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Analysis Of Secondary Metabolites And Antibiofilm Activity Of Ethanol Extract Symbiont Bacteria Isolate From Polycarpa Aurata In Barrang Lompo Island, Makassar City

Herwin[1,6], Gemini Alam[2,3*], Sartini[5], Nurhasni Hasan[4], Abdul Rahim[4], Herlina Rante[5], Risfah Yulianty[4], Latifah Rahman[4]

Pharmaceutical Sciences Doctoral Student, Faculty Of Pharmacy, Hasanuddin University, Makassar, 90245, Indonesia
Drug Discovery And Development Center, Institute Of Research And Community Service, Hasanuddin University, Makassar, 90245, Indonesia
Laboratory Of Pharmacognosy-Phytochemistry, Department Of Pharmaceutical Sciences And Technology, Faculty Of Pharmacy, Hasanuddin University, Makassar, 90245, Indonesia
Department Of Pharmaceutical Sciences And Technology, Faculty Of Pharmacy, Hasanuddin University, Makassar, 90245, Indonesia
Laboratory Of Microbiology, Department Of Pharmaceutical Sciences And Technology, Faculty Of Pharmacy, Hasanuddin University, Makassar, 90245, Indonesia
Laboratory Of Microbiology, Faculty Of Pharmacy, Universitas Muslim Indonesia, Makassar, 90231, Indonesia

Abstract
Background*: Polycarpa aurata is a marine biota from the ascidians contain bioactive compounds from the alkaloid group such as policarpaurin A, B dan C, tetracyclic pyridoacridine, pentacyclic pyridoacridines, segoline A, golongan peptide useful as anticancer, antiinflamatory and antibacterial.*
Materials and Methods*: Production of secondary metabolites with a variety of nitrogen source nutrients (meat, peptone, tryptone and yeast extract) by fermentation, analysis of chemical compounds groups by GC-MS, analysis of antibiofilm activity by Elisa Reader.*
Results*: The result of sencondary metabolites production from isolate symbiont bacteria Polycarpa aurata based on variations nitrogen source nutrients (tryptone, meat, yeast extract, peptone) obtained the heaviest weight is ethanol extract of AQ2-1 isolate with tryptone nutrition = 128.9mg.. Analysis of chemical compounds by GC-MS spectroscopy of ethanol extract AQ2-1 isolate is contains 130 groups of chemical compounds and there are 14 groups of compounds with the highest peaks, namely 2-Amino-1,3-propanediol ($C_3H_9NO_2$, BM=91), 2-Propenoic acid ($C_4H_6O_2$,86), 2-Furancarboxaldehyde (C_5H_4O,96), 3(2H)-Furanone ($C_{13}H_{22}O_2$,210), 2,3-dihydro-3,5-dihydroxy-6-methyl- ($C_6H_8O_4$,144), 5-Hydroxymethylfurfural ($C_6H_6O_3$,126), Beta-D-Glucopyranose ($C_6H_{10}O_5$, 162), Hexadecanoic acid ($C_{17}H_{34}O_2$,390), 9-Octadecenoic acid (Z)- ($C_{19}H_{36}O_2$,296), 12-cis-octadecadienoate ($C_{19}H_{34}O_2$, 294), cis-10-Nonadecenoic acid ($C_{20}H_{38}O_2$,310), Bis(2-Ethylhexyl)ester ($C_{22}H_{42}O_4$,370), 1,2-Benzenedicarboxylic Acid ($C_{20}H_{30}O_4$,334) and 2,6,10,15,19,23-hexamethyl- ($C_{30}H_{50}$,410). The result of analysis antibiofilm activity by Elisa Reader using concentration 1280 ppm, 640 ppm, 320 ppm, 160 ppm, 80 ppm, 40 ppm, 20 ppm, 10 ppm, 5 ppm, and 1 ppm obtained % inhibisi the largest against Gram-positive bacteria group at concentration 1280 ppm = 78.01%, namely against Streptococcus mutans ATCC 25175 bacteria and Gram-negative bacteria group = 56.83%, namely against Pseudomonas aeruginosa ATCC 27853 bacteria.*
Conclusion: *Based on the result of secondary metabolites analysis from ethanol extract AQ2-1 isolate of colourec-white Polycarpa aurata contains chemical compounds with antibiofilm activity against Gram-positive and Gram-negative bacteria.*
Key Word: *Symbiont Bacteria AQ2-1 Isolate, Polycarpa aurata, GC-MS, Antibiofilm*

