 m/z), then fragment C_9H_{17} +(125 m/z) releases C_4H_6 (54 m/z) to form +C_5H_{11} (71 m/z). The +C_5H_{11} fragment (71 m/z) was fragmented by releasing CH_2 (14 m/z) to form +C_4H_9 (57 m/z), +C_3H_7 (43 m/z), and +C_2H_5 (29 m/z). Another fragment of the 11-octadecenoate methyl compound ·+$C_{19}H_{36}O_2$ by releasing $C_{12}H_{25}$ (169 m/z) forms +$C_7H_{13}O_2$ (129 m/z). Fragment +$C_7H_{13}O_2$ (129 m/z) fragmented to form +$C_4H_7O_2$ (87 m/z) by releasing C_3H_6 (42 m/z), then fragment +$C_4H_7O_2$ (87 m/z) was fragmented by releasing CH_2 (14 m/z) to form +$C_3H_5O_2$ (73 m/z) and +$C_2H_3O_2$ (59 m/z).

Figure 8 is the mass spectra of the compound methyl decanoate ·+$C_{11}H_{22}O_2$ (186 m/z). The methyl decanoate compound is fragmented to form +$C_9H_{17}O_2$ (157 m/z) by releasing C_2H_5 (29 m/z). The $C_9H_{17}O_2$ +(157 m/z) fragment was fragmented by releasing CH_2 (14 m/z) to form +$C_8H_{15}O_2$ (143 m/z) and +$C_7H_{13}O_2$ (129 m/z). Fragment +$C_7H_{13}O_2$ (129 m/z) fragmented into +$C_5H_9O_2$ (101 m/z) by releasing C_2H_4 (28 m/z), then fragment +$C_5H_9O_2$ (101 m/z) fragmented into +$C_4H_7O_2$ (87 m/z) by releasing CH_2 (14 m/z). The +$C_4H_7O_2$ (87 m/z) fragment fragmented into +$C_2H_3O_2$ (59 m/z) by releasing C_2H_4 (28 m/z). Another fragment of the methyl decanoate compound ·+$C_{11}H_{22}O_2$ (186 m/z) can form +$C_{10}H_{22}O$ by removing CO (31 m/z), then forming +C_7H_{15} (155 m/z) by releasing C_3H_4O (56 m/z). Fragment +C_7H_{15} (155 m/z) forms +C_5H_{11} (71 m/z) by releasing C_2H_4 (28 m/z). +C_5H_{11} (71 m/z) fragmented releasing CH_2 (14 m/z) to form C_4H_9 +(57 m/z) and C_3H_7 +(43 m/z).

