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Phytochemical Compound, Antioxidant and Antibacterial Properties of *Sargassum* sp. Extracts from Pahawang Island, Lampung Province

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Abstract

Pahawang Island, located in Lampung Bay, has a variety of potential natural resources, one of which is macroalgae. One of the abundant brown macroalgae is Sargassum sp., with phytochemical components (flavonoids, steroids, tannins, and glycosides) that have potential as antioxidants, antibacterial, antitumor, anticancer, antifouling, and others. Sargassum sp. from Lampung waters, especially Pahawang Island still lacks scientific studies on its bioactive content and properties. This study aims to examine the phytochemical compounds (qualitatively and quantitatively), antioxidant activity (DPPH method) and antibacterial potential of Sargassum sp. extract (MIC/MBC method) against Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. The results of phytochemical assay showed that Sargassum sp. extract contained phenolic compounds, flavonoids, tannins, and saponins. Quantification results on total phenolic content were 0.56 mgGAE/g extract, total tannins were 4.27 mgTAE/g extract, total flavonoids were 14.19 mgQE/g extract, and total saponins were 5.54 %. Sargassum sp. extract classified as a very weak antioxidant with IC_{50} value of 964.10 ppm, and moderate antibacterial activity at a concentration of MIC/MBC 250/250 ppm against B. subtilis, E. coli, P. aeruginosa, and S. aureus

Keywords: *Antibacterial; Antioxidant; Phytochemical; Pahawang; Sargassum sp.*

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INTRODUCTION

Pahawang Island is one of the small islands in Lampung Bay, located in Marga Punduh District, Pesawaran Regency, Lampung Province. Pahawang has a variety of productive natural resources such as coral reefs, macroalgae, seagrass, mangroves, and

fisheries (Mardani et al., 2018). One of Pahawang Island's biodiversity that has the potential to be utilized for health is macroalgae. Based on several studies, brown macroalgae are known to contain secondary metabolites such as alkaloid compounds, glycosides, tannins, and steroids that are widely used in medicine and the pharmaceutical industry as a source of antioxidants.

Natural sources of antioxidants play an important role againts oxidative stress which related to aging process and degenerative diseases such as cancer, diabetes, cardiovascular disease, and alzheimer's (Gazali et al., 2018; Haerani et al., 2018). One type of brown macroalgae that is abundant in Indonesian waters, but still lacking in its utilization is *Sargassum* sp. (Zana et al., 2022). *Sargassum* sp. contains active compounds of steroids, alkaloids, phenols, and triterpenoids that function as antioxidants, antibacterial, antiviral, and antifungal. *Sargassum* sp. can also be used as antitumor, anticancer, anticholesterol, biofuel, biofertilizer, antifouling, and others (Pakidi & Suwoyo, 2017).

Research on the phytochemical content and potential of *Sargassum* sp. as an antioxidant, antibacterial, and anticancer from Indonesian waters, including *Sargassum* sp. from the West Coast of Aceh contains bioactive compounds such as alkaloids, phenols, and triterpenoids, and has potential as an antioxidant with IC_{50} values of 68.89 ppm - 239.51 ppm (Gazali et al., 2018). *Sargassum policystum* ethanolic extract from the waters of Kabung Island, West Kalimantan has a phenol content of 12.85 mg/g extract, antioxidant activity (IC_{50}) 98.903 ppm (classified as a strong antioxidant), and has the ability to inhibit the growth of pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli* (Safitri et al., 2021). Extract fraction of brown macroalgae *Sargassum* sp. from Bungin Permai Village, Southeast Sulawesi Province showed antibacterial activity with strong inhibition at a minimum inhibitory concentration (MIC) of 20 % against *Staphylococcus aureus* bacteria (Asmarani et al., 2017). *Sargassum plagyophyllum* extract from the coastal waters of Gunung Kidul, Yogyakarta has bioactive compounds of alkaloids, steroids, saponins, and phenolics that act as antibacterials as indicated by the inhibition zone against gram-positive bacteria *(Listeria monocytogenes)* and gram-negative bacteria *(Pseudomonas aeruginosa)* ranging from 2.67-4.67 mm and 4-6.67 mm, respectively (Sidauruk et al., 2021). *Sargassum polycystum* extract from Dompu beach, Nusa Tenggara Barat is a promising natural antioxidant and cervical anticancer agent because it contains four phytochemical components (flavonoids, steroids, tannins, and glycosides), antioxidant activity (IC_{50} of 298.3 µg/mL), and shows strong anticancer activity on HeLa cells (cervical cancer cells) with IC_{50} between 38.3 µg/mL - 112.8 µg/mL (Arsianti et al., 2020).