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I. Introduction

The diversity of marine biota in Indonesia as a source of raw materials for medicines has enormous potential, one of which comes from Ascidians type *Polycarpa aurata* with bioactive compounds as antibacterial, anti-inflammatory and anticancer[1]. Exploration of bioactive compounds from marine biota is currently starting to

be developed, both obtained from secondary metabolites of their hosts and from microorganisms that live in marine biota. These microorganisms have a major role in the production of bioactive compounds that are the same as their hosts [2]. Marine biota can be used as a source of raw materials for medicines with potential bioactive compounds, namely *Polycarpa aurata,* because it contains many bioactive compounds such as polycarpin compounds which are useful as antibacterials[3]. Other bioactive compounds from the alkaloid group such as poliaurin A and poliaurin B in vitro provide activity against human parasites, namely *Schistosoma mansoni* [1].

Bioactive compounds that have been identified as potential medicinal chemical compounds are from the alkaloid and peptide groups that are cytotoxic and have antibacterial activity. Ethanol extract of *Polycarpa aurata* by TLC-Bioautography and agar diffusion provides activity against *Escherichia coli* and *Salmonella thypi* bacteria with the chemical content of flavonoids, tannins, alkaloids, triterpenoids and saponins[4]. Bioactive compounds of *Polycarpa aurata* are reported to contain new alkaloid compounds, namely polycarpaurin compounds, tetracyclic pyridoacridines, pentacyclic pyridoacridines, segoline A, tetrahydro-β-carbolines, N-methyl β-carbolinium, and various types of alkaloids that have the potential to become secondary metabolites[5]**.**

The search for bioactive compounds in marine biota is due to the existence of various antibacterial and antibiotic drugs that continue to experience an increase in the number of drug resistance so the development of new drug compounds is needed[6]. With the increasing drug resistance, the need for drugs is very necessary for developing new bioactive compounds with strong therapeutics as an alternative to antibacterial or antibiotic drugs. These drug compounds can be obtained in the form of plant extracts with active compounds from the flavonoid, alkaloid, and glycoside groups that have been proven as antimicrobial compounds[7]. Resistance to antimicrobial products is caused by biofilms, natural molecular compounds can stop the growth of bacterial cells that have the ability to emit chemical signals from each bacterial gene. Bacteria cannot take part in the formation of biofilm characteristics and biofilm role activity if the gene is damaged[8]. These bioactive compounds can be obtained from both plants and marine biota such as the marine biota of the ascidians type *Polycarpa aurata.*

Secondary metabolites produced by marine biota as bioactive compound products can also be useful as antibiofilms because biofilms can protect bacteria from host defenses and biofilm cells can separate from each other and join other matrix systems. This makes it more difficult to suppress the population of biofilm cells compared to non-biofilm bacteria[9]. Biofilms are a collection of microorganisms that live together and bind strongly to biotic and abiotic surfaces in a humid environment and are covered by a polysaccharide matrix. To effectively clean the formed biofilm, antibiofilm and antimicrobial agents are needed that work with different mechanisms than antibiotics and disinfectants[10]. The use of bacteriophages is one effective method to control biofilm growth. Bacteriophages meet these requirements/needs because they are non-toxic, specific, and effective. Bacteriophages as antibiofilm agents based on several expert studies are considered effective in degrading polysaccharide compounds that make up the extracellular polymeric matrix of biofilms[11,12].

II. Material And Methods

Material

The research tools include an autoclave, sterile cabinet, 250 mL Erlenmeyer glass (Iwaki Pyrex®), incubator (memmert type UM200), LAF (Laminar Air Flow), analytical balance, UV-Vis Spectrophotometry, GC-MS Spectrophotometry. The research materials are Test Bacterial *(Escherichia coli* ATTC 25922, *Salmonella thypi* NCTC 786, *Vibrio cholerae* ATCC 25175, *Shigella dysenteriae*, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* ATCC 14990, *Bacillus subtilis* ATCC 6633, *Propionibacterium acnes*, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175), ethanol, Symbiont bakterial AQ2-1 isolate from *Polycarpa aurata*, Muller Hinton Agar (MHA) medium, Nutrient Agar (NA) medium, yeast extract (Merck 103753 500g), meat (Merck VM209979 500g), and peptone (Himedia RM001-500g), tryptone (Himedia M463-500g)**.**

