BIODIVERSITAS
Volume 25, Number 8, August 2024
Pages: 2669-2681

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250840

Morpho-anatomical characteristics, phytochemical and antibacterial potential of *Sargassum polycystum* collected along the southern coast of Java, Indonesia

MARIA DYAH NUR MEINITA[1,2], RISKI YULIA[1], MUHAMMAD NURSID[3], NUNUK ARIES NURULITA[4], DICKY HARWANTO[5], RIVIANI[1], RIYANTI[1♥]

[1]Faculty of Fisheries and Marine Sciences, Universitas Jenderal Soedirman. Jl. Dr. Soeparno, Komplek GOR Soesilo Soedarman, Karangwangkal, North Purwokerto, Banyumas 53122, Central Java, Indonesia. Tel.: +62-281-6596700, ♥email: riyanti1907@unsoed.ac.id

[2]Magister of Agricultural Biotechnology Study Program, Graduate School, Universitas Jenderal Soedirman. Jl. Dr. Soeparno, Komplek GOR Soesilo Soedarman, Karangwangkal, North Purwokerto, Banyumas 53122, Central Java, Indonesia

[3]Research Center for Marine and Inland Waters Bioindustry, National Research and Innovation Agency. Malaka, Pemenang, North Lombok 83352, West Nusa Tenggara, Indonesia

[4]Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto. Jl. KH. Ahmad Dahlan, Banyumas 53182, Central Java, Indonesia

[5]Faculty of Fishery and Marine Science, Universitas Diponegoro. Jl. Prof. H. Soedarto, S.H., Tembalang, Semarang, 50275, Central Java, Indonesia

Manuscript received: 9 December 2024. Revision accepted: 19 August 2024.

Abstract. *Meinita MDN, Yulia R, Nursid M, Nurulita NA, Harwanto D, Riviani, Riyanti. 2024. Morpho-anatomical characteristics, phytochemical and antibacterial potential of* Sargassum polycystum *collected along the south coast of Java, Indonesia. Biodiversitas 25: 2669-2681.* The southern coast of Java is one of the coastal areas in Indonesia with a high abundance of seaweed. *Sargassum polycystum*, a brown marine macroalga that is abundant in southern coast of Java, contains high secondary metabolites and exhibits promising potential as an antibacterial agent. There is lack of comprehensive study on morpho-anatomical characteristic and bioactivity of *S. polycystum*. Hence, this study investigated the morpho-anatomical characteristics, phytochemical compound content, and antibacterial activity of *S. polycystum* collected from three different beaches along the southern coast of Java Island against pathogenic bacteria *Bacillus megaterium*, *Micrococcus luteus*, and *Escherichia coli*. Specimen collection was done using purposive random sampling along southern coast of Java Island, followed by descriptive exploratory of morpho-anatomical characteristic, extraction, phytochemical analysis and antibacterial activity assay. Maceration with methanol solvent during 48 hours was used for extraction. The phytochemical analysis employed a qualitative descriptive method, while the antibacterial activity was assessed using the disc diffusion method (Kirby-Bauer). The morpho-anatomical characteristics observed of *S. polycystum* include the characteristic of a rough-textured stipe, round tapered serrated blade, small disc-shaped holdfast, alternate pinnate branching, cell wall, vacuole and cytoplasm. The morphology and anatomy of *S. polycystum* particularly the thallus and blade structure are strongly impacted by the environmental condition of southern coast of Java. Phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, and steroids in *S. polycystum* extract. Antibacterial assay demonstrated a medium to strong antibacterial activity against *B. megaterium* with bacteriostatic properties, strong to very strong antibacterial activity against *M. luteus*, and weak antibacterial activity against *E. coli*. Future research is required to examine how environmental conditions affect the composition and concentration of phytochemical compounds in *S. polycystum.*

Keywords: Antibacterial, morphology, phytochemicals, *Sargassum polycystum*

INTRODUCTION

Pathogenic bacteria can cause harmful diseases in humans. Recently, numerous efforts have been made to prevent bacterial pathogenicity, with one approach involving the use of synthetic antibacterials exhibiting bacteriostatic and bactericidal activities. However, the continuous administration of antibiotics can lead to resistance in pathogenic bacteria. Genes produced by bacteria can confer protection against the inhibitory effects of antibiotics due to their adaptation to continuous exposure (Munita and Arias 2016). Serwecińska (2020) has stated that bacterial resistance to drugs arises from the persistent use of antibiotics for infection treatment. Davis and Stout (1971) distinguished antibacterial properties into two types: bacteriostatic and bactericidal. Bacteriostatic refers to a compound that inhibits bacterial growth, while bactericidal pertains to an antibacterial compound that can kill bacteria without allowing further growth. Additionally, the effectiveness of an antibacterial compound is influenced by the inherent resistance properties of bacteria; Gram-positive bacteria generally exhibit higher susceptibility than Gram-negative bacteria due to differences in their cell wall complexity (Arguelles 2022). Gram-positive bacteria possess cell walls with peptidoglycan, making them more susceptible to antibiotics, whereas Gram-negative bacteria have less peptidoglycan and an outer membrane with complex polysaccharides. The use of synthetic drugs can lead to side effects in users, prompting the World Health Organization (WHO) to recommend the use of natural antibacterial alternatives to inhibit the growth of pathogenic bacteria. Previous studies explored marine resources including seaweed, tunicate and sponge as source of novel antibacterial compounds (Ayuningrum et al. 2019; Oktaviani et al. 2019; Riyanti et al. 2020).

Seaweed is one of the most abundant marine resources worldwide as well as in Indonesia, containing compounds with unique structures and potential applications in various sectors, including pharmacology (Meinita et al. 2021a, 2022a, 2023). *Sargassum* seaweed contained bioactive compounds such as fucosterol, phlorotannin and phloroglucinol, which showed numerous biological activities (Meinita et al. 2021b; Harwanto et al. 2022). The bioactivity of *Sargassum* seaweed compounds as antibacterials has been widely reported, including *S. polycystum* (Kok and Wong 2022; Manguntungi et al. 2022; Thiurunavukkarau et al. 2022), *S. filipendula*, and *S. hystrix* (Morales et al. 2008), as well as *S. ilicifolium* (Aftabuddin et al. 2021). The antibacterial activity of *Sargassum* is influenced by the content of secondary metabolite compounds (Meinita et al. 2022b). Secondary metabolite compounds are produced by organisms through the secondary metabolism of primary metabolites. For example, alkaloid compounds result from the synthesis of amino acids, flavonoids are synthesized from glucose, saponins and tannins are products of protein synthesis, and steroids are generated through the fat synthesis process (Rosa et al. 2019). Seaweed contains secondary metabolite compounds with the potential to act as antibacterials, including alkaloids, flavonoids, steroids/triterpenoids, and tannins (Arguelles 2022). The secondary metabolite compounds in *Sargassum* can inhibit the growth of bacteria, spanning from gram-positive bacteria to gram-negative bacteria (Li et al. 2018). The inhibition of bacterial growth by secondary metabolite compounds occurs because these compounds can bind and react with bacterial cells, causing damage to the enzyme system, protein synthesis, and ultimately leading to cell damage and death (Kordjazi et al. 2019).

Sargassum polycystum, the dominant species on Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach, are known as brown seaweed which contains secondary metabolite compounds with antibacterial potential (Palanisamy et al. 2018). The content of secondary metabolites in seaweed is influenced by environmental factors (Tanniou et al. 2013) and seasonal conditions (Abdala-Díaz et al. 2006), resulting in variations across different water areas. Additionally, this difference is caused by the adaptation and association of *S. polycystum* to its environment, with currents, salinity, and temperature identified as the factors most influencing seaweed morphology (Tanniou et al. 2013). Pollution levels in a body of water also influence the production of secondary metabolite compounds; increased pollution prompts seaweed to adapt by excreting and producing antibacterial compounds in greater quantities as a form of immunity against bacterial attacks (Zerrifi et al. 2018). This research examines the morphology, anatomical characteristics, phytochemical content, and antibacterial potential of *S. polycystum* seaweed found in Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach.

MATERIALS AND METHODS

Study site

Sampling was conducted at three different beaches along the southern coast of Java, Indonesia, i.e.: Karapyak Beach (108°45'29.37" E and 7°41'42.45" S), Sayang Heulang Beach (107°41'45.14" E and 7°40'8.76" S), and Sepanjang Beach (110°34'2.49" E and 8°8'13.86" S) (Figure 1). The sampling was conducted using purposive random sampling method that taken randomly with specific criteria based on the morphological characteristic (Etikan et al. 2016; Etikan and Bala 2017; Bhardwaj 2019; Nyimbili and Nyimbili 2024). Extraction and antibacterial testing of seaweed extract against bacteria *B. megaterium*, *M. luteus*, and *E. coli* were conducted at the Laboratory of Marine Biotechnology, Faculty of Fisheries and Marine Sciences, Universitas Jenderal Soedirman, Purwokerto. Phytochemical screening was performed at the Pharmaceutical Biology Laboratory, Universitas Jenderal Soedirman, Purwokerto Utara, Banyumas, Indonesia.