Currently, *Sargassum* sp. from Lampung waters has not been optimally utilized and there are still few studies on the content of bioactive compounds and their potential utilization. Based on the background above, *Sargassum* sp. is one of the natural resources with high potential for development in the health sector. Exploration of the potential of *Sargassum* sp. from the waters of Pahawang Island can support data on bioactive compounds and their potential bioactivity, especially from Lampung Province. So, the purpose of this study was to explore bioactive compounds from

extracts of brown macroalgae *Sargassum* sp. from the waters of Pahawang Island as antioxidants and antibacterials.

METHOD

The stages of this research were *Sargassum* sp. extraction, phytochemical test and quantification of total phenol, flavonoids, tannins and saponins content. This was followed by measurement of antioxidant activity (using the DPPH method), and antibacterial tests on four types of bacterial isolates (*Bacillus subtillis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). *Sargassum* sp. were collected from the waters of Pahawang Island around Jelarangan Hamlet -5.680736824186002, 105.23062163300901 (area marked in orange on the map in Figure 1).

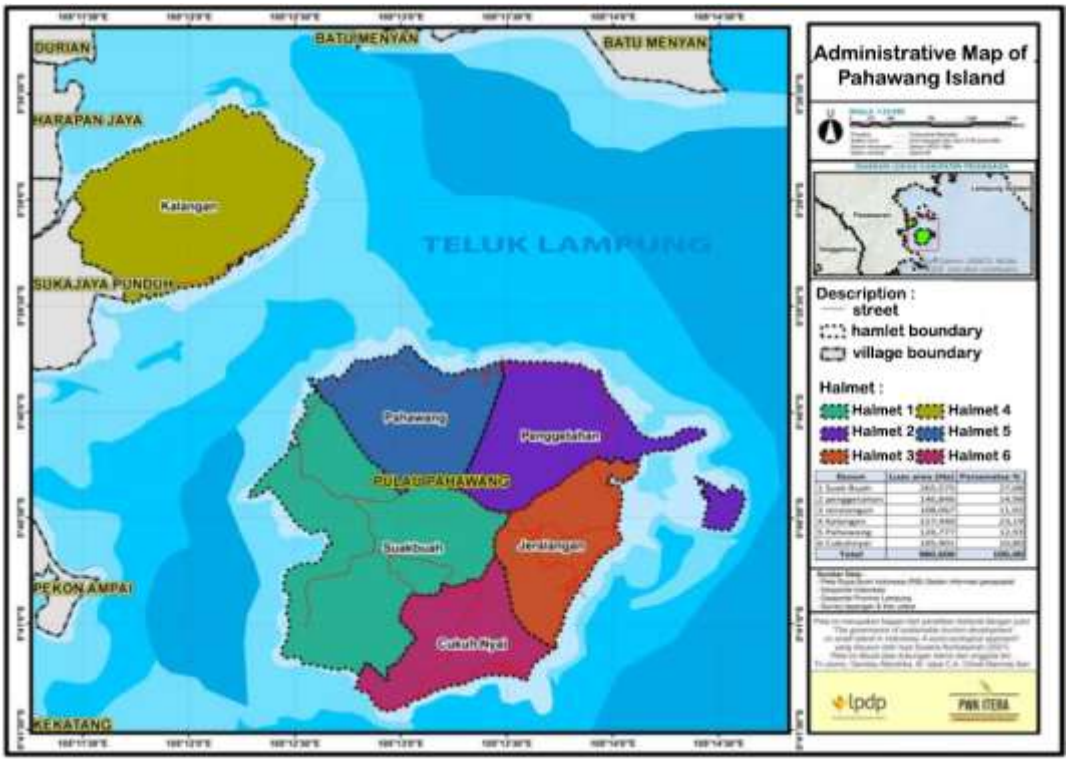

Figure 1. Pahawang Island Map
(copyright map: Isye Susana Nurhasanah and team 2021)