Isolation of Symbiont Bacteria *Polycarpa aurata*

The *Polycarpa aurata* obtained from Barrang Lompo Island, Makassar City with the coordinate point of *Polycarpa aurata* at position 119˚19.516'E" east longitude and $5^0$3.227'S south latitude at a depth of 4-15 meters. *Polycarpa aurata* samples were wet sorted by washing with seawater until samples were free of foreign substances. *Polycarpa aurata* samples were surface disinfected with 70% ethanol then 5.25% Sodium Hypochlorite for 1 minute and rinsed with sterile water for 1 minute. Samples were isolated from symbiont bacteria by cutting them into small pieces of approximately 1-2 cm then placed in a sterile petri dish containing MHA + Ketoconazole 0.05% medium, incubated at 37^0C for 1-3 days. Symbiont bacteria colonies were inoculated on MHA medium[13].

Purification of Symbiont Bacteria Isolates

The symbiont bacterial isolates as much as 1 ose were carried out aseptically using sterile ose wire, symbiont bacteria were scratched on the surface of sterile MHA medium in a sterile petri dish. The isolate was

purified using the *quadrant streak* method, incubated at 37^0C for 24 hours. The pure isolate obtained was inoculated on MHA slant medium in a sterile test tube as stock[14,15].

Antagonist Test of Symbiont Bacteria Isolates *Polycarpa aurata*

The symbiont bacteria isolates were subjected to antagonism tests by blocking the symbiont bacteria isolate *Polycarpa aurata* on MHA medium by cutting it using a sterile stainlessteel cylinder borer. Pieces of symbiont bacteria isolates were placed in a petri dish containing MHA medium and a suspension of test bacteria, namely *Escherichia coli* ATTC 25922, *Salmonella thypi* NCTC 786, *Vibrio cholerae* ATCC 25175, *Shigella dysenteriae*, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* ATCC 14990, *Bacillus subtilis* ATCC 6633, *Propionibacterium acnes*, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, incubated at 37^0C for 24 hours and the diameter of the clear zone around the pieces of the isolate was measured[16,17].

Production of Secondary Metabolites with Nutrient Variations by Fermentation

The symbiont bacteria isolates were fermented using a shaker at a speed of 200 rpm with various additions of substrate variations with nitrogen source content for the production of secondary metabolites from symbiont bacteria. The fermentation medium used contained maltose and meat extract in sterile distilled water. Variations in the addition of other nitrogen sources, meat extract was replaced with other nitrites, namely peptone, tryptone, yeast extract, each 0.3% (w/v) until the supernatant and mycelia were obtained. The supernatant was extracted with ethyl acetate and ethanol solvents 96% until the ethyl acetate extract and ethanol extract of symbiont bacteria were obtained[18].

Analysis of Chemical Compounds by GC-MS Spectroscopy

Chemical compound analysis by GC-MS, where the ethanol extract of AQ2-1 isolate of symbiont bacteria *Polycarpa aurata* was analyzed by GC-MS Perkin Elmer Turbo (Norwalk, CTO6859, and AS) with a capillary column measuring VF-5 ms, 30 mm x 0.25 mm (with a film thickness of 0.25 mm) consisting of 95% dimethylpolysiloxane with a carrier gas using helium at a flow rate of 0.5 mL/min. Sample analysis by injecting two µL and detecting by an ISQ 7000 mass detector with an injector temperature of 250^0C,, an ion source temperature of 2000C; mass range 50-500 amu, MS detection completed in 42 minutes[19,20]

Antibiofilm Activity of Ethanol Extract of Symbiont Bacteria Isolates[10,21,22]