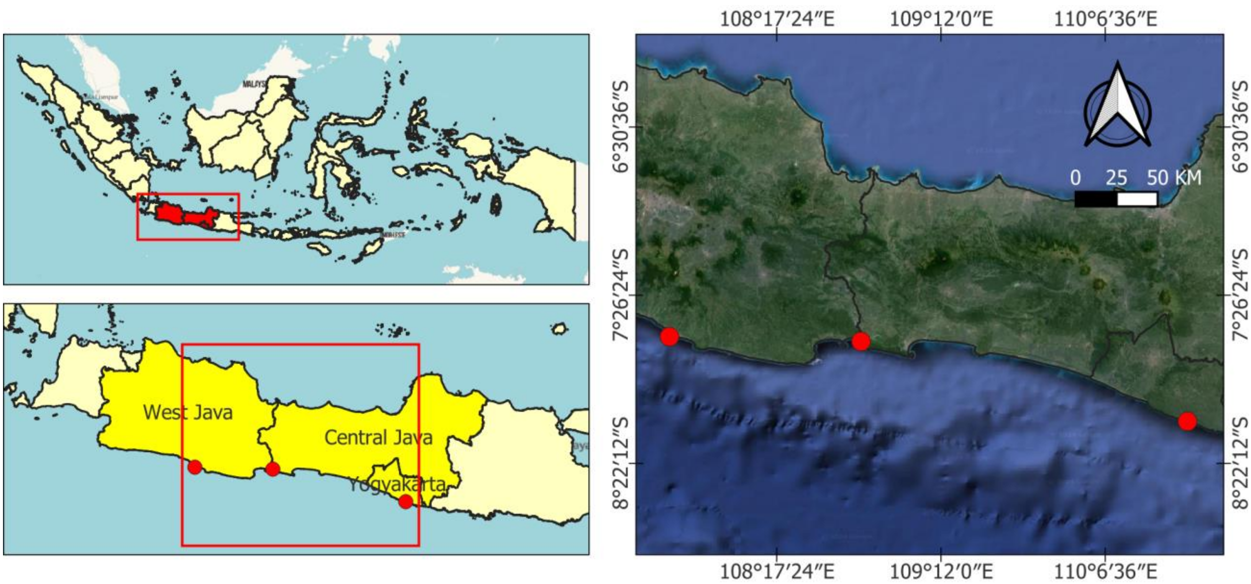

Figure 1. Sampling sites at three beaches on the southern coast of Java, Indonesia: A. Karapyak Beach; B. Sayang Heulang Beach; C. Sepanjang Beach

Sample preparation

Samples of *S. polycystum* obtained from the three beaches were cleaned of debris and salt. Subsequently, the samples were dried using the air-dry method. Afterward, the samples were crushed to become powdered simplicial.

Morphological identification

The morphological characteristics of *S. polycystum* were identified using the exploratory observation method (Marletta et al. 2023). *S. polycystum* cells were observed using a microscope. Sample preparation followed the procedure described by Widyartini et al. (2021) and Hernández-Cruz (2022). Samples were fixed in a 70% alcohol solution for 48 hours. Then, the sample was sliced as thinly as possible using a razor blade. The results of the incision were observed using a microscope by first dripping with distilled water. Observations were recorded, described, and also compared with references.

Sample extraction

Extraction was carried out using the maceration method with methanol as the solvent. The methanol ratio used was 1:30 (w/v). Maceration of *S. polycystum* was conducted for 1x48 hours (Arguelles et al. 2022; Haron et al. 2022). The residue resulting from the maceration process was re-macerated with the same solvent and time duration. The filtrate was evaporated until the methanol solvent evaporated, and a dry extract was obtained. The dry extract was diluted to a concentration of 10 mg/mL. The percentage yield of the extract was calculated using the following formula:

$$\%\,Yield = \frac{\text{Extract weight}}{\text{Sample weight}} \times 100\%$$

Phytochemical screening

Phytochemical testing was conducted using a qualitative descriptive method, with color change as an indicator for the presence of phytochemical compounds.

Alkaloids

The *S. polycystum* extract solution was placed on a test plate. Concentrated H_2SO_4 was added and stirred until a color change occurred. Subsequently, three plates were prepared, with 0.15 mL of Mayer's reagent, 0.15 mL of Wagener's reagent, and 0.15 mL of Dragendroff's reagent added. The presence of alkaloids in *S. polycystum* was indicated by the formation of an orange precipitate or a change in the color of the extract to brownish (Harborne 1998; Dubale et al. 2023; Nortjie et al. 2022).

Flavonoids

The flavonoid test, following Harborne (1998), Dubale et al. (2023) and Nortjie et al. (2022) with modification. One mililiter of the seaweed extract sample was mixed with 0.15 mL of concentrated HCl, followed by the addition of approximately 0.3 g of magnesium powder. A positive result was indicated by the appearance of a dark red color or the formation of a white precipitate.

Steroids/triterpenoids

The steroid test was performed by dissolving the extract in methanol. Subsequently, 0.25 mL of the solution was placed on a test plate and left for 15 minutes. Afterward, 0.1 mL of concentrated H_2SO_4 was added to the test plate. The presence of steroids was indicated by the formation of a blue color (Harborne 1998; Dubale et al. 2023; Nortjie et al. 2022).

Saponins

For the saponin test, 1 mL of the seaweed extract solution (concentration 10 mg/mL) was taken and placed in a test tube. Distilled water was added until all samples were submerged. The solution was then shaken for 2-3 minutes. A positive saponin result was indicated by the formation of stable foam (Harborne 1998; Dubale et al. 2023; Nortjie et al. 2022).

Tannin

In the tannin test, 0.25 mL of seaweed extract dissolved in methanol was transferred to a drip plate. Subsequently, 0.1 mL of 1% $FeCl_3$ solution was added. A positive tannin result was indicated by the formation of a bluish-black or green color (Harborne 1998; Dubale et al. 2023; Nortjie et al. 2022).

Cultivation of bacteria

Three pathogenic bacterial strains (*Bacillus megaterium* DSM32, *Micrococcus luteus* ATCC4698, and *Escherichia coli* K12) were cultured for antibacterial assay. The bacteria were grown using Nutrient Broth (Himedia) and incubated for 24 hours. After that, the bacteria that had been grown were transferred to solid media. The solid media were made of a mixture of 6.5 g of Nutrient Broth (Himedia) medium and 10 g of bacteriological agar (Himedia) which dissolved with distilled water to a volume of 500 mL. The media were sterilized in an autoclave at 121°C for 20 minutes. Then, 25 mL of the media was poured into a petri dish (Rajasekar et al. 2019). The bacteria *B. megaterium* DSM32, *M. luteus* ATCC4698, and *E. coli* K12 were streaked on the media and incubated for 24 hours (Chiao-Wei et al. 2011). The cultivated bacteria were used for antibacterial assay.

Antibacterial assay

Antibacterial assay was conducted using the agar diffusion method (Kirby Bauer). The inoculum pathogenic bacteria were streaked on solid medium. Ten microliters of *S. polycystum* extract (10 mg/mL), positive control and negative control were prepared on paper disc. Penicillin was used as the positive control, and methanol was used as the negative control. These paper discs were then placed on the solid media which contained the inoculum of pathogenic bacteria (*B. megaterium* DSM32, *M. luteus* ATCC4698, and *E. coli* K12). Antibacterial assay was determined by measuring the diameter of the inhibition zone around the paper disc after 24, 48, and 72 hours incubation (Fayzi et al. 2020).

RESULTS AND DISCUSSION

Morphological characteristics of *Sargassum polycystum*

Sargassum polycystum is an alga commonly found and dominant on several beaches in Indonesia, including Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach. Generally, *S. polycystum* exhibits a disc-shaped holdfast, a rough-textured stipe, a long, wide, and typically serrated blade, a bladder resembling a bubble, and a thallus that can attain a height of 7 meters **(**Table 1**).**

In this study, *S. polycystum* was found to have a yellowish-brown thallus with a slightly darker base tip, exhibiting an alternate pinnate branching type. The stipe is cylindrical in shape with a slightly rough texture. The stipe length of *S. polycystum* from Karapyak Beach ranged from 6 to 10 cm, while those from Sayang Heulang Beach and Sepanjang Beach have lengths ranging from 8 to 12 cm. The blade of *S. polycystum* from Karapyak Beach was rounded and smaller compared to those from Sayang Heulang Beach and Sepanjang Beach.

The observed differences in stipe and blade size are attributed to the adaptation of *S. polycystum* to its environment. Salinity is identified as one of the environmental factors influencing the morphological characteristics of *S. polycystum*. Kim et al. (2022) reported that seaweed living in aquatic environments with lower salinity tends to have shorter blades and stipes compared to seaweed in higher salinity environments. Consequently, the stipe and blade of *S. polycystum* from Karapyak Beach are shorter and smaller than those from Sayang Heulang Beach and Sepanjang Beach due to the lower salinity conditions in its habitat.

The length of the thallus of *S. polycystum* in this study ranged between 5-20 cm (Figure 2), a size notably shorter when compared to other studies on *S. polycystum*. Yip et al. (2018) reported thallus lengths ranging from 25-35 cm, while Yap-Dejeto et al. (2022) obtained *S. polycystum* with lengths of up to 75 cm. It is worth noting that thalli of *Sargassum* species can reach heights exceeding 1 meter. Sjøtun et al. (2021) documented *Sargassum muticum* thalli growing up to 1.5 m. In addition to its length, the thallus of *S. polycystum* in this study exhibited an alternate pinnate type, with growth alternately branching from the main branch and concentrated around its base. Notably, the thalli of *S. polycystum* were relatively longer than those of *Sargassum vulgare*. Robinson et al. (2012), in their research, mentioned that *S. vulgare* has short thallus conjunctions, typically ranging between 1 and 1.6 cm in size.

Table 1. Morphology and anatomy characteristics of *Sargassum polycystum* from the southern coast of Java, Indonesia: i.e. Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach

Morphological and anatomical characteristics	Location of *S. polycystum*		
	Karapyak	**Sayang Heulang**	**Sepanjang**
Branching	*Pinnate alternate*	*Pinnate alternate*	*Pinnate alternate*
Thallus color	Yellowish brown, and slightly darker at the base	Yellowish brown, and slightly darker at the base	Yellowish brown, and slightly darker at the base
Stipe	Cylindrical in shape, rough texture, 6-10 cm long	Cylindrical in shape, rough texture, 8-12 cm long	Cylindrical in shape, rough texture, 8-12 cm long
Blade	Elongated round, serrated edges, between 1.5-4.5 cm long, 0.5-1.3 cm wide, black spots	Elongated oval, serrated edge, length between 1.5-4.5 cm, width 0.5-1.3 cm, black spots	Long serrated, between 2-5 cm long, 0.3-1.0 cm wide, black spots
Holdfast	Small disc	Small disc	Small disc
Cell wall	Alginate as the main constituent	Alginate as the main constituent	Alginate as the main constituent
Vacuoles	Large in size, filled with liquid	Large in size, filled with liquid	Large in size, filled with liquid

Figure 2. Thallus of *Sargassum polycystum* collected from from the southern coast of Java, Indonesia: i.e. Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach

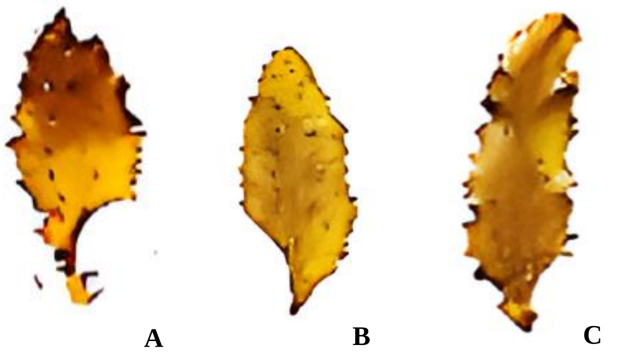

Figure 3. Blade of *Sargassum polycystum* collected from the southern coast of Java, Indonesia: i.e. Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach

The shape of the *S. polycystum* blade in this study varies and includes round, oval, and elongated shapes (Figure 3). The variability in the shape of the *S. polycystum* blade can be attributed to its growth time and environmental conditions. This finding aligns with a study conducted by Sjøtun et al. (2021), which observed *S. polycystum* blades exhibiting a round, elongated, and oval shape with folded edges and sloping ends. Additionally, Yip et al. (2018) noted in their research that *S. polycystum* displays a blade with a round and elongated shape, featuring a sloping tip, folded edge, and the presence of black spots.

