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Anatomical Structure of *Sargassum Polycystum* Thallus from Menganti and Karimunjawa Beaches, Central Java Indonesia

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Abstract: Observation of anatomical characteristics can help in identifying and supporting information related to alginate productivity. This study aims to determine the anatomical structure of the thallus forms *Sargassum polycystum* and the structural differences in the shape of the thallus from Menganti and Karimunjawa beaches. The anatomical structure of the thallus, which shows the presence of polysaccharides, is a scientific novelty to conclude that the thallus contains alginate so that it can be explored as a sustainable alginate resource. Knowing the structure of the epidermis, cortex, and medulla in the thallus can identify the presence of polysaccharides to show which parts of the talus contain alginates. Scanning Electron Microscopy (SEM) provides a more transparent and more detailed picture of the anatomical structure of the Scanning Electron Microscopy axes, leaves, and vesicles since it has a resolving power more significant than a light microscope. The preparation method uses the Simple Air-Drying preparation technique without pretreatment. The parameters observed were the thickness of the thallus, the thickness of the epidermis, and the cortex's thickness. The data were analyzed descriptively to describe the anatomical structure. A t-test was conducted to determine the differences in the thallus' anatomical structure from Menganti and Karimunjawa beaches. The research results on the anatomical structure of *S. polycystum* from Menganti and Karimunjawa beaches show that the shape of the thallus axes and leaves consists of the epidermis, cortex, and medulla. In contrast, the vesicular thallus consists of the epidermis and cortex layers. From the structure of the epidermis, the presence of polysaccharides can be identified. The thickness of the epidermis from Menganti Beach was higher than that from Karimunjawa Beach. In the thallus axes and vesicles, the epidermal layer is thicker than the leaf thallus, which is assumed to contain more alginate.

Keywords: *Sargassum polycystum*, anatomy, scanning electron microscopy, epidermis, alginate.

体能对女体育教师职业倦怠及心理健康的影响印度尼西亞中爪哇門甘蒂和卡里蒙賈瓦海灘馬尾藻多囊藻的解剖結構

摘要：觀察解剖特徵有助於識別和支持與藻酸鹽生產力相關的信息。本研究旨在確定馬尾藻多囊藻的解剖結構以及來自門甘蒂和卡里蒙賈瓦海灘的藻體形狀的結構差異。菌體的解剖結構顯示了多醣的存在，這是一項科學創新，可以得出結論，菌體含有藻酸鹽，因此可以作為可持續的藻酸鹽資源進行探索。了解菌體中表皮、皮層和髓質的結構可以識別多醣的存在，以顯示距體的哪些部分含有藻酸鹽。掃描電子顯微鏡提供了比光學顯微鏡更重要的分辨能力，因此可以提供更透明、更詳細的骨軸、葉和囊泡解剖結構圖片。製備方法採用簡易風乾製備工藝，無需預處理。觀察到的參數是葉狀體的厚度、表皮的厚度和皮質的厚度。對數據進行描述性分析以描述解剖結構。進行了 t 檢驗以確定來自門甘蒂和卡里蒙賈瓦海灘的葉狀體解剖結構的差異。對門甘蒂和卡里蒙賈瓦 海灘多囊藻解剖結構的研究結果表明，葉狀體

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軸和葉的形狀由表皮、皮層和髓質組成。相比之下，囊泡葉狀體由表皮層和皮質層組成。從表皮的結構可以確定多醣的存在。門甘蒂海灘的表皮厚度高於卡里蒙賈瓦海灘的表皮厚度。在葉狀體軸和囊泡中，表皮層比葉狀體厚，假設葉狀體含有更多的藻酸鹽。

关键词：马尾藻多囊，解剖學，扫描电子显微镜，表皮，藻酸鹽。

1. Introduction

Sargassum polycystum C. Agardh grew in shallow and moderate waters or attached to rocks [1]. The *Sargassum* has a specialized thallus shape, making it easy to distinguish between sections. The base or axis is generally cylindrical and branched but straighter with shorter internodes. Each branch has an air bubble (bladder) that is round or called the vesicle's thallus. The axis's base is the leaf thallus that grows sideways [2-5]. Although the shape is morphologically different, it cannot clearly distinguish between parts. The thallus is not a real organ but is an axis with various shapes. The thallus substance varies, including soft, hard, lime, stringy, and cartilaginous [1].