Sargassum sp. Extraction

The collected *Sargassum* sp. was cleaned of trash and other seaweed and then washed thoroughly with fresh water. *Sargassum* sp. was dried for 5-7 days and protected from direct sunlight. The dried *Sargassum* sp. was ground using a blender into powder. 300 g of *Sargassum* sp. powder was macerated with 96% ethanol (W/V ratio 1:5) for 72 hours in a glass jar. The macerate was evaporated using a rotary evaporator with a temperature of 40°C and a rotation speed of 30 rpm to obtained crude extract (Ermawati et al., 2023).

Phytochemical Test and Quantification of Total Phenol, Tannin, Flavonoids, and Saponins Content

The total phenolic content was determined by dissolving 5 mg of the extract in 2 mL of 96% ethanol, followed by the addition of 5 mL of distilled water and 0.5 mL of 50% Folin-Ciocalteu reagent. The mixture was incubated for 5 minutes, then 1 mL of 5% Na_2CO_3 was added. After homogenization, the solution was incubated for one hour, and the absorbance was measured at 745 nm using a spectrophotometer. Gallic acid was used as the standard for phenolic determination, with a standard curve prepared from various concentrations of 5, 10, 15, 20, and 25 ppm. Total phenol content was calculated from the regression equation on the gallic acid curve in mgGAE/g extract (Safitri et al., 2021).

The total tannin content was determined by dissolving 100 mg of the extract in 10 mL of methanol and filtering the solution. Distilled water was then added to bring the total volume to 10 mL. 1 mL sample was mixed with 0.1 mL of Folin-Ciocalteu reagent and homogenized, then allowed to sit for 5 minutes. Next, 2 mL of 20% Na_2CO_3 was added, and the solution was diluted to 10 mL with distilled water. After incubating the sample at room temperature for 30 minutes, the absorbance was measured at 760 nm. Tannic acid was used as the standard for tannin determination, and a standard curve was prepared with concentrations of 0.5, 1, 1.5, 2, and 2.5 ppm. Total tannin content was calculated from the regression equation on the tannic acid curve in mgTAE/g extract (Handayani et al., 2020).

The total flavonoid content was determined by dissolving 10 mg of the extract in 10 mL of methanol. 1 mL sample was then mixed with 3 mL of methanol, 0.2 mL of 10% $AlCl_3$, and 0.2 mL of 1 M CH_3COONa, followed by the addition of distilled water to a final volume of 10 mL. The mixture was incubated for 30 minutes, and the absorbance was measured at 431 nm using a spectrophotometer. Quercetin was used as the standard for flavonoid determination, and a standard curve was created with concentrations of 10, 20, 30, 40, and 50 ppm. Total flavonoid content was calculated from the regression equation on the quercetin curve in mgQE/g extract (Styawan & Rohmanti, 2020).

The total saponin content was determined by weighed 10 mg of the sample and adding 5 mL of water. The mixture was homogenized using a vortex for 5 minutes. Next, 50 µL of anisaldehyde was added, and the sample was shaken and left to stand for 10 minutes. Then, 2 mL of 50 % H_2SO_4 was added, and the mixture was heated in a water bath at 60 °C for 10 minutes. After cooling, distilled water was added to bring the total volume to 10 mL in a measuring flask. Standard dilutions of quillaja bark were prepared at concentrations of 6.25, 12.5, 25, 50, 100, and 200 ppm. The absorbance was measured at 435 nm using a spectrophotometer (Handayani et al., 2020).