A total of 100 µL of liquid LB media, Variations in sample concentrations are 1 ppm, 5 ppm, 10 ppm, 20 ppm, 40 ppm, 80 ppm, 160 ppm, 320 ppm, 640 ppm, and 1280 ppm of ethanol extract of symbiont bacteria isolates are mixed and put into each well of the microplate, covered and incubated at 37^0C for 48 hours. The mixture is discarded, and the microplate is washed with water and dried. The microplate is stained with 200 µL of 1% crystal violet, and incubated for 15 minutes at room temperature, the dye is discarded, the microplate is washed with clean water and dried. After drying, 200 µL of 96% ethanol is put into the microplate and incubated for 48 hours at 37^0C. Optical density (OD) is measured using a microplate reader at 595 nm. The test was replicated three times and the percentage of biofilm inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{Negative Control OD} - \text{Sample Treatment OD}}{\text{Negative Control OD}} \times 100\ \%$$

III. Result

Preparation of Samples

Polycarpa aurata is a marine biota classified under the tunicate category, sourced from Barrang Lompo Island in the city of Makassar. The Polycarpa aurata sample used was the white variaty (**Figure 1**).

Figure 1. White-coloured *Polycarpa aurata* from Barrang Lompo Island, Makassar City

Isolaton of Symbiont bacteria white-coloured *Polycarpa aurata*

The result of isolation of symbiont bacteria white-coloured *Polycarpa aurata* using NA medium with destiled water and sea water as a solvent, obtained 7 isolates of symbiont bacteria (5 isolates with sea water a solvent and 2 isolates with aquadest a solvent). The result of isolates purification obtained pure isolates with isolate code AL2-1, AL2-2, AL2-3, AL2-4, AL2-5, AQ22-1 and AQ2-2.

Antagonist Test

The results of antagonist test againt pathogenic bacterial, namely against *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Staphylicoccus epidermidis* ATCC 14990, *Propionibacterium acnes*, *Streptococcus mutans* ATCC 25175, *Eschericia coli* ATTC 25922, *Shygella dysenteriae*, *Pseudomonas aeruginosa* ATCC 27853, *Vibrio cholerae* ATCC 25175, and *Salmonella thypi* NCTC 786 bacteria showed that AQ2-1 isolate symbiont bacteria of white-coloured *Polycarpa aurata*, avtive to 10 pathogenic bacterial with inhibition zona diameter the largest against *Vibrio cholerae* ATCC 25175 bacteria = 26.47 mm.

Production of Secondary Metabolites with Nutrient Variations by Fermentation

Symbiont bacteria AQ2-1 isolate *Polycarpa aurata* produced secondary metabolites by fermentation using a variety of nitrogen sources, namely meat nutrition, peptone, tryptone and yeat extract. The result of the production secondary metabolites isolate AQ2-1 *Polycarpa aurata* (white) by fermentation obtained the best was tryptone with an optimizatiobn time of 30 hours with a fermentation speed is 200 rpm in a volume of 100 mL nutrient with the weight of the heaviest of ethanol extract was trypyone = 128.9 mg (Figure 2).

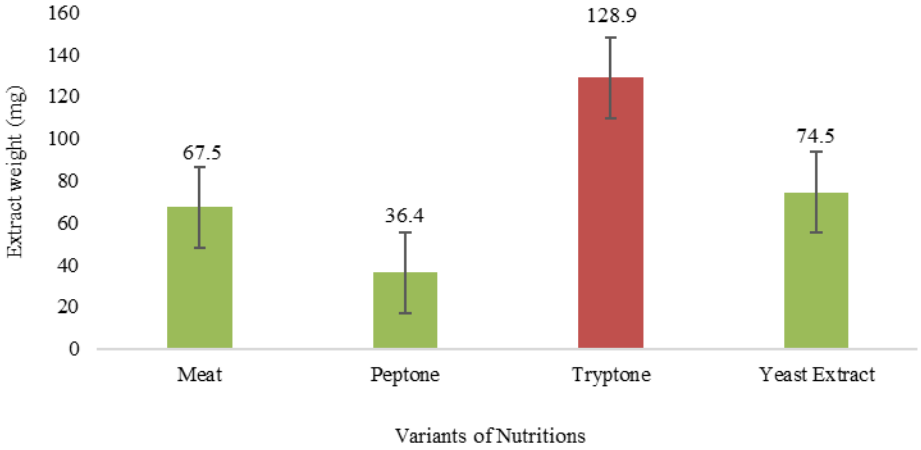


Figure 2. The result of weight extract AQ2-1 Isolate based on nutrition variety

Analysis GC-MS Spectroscopy

GC-MS Spectroscopy is a spectroscopic tool used to analyze secondary metabilites in medicinal plants such as alkaloids, steroids, flavonoids, and others. This method is simple and effective in separating the components of chemical compounds that are still mixed. GC-MS can also identify bioactive compounds that can be used as medicinal chemical compounds. The results of GC-MS analysis of the ethanol extract of isolate AQ2-1 there are 130 chemical compounds and obtained 14 chemical compounds with the highgest peaks (Table 1; Figure 3).