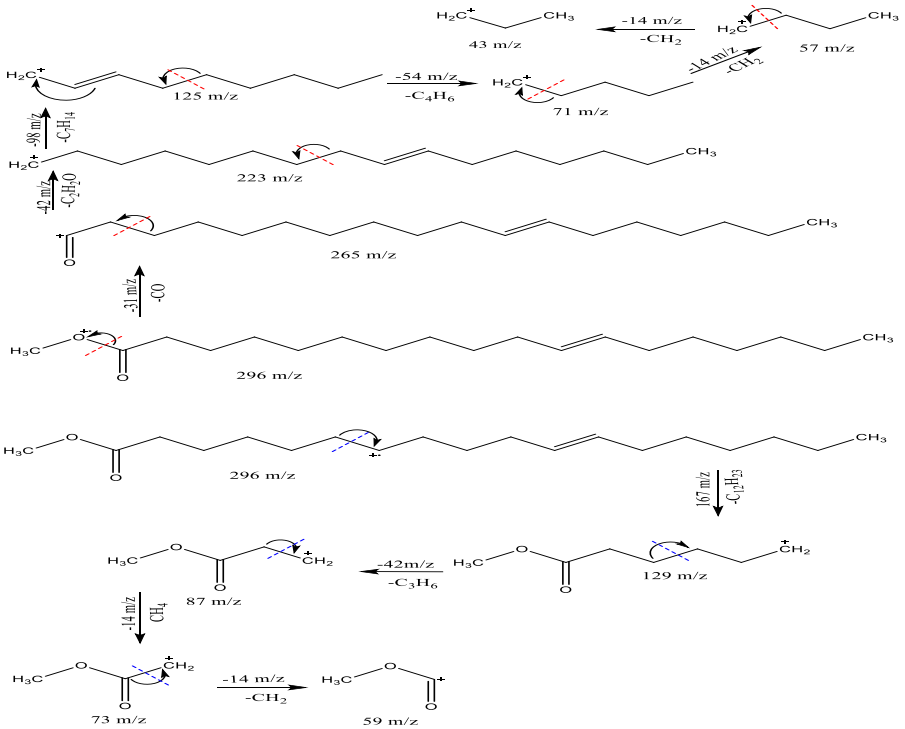


Figure 7. Schematic Diagram on The Fragmentation of Methyl 11- Octadecenoate Compounds

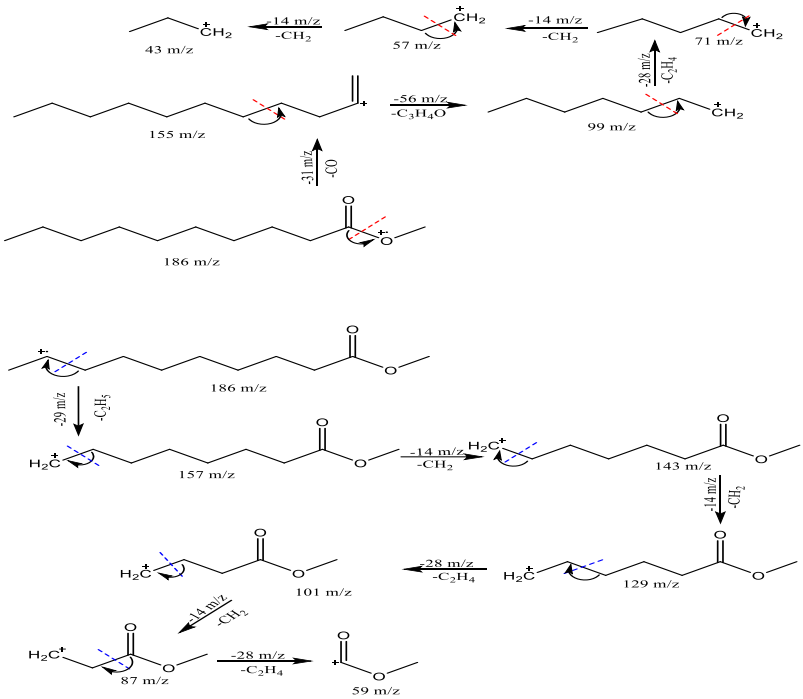
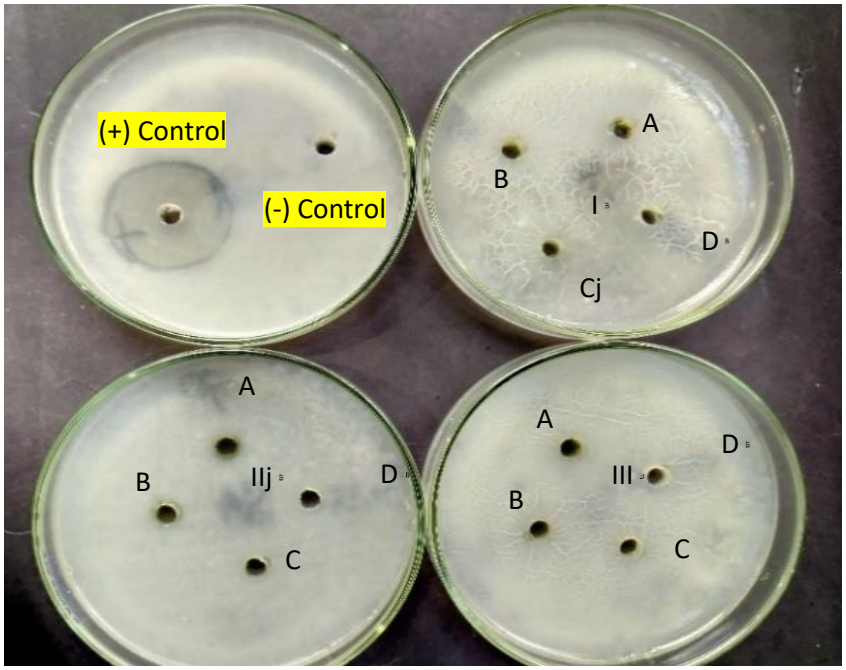