Antioxidant activity

Antioxidant test was conducted using DPPH (2,2-diphenyl-1-picrylhydrazyl) method. 2 mg of DPPH was dissolved in 10 ml of methanol to obtain a concentration of 50 µg/ml, then the absorbance was measured at a wavelength of 517 nm. A total of 1 ml of 100 µg/ml DPPH solution was put in a test tube then added 2 ml of methanol,

homogenized and incubated for 30 minutes and then the absorbance was measured at the 517 nm wavelength. Furthermore, 25 mg of the *Sargassum* sp. extract was dissolved in 25 ml of methanol to obtain a concentration of 1000 µg/ml. The next step was dilution to obtain concentrations of 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml. The same was done for ascorbic acid as a positive control, making solutions with concentrations of 2.5 µg/ml, 5 µg/ml, 7.5 µg/ml, 10 µg/ml, and 12.5 µg/ml. 1 ml of sample solution (*Sargassum* sp. extract and ascorbic acid) was put into a test tube then added 1 ml of DPPH 50 µg/ml and diluted with 2 ml of methanol then homogenized. Each solution was incubated for 30 minutes and then the absorbance was measured at the optimum wavelength of 517 nm using a spectrophotometer (A & E Lab.). Antioxidant activity was calculated using the equation :

$$\% \text{ inhibition} = \frac{(\text{Absorbance of the control} - \text{Absorbance of the sample})}{\text{Absorbance of the control}} \times 100\ \%$$

The results of % inhibition are then substituted into the linear equation (y = ax+b). The resulting linear equation is then used to obtain the value of the antioxidant strength of *Sargassum* sp. (IC_{50}) (Khairani et al., 2024).

Antibacterial activity by Determination of Minimum Inhibitory/Bactericidal Concentration (MIC/MBC) value using 96-well microplate

The MIC value was calculated using the serial dilution standard test method. The test bacteria used were *Bacillus subtillis* ATCC 6633, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* ATCC 25923 (Collection of Laboratorium Mikrobiologi dan Biokimia, Pusat Riset Bahan Baku Obat dan Obat tradisional, BRIN, Serpong). A total of 20 µL of sample at an initial concentration of 5,000 ppm in 99 % methanol solvent was added to a sterile 96-well microplate. The mixture was then added with 180 µL of Mueller Hinton Broth (MHB) media and serial dilution was carried out in each test well. Test bacteria were then prepared by inoculating in 0.85 % NaCl and adjusted to McFarland standard 0.5 (equivalent to a cell number of 1×10^8 CFU/mL), then the bacteria were put in each well as much as 100 µL. The concentration of extract in each well's row of microplate was 250 ppm , 125 ppm, 62.5 ppm, 31.25 ppm, 15.625 ppm, 7.8125 ppm, 3.90625 ppm, and 1.953125 ppm. The test plate was then incubated at 37 °C for 24 hours. The MIC value was determined as the lowest concentration of extract that can suppress bacterial growth, observed from the test wells that appeared clear. The MBC value was determined as the lowest concentration of extract that was able to kill the test bacteria analysed using growing test results from the well - MIC value. Tetracycline was used as positive control, and methanol 99 % as negative control (Priyanto et al., 2023).

RESULT AND DISCUSSION

The results of phytochemical assay showed that *Sargassum* sp. extract contained phenolic compounds, tannins, flavonoids, and saponins. The quantification

results of total phenolic content were 0.56 mgGAE/g extract, total tannins were 4.27 mgTAE/g extract, total flavonoids were 14.19 mgQE/g extract, and total saponins were 5.54 %, as shown in Table 1.