Sargassum polycystum possesses a holdfast that serves to attach the thallus to the substrate. The holdfast of *S. polycystum* observed in this study had a disc shape (Figure 4). This type of holdfast shape is well-suited for algae that inhabit coral rock substrates (Sargazi 2021). However, the size of the holdfast in *S. polycystum* can undergo changes as it adapts to ocean currents and water movement. Increased current strength is associated with larger holdfasts in *Sargassum*. Furthermore, both current and water movements impact the size of the stipe and blade in *Sargassum* as part of its adaptation. Kim et al. (2022) noted that in areas with strong currents, *Sargassum* exhibits a shorter thallus size, but with stipes that are more robust and smaller branches, indicative of its adaptation to the environment.

The *S. polycystum* specimens in this study exhibited cell sizes ranging from 50-150 µm (Figure 5), which differs slightly from the findings of Andrade-Sorcia and Riosmena-Rodríguez (2011), who reported *Sargassum* cell sizes around 100-200 µm. The cell walls of *Sargassum* are composed of two layers, with the inner layer containing cellulose and the outer layer formed from alginate as the main constituent. The presence of alginate in *Sargassum* serves to provide flexibility against ocean currents (Deniaud-Bouët et al. 2014). Additionally, the vacuoles in *Sargassum* are relatively large and contain cytoplasmic fluid. The cytoplasmic fluid surrounding the cells aids *S. polycystum* in regulating the absorption of substances and compounds into the cells. Its absorption ability is attributed to the dominance of potassium, which binds oxygen and removes toxic substances from the cells (Vasuki et al. 2020).

Figure 4. Holdfast of *Sargassum polycystum* collected from the southern coast of Java, Indonesia: i.e. Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach

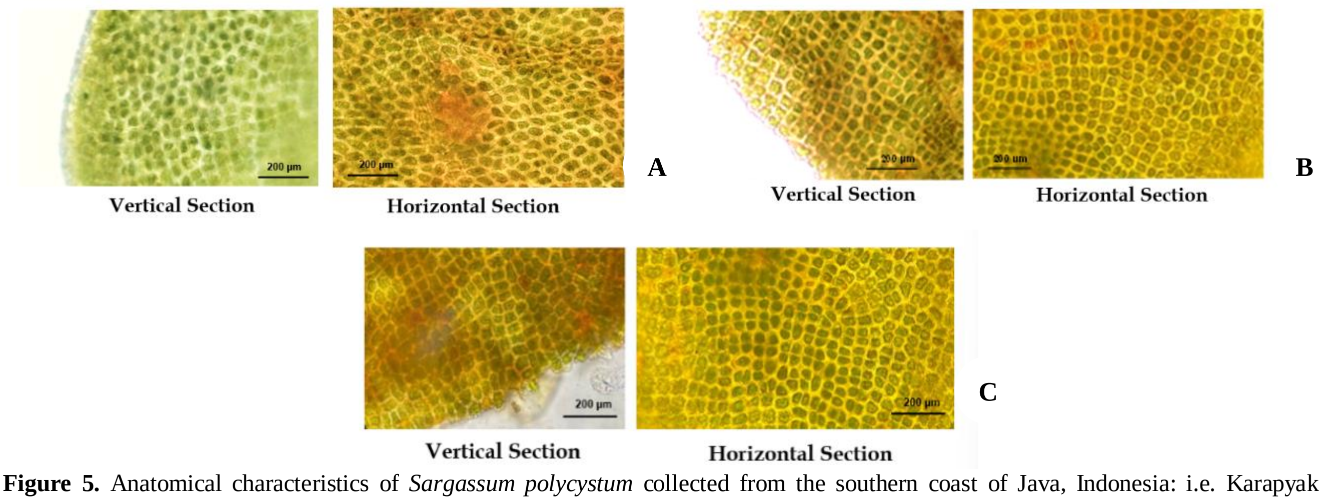

Figure 5. Anatomical characteristics of *Sargassum polycystum* collected from the southern coast of Java, Indonesia: i.e. Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach

Phytochemical analysis of *Sargassum polycystum* extract

Phytochemical compounds are secondary metabolites that play a role in defense against environmental stresses, including bacterial pathogenesis. The inhibitory effect of secondary metabolite compounds on bacterial growth determines the antibacterial activity of *Sargassum* sp. (Dixit et al. 2018). Phytochemical compounds in seaweed inhibit bacterial growth by disrupting the action of peptidoglycan in bacterial cells (Saleh et al. 2019). The results of the phytochemical screening of *S. polycystum* extract can be seen in Table 2.

In this study, the phytochemical contents identified in *S. polycystum* were alkaloids, flavonoids, steroids, saponins, and negative test result for tannins compounds. *S. polycystum* is known as a source of metabolite compounds such as alkaloids, flavonoids, tannins, saponins, and steroids (Arsianti et al. 2020; Kok and Wong 2022; Thiurunavukkarau et al. 2022). Furthermore, the presence of seaweed phytochemical compounds can be shown according to color change after treatment. Furthermore, color difference means stronger seaweed phytochemical compound (Sobuj et al. 2024).

Alkaloid compounds

Table 2 demonstrates that *S. polycystum* from the three beaches tested were positive for alkaloids. According to Harborne (1998), Dubale et al. (2023) and Nortjie et al. (2022), the formation of an orange precipitate or a change in color to dark brown serves as an indicator of positive alkaloid content. The test results are illustrated in Figures 6-8.

Alkaloids are secondary metabolite compounds in *S. polycystum* produced through the synthesis of amino acids. Various types of algae, including *S. polycystum*, have been found to synthesize alkaloids. Positive alkaloid content has also been identified in *S. polyphyllum* (Arunkumar et al. 2023), *S. vulgare* (Saleh et al. 2019), and the alkaloids in *S. cristaefolium* are known to inhibit the growth of pathogenic bacteria by disrupting the bacteria's DNA, potentially leading to cell lysis (Dewinta et al. 2023).

Flavonoid compounds

The results for flavonoid compounds indicated positive outcomes for all samples, as evidenced by a change in orange color or the appearance of white deposits (Harborne 1998; Dubale et al. 2023; Nortjie et al. 2022), as shown in Figure 9.

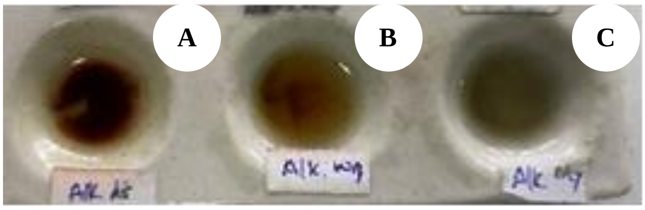

Figure 6. Alkaloid test of *Sargassum polycystum* collected from Karapyak Beach, southern coast of Java, Indonesia using (A) Dragendroff, (B) Wagener, (C) Mayer indicator

Figure 7. Alkaloid test of *Sargassum polycystum* collected from Sayang Heulang Beach, southern coast of Java, Indonesia using (A) Dragendroff, (B) Wagener, (C) Mayer indicator

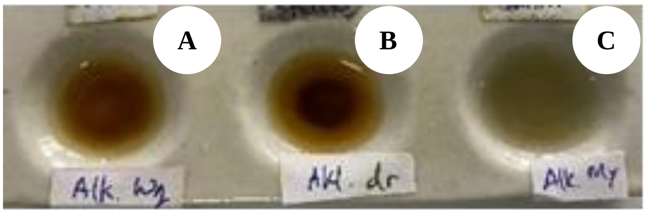

Figure 8. Alkaloid test of *Sargassum polycystum* collected from Sepanjang Beach, southern coast of Java, Indonesia using (A) Dragendroff, (B) Wagener, (C) Mayer indicator

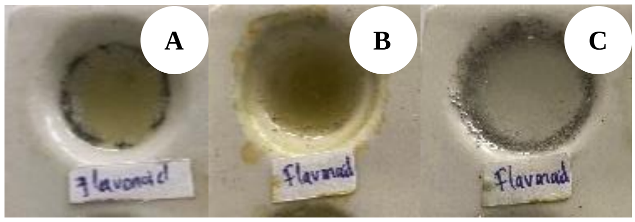

Figure 9. Flavonoid test of *Sargassum polycystum* collected from (A) Karapyak Beach, (B) Sayang Heulang Beach and (C) Sepanjang Beach

Table 2. Phytochemical content of *Sargassum polycystum* collected from the southern coast of Java, Indonesia: i.e. Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach

Phytochemical compounds	Location of *S. polycystum*			Indicator
	Karapyak	Sayang Heulang	Sepanjang	
Alkaloids	+++	+	++	Orange or brown
Flavonoids	++	+	+++	Orange or white
Steroids	+	+++	+	Bluish
Saponins	+	+	+	Bubbly
Tannin	-	-	-	Blue/green

Note: Extracts have no reaction towards reagent (-); extracts have a weak reaction towards reagent (+); Extracts have a reaction towards reagent (++); Extracts have a very strong reaction towards reagent (+++)

Most algae, including *Sargassum* algae, contain flavonoid compounds. *Sargassum* has been widely utilized as a source of flavonoid compounds known for their antibacterial properties. Nofal et al. (2023) obtained positive results in testing flavonoids in *S. muticum*, and Thiurunavukkarau et al. (2022) also reported positive outcomes in flavonoid testing. The positive results of this flavonoid test indicate that *Sargassum* holds potential as an alternative antibacterial agent. Flavonoids can inhibit bacterial cell replication by damaging the bacterial cell membrane, halting replication and protein synthesis, ultimately leading to cell lysis or death (Scania and Chasani 2021).

