Effectiveness of iodine derived from *Eucheuma spinosum* against the bacteria *Staphylococcus aureus* and *Salmonella* sp.

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Abstrak

Rumput laut (*Eucheuma spinosum*) adalah sumber utama karagenan, selain itu juga mengandung iodium yang dapat menghambat aktivitas dan pertumbuhan bakteri. Iodium yang terkandung di dalam rumput laut merupakan hasil interaksi dengan lingkungan laut yang kaya iodium. Penelitian dilaksanakan mulai bulan Juni sampai 2022, dengan menggunakan iodium hasil ekstraksi rumput laut untuk bahan uji aktivitas antibakteri (*Staphylococcus aureus* dan *Salmonella* sp). Uji aktivitas antibakteri dilakukan selama 48 jam. Konsentrasi iodium diketahui dengan menggunakan metode titrasi, sedangkan uji aktivitas antibakteri menggunakan metode *Disc Diffusion Test.* Hasil penelitian menunjukkan bahwa ekstrak *Eucheuma spinosum* mengandung iodium 0,38% - 1,22%. Hasil uji aktivitas antibakteri iodium rumput laut dengan konsentrasi 1,22% menunjukkan rata-rata diameter zona hambat 11,5 mm (*Staphylococcus aureus*), dan 12 mm (*Salmonella* sp.). Hasil penelitian membuktikan bahwa iodium yang berasal dari ekstrak *Eucheuma spinosum*, mempunyai daya hambat yang kuat terhadap aktivitas dan pertumbuhan bakteri *Staphylococcus aureus* dan *Salmonella* sp.

Kata kunci: *antibakteri, Eucheuma spinosum, iodium, rendemen, zona hambat*

Abstract

Seaweed is the primary source of carrageenan, but it also contains iodine, which can inhibit bacterial activity and growth. The iodine contained in seaweed results from interaction with the marine environment rich in iodine. The research was conducted from June to November 2022 and used iodine from seaweed extraction to test antibacterial activity (Staphylococcus aureus and Salmonella sp). The antibacterial activity test was carried out for 48 hours. The iodine concentration was determined using titration, while the antibacterial activity test used the Disc Diffusion Test. The research showed that Eucheuma spinosum extract contained iodine, 0.38% - 1.22%. The antibacterial activity of seaweed iodine test results revealed an average inhibition zone diameter of 11.5 mm for Staphylococcus aureus and 12 mm for Salmonella sp. This research shows that the iodine in Eucheuma spinosum extract has an inhibitory solid impact on the activity and growth of Staphylococcus aureus and Salmonella sp.

Keywords: *antibacteria, Eucheuma spinosum, iodine, inhibition zone, rendement*

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Introduction

Seaweed is one of Indonesia's mainstay seafood commodities. Seaweed cultivation has excellent opportunities for the economic development of coastal communities, and *Eucheuma* sp. is a seaweed widely cultivated in Madura. (Fatmawati & Wahyudi 2016). It was noted that the potential land for seaweed cultivation is 16,420 ha, and only 372 ha (2.27%) has been utilized. Due to the high demand for industrial raw materials, seaweed cultivation continues to grow today (Priono, 2016). Seaweed *Eucheuma* sp. is much needed for industry because it has a relatively high carrageenan content.

Another ingredient in *Eucheuma* sp. is antibacterial compounds, which can be obtained from bioactive compounds through extraction. (Akib *et al.* 2019; Yusvantika *et al.* 2022). According to research Santika *et al.* (2019), *Eucheuma* sp. it contains flavonoids that function as antibacterials against *Staphylococcus aureus* and *Salmonella* sp.

This is the basis for characterizing antibacterial compounds as raw materials for alcohol-free disinfectants. Research result Damongilala *et al.* (2021), found that 100 grams of *Eucheuma* sp contained an iodine content of 409.35 ppm (0.0409%). Iodine has proven to be very effective in small-scale air disinfection processes. Two drops of iodine (0.1 ml) in ethanol solution effectively disinfect 1 liter of clear water. Natural iodine compounds compared to synthetic iodine are relatively stable, have a long shelf life, can effectively kill almost all types of bacteria and viruses, besides that iodine is also non-corrosive and easily dispersed. Based on the advantages of iodine, it is very feasible to use *Eucheuma spinosum* as a new source of natural disinfectant obtained from Madura's marine biological resources.