Table 1. Results of GC-MS Spectroscopy Analysis of AQ2-1 Isolate Ethanol Extract

Peak	Real Time	Compounds Name	Area %	Molecular Formula	Molecular Weight
4	3.571	2-Amino-1,3-propanediol	1.34	$C_3H_9NO_2$	91
12	4.370	2-Propenoic acid	0.55	$C_4H_6O_2$	86
18	5.063	2-Furancarboxaldehyde	1.68	C_5H_4O	96
31	6.946	3(2H)-Furanone	1.34	$C_{13}H_{22}O_2$	210
51	11.064	2,3-dihydro-3,5-dihydroxy-6-methyl-	3.79	$C_6H_8O_4$	144
58	12.623	5-Hydroxymethylfurfural	10.99	$C_6H_6O_3$	126
80	17.317	Beta-D-Glucopyranose	53.53	$C_6H_{10}O_5$	162
88	21.061	Hexadecanoic acid	2.06	$C_{17}H_{34}O_2$	390
99	25.067	9-Octadecenoic acid (Z)-	1.25	$C_{19}H_{36}O_2$	296
101	26.119	12-cis-octadecadienoate	1.45	$C_{19}H_{34}O_2$	294
104	27.596	cis-10-Nonadecenoic acid	0.53	$C_{20}H_{38}O_2$	310
112	31.203	Bis(2-Ethylhexyl) Ester	0.57	$C_{22}H_{42}O_4$	370

117	33.864	1,2-Benzenedicarboxylic Acid	1.60	$C_{20}H_{30}O_4$	334
125	38.325	2,6,10,15,19,23-hexamethyl-	0.58	$C_{30}H_{50}$	410