Saponin compound

Saponin content tested were positive in all *S. polycystum* samples from the three beaches. The positive saponin content, according to Harborne (1998), Dubale et al. (2023) and Nortjie et al. (2022), is indicated by the presence of stable foam in the solution (Figure 10).

Positive test results for tannins in *S. polycystum* were also reported in the research conducted by Kok and Wong (2022). Sivakumar et al. (2018) found positive results for saponin content in *Sargassum binderi*, indicating the presence of antibacterial compounds within the *Sargassum genus*. Saponin in *S. polycystum* inhibits bacterial growth by impeding enzyme activity in bacteria, thereby hindering protein and DNA synthesis in bacterial cells, ultimately leading to cell lysis and death (Lopes et al. 2012).

Tannin compounds

Figure 11 reveals negative tannin test results for all *S. polycystum* samples from the three beaches. Positive tannin results, according to Harborne (1998), Dubale et al. (2023), Nortjie et al. (2022), are indicated by a change in color to bluish or bluish-green.

The results of this test do not align with the research conducted by Metwally et al. (2020), who obtained positive results for tannins in *Sargassum*. Tannin is one of the compounds that plays a role in the immune system of algae, specifically *Sargassum*. Tannin inhibits bacterial growth by reacting with enzymes and disrupting the protein working system in bacterial cells, leading to cell damage and death (Lopes et al. 2012). Tannin is one of the compounds that play a role in the immune system of algae, specifically *Sargassum*, where tannin will inhibit bacterial growth by reacting with enzymes and damaging the protein working system in bacterial cells which will cause cell damage and death (Lopes et al. 2012).

Steroid compounds

Steroids were found to be positive in all *S. polycystum* samples from all three beaches. According to Harborne (1998), Dubale et al. (2023) and Nortjie et al. (2022), positive steroid content is indicated by a bluish color change (Figure 12).

Steroids are bioactive compounds that also play a role in the algae's immune system. The positive results of steroid testing align with research by Manguntungi et al. (2022), who found that *S. polycystum* contains steroids, making it suitable for use as an antibacterial agent. Steroids inhibit bacterial growth by disrupting protein function, inactivating enzymes, and producing hydrogen peroxide, leading to cell death (Arunkumar et al. 2023).

Antibacterial activity of Sargassum polycystum *against pathogenic bacteria*

The results of the antibacterial test of *S. polycystum* extract from Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach against *B. megaterium*, *M. luteus*, and *E. coli* are shown in Figures 13-14. Figure 13 displays the mean of the inhibitory zone from the antibacterial test of *S. polycystum* against each pathogenic bacteria, while Figure 14 shows the disc-diffusion test results from *S. polycystum* against each pathogenic bacteria.

The antibacterial activity of *S. polycystum* according to its strength can be classified as weak (+), moderate (++), strong (+++), and very strong (++++) as shown in Table 3. Afrin et al. (2023) classified that *Sargassum* has strong ability to inhibit bacteria growth according to the present of inhibitory zone in agar diffusion method. The strength of antibacterial activity can be seen by its inhibitory zone where the bigger inhibitory zone means stronger antibacterial activity (Park et al. 2023).

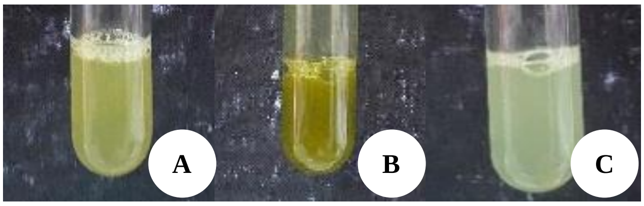

Figure 10. Saponin test of *Sargassum polycystum* collected from the southern coast of Java, Indonesia: i.e. Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach

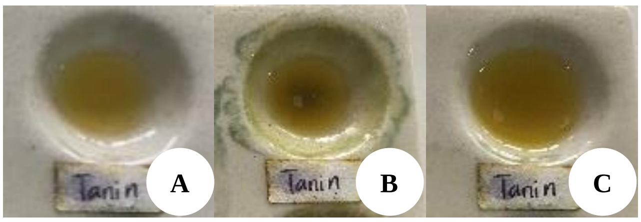

Figure 11. Tannin test of *Sargassum polycystum* collected from the southern coast of Java, Indonesia: i.e. Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach

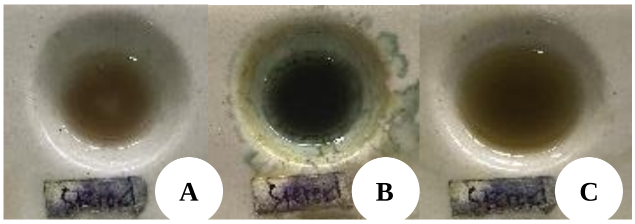

Figure 12. Steroid test of *Sargassum polycystum* collected from the southern coast of Java, Indonesia: i.e. Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach

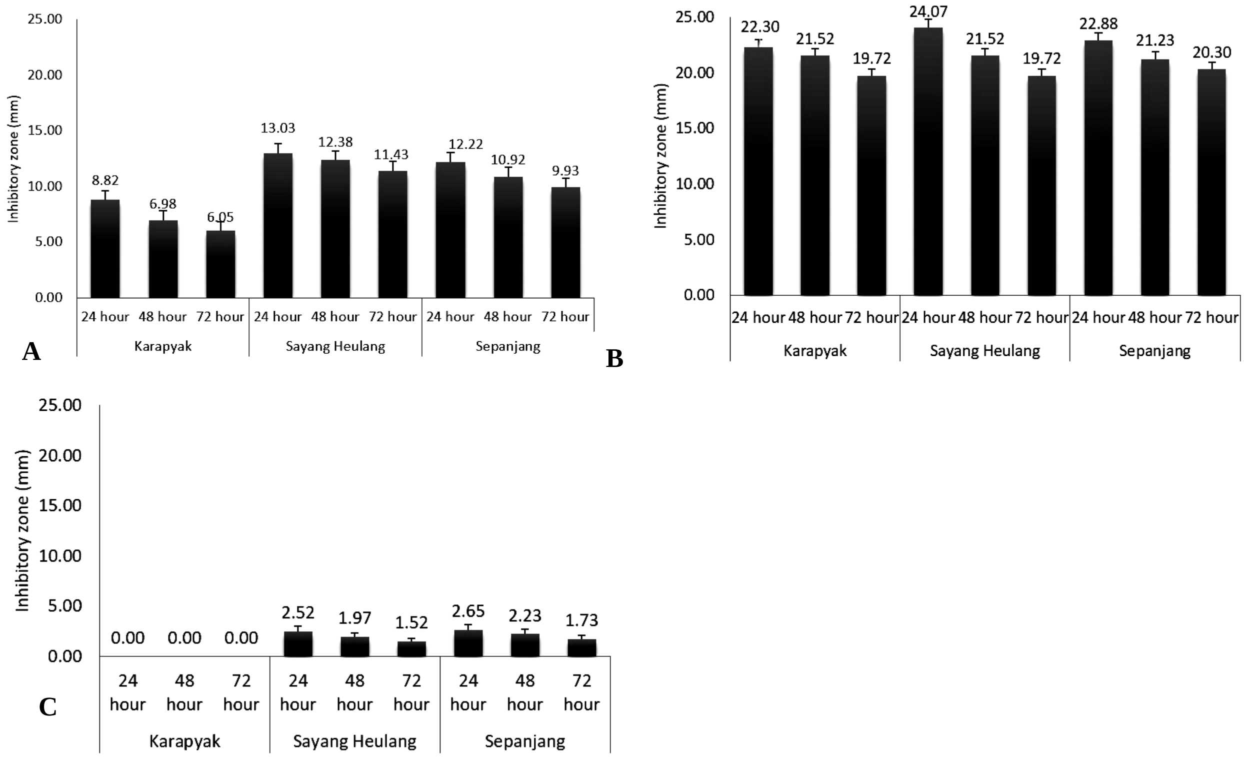

Figure 13. Antibacterial activity of *Sargassum polycystum* extracts from the southern coast of Java, Indonesia: i.e. Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach against: A. *B. megaterium* DSM32, B. *M. luteus* ATCC4698, and C. *E. coli* K12. Values represent mean of inhibitory zone in millimeter (mm) ± SD (n≥3)

Table 3. Antibacterial activity of *Sargassum polycystum* extracts from the southern coast of Java, Indonesia: i.e. Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach against *B. megaterium* DSM32, *M. luteus* ATCC4698, and *E. coli* K12

Bacteria	*S. polycystum*		
	Karapyak	Sayang Heulang	Sepanjang
B. megaterium	++	+++	+++
M. luteus	++++	++++	++++
E. coli	-	+	+

Note: Inhibition zone 0 mm categorized as inactive (-); < 5 mm categorized as weak active (+); between 5-10 mm categorized as moderately active (++); 10-20 mm categorized as active (+++); >20 mm categorized as highly active (++++)

Sargassum is an alginophyte which rich in bioactive compounds such as fucoxanthin, flavonoids, sterols (Afreen et al. 2023; Alvarado et al. 2023). The physicochemical content of *Sargassum* sp. can be developed as an antibacterial agent, since *Sargassum* extract revealed the antibacterial bioactivity against *Staphylococcus aureus, Bacillus subtilis, Proteus mirabilis*, and *Escherichia coli* growth (Abdi et al. 2022). Furthermore, *Sargassum* also can inhibit *Vibrio* growth (Villegas-Silva et al. 2022) *E. faecalis, S aureus,* and *S. epidermidis* (Alvarado et al. 2023). *Sargassum* species also have potential and promising resources to be developed in the pharmaceutical industry due to their bioactive compound and ability to inhibit bacterial growth. Previous studies showed *Sargassum* can inhibit *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes,* and *Klebsiella pneumoniae* bacteria (Albratty et al. 2021). *Sargassum* was effective in inhibiting the growth of *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae,* and *Salmonella typhi* (Trivedi et al. 2021). *Sargassum* also can inhibit bacterial growth prevent coral disease and able to improve the health status of commercially important coral species (Ahmed et al. 2022).