The use of *Eucheuma spinosum* refers to the results of previous research that mostly used other seaweed species. Kereh *et al.* (2018), tested the inhibitory power of *Sargassum* sp. extract. (*S. echinocarpum*, *S. duplicatum* and *S. polycystum*) taken from Jepara waters against *E. coli* and *S. aureus*. Study Maduriana & Sudira (2009), found differences in the diameter of inhibitory power in Gram-positive and Gram-negative bacteria which are closely related to the cell wall structure of the bacteria. This is based on the results of tests on the inhibitory power of *Eucheuma cottoni* crude extract against the pathogenic bacteria *Staphylococcus aureus* and *Salmonella* sp. *Eucheuma cottoni* crude extract was able to inhibit the growth of test bacteria at concentrations of 0.1% and 0.5%. The largest inhibitory diameter was found in *Staphylococcus aureus* colonies (10.5 mm), while a concentration of 1.5% produced an inhibitory diameter in *Salmonella typhi* colonies of 8.75 mm. Research result Fattah *et al.* (2013) they have proved that crude extract of *Eucheuma spinosum* could inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. The results of the research above demonstrate that natural seaweed extract can inhibit bacterial growth, so this research focuses more on the ability of iodine as an antibacterial, which was tested on *Staphylococcus aureus* and *Salmonella* sp..

Methods

From June to November of 2022, the study was carried out at Trunojoyo University's Basic Sciences Laboratory in Madura. Seaweed (*Eucheuma spinosum*) is harvested from the seaweed growing center located in Saronggi District, Sumenep Regency, using raw materials.

Tools used include blender, spray bottle, bunsen, burette, petri dish, glass funnel, erlenmeyer, beaker, measuring cup, scissors, hot plate, digital caliper, net, oce needle, filter paper, measuring flask, oven, tweezers, dropper pipette, sanoclave, orbital rotator shaker, spatula, spreader glass, test tube, and digital scale. The materials used are 70% alcohol, aluminum foil, starch, distilled water, Chloroform ($CHCl_3$), bacteria (*Staphylococcus aureus* and *Salmonella* sp.), seaweed (*Eucheuma spinosum*), opaque paper, label paper, iodine solution, NA (Nutrient Agar), Sodium Chloride (NaCl), Sodium Thiosulfate ($Na_2S_2O_3$), paper disc, plastic warp, spirtus, and Yodor Vex.

The research began by washing *Eucheuma spinosum* to remove lime and then drying it in the sun to dry. Extraction was done by first grinding 50 g using a blender, followed by maceration using 100 ml chloroform in a ratio of 1:2 (w/v). During the maceration process, the sample

was shaken using an orbital rotary shaker for 72 hours at a speed of 120 rpm. The resulting solution is then filtered and heated to separate the pure extract from the solvent.

The iodine concentration was then calculated using titration, with 1% starch solution and 0.1N $Na_2S_2O_3$ solution. The solution was made by weighing 6.25 g of $Na_2S_2O_3$ and adding 100 ml of distilled water. Pour into a measuring flask and store in a dark place. Add 1 ml of 0.1N iodine to the homogeneous solution, then add 1% starch until the color changes to brownish yellow + 5 drops. According Slamet & Bambang (2002), measurement of iodine concentration using titration is calculated using the following formula:

$$Num.Iodine = \frac{vol.titration\ (blank\ liquid - sample)}{vol.sample} \times thiosulphate\ concentration \times 12.691$$

$$Iodine\ concentration\ \frac{Num.\ iodine}{Dry\ sample\ weight} \times 100\%$$

Bacterial culture media was made by weighing 0.42 mg NA in 15 ml of distilled water at a rate of 28 g per 1000 ml, then putting it in an Erlenmeyer flask tightly covered with aluminum foil so that the NA did not spill (Sakul *et al.* 2020). The NA media was then sterilized at 121ºC for 30 minutes, and then poured into 5 ml test tubes. Na media production is calculated using the following dilution formula:

$$V_1 \text{ x } M_1 = V_2 \text{ x } M_2$$

Test of the inhibitory power of *Eucheuma spinosum* iodine against *Staphylococcus aureus* and *Salmonella* sp compared with the control solution (yodor vex). Mattulada *et al.* (2018) says observations of the activity of bacteria treated with iodine were carried out for 24 hours at a temperature of 37ºC. Haris *et al.* (2013) says calculation of resistance using the following formula:

$$Barrier\ Zone\ Diameter = \frac{(D1-Dp)+(D2-Dp)}{2}$$

Results and discussion

Extract rendement *Eucheuma spinosum*

The rendement of *Eucheuma spinosum* extract is the result of the extraction process using chloroform solvent (Table 1). Based on research by Maligan *et al.* (2015), the chloroform solvent was chosen because the chloroform fraction was greater than the yield of other fractions. The yield results indicate that the content of semipolar compounds is greater than polar and nonpolar compounds.

Table 1. Yield of crude extract of *Eucheuma spinosum*

Numb.	Name	Unit
1	Dry weight *Eucheuma spinosum*	50 g
2	Extraction results	23.5 g
3	Rendement	47 %

(Source: Researcher data, 2022)

Table 1 shows that the results of maceration for 72 hours with chloroform solvent obtained a crude extract with a yield of 47%. The chloroform solvent used was 100 ml with a ratio of 1:2 (w/v) (Dhanraj *et al.* 2009). The extraction method, solvent used, solvent ratio, temperature, and length of maceration time influence the high yield value. The lower temperature will cause a decrease in the yield value. The yield value is high if the temperature during evaporation is constant at 40ºC (Shofikha, 2017). According to research by Mardiyah *et al.* (2014), every 50 g of seaweed can produce 9.17% crude extract if using ethanol as a solvent. Referring to the research results above, the yield value of the crude extract produced in this research is relatively high.

Iodine levels

Following the extraction of the crude extract, filter paper is used to separate the crude fiber from iodine, resulting in the wet weight. The titration method was used to conduct the iodine concentration test in this study, and the test's findings are shown in Table 2.

Table 2. Results of iodine concentration calculation

Numb.	Name	Results
1	Colour changes after adding the blank liquid	4.8 ml
2	Num. Iodine	0.61
3	Iodine level	1.22 %

(Source: Researcher data, 2022)

The test results as shown in Table 2 show that the color change occurred after adding 4.8 ml of blank liquid. The color change occurs from brownish yellow to clear or colorless. According to research by Linda (2018), red seaweed contains iodine at a concentration of 0.1-1%. Based on this range, it shows that the test results in this study (1.22%) were higher than in previous studies. Iodine is produced after the evaporation process. Agustin & Ismiyati (2015) says evaporation is the process of separating the solvent from the pure extract. Research using evaporation treatment using an oven at 40ºC has been proven to produce relatively high iodine concentrations.

Iodine has polar properties, so that it can dissolve well in chloroform. According to research by Hildianti (2016), stirring for a long time during maceration causes an increase in osmosis between the extracted material and the solvent. This causes dissolution into the *Eucheuma spinosum* tissue through a screen effect to occur optimally so that the cell glands produce a high concentration of iodine.

Antibacterial activity test for *Staphylococcus aureus* and *Salmonella* sp.

By measuring the clear zone that developed around the papare disk, which demonstrated that there was inhibitory activity against bacterial growth, the antibacterial activity test findings were determined (Figure 1)

