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ANTIBACTERIAL ACTIVITY OF *Sargassum* sp. IN INHIBITING *Vibrio* sp. ISOLATED FROM CANTANG GROUPER (*Epinephelus fuscoguttatus x Epinephelus lanceolatus*)

Aktivitas Antibakteri *Sargassum* sp. Dalam Menghambat *Vibrio* sp. Terisolasi Dari Kerapu Cantang (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*)

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ABSTRACT

Treatment of vibriosis using natural ingredients such as *Sargassum* sp. extract in cantang grouper cultivation activities needs to be studied. Testing the effectiveness of solvents in producing phytochemical compounds as antibacterials and modifying the extraction process to make it simpler aims to be replicated by farmers in the field. The tested bacterial isolates were isolated directly from cantang grouper fish showing symptoms of vibriosis from fish farmers in Senggarang Besar, Tanjungpinang City. Testing the antibacterial activity of *Sargassum* sp. extract in inhibiting the growth of *Vibrio* sp. was carried out through an inhibition zone test using the paper disc diffusion method (Kirby-Bauer method). The results showed that the use of 70% alcohol solvent in the extraction process resulted in a clearer and better inhibition zone diameter of 13 mm at the highest treatment concentration (10%) compared to the extract using 96% alcohol solvent at the same concentration of 11.67 mm. It should be noted that the inhibition zone formed is classified as partial because it is not clear or completely clear due to the presence of thin bacterial growth in the zone even though the circle shape of the inhibition zone is quite clear. The use of 70% alcohol solvent can be used for the extraction process of *Sargassum* sp. considering that it is cheaper and the results are better than 96% alcohol based on the results shown through this study.

Keywords: Antibacterial, Cantang Grouper, Extraction, *Sargassum* sp., *Vibrio* sp.

ABSTRAK

Penanganan vibriosis menggunakan bahan alami seperti ekstrak *Sargassum* sp. pada kegiatan budidaya ikan kerapu cantang perlu diteliti. Pengujian efektifitas pelarut dalam menghasilkan senyawa fitokimia sebagai antibakeri dan modifikasi proses ekstraksi menjadi lebih sederhana bertujuan agar dapat ditiru oleh pembudidaya dilapangan. Isolat bakteri uji diisolasi langsung dari ikan yang memiliki gejala vibriosis dari ikan kerapu cantang pembudidaya di Senggarang Besar, Kota Tanjungpinang. Pengujian aktivitas antibakteri dari ekstrak *Sargassum* sp. dalam menghambat pertumbuhan *Vibrio* sp. dilakukan melalui uji zona hambat metode difusi kertas cakram (metode Kirby-Bauer). Hasil penelitian menunjukkan penggunaan pelarut alkohol 70% pada proses ektraksi memberikan hasil diameter zona hambat yang jernih dan lebih baik yaitu 13 mm pada konsentrasi tertinggi dari perlakuan (10%) dibandingkan dengan ekstrak yang menggunakan pelarut alkohol 96% pada konsentrasi yang sama yaitu 11.67 mm dengan catatan zona hambat yang terbentuk tergolong parsial karena tidak jernih atau bening sempurna akibat masih terdapat pertumbuhan bakteri yang tipis di dalam zona meskipun bentuk lingkaran zona hambat terlihat cukup jelas. Penggunaan pelarut alkohol 70% dapat digunakan untuk proses ekstraksi Sargassum sp. dengan pertimbang harga yang lebih murah dan hasil yang lebih baik dibandingkan alkohol 96% berdasarkan hasil yang ditunjukkan melalui penelitian ini.

Kata Kunci: Antibakteri, Ekstraksi, Kerapu Cantang, *Sargassum* sp., *Vibrio* sp.

INTRODUCTION

The cultivation of cantang grouper (Epinephelus fuscoguttatus × Epinephelus lanceolatus) is highly sought after by fish farmers in the Riau Islands. This fish commands a high price when live and is marketed to Singapore, Malaysia, and Hong Kong. Buying vessels legally transport the fish directly to large holding facilities in Natuna and the Anambas Islands (Husin et al., 2023). In 2026, live fish sales from Natuna to Hong Kong reached 1.2 billion, dominated by grouper (Sirait and Rahmawati, 2026). Although the waters in the Riau Islands are very conducive to cantang grouper cultivation, this fish farming activity is not without problems, such as disease outbreaks.