Antibacterial activity of *Sargassum polycystum* against *B. megaterium* DSM32

The antibacterial test results for *Sargassum polycystum* extracts from Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach against *B. megaterium* bacteria are illustrated in Figure 14. According to the inhibition strength categories of bacterial growth (Davis and Stout 1971), *S. polycystum* from Karapyak Beach exhibits a moderate inhibition zone against *B. megaterium,* while *S. polycystum* from Sayang Heulang Beach and Sepanjang Beach are classified as having a strong inhibition zone. The strong antibacterial activity observed at Sayang Heulang Beach and Sepanjang Beach is attributed to their distinct environmental associations. Zerrifi et al. (2018) demonstrated that seaweed in more extreme conditions has higher metabolite content due to its adaptive capacity.

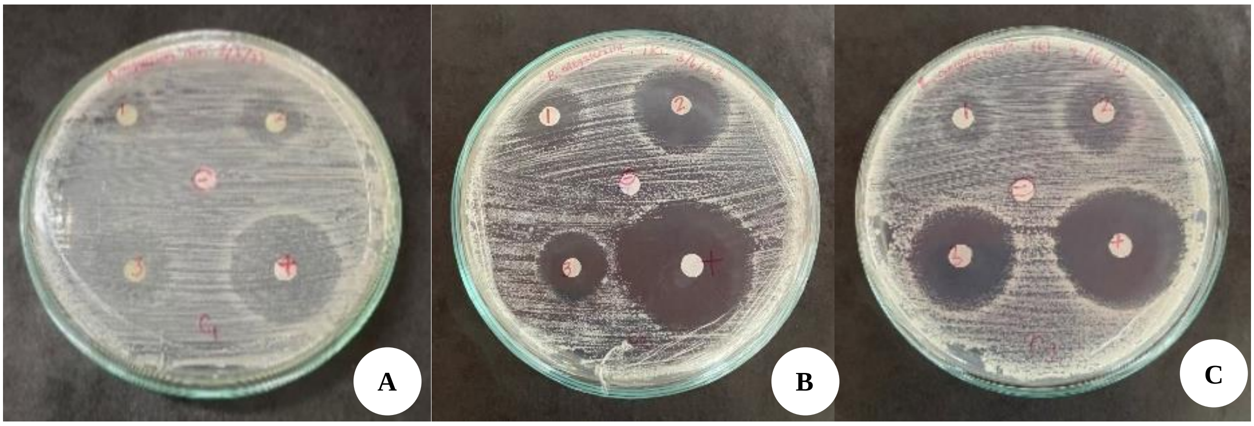

Figure 14. Inhibition zone of *Sargassum polycystum* collected from from the southern coast of Java, Indonesia: i.e. Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach against *B. megaterium* DSM32

Sargassum polycystum originating from three different beaches, exhibited a reduction in the inhibition zone with increasing observation time, suggesting that the *S. polycystum* extract possesses bacteriostatic properties. This contrasts with the findings of Rattaya et al. (2015), who reported no antibacterial activity of *S. polycystum* against *Bacillus* bacteria. The discrepancy is attributed to variations in the concentration of the *S. polycystum* extract used in the studies. Interestingly, the results align with the research conducted by Kok and Wong (2022), which demonstrated that *S. polycystum* displays antibacterial activity against pathogenic bacteria of the *Bacillu*s genus, including *B. subtilis* and *B. cereus*. Similar positive outcomes were reported by Chiao-Wei et al. (2011) and Yip et al. (2018), highlighting the antibacterial efficacy of *S. polycystum* against *Bacillus* bacteria, specifically *B. cereus.*

Sargassum, a diverse algae with bioactive compounds, plays a role in various pharmacological sectors, particularly as an antibacterial agent against Gram-positive bacteria (*Bacillus*). Moheimanian et al. (2023) demonstrated strong antibacterial activity of *S. boveanum* methanol extract against *B. subtilis*. Similarly, Bakar et al. (2019) reported positive antibacterial results for *Sargassum granuliferum* against *B. subtilis*. Park et al. (2023) highlighted the inhibitory role of phlorotannin in *Sargassum* against *B. cereus*, supporting Lopes et al. (2012) who also found that phlorotannin reacted with bacterial enzymes and proteins, damaging the cell wall and causing lysis. *S. boveanum*, another *Sargassum* type, exhibits antibacterial activity against *Bacillus* bacteria (Shanmughapriya et al. 2008). The methanol extract of *S. muticum* displays strong antibacterial activity against *Bacillus*, attributed to its flavonoid content (Nofal et al. 2023). According to Mughal et al. (2006), flavonoids demonstrated significant inhibitory capabilities *against B. subtilis*. Furthermore, Ávila et al. (2008) revealed that the hydrophobic group of flavonoids has a significant role in increasing the lipophilicity of the chalcone and allowing its entry into the bacterial cell membrane. *Sargassum*'s antibacterial activity against Bacillus is also evident in *S. granuliferum*, attributed to its sterol content that maintains membrane structure and disrupts bacterial cell permeability (Bakar et al. 2019).

Antibacterial activity of *Sargassum polycystum* against *Micrococcus luteus* ATCC4698

The antibacterial test results of *S. polycystum* extract from Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach against *M. luteus* bacteria can be seen in Figure 15. The antibacterial activity of *S. polycystum* from these beaches against *M. luteus* exhibits inhibitory strength classified as very strong based on the antibacterial strength category according to Davis and Stout (1971).

The very strong antibacterial activity of *S. polycystum* against *M. luteus* is attributed to *M. luteus* being inherently weak against several antibacterial compounds from seaweed (Rajasekar et al. 2019). Munita and Arias (2016) asserted that each bacterium has different susceptibility to the physical and chemical properties of antibacterial compounds. Additionally, resistance to antimicrobial compounds can be inherent to the microorganism, causing varied responses to antibacterials even among Gram-positive bacteria, as seen with *M. luteus* and *B. megaterium.*

The antibacterial activity of *S. polycystum* against *M. luteus* displayed bacteriostatic properties, as evidenced by a decrease in the diameter of the clear zone over time. These results align with research by Thiurunavukkarau et al. (2022), indicating strong antibacterial activity. Furthermore, Bolaños et al. (2017) demonstrated that the phenolic content of phlorotannin in *Sargassum* plays a crucial role in antibacterial activity against *M. luteus*, especially in *S. polycystum* extract, which exhibited the most potent antibacterial activity. Kim et al. (2014) also emphasized the positive correlation between the presence of phenolic compounds in *Sargassum* and antibacterial activity, attributing it to the ability of phenolic compounds to react with Reactive Oxygen Species (ROS), causing oxidative damage to DNA and proteins.

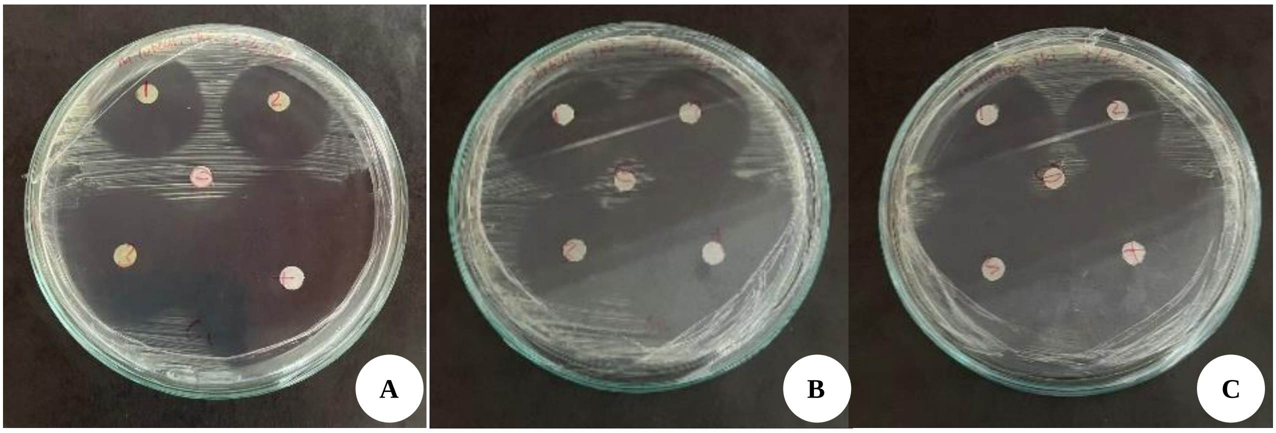

Figure 15. Disc plate result of inhibition zone of *Sargassum polycystum* collected from the southern coast of Java, Indonesia: i.e. Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach against *M. luteus* ATCC4698

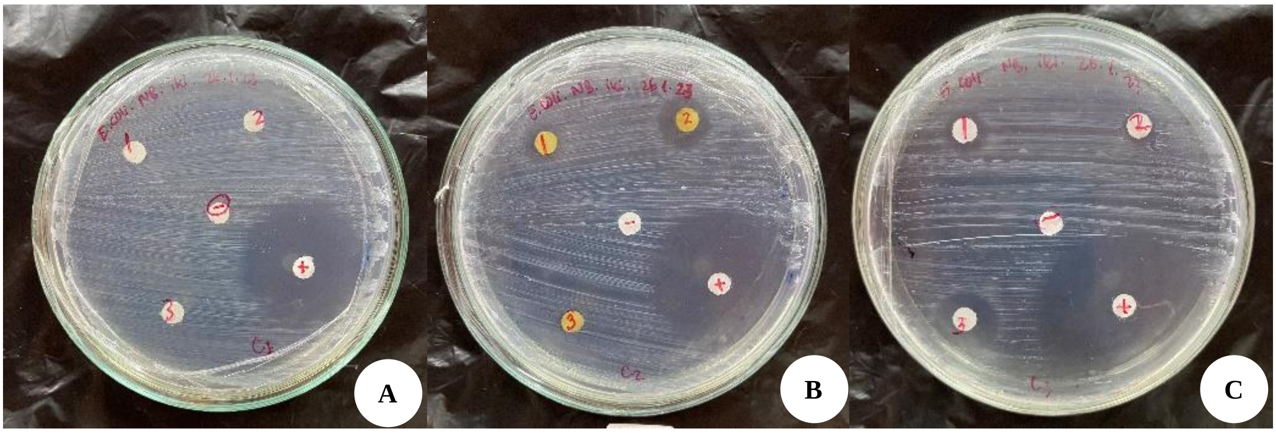

Figure 16. Inhibition zone of *Sargassum polycystum* collected from the southern coast of Java, Indonesia: i.e. Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach against *E. coli* K12

Wu et al. (2013) revealed that flavonoids can inhibit DNA gyrase of pathogen bacteria. The bacterial replication process depends mostly on DNA gyrase, and some flavonoids prevent this enzyme from relieving the strain that results from double-strain DNA during DNA replication. According to Baleta et al. (2017), sterol and tannins were also discovered in the extracts of *S. crassifolium* and *S. oligocystum*, which were tested for antibacterial activity against *M. luteus* and demonstrated their bioactivity. These previous studies showed similar results compare to this study. Tannin affects the membranes of microorganisms, and its toxicity may be related to tannins'complexation of metal ions.