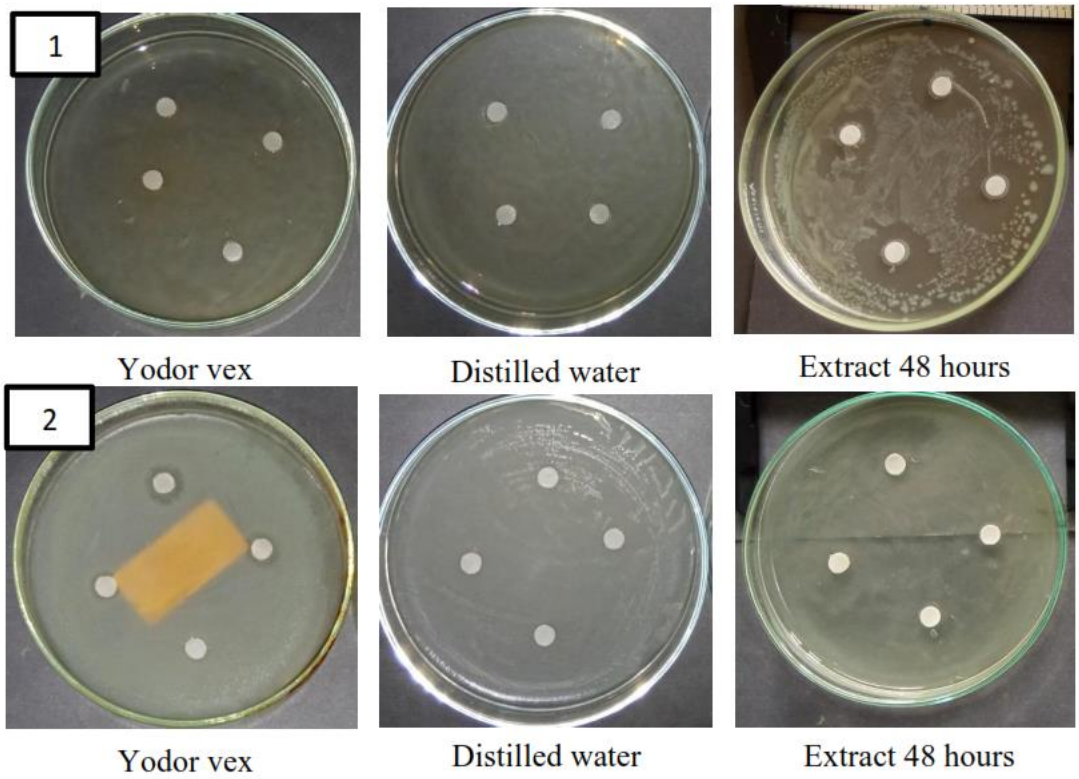

Figure 1. Antibacterial activity test results: 1 *Staphylococcus aureus* dan 2 (*Salmonella* sp.).

The clear zone seen in Figure 1 is evidence of iodine's ability to inhibit the growth of *Staphylococcus aureus* and *Salmonella* sp. bacteria. The diameter of the clear zone formed was then measured using a digital caliper, and the results are presented in Table 3. The measurement results showed that the positive control (yodor vex) and negative control (Distilled water) were included in the category of having weak inhibitory power, while iodine from *Eucheuma spinosum* was included in the strong category in the inhibition test for the bacteria *Staphylococcus aureus* and *Salmonella* sp.

The test was carried out by diffusing 10µl of the extract. The antibacterial activity test of *Eucheuma spinosum* against *Staphylococcus aureus* based on research by Serment *et al.* (1970), showed the average diameter of the bacterial inhibition zone was 4.00 mm (200µg=0.2 µl), which was included in the weak category. The research results of Rostinawati *et al.* (2017) stated that the antibacterial activity test on *Salmonella* sp. with a concentration of 125,000 µl produced an average diameter of the bacterial inhibition zone of 15.20 mm.

Table 3. Results of resistance diameter measuring

Bacteria	Treatment	Repetition				Avarage (mm)	Category
		1	2	3	4		
Staphylococcus aureus	Yodor vex	6.35	3.9	2.8	4	3.9	weak
	Distilled water	0.00	0.3	0.75	0.10	0.3	weak
	Extract oven 48 hours	8.50	11.5	11.5	9.05	11.5	strong
Salmonela sp.	Yodor vex	1.80	1.05	0.65	1.65	1.3	weak
	Distilled water	0.10	0.10	0.00	0.00	0.0	weak
	Extract oven 48 hours	13.50	18.50	6.6	9.5	12	strong

(Source: Researcher data, 2022)

The results of this research succeeded in proving that iodine derived from *Eucheuma spinosum* has a strong inhibitory effect on the growth of *Staphylococcus aureus* and *Salmonella* sp. bacteria. The concentration of iodine contained in *Eucheuma spinosum* has the ability to break down bacterial cell walls so that the growth of pathogenic bacteria is inhibited.

Conclusion

The research results prove that iodine derived from *Eucheuma spinosum* extract has a strong inhibitory effect (11.5 mm) on the activity and growth of the bacteria *Staphylococcus aureus* and *Salmonella* sp.

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