Vibriosis is one disease that frequently affects cantang grouper (Mohamad et al., 2019; Putra et al., 2025). This disease also frequently affects crustaceans and shellfish. In grouper, this disease is caused by bacteria from the Vibrio genus, such as Vibrio alginolyticus. Infected fish exhibit symptoms such as red spots on the body and fins. Furthermore, blood-red clots appear in the oral cavity (Figure 1), which are frequently identified in cantang grouper farmed in Madong Village and Senggarang Besar, Tanjungpinang City. Isolation results showed orange vibrio growth on TCBS (Thiosulfate-Citrate-Bile Salts-Sucrose) media. This condition is known as a silent killer because the fish's external appearance appears normal, but the mouth cavity is covered in red clots, which cause the fish to lose their appetite and ultimately die, often undetected by the farmer.

Deaths due to disease can be detrimental to farmers if mortality is high. Treating the disease using effective, inexpensive, and readily available natural ingredients is highly recommended. This is because using natural ingredients is more environmentally friendly and has no side effects. Meanwhile, long-term, unregulated use of synthetic antibiotics can trigger resistance in the pathogen. Sargassum sp. is a readily available brown seaweed. During certain seasons, large numbers of Sargassum sp. are stranded on beaches. Sargassum sp. is known locally as "Rengkam" by the Malay people of the Riau Islands. Several studies have shown that extracting Sargassum sp. can extract phytochemical compounds that can function as natural antibacterials, such as saponins, tannins, phenols, alkaloids, and flavonoids (Harharah et al., 2021; Akbar et al., 2022; Sari et al., 2024; Afrin et al., 2025).

Sargassum sp.'s antibacterial activity has been tested against several types of bacteria, including Vibrio harveyi (Naina et al., 2019), Staphylococcus epidermidis (Harharah et al.,

2021), Staphylococcus aureus (Widowaty, 2018; Akbar et al., 2022), and Escherichia coli (Sari et al., 2024). Even Sargassum sp. extract using 96% ethanol solvent has been tested through feed to observe the phagocytic activity of white snapper (Lates calcarifer) infected with Vibrio alginolyticus (Azuwarita et al., 2021). Not only for fish health, Sargassum sp. flour has also been studied for the growth of various groupers (Annisa et al., 2018). Although the use of Sargassum sp. extract as an antibacterial to treat Vibrio sp. has been recorded. In this study, the antibacterial activity of Sargassum sp. focused on observing the results of extraction using different types of solvents and modifying the Sargassum sp. preparation process that is more applicable for imitation at the farmer level. This study compared the results of extraction using 96% alcohol solvents with 70% alcohol through an economic approach, such as price and accessibility to obtain solvents in the Riau Islands region, as well as a simpler extract preparation method that does not require sophisticated equipment. In addition, the test bacteria used in this study were Vibrio sp. isolated directly from farmed fish that have symptoms of vibriosis.

(a) (b)

Figure 1. Red lumps in the mouth cavity of the cantang grouper (in a dead condition) which is cultivated in Madong Village (a) and Senggarang Besar (b) Tanjungpinang City (Source: Personal Documentation, 2026)

RESEARCH METHODS

This research was conducted in July 2026. Sargassum sp. was obtained from the shores of Tanjung Siambang, Tanjungpinang City, Riau Islands. Vibrio sp. bacterial isolates were obtained from the isolation of red lumps in the oral cavity of cantang grouper fish owned by farmers in Senggarang Besar. The process of preparing Sargassum sp. extracts and antibacterial tests were carried out at the Marine Aquaculture Laboratory, Faculty of Marine Sciences and Fisheries, Raja Ali Haji Maritime University. The stages of activity in this research are as follows:

1. Preparation of Sargassum sp. Extract

The obtained Sargassum sp. was thoroughly washed using running water to remove any adhering sand and dirt. It was then immediately drained by hanging it on a wooden pole, away from direct sunlight, for approximately 2 hours, until no water dripped. To expedite the drying process, the Sargassum sp. was oven-dried at 50°C for 15 hours, resulting in a semi-dry state. This process used 500 grams of drained Sargassum sp., yielding 116 grams of semi-dry Sargassum sp. Without an oven, drying takes 3-4 days without direct sunlight. The 50 grams of semi-dry Sargassum sp. was then roughly chopped with scissors and blended in a blender until approximately 45 grams were obtained.

