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Antibacterial activities of ethyl asetat fraction *Sargassum Polycystum* on the growth of *Staphylococcus Aureus* ATCC 25923

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Abstract
Secondary metabolites found in *Sargassum polycystum* include flavonoids, alkaloids, saponins, steroids, and triterpenoids, all of which have antibacterial properties. *Staphylococcus aureus* is one of the pathogenic bacteria that can infect humans. The aim of this work was to establish the antibacterial activity of the ethyl acetate fraction against *Staphylococcus aureus* bacteria and to determine the secondary metabolite compounds in *Sargassum polycystum* ethyl acetate fraction. The liquid-liquid partition method is used to obtain the ethyl acetate fraction. The diffusion method (*Well Diffusion Method*) is the research technique used to study antibacterial activity. According to the results of the phytochemical screening, *Sargassum polycystum* positive contained steroids in the ethyl acetate fraction. The antibacterial activity study's findings revealed readings of the inhibitory negative control zone diameter of 0.00 mm and the positive control diameter of 34.46 mm. Five different concentrations were used in the control test: 0.2, 0.4, 0.8, 1, 2, and 2% (b/v). The average measurement findings are less than 5 mm. It is believed that secondary steroid metabolites are in charge of *Staphylococcus aureus*'s antibacterial action. The *Sargassum polycystum*'s ethyl acetate fraction has a weak antibacterial activity, that is less than 5 mm.

Keywords: *Sargassum polycystum*, ethyl acetate fraction, *Staphylococcus aureus*, antibacterial, *Well Diffusion Method*

Introduction

One of the reasons why diseases are so common in tropical areas, notably Indonesia, is infection. Infections in humans are brought on by pathogenic microorganisms like *Staphylococcus aureus*. Treatment with antibiotics is a technique that is frequently used to stop and treat bacterial infections on the skin (Hidayah *et al*., 2016) [7]. Penicillin is an antibiotic frequently used to treat bacterial infections brought on by the *Staphylococcus aureus* bacteria. 2011 (Permenkes). Garba *et al*. (2017) [4] reported that the penicillin group encounters resistance nowadays.

Primary and secondary metabolites are both present in *Sargassum polycystum*. Along with its primary components, seaweed also contains secondary metabolites that may be bioactive, such as flavonoids, alkaloids, saponins, steroids, and triterpenoids that have antibacterial, antiviral, antifungal, and cytostatic properties. (Kusumaningrum *et al*., 2007) [9].

Following an *in vitro* investigation, it was discovered that the brown algae extract from *Sargassum* sp. has the capacity to prevent the maximal growth of a number of pathogenic bacteria, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella* typhi and *E.coli* (Alamsjah, 2011) [1]. There have been numerous research done to evaluate antibacterial activity. The presence of secondary metabolite molecules in a natural material affects its antibacterial effectiveness (Alfiyaturohmah, 2014) [2].

There has been a lot of investigation into the antibacterial properties of *Sargassum polycystum*. The brown algae *Sargassum polycystum* C. Agardh was used in the study by Enida (2011) [3] to investigate the antibacterial activity of n-hexane extract, ethyl acetate, and ethanol against *Staphylococcus aureus* bacteria by diffusion agar. According to the study's findings, brown algae ethyl acetate extract can prevent the growth of *S. aureus* bacteria at a concentration of 70 mg/mL (14.78 mm), while minimum inhibitory concentration of *S. aureus* is present at a concentration of 30 mg/mL (8.14) mm (Hanapi, 2013) [5].

Research by Widowati *et al*. (2014) [15] demonstrated that *Sargassum polycystum* ethyl acetate extract can offer antibacterial activity against *Staphylococcus aureus* bacteria distinguished by the existence of a inhibition zone by using a gradual maseration method. Another study by Khoiriyah *et al*. (2014) [8] demonstrated that the ethyl acetate fraction of *Sargassum vulgare* can inhibit the growth of the bacteria. This study used the disc method to test the antibacterial activity of the *Sargassum vulgare* ethyl acetate fraction against the *Staphylococcus aureus* bacterium.

Staphylococcus aureus from *Sargassum polycystum* is tested for antibacterial activity using the diffusion method with the well diffusion method, which can be detected by the presence of a inhibition zone. *Staphylococcus aureus* can be used as a positive control, that uses chloramphenicol, to determine how well the ethyl acetate component of *Sargassum polycystum* inhibits the development of bacterium. Chloramphenicol is susceptible to the bacterium *Staphylococcus aureus* (Garba *et al*., 2017) [4]. The mechanism by which chloramphenicol inhibits bacterial activity is the same as that of a steroid found in the secondary metabolite of *Sargassum polycystum*.