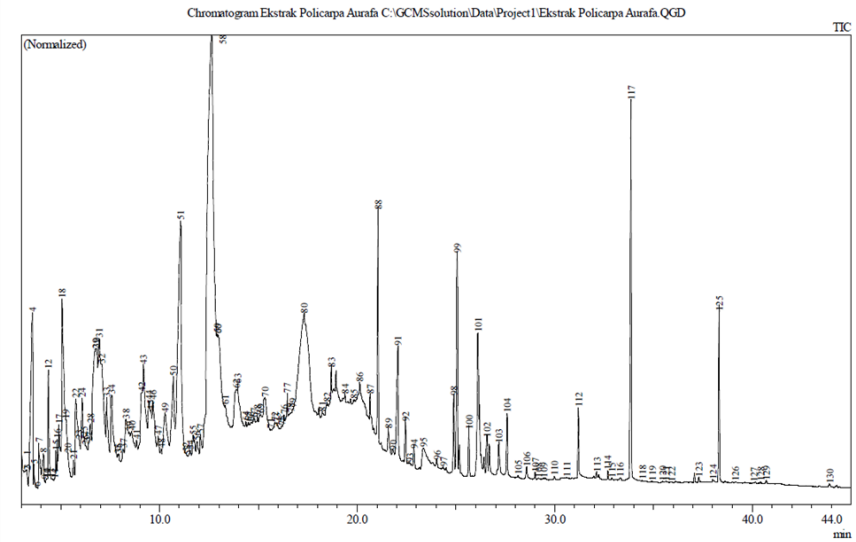


Figure 3. Chromatogram GC-MS Spectroscopy of Ethanol Extract AQ2-1 Isolate

Spectrophotometry GC-MS used to analyze of chemical compounds in the discovery of bioactive compound such as flavonoid group, tannin group, steroids group, alkaloids group, and others chemical compounds. The results of the GC-MS spectroscopy analysis of ethanol extract symbiont bacteria of isolate AQ2-1 *Polycarpa aurata* there is 130 chemical compounds and obtained 14 compound components with the highest peak, namely the 4st compound component, namely 2-Amino-1,3-propanediol ($C_3H_9NO_2$, 91), the 12th compound, namely 2-Propenoic acid ($C_4H_6O_2$; 86), the 18th compound is 2-Furancarboxaldehyde (C_5H_4O; 96), the 31th compounds is 3(2H)-Furanone ($C_{13}H_{22}O_2$; 210), the 51th compound is 2,3-dihydro-3,5-dihydroxy-6-methyl-($C_6H_8O_4$; 144), the 58th compound is 5-Hydroxymethylfurfural ($C_6H_6O_3$; 126), the 80th compound is Beta-D-Glucopyranose ($C_6H_{10}O_5$; 162), the 88th compound is Hexadecanoic acid ($C_{17}H_{34}O_2$; 390), the 99th compounds is 3(2H)-Furanone 9-Octadecenoic acid (Z)- ($C_{19}H_{36}O_2$; 296), the 101th compound is 12-cis-octadecadienoate ($C_{19}H_{34}O_2$; 294), the 104th compound is 5-Hydroxymethylfurfural cis-10-Nonadecenoic acid ($C_{20}H_{38}O_2$; 310), the 112th compound is Bis (2-Ethylhexyl) Ester ($C_{22}H_{42}O_4$; 370), the 117th compound is 1,2-Benzenedicarboxylic Acid ($C_{20}H_{30}O_4$; 334) and the 125th compounds is 2,6,10,15,19,23-hexamethyl- ($C_{30}H_{50}$; 410) (Figure 4).

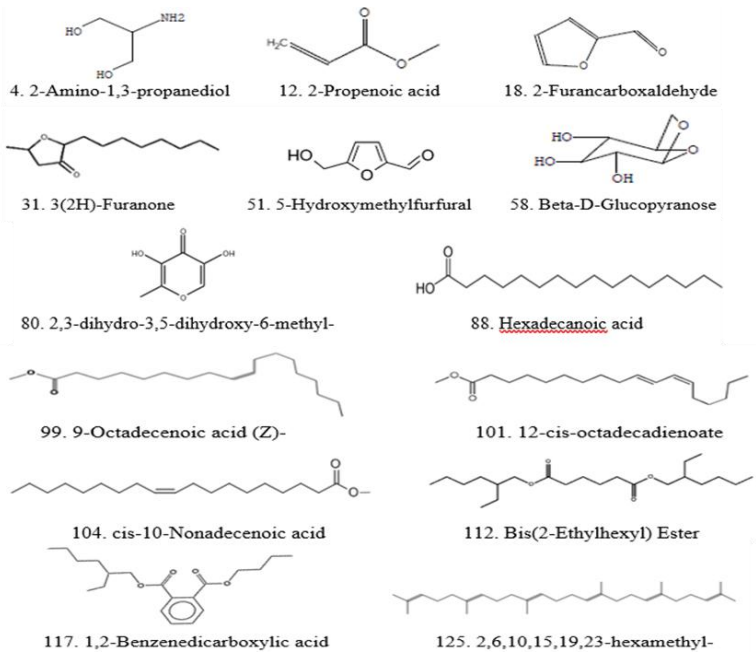
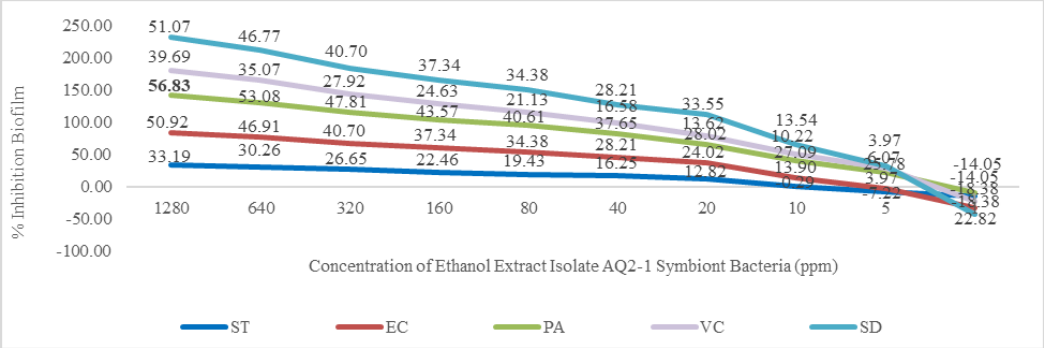