Table 1. Quantitative phytochemical test of *Sargassum* sp. from Pahawang Island

Quantitative phytochemical test	Value
Total phenolic content	0,56 mgGAE/g extract
Total tannin content	4,27 mgTAE/g extract
Total flavonoids content	14,19 mgQE/g extract
Total saponins content	5,54 %

Sargassum sp. ethanol extract from Pahawang Island, has the same bioactive content as *Sargassum polycystum* extract taken from the Sumenep area Madura (based on Riwanti (2019), which contains alkaloid compounds, glycosides, steroids/triterpenoids, saponins, flavanoids, polyphenols, and tannins (tested qualitatively). Ethanol extract of *Sargassum* sp. from the waters of Temajo Island, West Kalimantan contains alkaloid, saponin and steroid compounds (which was tested qualitatively) (Noyanti et al., 2023). *Sargassum* sp. originating from the West Coast of Aceh also contains bioactive compounds such as alkaloids and triterpenoids, which was tested qualitatively), and total phenol content 563.22 mgGAE/g extract (Gazali et al., 2018). Another results from Widyaswari et al., (2024) research *Sargassum* from coastal water of Makassar City, South Sulawesi, Indonesia, *S. polycystum* contains alkaloids, flavonoids, phenol hydroquinone, and saponins (qualitatively), also total phenol content 365.96 mgGAE/g extract, while *S. ilicifolium* contains flavonoids, phenol hydroquinone, tannins, and steroids (qualitatively), also total phenol content 382.94 mgGAE/g extract.

Table 2. Antioxidant activity of *Sargassum* sp. from Pahawang Island

Sample	Antioxidant activity (IC_{50}) µg/ml (ppm)
Sargassum sp. extract	964.10
Ascorbic acid (*positive control*)	5.32

The antioxidant activity test results of *Sargassum* sp. ethanolic extract showed an Inhibitor Concentration 50 % (IC_{50}) value of 964.10 µg/ml (shown in Table 2), which is categorized as a very weak antioxidant. On the other hand, ascorbic acid as a positive control has an antioxidant activity of 5.32 µg/ml which is categorized as a very strong antioxidant. The antioxidant strength is grouped into the following classifications based on (Molyneux, 2004) strong antioxidant (IC_{50} < 50 µg/ml), moderately strong (IC_{50} 50-100 µg/ml), moderate (IC_{50} 101-250 µg/ml), weak (IC_{50} 250-500 µg/ml), and very weak (IC_{50} > 500 µg/ml). Another study Gazali et al., (2018) showed that ethanol extract of *Sargassum* sp. from West Coast of Aceh has moderate antioxidant activity with IC_{50} value of 239.51 µg/ml. Arsianti et al., (2020) reported that Ethanol extract of *S. polycystum* from Dompu beach, Nusa Tenggara Barat was assigned to have a weak antioxidant activity with IC_{50} value of 298.3 µg/ml.

The total level of phenolic compounds contained in *Sargassum* sp. extract from Pahawang is quite low at 0.56 mgGAE/g extract. This resulted in the antioxidant activity contained in *Sargassum* sp. extract included into the category of very weak (IC_{50} of 964.10 ppm). According to Molyneux (2004), if a material has a high content of phenol compounds, the material also exhibits a high level of antioxidant activity. The total phenol content and antioxidant activity in this study were lower than the phenol content of *Sargassum policystum* from the waters of Pulau Kabung, West Kalimantan at 12.85 mg/g extract, with antioxidant activity (IC_{50}) 98.903 ppm (classified as a strong antioxidant) (Safitri et al., 2021). Total phenolics and antioxidant activity in this study were also lower than the total phenolics of several types of *Sargassum* species from Simeulue Waters, Aceh Province with total phenolics and antioxidant activity values, namely *S. binderi* 9.02 mgGAE/g extract, IC_{50} 74.7 ppm; *S. crassifolium* 8.71 mgGAE/g extract, IC_{50} 87.5 ppm; *S. muticum* 8.72 mgGAE/g extract, IC_{50} 88.3 ppm; *S. granuliferum* 8.13 mgGAE/g extract, IC_{50} 96.5 ppm, and *S. fluitans* 7.45 mgGAE/g extract, IC_{50} 152.4 ppm (Erniati et al., 2024).