Figure 8. Schematic Diagram on the Fragmentation of Methyl Decanoate Compounds

3.7. Bacterial Activity Test

The antibacterial activity test of brown seaweed *P. minor* from Singkawang waters was carried out using the well-diffusion method. Activity test in this study used concentrations of 50 µg/µL, 25 µg/µL, 12.5 µg/µL, and 12.5 µg/µL. The test results showed no inhibitory activity in the methanol extract and *n*-hexane fraction against *S. aureus* and *E. coli* bacteria with no clear zones around the wells (Figure 9 and Figure 10). Whereas the positive control that used tetracycline had inhibition against *S. aureus and E. coli* bacteria. Tetracycline is an antibiotic that has the ability to inhibit cell wall synthesis (Nasronudin, 2011).

Four factors that affect antibacterial activity, namely the concentration of the extract, the content of metabolites, the type of bacteria that is inhibited, and the diffusion power of the extract (Jawetz, *et al*, 1996). Based on the test results, the antibacterial activity can be affected by the diffusion power of the extract. The methanol extract and the n-hexane fraction of *P. minor* in the well (Well) will inhibit the growth of bacteria by diffusing into the media containing the test bacteria. The type of bacteria and concentration can affect diffusion. In addition, the thickness of the media and the diameter of the wells (Well) can affect the rate of diffusion of metabolites.



(a)