Antibacterial activity of *Sargassum polycystum* against *Escherichia coli* K12

The antibacterial test results of *S. polycystum* extracts from Karapyak Beach, Sayang Heulang Beach, and Sepanjang Beach against *E. coli* bacteria can be seen in Figure 16. The antibacterial activity of *S. polycystum* against *E. coli* exhibited a lower inhibition zone compared to *B. megaterium*, and *S. polycystum* from Karapyak Beach did not show any inhibition zone. According to the antibacterial strength category by Davis and Stout (1971), *S. polycystum* originating from Sepanjang Beach and Sayang Heulang Beach has weak inhibitory power against the growth of *E. coli* bacteria. This is attributed to *E. coli* being a Gram-negative bacteria with a more complex cell wall, making it more resistant to antibacterial compounds.

Figure 13 shows that the inhibition zone decreases as the observation time increases. This decrease in the inhibition zone indicates the bacteriostatic properties of the *S. polycystum* extract, where the extract does not have the ability to kill but only inhibits the growth of *E. coli* bacteria. This aligns with research by Ghazali et al. (2021), stating that *S. polycystum* does not exhibit strong inhibitory power against *E. coli*, except at high extract concentrations. The results corroborate with Yip et al. (2018), showing bacteriostatic properties of *S. polycystum* against *E. coli*. Kok and Wong (2022) also suggested *S. polycystum* as a potential alternative for inhibiting *E. coli* pathogenesis.

The *Sargassum* genus algae exhibit highly active antibacterial activity against *E. coli*. Notably, research by Li et al. (2018) demonstrated that *S. fusiforme* extract possesses strong antibacterial activity against *E. coli* bacteria. Despite *E. coli* being classified as a Gram-negative bacterium with a higher cell wall complexity than Gram-positive bacteria, the robust antibacterial activity of *Sargassum* against it indicates the potential of algae from the *Sargassum* genus as effective antibacterial agents. The substantial antibacterial activity of *Sargassum* is likely attributed to the high flavonoid content in the produced extract. Flavonoids, commonly found in *Sargassum*, play a crucial role in inhibiting bacterial growth by disrupting bacterial cells and damaging cell membranes through the hydrogen bonds they create, leading to bacterial cell lysis (Arunkumar et al. 2023).

Sargassum's ability to inhibit the growth of *E. coli* was also validated by Sivagnanam et al. (2015), who asserted that the antibacterial activity of *S. japonica* and *S. horneri* was driven by the content of phenolic compounds and the presence of fucoxanthin. Moni et al. (2018) further demonstrated the antibacterial activity of *S. binderi* seaweed against *E. coli* and other pathogenic bacteria. Antibacterial activity was also observed in *S. polycystum* against *E. coli*, falling into the strong category due to the content of flavonoids, steroids, and terpenoids (Manguntungi et al. 2022; Moni et al. 2018). *S. polyphyllum* also exhibits antibacterial activity against *E. coli* (Arunkumar et al. 2023; Kok and Wong 2022; Moheimanian et al. 2023). *S. polycystum* from Karapyak Beach, Sayang Heulang, and Sepanjang demonstrates moderate to very strong antibacterial activity with bacteriostatic properties against *B. megaterium* and *M. luteus* bacteria, and weak antibacterial activity against *E. coli* with bacteriostatic properties. *S. polycystum* holds potential as a candidate to be developed as a new antibacterial source.

In conclusion, *S. polycystum* exhibits morphology and anatomy highly influenced by its environment, especially the thallus and blade structure. *S. polycystum* contains the phytochemical compounds including alkaloids, flavonoids, steroids and saponins. The content of alkaloids, flavonoids, steroids and saponins of *S. polycystum* varies in different sampling areas. The highest alkaloid compounds where found in *S. polycystum* from Karapyak Beach, while *S. polycystum* from Sayang Heulang showed the highest steroid content. On the other hand, *S. polycystum* from Sepanjang Beach had the highest flavonoid content. Future study is needed to investigate the effect of environmental factors on the phytochemical compound content and composition in *S. polycystum.* The phytochemical compounds found in *S. polycystum* can be utilized as antibacterial agents against *B. megaterium* DSM32, *M. luteus* ATCC4698, and *E. coli* K12, given their ability to destroy and disrupt bacterial cell synthesis. *S. polycystum* showed strong activity to inhibit *M. luteus* ATCC4698, but showed a low activity to inhibit *E. coli* K12.

ACKNOWLEDGEMENTS

The authors express gratitude for the support provided by the *Penelitian Kerjasama Dalam Negeri* (PKDN) 2023 research grant No. 3.54/UN23.35.5/PT.01/VII/2023, Ministry of Education, Culture, Research, and Technology, Republic of Indonesia.

REFERENCES

Abdi G, Karande VC, Mohammed A, Tarighat MA, Goh KW, Kari ZA, Wei LS, Zain MRAM, Mohammadi M, Lee GE, Barwant MM. 2022. Pharmacological potential of *Sargassum* sp. of West Coast of Maharashtra Kunkeshwar, India. Front Mar Sci 9: 1011218. DOI: 10.3389/fmars.2022.1011218.

Afrin F, Ahsan T, Mondal MN, Rasul MG, Afrin M, Silva AA, Yuan C, Shah AKMA. 2023. Evaluation of antioxidant and antibacterial activities of some selected seaweeds from Saint Martin's Island of Bangladesh. Food Chem Adv 3: 100393. DOI: 10.1016/j.focha.2023.100393.

AftabUddin S, Siddique MAM, Habib A, Akter S, Hossen S, Tanchangya P, Abdullah Al M. 2021. Effects of seaweeds extract on growth, survival, antibacterial activities, and immune responses of *Penaeus monodon* against *Vibrio parahaemolyticus*. Ital J Anim Sci 20 (1): 243-255. DOI: 10.1080/1828051X.2021.1878943.

Ahmed N, Mohamed HF, Xu C, Sun X, Huang L. 2022. Novel antibacterial activity of *Sargassum fusiforme* extract against coral white band disease. Electron J Biotechnol 57: 12-23. DOI: 10.1016/j.ejbt.2022.03.002.

Albratty M, Bajawi AAM, Marei TMH, Alam MS, Alhazmi HA, Najmi A, ur Rehman Z, Moni SS. 2021. Spectral analysis and antibacterial potential of bioactive principles of *Sargassum Crassifolium* J. Agardh from red sea of Jazan Origin. Saudi J Biol Sci 28 (10): 5745-5753. DOI: 10.1016/j.sjbs.2021.06.017.

Alvarado-Sansininea JJ, Tavera-Hernández R, Jiménez-Estrada M, Coronado-Aceves EW, Espitia-Pinzón CI, Díaz-Martínez S, Hernández-Anaya L, Rangel-Corona R, Avila-Ortiz AG. 2023. Antibacterial, antidiabetic, and toxicity effects of two brown algae : *Sargassum buxifolium* and *Padina gymnospora*. Intl J Plant Biol 14 (1): 63-76. DOI: 10.3390/ijpb14010006.

Andrade-Sorcia G, Riosmena-Rodríguez R. 2011. Vegetative and reproductive anatomy of *Sargassum lapazeanum* (Fucales: Sargassaseae) in the south-western Gulf of California, Mexico. Algae 26 (4): 327-331. DOI: 10.4490/algae.2011.26.4.327.

Arguelles EDLR. 2022. Chemical composition and in vitro study of antioxidant and antibacterial activities of *Sargassum oligocystum* Montagne (Sargassaceae, Ochrophyta). Asian J Agric Biol 2022 (4): 202105209. DOI: 10.35495/ajab.2021.05.209.

Arsianti A, Bahtiar A, Wangsaputra VK, Azizah NN, Fachri W, Nadapdap LD, Fajrin AM, Tanimoto H, Kakiuchi K. 2020. Phytochemical composition and evaluation of marine algal *Sargassum polycystum* for antioxidant activity and in vitro cytotoxicity on hela cells. Pharm J 2020 12 (1): 88-94. DOI: 10.5530/pj.2020.12.14.

Arunkumar M, Suhashini K, Mahesh N, Ravikumar R. 2023. Quorum quenching and antibacterial activity of silver nanoparticles synthesized from *Sargassum polyphyllum*. Bangladesh J Pharmacol 9 (1): 54-59. DOI: 10.3329/bjp.v9i1.17301.

Ávila HP, Smânia ED, Delle Monache F, Júnior AS. 2008. Structure–activity relationship of antibacterial chalcones. Bioorg Med Chem 16 (22): 9790-9794. DOI: 10.1016/j.bmc.2008.09.064.

Ayuningrum D, Liu Y, Riyanti, Sibero MT, Kristiana R, Asagabaldan MA, Wuisan ZG, Trianto A, Radjasa OK, Sabdono A, Schäberle TF. 2019. Tunicate-associated bacteria show a great potential for the discovery of antimicrobial compounds. PLoS One 14 (3): e0213797. DOI: 10.1371/journal.pone.0213797.

Bakar K, Mohamad H, Tan HS, Latip J. 2019. Sterols compositions, antibacterial, and antifouling properties from two Malaysian seaweeds: *Dictyota dichotoma* and *Sargassum granuliferum*. J Appl Pharm Sci 9 (10): 47-53. DOI: 10.7324/JAPS.2019.91006.