Based on the information provided above, the study's objective is to use the well diffusion method to assess the antibacterial activity of the ethyl acetate fraction of *Sargassum polycystum* against the bacteria *Staphylococcus aureus*. This research is expected to add information regarding the antibacterial activity of Sargassum polycystum against *Staphylococcus aureus*.

Materials and method

Materials

Sargassum polycystum from Sumenep Island, aquadest, ethanol 96%, n-hexane (Merck, Germany), ethyl acetate (Merck, Germany), *Staphylococcus aureus* ATCC 25923 (Balai Besar Laboratorium Kesehatan, Indonesia), nutrient agar, chloramphenicol (Merck, Germany)

Methods

Sample extract preparation

The maseration method is used to extract *Sargassum polycystum* powder with 1:4 ratio using 96% ethanol. The maseration extraction repeated 3 times for 24 hours then use the rotary evaporator.

Sample fraction preparation

Three different solvents—aquadest, n-hexane, and ethyl acetate—are used during the fractionation procedure. Aquadest is used to first dilute the 96% ethanol extract when it is put into a separator funnel. As many times as necessary to produce a clear n-hexane phase, add the equal volume of n-hexana and shake strongly. Ethyl acetate is mixed with the aquadest fraction, strongly shaken, and repeated until a clear ethyl acetate is produced.

The concentrations of ethyl acetate fractions used are 0.2%; 0.4%; 0.8%; 1.2% and 2% (b/v)

Sterilization of equipment

The tools used in the study of this antibacterial activity were first sterilized. The glass tools are sterilized in the oven at a temperature of 170°C for ± 2 hours, the oxygen needles and pinsets are burned with direct burning and the media are sterilised in an autoclave at 121 °C for 15 minutes.

Bacteria media nutrition agar (NA)

Using beaker glass, dissolve 0.46 grams of nutrition agar (NA) in 20 mL of aquadest. The mixture is then stirred until smooth using a stirrer placed over a water boiler. Each of sterile reaction tubes is filled with a total of 5 mL and covered with aluminum foil. The base layer and the seed layer are the two layers used in the activity test using nutritional agar (NA). The base layer is created by weighing Nutrient Agar (NA) at 2.3 grams and using the Erlenmeyer method to dissolve it in 100 ml of water (23 g/1000 ml). 5.75 grams of NA are weighed out and then dissolved in 250 ml of aquade (23 g/1000) to create the seed layer.

Preparation of *Staphylococcus aureus*

(Misna *et al*., 2016) [11] To make a suspension solution of *Staphylococcus aureus* bacteria, 1 bacterial was inserted into a reaction tube containing 10 ml of a 0.9% physiological NaCl solution, with pure *Staphylococcus aureus* within the reaction tubes matched to homogeneous, then equated to the McFarland standard.

Antibacterial activity test

Staphylococcus aureus suspension is inserted using a cotton swap into the surface of the medium until well is produced on the medium to test the antibacterial activity. A 20 μL fraction of *Sargassum polycystum*, is used to fill the wells with ethyl acetate in the following concentrations: 0.2%, 0.4%, 0.8%, 1.2%, and 2% (b/v). Ethyl acetate is used for negative control and 0,1% chloramphenicol solution is used for positive control. After 24 hours of incubation at 37°C, it was monitored and measured to see if a inhibition zone had formed.

Results and discussion

The result of extraction and fractionation process was shown in Table 1 and Table 2.

Table 1: Extraction yield of *Sargassum polycystum*

Name	Dry Powder (g)	Extract (g)	Yield Value (%)
Sargassum polycystum	7000	219,1503	3,1307

Table 2: Fractination yield of *Sargassum polycystum*

Name	Extract (g)	Fraction(g)	Yield Value (%)
Sargassum polycystum	89,9034	2,2929	2,5504

A low yield percentage indicates the bioactive components contained in it are also low (Lantah *et al.*, 2017) [10]. The amount of fraction by the ethyl acetate solvent has a relatively small amount of yield. This is possible by the minimum amount of semi-polar content that can be taken by ethyl acetate. (Pratiwi *et al*., 2016) [14].