The extraction process used a maceration method with 96% and 70% alcohol solvents. A total of 20 grams of coarsely chopped Sargassum sp. was put into an Erlenmeyer flask and then added with 200 ml of each solvent (ratio 1:10 (w/v)). After stirring and mixing thoroughly, the Erlenmeyer flask was tightly closed using aluminum foil. Next, store the

Erlenmeyer flask in a place that is not exposed to light for 2 days and shake it occasionally (approximately every 6 hours) so that the coarsely chopped Sargassum sp. is mixed with the solvent. After maceration for 2 days, it was filtered using a cloth filter and squeezed until no solvent drips. Next, the filtered results were placed in a petri dish to accelerate the alcohol evaporation process at room temperature. In this process, the petri dish was also stored in a place that was not exposed to light. This process takes approximately 18 hours for the alcohol to evaporate, which is indicated by the absence of a pungent alcohol odor. Under these conditions, a thick extract was formed in the petri dish which was then collected using an iron spatula and stored in a dark glass bottle to become a stock of Sargassum sp. extract in the next antibacterial test.

Figure 2. Preparation process for coarsely chopped semi-dry Sargassum sp. and maceration using 96% alcohol (greenish liquid) and 70% alcohol (brown liquid), as well as the alcohol evaporation process using a Petri dish.
(Source: Personal Documentation, 2026)

2. Agar Media Preparation

The agar media used in this study consisted of TCBS (Thiosulfate-Citrate-Bile Salts-Sucrose) media for isolating test bacteria from fish that had symptoms of vibriosis. NA (Nutrient Agar) media was added with 2% pure NaCl for the antibacterial test using the disc paper diffusion method (Kirby-Bauer method). Both were prepared into sterile petri dishes.

3. Isolation of Vibrio sp. Bacteria

A sterile loop was applied to the surface of the red lumps found in the oral cavity of the cantang grouper. The loop was then dipped and shaken in a sample bottle containing 10 ml of 0.9% NaCl solution. Then, 100 µl was taken using a microtip and micropipette and dripped onto the surface of the TCBS medium, then spread aseptically using a triangular glass rod. After that, the incubation was carried out at room temperature for 20 hours. The growing bacterial colonies were then transferred to previously prepared NA media as a test bacterial stock.

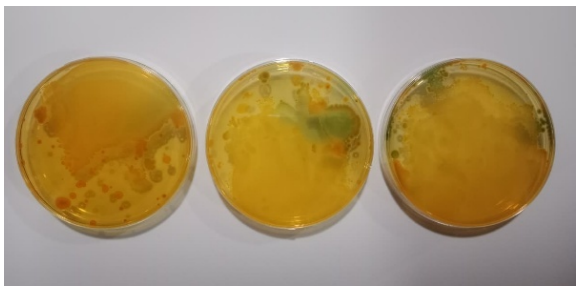

Figure 3. Results of isolating red lumps found in the oral cavity of cantang grouper fish on TCBS media (Source: Personal Documentation, 2026)

4. Antibacterial Test of Sargassum sp. Extract

The Sargassum sp. extracts to be tested had concentrations of 10%, 5%, and 2.5%. Each viscous extract was taken as 0.2 grams and dissolved in 2 ml of 96% and 70% alcohol, respectively, separately in glass bottles. Serial dilutions were then performed by transferring 1 ml of each extract to another bottle containing 1 ml of 96% or 70% alcohol to obtain a 5% concentration, followed by further dilution to obtain a 2.5% concentration. The paper discs used were Whatman No. 1 paper, cut into small pieces using a hole punch to obtain a paper diameter of 0.6 mm. The paper discs were then stored in glass petri dishes, wrapped in paper, and then sterilized in an autoclave and oven at 50°C overnight until dry to ensure sterility for use in the antibacterial test of the Sargassum sp. extract.