The difference in species can produce different antioxidant activity because each species has different morphology, metabolism, and environmental adaptability so that the bioactive compounds contained therein are also different. Each seaweed species has differences in taking nutrients from the environment. Various abiotic factors can affect the ability of seaweed to absorb nutrients from the environment that will affect metabolism (Erniati et al., 2024; Roleda & Hurd, 2019). In addition, total phenol content and antioxidant activity are also influenced by the selection of the type of solvent in the extraction process. Research by Gazali et al., (2018) explained that *Sargassum* sp. from the waters of Lhok Bubon Beach, West Aceh Regency extracted using three different types of solvents (ethanol, n-hexane, and ethyl acetate), produced different total phenol values and antioxidant activity. *Sargassum* sp. extract with ethyl acetate solvent had the highest total phenol value and antioxidant activity, compared to the extract using ethanol and n-hexane solvents. Ethyl acetate solvent contains more isoflavone compounds both non-polar (aglycone) and polar (glycone), ethyl acetate extract gives the best results compared to ethanol extract and n-hexane extract.

Based on the results in this study, the bioactive compounds in the ethanol extract of *Sargassum* sp. from Pahawang Island may not be extracted completely, so the value of the total content of bioactive compounds is low and the antioxidant activity is very weak. It may be necessary to optimize the solvent. According to Riwanti & Juniar (2019), *Sargassum* sp. extract has strong antioxidant activity using methanol as a solvent and contains phenolic compounds, flavonoids, and carotenoids which contribute the most to antioxidant activity, compared to aqueous solvents, ethanol, aceton, ethyl acetate, chloroform, petroleum ether, and n-hexane.

Table 3. Antibacterial activity of *Sargassum* sp. from Pahawang Island

Sample	Bacteria (MIC/MBC ppm)			
	B. subtilis	*E. coli*	*P. aeruginosa*	*S. aureus*
Sargassum sp. extract	250	250	250	250
Tetracycline (positive control)	3.125	3.125	3.125	3.125

Then the antimicrobial tests were carried out on several pathogenic bacteria, namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* with the calculation of the Minimum Inhibitory/Bactericidal Concentration (MIC/MBC) of *Sargassum* sp. extract were shown in Figure 2 and Table 3. This *Sargassum* sp. extract has moderate potential in inhibiting pathogenic bacteria at a concentration of 250 ppm based on turbidity showed on 96-well microplate. The MIC value was determined as the lowest concentration of extract that could suppress bacterial growth, observed from the test wells that appeared clear. In this study, the test wells were clear at a concentration of 250 ppm for all four test bacteria (Figure 2). Based on (Kuete, 2010), the antibacterial activity of the extract is classified into significant (MIC values of < 100 ppm), moderate (100 < MIC ≤ 625 ppm), and weak (MIC values > 625 ppm) .