Figure 4. Chemical Structure in Ethanol Extract of Isolate AQ2-1

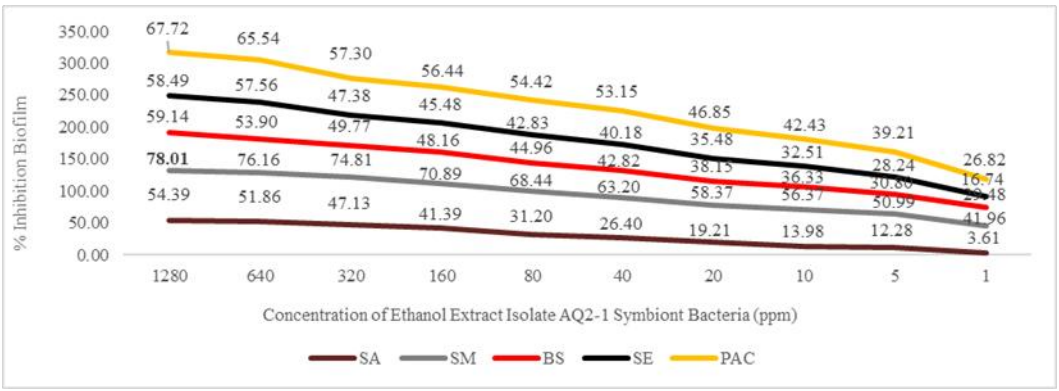
Antibioflm Test

The result of antibiofilm activity test ethanol extract AQ2-1 isolate of symbiont bacteria *Polycarpa aurata* by *Elisa Reader* using concentration of 1280 ppm, 640 ppm, 320 ppm, 160 ppm, 80 ppm, 40 ppm, 20 ppm, 10 ppm, 5 ppm, and 1 ppm obtained the largest % inhibition in the Gram-negative bacteria group was against *Pseudomonas aeruginosa* ATCC 27853 bacteria = 56.83% (Figure 5) and Garm-positive bacteria group obtained the largest % inhibition was against *Streptococcus mutans* ATCC 25175 bacteria = 78.01% at 1280 ppm concentration (Figure 6).



Description: EC = *Eschericia coli* ATTC 25922, SD = *Shygella dysenteriae*, PA = *Pseudomonas aeruginosa* ATCC 27853, VC =*Vibrio cholerae* ATCC 25175 ST = *Salmonella thypi* NCTC 786

Gambar 5. Antibiofilm activity of Ethanol extract AQ2-1 against Gram-negative bacteria



Description: BS = *Bacillus subtilis* ATCC 6633, SA =*Staphylococcus aureus* ATCC 25923, SE =*Staphylicoccus epidermidis* ATCC 14990, Pac =*Propionibacterium acnes*, SM = *Streptococcus mutans* ATCC 25175

Gambar 6. Antibiofilm activity of Ethanol extract AQ2-1 against Gram-positive bacteria

IV. Discussion

The use of nutrient variations in fermentation media for secondary metabolite production is greatly influenced by various nutrient sources, both from carbon, nitrogen, oxygen, oxygen or other elements. The influence of nutrient sources such as from nitrogen sources meat, peptone, tryptone and yeast extract will affect secondary metabolite products and the activity of bioactive compounds in symbiont bacteria. The variation of nitrogen sources, the weight of the extract produced is greatly influenced by each isolate of symbiont bacteria in each marine biota. The weight of the AQ2-1 isolate extract was obtained from the weight of meat nutrients = 67.5 mg, peptone = 36.4 mg, tryptone 128.9 mg and yeast extract = 74.5 mg (fig. 3). Nitrogen is the main element in cell development, especially in producing secondary metabolite compounds. In general, the media is a media with a nitrate composition as a nitrogen source. This media is very suitable for growing symbiont bacteria and symbiont molds with the aim of obtaining secondary metabolites, especially those that have activity as a source of bioactive compounds[23].