Baleta FN, Bolaños JM, Ruma OC, Baleta AN, Cariel JD. 2017. Phytochemicals screening and antimicrobial properties of *Sargassum oligocystum* and *Sargassum carssifolium* extract. J Med Plants Stud 5 (1): 382-387.

Bhardwaj P. 2019. Types of sampling in research. J Pract Cardiovasc Sci 5 (3): 157-163. DOI: 10.4103/jpcs.jpcs_62_19.

Bolaños JM, Baleta FN, Cairel JD. 2017. Antimicrobial properties of *Sargassum* spp. (Phaeophyceae) against selected aquaculture pathogens. Intl J Curr Microbiol App Sci 6 (2): 1024-1037. DOI: 10.20546/ijcmas.2017.602.115.

Hernández-Cruz K, Jiménez-Martínez C, Perucini-Avendaño M, Mateo Cid LL, Perea-Flores MdJ, Gutiérrez-López GF, Dávila-Ortiz G. 2022. Chemical and microstructural characterization of three seaweed species from two locations of Veracruz, Mexico. Food Sci Technol 42: e41421. DOI: 10.1590/fst.41421.

Davis WW, Stout TR. 1971. Disc plate method of microbiological antibiotic assay. Appl Environ Microbiol 22 (4): 659-665. DOI: 10.1128/am.22.4.659-665.1971.

Deniaud-Bouët E, Kervarec N, Michel G, Tonon T, Kloareg B, Hervé C. 2014. Chemical and enzymatic fractionation of cell walls from Fucales: Insights into the structure of the extracellular matrix of brown algae. Ann Bot 114 (6): 1203-1216. DOI: 10.1093/aob/mcu096.

Dewinta AF, Susetya IE, Khairunnisa, Suriani M, Addina S, Fadhilah A. 2023. Alginate profile, antioxidant, and antibacterial activities of brown algae *Sargassum cristaefolium* from Pane Island, North Sumatera. Sci J Fish Mar 15 (2): 331-345. DOI: 10.20473/jipk.v15i2.41621.

Abdala-Díaz RT, Cabello-Pasini A, Pérez-Rodríguez E, Álvarez RMC, Figueroa FL. 2006. Daily and seasonal variations of optimum quantum yield and phenolic compounds in *Cystoseira tamariscifolia* (Phaeophyta). Mar Biol 148: 459-465. DOI: 10.1007/s00227-005-0102-6.

Dixit DC, Reddy CRK, Balar N, Suthar P, Gajaria T, Gadhavi DK. 2018. Assessment of the nutritive, biochemical, antioxidant and antibacterial potential of eight tropical macroalgae along Kachchh Coast, India as human food supplements. J Aquat Food Prod Technol 27 (1): 61-79. DOI: 10.1080/10498850.2017.1396274.

Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. 2023. Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. J Exp Pharmacol 15: 51-62. DOI: 10.2147/JEP.S379805.

Etikan I, Bala K. 2017. Combination of probability random sampling method with non probability random sampling method (sampling versus sampling methods). Biometrics Biostat Intl J 5 (6): 210-213. DOI: 10.15406/bbij.2017.05.00148.

Etikan I, Musa SA, Alkassim RS. 2016. Comparison of convenience sampling and purposive sampling. Am J Theor Appl Stat 5 (1): 1-4. DOI: 10.11648/j.ajtas.20160501.11.

Fayzi L, Askarne L, Cherifi O, Boufous EH, Cherifi K. 2020. Comparative antibacterial activity of some selected seaweed extracts from Agadir Coastal Regions in Morocco. Intl J Curr Microbiol Appl Sci 9 (6): 390-399. DOI: 10.20546/ijcmas.2020.906.051.

Nyimbili F, Nyimbili L. 2024. Types of purposive sampling techniques with their examples and application in qualitative research studies. Br J Multidisciplinary Adv Stud 5 (1): 90-99. DOI: 10.37745/bjmas.2022.0419.

Ghazali M, Zaki M, Hidayati E. 2021. Antibacterial activity of methanol extract of *Sargassum polycystum* on *Escherichia coli* and *Staphylococcus aureus*. J Biologi Tropis 21 (1): 199-205. DOI: 10.29303/jbt.v21i1.2485.

Harborne JB. 1998. Textbook of phytochemical methods. A Guide to Modern Techniques of Plant Analysis. 3rd Edition, Chapman and Hall Ltd, London.

Haron FK, Shah MD, Yong YS, Tan JK, Lal MTM, Maran BAV. 2022. Antiparasitic potential of methanol extract of brown alga metabolite profiling. Diversity 14 (10): 796. DOI: 10.3390/d14100796.

Harwanto D, Negara BFSP, Tirtawijaya G, Meinita MDN, Choi J-S. 2022. Evaluation of toxicity of crude phlorotannins and phloroglucinol using different model organisms. Toxins 14 (5): 312. DOI: 10.3390/toxins14050312.

Kim DH, Kim KBWR, Cho JY, Ahn DH. 2014. Inhibitory effects of brown algae extracts on histamine production in mackerel muscle via inhibition of growth and histidine decarboxylase activity of *Morganella morganii*. J Microbiol Biotechnol 24 (4): 465-474. DOI: 10.4014/jmb.1309.09071.

Kim S, Choi SK, Van S, Kim ST, Kang YH, Park SR. 2022. Geographic differentiation of morphological characteristics in the brown seaweed *Sargassum thunbergii* along the Korean Coast: a response to local environmental conditions. J Mar Sci Eng 10 (4): 549. DOI: 10.3390/jmse10040549.

Kok JM-L, Wong C-L. 2022. *In vitro* properties of methanol extract and sodium alginate of *Sargassum polycystum* C. Agardh brown seaweed collected from Malaysia. Trop Life Sci Res 33 (1): 55-76. DOI: 10.21315/tlsr2022.33.1.4.

Kordjazi M, Etemadian Y, Shabanpour B, Pourashouri P. 2019. Chemical composition antioxidant and antimicrobial activities of fucoidan extracted from two species of brown seaweeds (*Sargassum ilicifolium* and *S. angustifolium*) around Qeshm Island. Iran J Fish Sci 18 (3): 457-475. DOI: 10.22092/IJFS.2018.115491.

Li Y, Sun S, Pu X, Yang Y, Zhu F, Zhang S, Xu N. 2018. Evaluation of antimicrobial activities of seaweed resources from Zhejiang Coast, China. Sustainability 10 (7): 2158. DOI: 10.3390/su10072158.

Lopes G, Sousa C, Silva LR, Pinto E, Andrade PB, Bernardo J, Mouga T, Valentão P. 2012. Can phlorotannins purified extracts constitute a novel pharmacological alternative for microbial infections with associated inflammatory conditions? PLoS ONE 7 (2): e31145. DOI: 10.1371/journal.pone.0031145.

Manguntungi B, Sari AP, Ariandi A, Vanggy LR, Erlangga R, Mahesa MA. 2022. Secondary metabolite of Sumbawa algae and its potential as a natural preservative. Biotropia 29 (2): 95-102. DOI: 10.11598/btb.2022.29.2.1625.

Marletta G, Lombardo A, Serio D, Bianchelli S. 2023. Diversity of Fucales (Ochrophyta, Phaeophyceae) along the Coasts of Lipari and Vulcano (Aeolian archipelago), Tyrrhenian Sea (Central Mediterranean Sea). J Mar Sci Eng 11 (12): 2222. DOI: 10.3390/jmse11122222.

Meinita MDN, Akromah N, Andriyani N, Setijanto, Harwanto D, Liu T. 2021a. Molecular identification of *Gracilaria* species (Gracilariales, Rhodophyta) obtained from the South Coast of Java Island, Indonesia. Biodiversitas 22 (7): 3046-3056. DOI: 10.13057/biodiv/d220759.

Meinita MDN, Harwanto D, Amron, Hannan Md.A, Jeong G-T, Moon IS, Choi J-S. 2023. A concise review of the potential utilization based on bioactivity and pharmacological properties of the genus *Gelidium* (Gelidiales, Rhodophyta). J Appl Phycol 35: 1499-1523. DOI: 10.1007/s10811-023-02956-7.

Meinita MDN, Harwanto D, Choi J-S. 2022a. A concise review of the bioactivity and pharmacological properties of the genus *Codium* (Bryopsidales, Chlorophyta). J Appl Phycol 34: 2827-2845. DOI: 10.1007/s10811-022-02842-8.

Meinita MDN, Harwanto D, Choi J-S. 2022b. Seaweed exhibits therapeutic properties against chronic diseases: An overview. Appl Sci 12 (5): 2638. DOI: 10.3390/app12052638.

Meinita MDN, Harwanto D, Tirtawijaya G, Negara BFSP, Sohn J-H, Kim J-S, Choi J-S. 2021b. Fucosterol of marine macroalgae: Bioactivity, safety and toxicity on organism. Mar Drugs 19 (10): 545. DOI: 10.3390/md19100545.

Metwally MA, Ali S, Khatab I, El-Sayed MK. 2020. Antibacterial potential of some seaweeds species to combat biofilm-producing multi-drug resistant *Staphylococcus aureus* of Nile tilapia. Egypt J Bot 60 (1): 9-24. DOI: 10.21608/ejbo.2019.6829.1275.

Moheimanian N, Mirkhani H, Purkhosrow A, Sohrabipour J, Jassbi AR. 2023. *In vitro* and *in vivo* antidiabetic, α-Glucosidase inhibition and antibacterial activities of three brown algae, *Polycladia myrica, Padina antillarum, and Sargassum boveanum,* and a red alga, *Palisada perforata* from the Persian Gulf. Iran J Pharm Res 22 (1): e133731. DOI: 10.5812/ijpr-133731.

Moni SS, Alam MF, Safhi MM, Jabeen A, Sanobar S, Siddiqui R, Moochikkal R. 2018. Potency of nano-antibacterial formulation from *Sargassum binderi* against selected human pathogenic bacteria. Braz J Pharm Sci 54 (4): e17811. DOI: 10.1590/s2175-97902018000417811.

Morales JL, Cantillo-Ciau ZO, Sánchez-Molina I, Mena-Rejón GJ. 2008. Screening of antibacterial and antifungal activities of six marine macroalgae from coasts of Yucatán Peninsula. Pharm Biol 44 (8): 632-635. DOI: 10.1080/13880200600897569.