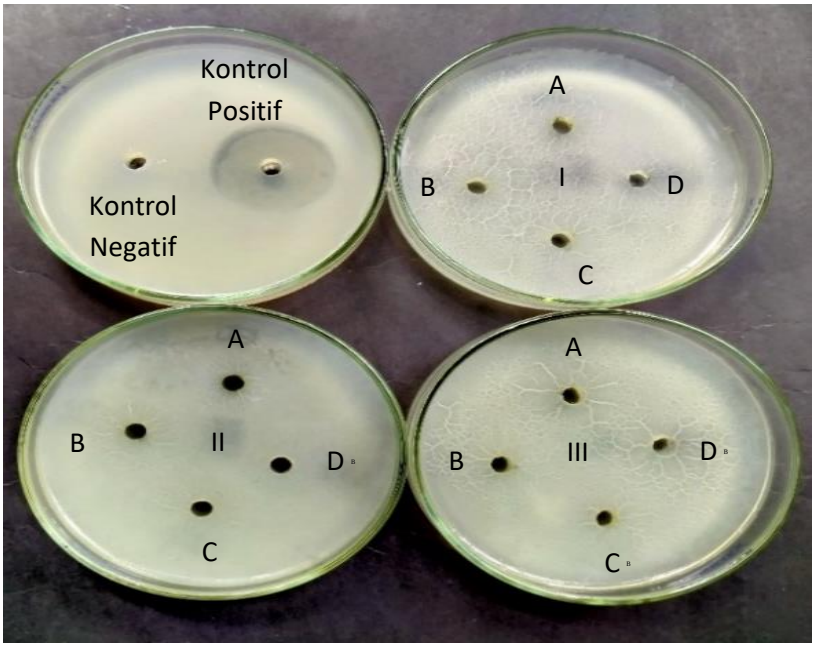
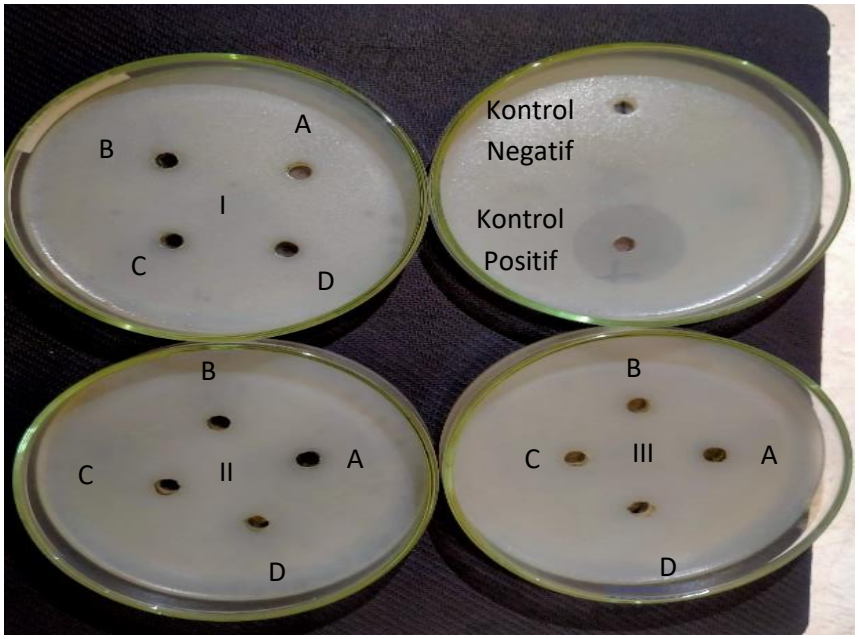
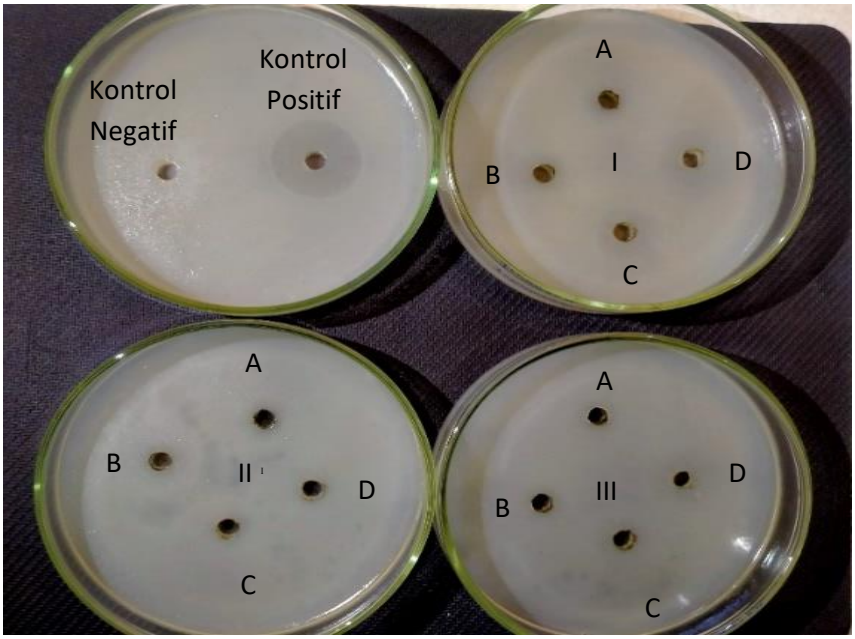


Figure 9. Results of antibacterial activity test of *Padina minor* methanol extract against bacteria (a) *Stahphylococcus aureus* and (b) *Escherichia coli* (A) 50 µg/ µL (B) 25 µg/ µL (C) 12.5 µg/ µL (D 6.25 µg/ µL



(a)



(b)

Figure 10. Results of the antibacterial activity test of the Padina minor n-hexane fraction against bacteria (a) *Stahphylococcus aureus* and (b) *Escherichia coli* (A) 50 µg/ µL (B) 25 µg/ µL (C) 12.5 µg/ µL (D) 6.25 µg/ µL

5. CONCLUSION

Based on the research that has been done, it can be concluded that: a. The results of characterization using the FTIR spectrum on the F_2RW_3 fraction identified the absorption of O-H hydroxyl groups (345.65 cm^{-1}), C=O (1743.65 cm^{-1}), C=C bonds (1633.71 cm^{-1}), C-H (2926.01; 2854.0, 1438.9 cm^{-1} and 723.31 cm-1), and C-O-C bonds (1170.79 cm^{-1}). While the results of the GC-MS analysis showed that the F_2RW_4 fraction had 2 main compounds, namely Methyl 11-Octadecenoate and Methyl Decanoate. The activity test results on the methanol extract and *n*-hexane fraction against *Staphylococcus aureus* and *Escherichia coli* were inactive.

6. LIST OF REFERENCES

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