The compounds above are included in the group of carboxylic, ester, alcohol, phenolic and aldehyde compounds. The group of phenolic compounds plays an important role in its bioactivity as an antioxidant, antibacterial and anticancer[24]. The mechanism of inhibition by secondary metabolites on bacterial growth can be through four mechanisms, namely, inhibition of peptidoglycan synthesis, inhibition of cell membrane permeability function, destruction of structural and functional protein structures and DNA structures[25]. The antimicrobial mechanism by secondary metabolites is known to interfere with the process of bacterial cell membrane synthesis so that the nature of the cell membrane becomes permeable and causes the cytoplasm to

easily exit the cell. Because of this, microbes cannot survive[26]. Phenolic compounds found in flavonoids can cause cell walls to shrink and damage membrane proteins, thereby disrupting the permeability of bacterial cell membranes[27]. The mechanism of other compounds such as tannins also has the potential to interfere with the synthesis of bacterial cell wall peptidoglycan and is followed by damage to the cell membrane so that bacterial cell viability decreases, while flavonoids are reported to damage the gyrase enzyme in the DNA synthesis process[28]. Alkaloid and saponin compounds as antibacterials, namely by inhibiting cell wall synthesis and causing cell lysis through a decrease in the surface tension of the cell membrane due to damage to the structure that plays a role in peptidoglycan synthesis[29].

Streptococcus mutans is a group of Gram-positive bacteria have a greater % inhibition than *Pseudomonas aeruginosa*, which is a group of Gram-negative bacteria group bacteria while other bacteria have a smaller % inhibition. *Streptococcus mutans* and *Pseudomonas aeruginosa* bacteria with the largest % inhibition because these bacteria can form aggregates and have a relatively strong binding force on the surface of the formed biofilm. Biofilms from *Streptococcus mutans* and *Pseudomonas aeruginosa* bacteria are very difficult to remove during washing because they produce extracellular polysaccharide compounds[30]. One of the group of bacteria that often infection them is salmonella, streptococcus and other bacteria. Salmonella bacteria are examples of bacteria that most often infect humans in the digestive tract through contaminated food and drink[31]. The complex structure of biofilms can protect the bacteria that form them from antibacterial drugs and antibiotics[32]

Biofilm is one of parameters to determine the pathogenic pathway of a bacteria. The formation of this biofilm, can also cause bacteria to be protected in a polysaccharide matrix so that they cannot be reached by the host's immune sustem [33]. Bacterial adhesion followed by colonization will trigger the formation of biofilm and is a sign of the onset of disease pathogenesis. Bacteria that have adhesion and colonization capabilities and defense against phagocytosis are more virulent than bacteria in general [34]. Biofilm formation is one of the determinants for identifying pathogenic strains of bacteria. Every bacteria has the potential to form a biofilm, which can triger virulence, namely the formation of biofilm by *Staphylococcus aureus* causes changes in gene transcription from plantonic cells that are resistant to the host's immune system because antibodies are not optimal in the process of phagocytosis and elimination of cell growth, so that host cells become a good place for *Staphylococcus aureus* to live and develop [35]. The biofilm mechanism in *Pseudomonas aeruginosa* bacteria can be inhibited by disrupting and preventing the formation of biofilms, namely by using disinfectants and natural bioactive compounds to inhibit biofilm formation[36]. Natural compounds such as flavonoids can inhibit the formation of biofilms which show their antibacterial properties against *Pseudomonas aeruginosa*, *Eschericia coli* and *Salmonella typhi.* Bacterial cells in the growing matrix will produce chemical signals that help form biofilm characteristics such as becoming more mature and increasing coordination of biofilm activity[8]. The flavonoid content in has the ability to stop biofilm formation by stopping the activity of bacterial gene expression[37].

V. Conclusion

The results of the analysis chemical compounds of ethanol extract AQ2-1 isolate symbiont bacteria *Polycarpa aurata* there is 130 chemical compounds and obtained 14 compound components with the highest peak. The results of antibiofilm activity analysis by *Elisa reader* obtained % inhibition the largest against the Gram-positive bacteria group = 78.01% at a concentration of 1280 ppm, namely against *Streptococcus mutans ATCC* 25175 bacteria and the Gra-negative bacteria group = 56.83%, namely against *Pseudomonas aeruginosa* ATCC 27853 bacteria.

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