Preparation of bacterial suspensions for the antibacterial test was carried out by dissolving several bacterial colonies growing on NA media in 10 ml of 0.9% NaCl solution. Shake using a vortex until dissolved and form a turbidity equivalent to a 0.5 McFarland solution. After that, take 100 µl using a microtip and micropipette, drop it on the surface of the NA media, then spread it using a triangular glass rod aseptically and wait until the agar surface is dry. Sterile disc paper is taken using sterile tweezers and placed on an empty, sterile petri dish to be dripped with Sargassum sp. extract according to the prepared test dose of 10 µl. Next, wait until it is completely absorbed, does not drip, and does not smell of alcohol, then stick it on the surface of the NA agar that has been spread with bacteria previously. This test was carried out three times. Then the petri dish was incubated at room temperature for 18-20 hours. Antibacterial activity was observed through the formation of an inhibition zone or clear zone that formed around the disc paper.

5. Data Analysis

The diameter of the inhibition zone formed was measured using a ruler. The data were tabulated using Microsoft Excel and presented in tabular form. The data were analyzed descriptively based on the level categories according to Davis & Stout (1971).

RESULT

The antibacterial activity test results of Sargassum sp. extracts extracted using 96% and 70% alcohol solvents can be seen from the diameter of the inhibition zone formed by the Kirby-Bauer disc diffusion method, as presented in Table 1 and Figure 2. The treatment at the highest concentration showed the best results compared to treatments at lower concentrations. The best treatment was found with the extraction using 70% alcohol solvent at a concentration of 10%.

Table 1. Diameter of the inhibition zone in the antibacterial test of Sargassum sp. extract against Vibrio sp.

Concentration	Replication	Inhibition Zone (mm)	
		Alkohol 96%	Alkohol 70%
10%	1	12.00	13.00
	2	12.00	12.00
	3	11.00	14.00
	Average	**11.67***	**13.00**
5%	1	11.00	9.00
	2	12.00	8.00
	3	11.00	9.00
	Average	**11.33***	**8.67**
2.5%	1	13.00	8.00
	2	11.00	8.00
	3	12.00	9.00
	Average	**12.00***	**8.33**

Control Positif (Alkohol)	1	7.00	6.00
	2	7.00	7.00
	3	8.00	7.00
	Average	**7.33**	**6.67**

Description: * partial inhibition zone is not perfectly clear.

The diameter of the inhibition zone formed in the antibacterial test using the disc paper diffusion method (Kirby-Bauer method) in this study showed perfectly clear results in the Sargassum sp. extract using 70% alcohol solvent. Meanwhile, the extract using 96% alcohol solvent was not very clear because there was still thin bacterial growth in the inhibition zone.

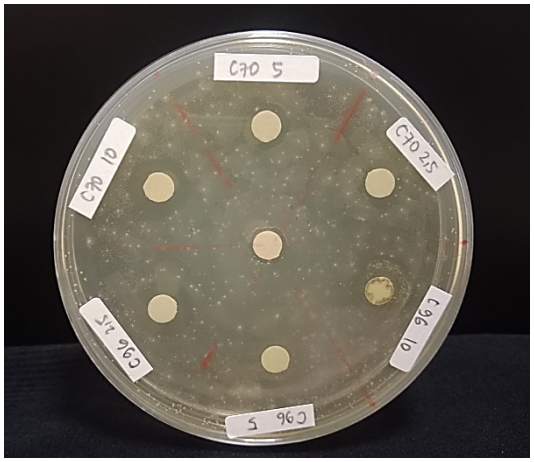

Figure 4. Inhibition zone in the antibacterial test of Sargassum sp. extract against Vibrio sp. in vitro. Caption: C: Sargassum sp. shreds; 70 and 96: Alcohol concentration; 2.5, 5, and 10: Sargassum sp. extract concentration (Source: Personal Documentation, 2026)

DISCUSSION

The red lumps in the mouth cavity of cantang grouper fish are caused by vibriosis, as evidenced by isolation results on TCBS media. The dominant growth of orange colonies indicates that this vibriosis is likely caused by the bacterium Vibrio alginolyticus. Vibrios that form orange colonies on TCBS media include Vibrio alginolyticus and Vibrio cholerae (Nepal et al., 2026). This possibility is also based on several reports indicating that vibriosis in cantang grouper fish is often caused by Vibrio alginolyticus (Dahlia et al., 2017).