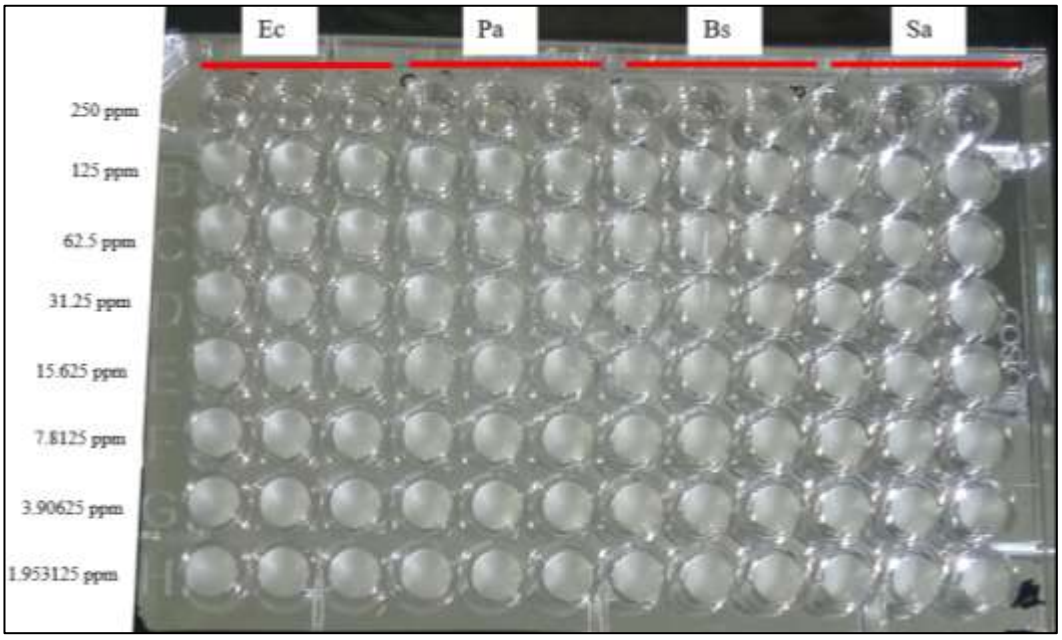

Figure 2. Antibacterial activity MIC value using 96-well microplate
(Code Ec: *Escherichia coli*, Pa: *Pseudomonas aeruginosa*, Bs: *Bacillus subtillis*, and Sa: *Staphylococcus aureus*)

The ethanol extract of *Sargassum* sp. from Pahawang Island has potential as an antibacterial. This extract can inhibit pathogenic bacteria with a minimum inhibitory/bacterisidal concentration of 250 ppm. This study is in line with other studies, the ethanolic extract of *Sargassum policystum* from the waters of Kabung Island, West Kalimantan has the ability to inhibit the growth of pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli* (Safitri et al., 2021). *Sargassum crassifolium* from Karimunjawa Islands, Jepara extracted using different solvents showed antibacterial activity against *P. aeruginosa* and *S. aureus*. *Sargassum crassifolium* extracts from diethyl ether solvent had the best bacteriostatic activity against both test bacteria (MIC *P. aeruginosa* 4 ppm; MIC *S. aureus* 5 ppm) compared to extracts with other solvents (methanol, ethanol, and chloroform) (Setyati et al., 2020). Other studies showed the antibacterial activity of *Sargassum aquifolium* ethanolic extract against

Staphylococcus aureus, Escherichia coli, and P. aeruginosa with minimum inhibitory concentration are 256 µg/mL (ppm), 512 µg/mL (ppm), and 512 µg/mL (ppm) respectively (Bamunuarachchi et al., 2021).

S. polycystum originating from Teluk Kemang and Cape Rachado had antibacterial activity against gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus, Bacillus cereus*). n-Hexane extracts of *S. polycystum* exhibited promising bacteriostatic agents against *B. cereus* with MIC values of 0.065 mg/ml (Wei et al., 2011). Extract fraction of brown macroalgae *Sargassum* sp. from Bungin Permai Village, Southeast Sulawesi Province showed antibacterial activity with strong inhibition at a minimum inhibitory concentration (MIC) concentration of 20 % against *Staphylococcus aureu*s bacteria (Asmarani et al., 2017). *Sargassum plagyophyllum* extract from the coastal waters of Gunung Kidul, Yogyakarta has bioactive compounds of alkaloids, steroids, saponins, and phenolics that act as antibacterials as indicated by the inhibition zone against gram-positive bacteria (*Listeria monocytogenes*) and gram-negative bacteria (*Pseudomonas aeruginosa*) (Sidauruk et al., 2021).

CONCLUSION

Sargassum sp. extract from the waters of Pahawang Island contained phenolic compounds, flavonoids, tannins, and saponins. Quantification results on total phenolic content were 0.56 mgGAE/g extract, total tannins were 4.27 mgTAE/g extract, total flavonoids were 14.19 mgQE/g extract, and total saponins were 5.54 %. *Sargassum* extract classified as a very weak antioxidant with IC_{50} value of 964.10 ppm, and moderate antibacterial activity at a concentration of MIC/MBC 250/250 ppm against *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. aureus*.

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