Mughal UE, Ayaz M, Hussain Z, Hasan A, Sadiq A, Riaz M, Malik A, Hussain S, Choudhary MI. 2006. Synthesis and antibacterial activity of substituted flavones, 4-thioflavones and 4-iminoflavones. Bioorg Med Chem 14 (14): 4704-4711. DOI: 10.1016/j.bmc.2006.03.031.

Munita JM, Arias CA. 2016. Mechanisms of antibiotic resistance. Appl Environ Microbiol 4 (2): VMBF-0016-2015. DOI: 10.1128/microbiolspec.VMBF-0016-2015.

Nofal A, Azzazy M, Ayyad S, Abdelsalm E, Abousekken MS, Tammam O. 2023. Evaluation of the brown alga, *Sargassum muticum* extract as an antimicrobial and feeding additives. Braz J Biol 84: e259721. DOI: 10.1590/1519-6984.259721.

Nortjie E, Basitere M, Moyo D, Nyamukamba P. 2022. Extraction methods, quantitative and qualitative phytochemical screening of medicinal plants for antimicrobial textiles: A review. Plants 11 (15): 2011. DOI: 10.3390/plants11152011.

Oktaviani DF, Nursatya SM, Tristiani F, Faozi AN, Saputra RH, Meinita MDN, Riyanti. 2019. Antibacterial activity from seaweeds *Turbinaria ornata* and *Chaetomorpha antennina* against fouling bacteria. IOP Conf Ser: Earth Environ Sci 255: 012045. DOI: 10.1088/1755-1315/255/1/012045.

Palanisamy S, Vinosha M, Rajasekar P, Anjali R, Sathiyaraj G, Marudhupandi T, Selvam S, Prabhu NM, You S. 2018. Antibacterial efficacy of a fucoidan fraction (Fu-F2) extracted from *Sargassum polycystum*. Intl J Biol Macromol 125: 485-495. DOI: 10.1016/j.ijbiomac.2018.12.070.

Park J-S, Han J-M, Shin Y-N, Park Y-S, Shin Y-R, Park S-W, Roy V-C, Lee H-J, Kumagai Y, Kishimura H, Chun B-S. 2023. Exploring bioactive compounds in brown seaweeds using subcritical water: A comprehensive analysis. Mar Drugs 21 (6): 328. DOI: 10.3390/md21060328.

Rajasekar T, Shamya M, Joseph J. 2019. Screening of phytochemical, antioxidant activity and anti-bacterial activity of marine seaweeds. Intl J Pharm Pharm Sci 11 (1): 61-66. DOI: 10.22159/ijpps.2019v11i1.29119.

Afreen AB, Rasool F, Fatima M. 2023. Bioactive properties of brown seaweed, *Sargassum wightii* and its nutritional, therapeutic potential and health benefits: A review. J Environ Biol 44 (2): 146-158. DOI: 10.22438/jeb/44/2/MRN-5081.

Rattaya S, Benjakul S, Prodpran T. 2015. Extraction, antioxidative, and antimicrobial activities of brown seaweed extracts, *Turbinaria ornata* and *Sargassum polycystum*, grown in Thailand. Intl Aquat Res 7: 1-16. DOI: 10.1007/s40071-014-0085-3.

Riyanti, Marner M, Hartwig C, Patras MA, Wodi SIM, Rieuwpassa FJ, Ijong FG, Balansa W, Schäberle TF. 2020. Sustainable low-volume analysis of environmental samples by Semi-Automated Prioritization of Extracts for Natural Product Research (SeaPEPR). Mar Drugs 18 (12): 649. DOI: 10.3390/md18120649.

Robinson NM, Galicia-Garcia C, Okolodkov YB. 2012. New records of green (Chlorophyta) and brown algae (Phaeophyceae) for Cabezo Reef, National Park Sistema Arrecifal Veracruzano, Gulf of Mexico. Acta Bot Mex 101: 11-48.

Rosa GP, Tavares WR, Sousa PMC, Pagès AK, Seca AML, Pinto DCGA. 2019. Seaweed secondary metabolites with beneficial health effects: An overview of successes in *in vivo* studies and clinical trials. Mar Drugs 18 (1): 8. DOI: 10.3390/md18010008.

Saleh B, Al-Hallab L, Al-Mariri A. 2019. Seaweed extracts effectiveness against selected Gram-negative bacterial isolates. Biol Sci - PJSIR 62 (2): 101-110. DOI: 10.52763/PJSIR.BIOL.SCI.62.2.2019.101.110.

Sargazi F. 2021. Morphological diversity of *Sargassum* species of Oman Sea Coasts. Iran J Bot 27: 62-70. DOI: 10.22092/ijb.2021.353924.1317.

Scania AE, Chasani AR. 2021. The anti-bacterial effect of phenolic compounds from three species of marine macroalgae. Biodiversitas 22 (6): 3412-3417. DOI: 10.13057/biodiv/d220649.

Serwecińska L. 2020. Antimicrobials and antibiotic-resistant bacteria: A risk to the environment and to public health. Water 12 (12): 3313. DOI: 10.3390/w12123313.

Shanmughapriya S, Manilal A, Sujith S, Selvin J, Kiran GS, Natarajaseenivasan K. 2008. Antimicrobial activity of seaweeds extracts against multiresistant pathogens. Ann Microbiol 58: 535-541. DOI: 10.1007/BF03175554.

Sivagnanam SP, Yin S, Choi JH, Park YB, Woo HC, Chun BS. 2015. Biological properties of fucoxanthin in oil recovered from two brown seaweeds using supercritical CO_2 extraction. Mar Drugs 13 (6): 3422-3442. DOI: 10.3390/md13063422.

Sjøtun K, Armitage CS, Eilertsen M, Todt C. 2021. Fauna associated with non-native *Sargassum muticum* (Fucales, Phaeophyceae) vary with thallus morphology and site type (sounds and bays). Mar Biol Res 17 (5-6): 454-466. DOI: 10.1080/17451000.2021.1994999.

Sobuj MKA, Shemul MS, Islam MS, Islam MA, Mely SS, Ayon MH, Pranto SM, Alam MS, Bhuiyan MS, Rafiquzzaman SM. 2024. Qualitative and quantitative phytochemical analysis of brown seaweed *Sargassum polycystum* collected from Bangladesh with its antioxidant activity determination. Food Chem Adv 4: 100565. DOI: 10.1016/j.focha.2023.100565.

Tanniou A, Vandanjon L, Incera M, Leon S, Husa V, Le Grand J, Nicolas JL, Poupart N, Kervarec N, Engelen A, Walsh R, Guerard F, Bourgougnon N, Stiger-Pouvreau V. 2013. Assessment of the spatial variability of phenolic contents and associated bioactivities in the invasive alga *Sargassum Muticum* sampled along its European range from Norway to Portugal. J Appl Phycol 26: 1215-1230. DOI: 10.1007/s10811-013-0198-x.

Thiurunavukkarau R, Shanmugam S, Subramanian K, Pandi P, Muralitharan G, Arokiarajan M, Kasinathan K, Sivaraj A, Kalyanasundaram R, AlOmar SY, Shanmugam V. 2022. Silver nanoparticles synthesized from the seaweed *Sargassum polycystum* and screening for their biological potential. Sci Rep 12: 14757. DOI: 10.1038/s41598-022-18379-2.

Trivedi S, Alshehri MA, Aziz AT, Panneerselvam C, Al-Aoh HA, Maggi F, Sut S, Dall'Acqua S. 2021. Insecticidal, Antibacterial and dye adsorbent properties of *Sargassum muticum* decorated nano-silver particles. S Afr J Bot 139: 432-441. DOI: 10.1016/j.sajb.2021.03.002.

Vasuki S, Kokilam G, Babitha D. 2020. Mineral composition of some selected brown seaweeds from Mandapam Region of Gulf of Mannar, Tamil Nadu. Indian J Geo-Mar Sci 49 (1): 63-66. DOI: 10.56042/ijms.v49i01.68042.

Villegas-Silva VA, Muñoz-Ochoa M, Rodríguez-Montesinos YE, Arvizu-Higuera DL. 2022. Partial characterization and biological activity of *Sargassum Lapazeanum* (Fucales, Phaeophyceae). Hidrobiologica 32 (3): 365-372. DOI: 10.24275/uam/izt/dcbs/hidro/2022v32n3/Munoz.

Chiao-Wei C, Siew-Ling H, Ching-Lee W. 2011. Antibacterial activity of *Sargassum polycystum* C. Agardh and *Padina australis* Hauck (Phaeophyceae). Afr J Biotechnol 10 (64): 14125-1413. DOI: 10.5897/AJB11.966.

Widyartini DS, Widodo P, Susanto AB. 2017. Thallus variation of *Sargassum polycystum* from Central Java, Indonesia. Biodiversitas 18 (3): 1004-1011. DOI: 10.13057/biodiv/d180319.

Wu T, He M, Zang X, Zhou Y, Qiu T, Pan S, Xu X. 2013. A structure–activity relationship study of flavonoids as inhibitors of *E. coli* by membrane interaction effect. Biochim Biophys Acta Biomembr 1828 (11): 2751-2756. DOI: 10.1016/j.bbamem.2013.07.029.

Yap-Dejeto LG, Fabillo M, Sison-Mangus M. 2022. Biodiversity of *Sargassum* (Fucales, Sargassaceae) from Eastern Samar (Philippines) inferred from nuclear ribosomal Internal Transcribed Spacer (ITS) sequence data. Appl Phycol 3 (1): 422-434. DOI: 10.1080/26388081.2022.2119164.

Yip ZT, Quek RZB, Low JKY, Wilson B, Bauman AG, Chou LM, Todd PA, Huang D. 2018. Diversity and phylogeny of *Sargassum* (Fucales, Phaeophyceae) in Singapore. Phytotaxa 369 (3): 200-210. DOI: 10.11646/phytotaxa.369.3.3.

Zerrifi SEA, Khalloufi FE, Oudra B, Vasconcelos V. 2018. Seaweed bioactive compounds against pathogens and microalgae: Potential uses on pharmacology and harmful algae bloom control. Mar Drugs 16 (2): 55. DOI: 10.3390/md16020055.