The use of different solvents to extract coarsely chopped semi-dried Sargassum sp. yielded visually different results. The different solvents produced distinct colors, as seen in Figure 2. 96% alcohol produced a greenish liquid, while 70% alcohol produced a brownish one. Extract preparation using the maceration method is quite easy to replicate for fish farmers (Rismiyati et al., 2025). In terms of price, 96% alcohol is more expensive than 70% alcohol, so the effectiveness of the extract in inhibiting the growth of Vibrio sp. was tested through an antibacterial test as a basis for assessing the appropriate extraction solvent for producing phytochemical compounds with antibacterial activity. This test can be seen from the diameter of the inhibition zone formed using the paper disc diffusion method (Kirby-Bauer method), as shown in Figure 4.

The inhibition zone diameter appeared clearer and wider, reaching 13 mm in the Sargassum sp. extract using 70% alcohol at the highest extract concentration, 10%. In the extract using 96% alcohol, a fairly clear, circular zone of inhibition was observed for all concentrations, at 11-12 mm. However, thin bacterial growth was still visible within the zone, so the inhibition zone was not perfectly clear and was categorized as a partial inhibition zone.

Tests were also conducted with 96% and 70% alcohol, which also produced inhibition zones, but not as large as those in the treatment with Sargassum sp. extract. This demonstrates the size of the inhibition zone diameter formed by the influence of the phytochemical compounds that function as antibacterials. The diameter of the inhibition zone formed categorizes the active compounds from Sargassum sp. as strong, based on the Davis and Stout (1971) standard, because the inhibition zone diameter formed is ≥ 10 mm (Emelda et al., 2021; Rahmi et al., 2026).

Based on the results of this study, it can be seen that 70% alcohol solvent produces better inhibition zone results than 96% alcohol solvent in the antibacterial test using the paper disc diffusion method. The phytochemical compounds extracted during the extraction process are not due to the high alcohol concentration used. Rather, the polar and non-polar nature of the active compounds to be extracted determines the solvent used (Riskiana et al., 2012; Handayani et al., 2026). Several phytochemical compounds from Sargassum sp. that have antibacterial functions include tannins, saponins, phlorotannins (phenolic compounds typical of brown algae), and alkaloids. Tannins, phlorotannins, and alkaloids are readily soluble in water and are more effectively extracted using heating methods such as infusions or decoctions. However, extractions using water or distilled water as a solvent tend to be less durable and must be applied immediately. Therefore, the 30% water content of 70% alcohol significantly improves the extraction of antibacterial compounds from Sargassum sp. compared to 96% alcohol, which only contains 4% water.

The formation of an inhibition zone occurs in several stages. First, phytochemical compounds from the extract diffuse from the paper disc into the pores of the agar medium. Second, as the extract spreads, it interacts with the bacterial cell wall. Third, phytochemical compounds that function as antibacterials, such as tannins and saponins, begin to inhibit and even kill bacteria by damaging the cell wall, disrupting membrane permeability, denaturing proteins, and inhibiting nucleic acid synthesis. This mechanism causes cell leakage and bacterial death. Each active compound has a different target or method of inhibiting bacteria. Tannins have the ability to disrupt peptidoglycan formation, causing the cell wall to shrink and inactivating enzymes and bacterial cell adhesion proteins (Rinjani et al., 2025). Saponins can cause cell membrane instability and damage because they function as surfactants that reduce the surface tension of the cell membrane (Pangestuti et al., 2017). The imperfect circular shape of the inhibition zone can be caused by several factors, such as the paper disc being less than perfectly round and the disc's surface not being completely attached to the agar medium, resulting in uneven diffusion of the extract. Furthermore, the thickness and uneven surface of the agar medium will also affect the diffusion of the extract and the resulting inhibition zone.

CONCLUSION

Different solvents yielded different results in antibacterial tests of Sargassum sp. extract in inhibiting the growth of Vibrio sp. bacteria. 70% alcohol solvent tended to be better than 96% solvent. The diameter of the inhibition zone formed was ≥10 mm and was categorized as having strong inhibitory power. A simpler extract preparation method was still able to demonstrate the antibacterial activity of Sargassum sp., making it possible for farmers to replicate it in the field. Concentration toxicity testing on organisms such as artemia is needed in further research to ensure that the extract is not toxic when applied to fish.

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