Phytochemical screening was performed in this study to determine secondary metabolite are present in *Sargassum polycystum.* The result study show that Ethyl acetate fraction of *Sargassum polycystum* contains steroid. Research conducted by Khoiriyah *et al*. (2014) [8], which found that the fraction of ethyl acetate *Sargassum vulgare* had steroid content with antibacterial activity against the growth of *Staphylococcus aureus* and *Escherichia coli*. In addition to being able to remove some of the hydrophilic components from the aquatic phase, ethyl acetate can be used to remove lipophilic components from the water phase (Hardiningtyas *et al*., 2014) [6].

Table 3: Inhibition zone ethyl acetate fraction of *Sargassum polycystum*

Concentration of fraction (% b/v)	Inhibition zone (mm)			Average ± SD (mm)	Interpretation
	Replication				
	1	2	3		
0,2	0,00	0,00	0,00	0 ± 0	Weak
0,4	0,00	0,00	0,00	0 ± 0	Weak
0,8	3,03	2,98	2,83	2,95 ± 0,11	Weak
1,2	3,77	2,73	2,03	2,84 ± 0,88	Weak
2,0	5,10	2,03	2,27	3,13 ± 1,71	Weak
Positive Control Chloramphenicol 0,1% (b/v)	36,98	34,30	32,10	34,46 ± 2,44	Very Strong
Negative control	0,00	0,00	0,00	0 ± 0	Weak

The presence of a clear zone surrounding the well serves as evidence that this approach contains antibacterial activity. Because the active compounds interact not only on the upper surface of the nutrients but also at the bottom, diffusion has the benefit that it is simpler to assess the area of the inhibition zone that is generated.

Concentrations of 0.8%; 1.2% and 2% indicated antibacterial activity characterized by the presence of a inhibition zone

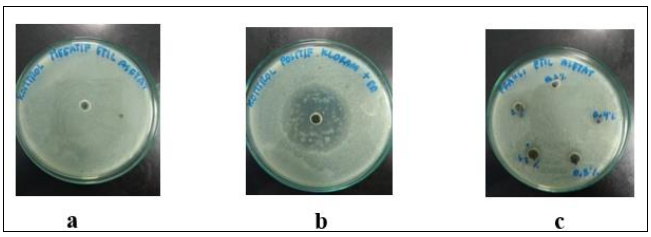

Fig 1: (a). Negative control (b) Positive control of Chloramphenicol (c) Ethyl acetate fraction of *S.polycystum*

Ethyl acetate, the solvent, is employed as the negative control. The diameter of the negative control inhibition zone is measured to be 0 mm, proving that ethyl acetate has no effect on *Staphylococcus aureus* bacteria and that the formed fraction's barrier area is made entirely of the metabolite compounds present in the fraction and is unaffected by the solvent used. Chloramfenicol 0.1% is the positive control substance. Chloramphenicol is susceptible to the bacterium *Staphylococcus aureus*, claim Garba *et al*. (2017) [4]. By blocking the extension of the protein chain by reducing the activity of the peptidyl transferase enzyme on the bacterial ribosome, the antibacterial drug chloramphenicol prevents the development of bacteria.

The secondary metabolite compound contained in the ethyl acetate fraction of *Sargassum polycystum* is a steroid. Steroids have a mechanism to inhibit bacteria by damaging bacterial cell membranes by increasing cell permeability, resulting in cell leakage followed by the exit of intercellular material. (Pramesti *et al*., 2017) [13]. Steroid secondary metabolite compounds are thought to be responsible for antibacterial activity against *Staphylococcus aureus* bacteria (Pramesti *et al*., 2017) [13]. Steroid compounds are thought to be responsible for antibacterial activity against *Staphylococcus aureus* bacteria.

Steroid substances interact with the phospholipids in bacterial cell membranes because of their lipophilic structure. Membrane integrity decreases and membrane morphology changes as a result of this process, resulting in fragile membranes and lysis cells (Pramesti *et al*., 2017) [13]. Since chloramphenicol's hydroxyl group and the hydroxyl group in the chemical structure of the steroid are similar, it is thought that the hydroxil group is what gives the steroid its capacity to stop *Staphylococcus aureus* from growing.

Conclusion

The ethyl acetate fraction of *Sargassum polycystum* has weak antibacterial activity against *Staphylococcus aureus ATCC* 25923

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