S. polycystum contains alginate and iodine compounds used in the food, pharmaceutical, cosmetic and textile industries [6, 7]. S. polycystum also contains active steroid compounds, alkaloids, phenols, and triterpenoids with antibacterial, antiviral, and antifungal properties [8]. *S. polycystum* is an alginate-producing brown seaweed widely grown in Menganti and Karimunjawa beaches. The lack of development and fluctuating growth factors in the area has resulted in suboptimal seaweed growth. The range of salinity changes often causes seaweed growth to slow down [9]. Polluted environmental conditions and the availability of nutrients can also lead to morphological and cytological changes, affecting the growth and reproduction of seaweed [10].

Environmental conditions for growing in different places can change the anatomical character of plants [11]. The research results by [5] stated that growth factors such as pH, temperature, and salinity at Karimunjawa and Menganti beaches were related to several morphological characteristics of the thallus *S. polycystum*, such as the shape, length, width, and color of the thallus. Furthermore, *S. polycystum thallus* originating from Karimunjawa is more varied than that from Menganti Beach.

The shape of the thallus can affect the amount of alginate content. According to [10], alginate is contained in the thallus cell wall. The percentage of alginate content in young shoots is smaller than that in old thalli. Axes have thick cell walls compared to leaves and vesicles. [12] found that the alginate compounds formed in the cell walls of *Sargassum* seaweed reach 40% of the total dry weight and play an essential role in maintaining thallus tissue.

The anatomical structure of each seaweed species thallus is different, even though they are from the same genus. Brown seaweed has a complex morphology with anatomical differentiation seen from the development of its parenchyma tissue. Differentiation in vascular plants at the tissue level also occurs in many seaweed groups, especially in the members of Fucales and Laminariales. Cells are morphologically and functionally different in terms of photosynthesis, photosynthate storage, transport, and reproduction. The same tissue in other thallus parts can have different cytological properties.

Scanning electron microscopy (SEM) is a tool for observing the surface of a sample with a magnification of times. Unlike a light microscope, which uses light as its energy source, SEM makes use of a beam of electrons that have a short wavelength and are scattered onto the surface of the sample to obtain images with better resolution [13]. The power possessed by the SEM resolution is greater than that of the light microscope, so using the tool in this study is expected to describe the sample more clearly and in better detail.

SEM was used to observe the anatomical structure of the thallus *S. polycystum* at the axis thallus, vesicle thallus, and leaf thallus. Seaweed samples were taken from Menganti and Karimunjawa beaches and were then compared to determine whether there was a difference in the anatomical structure. Sampling seaweed in Menganti and Karimunjawa beaches confirmed the presence of *S. polycystum*, which still grows wild and has not been well maintained, and the potential development of sustainable seaweed in these two places. Based on the text above, the anatomical structure of the different thallus *S. polycystum* and the differences in the thallus *S. polycystum* between Menganti and Karimunjawa beaches are discussed.

Anatomy is an individual's phenotype or outward appearance resulting from the interaction between the genotype and the environment [14]. The information encoded by the genome, DNA, and the interaction with the domain result in different forms and functions [15]. As described by [16], the epidermal layer can provide information about the presence of polysaccharides, indicating the alginate content. The cortex is between the mesoderm and medulla composed of parenchymal cells, and functions as a food reserve store. The medulla is in the middle of the cross-section of the thallus and forms axes and leaves with thickened walls and an elongated shape. The medulla plays a role in the water and metabolite transport system.

2. Material and Methods

The research material was thallus seaweed S. polycystum from Menganti and Karimunjawa beaches. Iceboxes, tweezers, razors, plastic flips, stationery, freezers, SEM, and computer equipment were used. *S. polycystum* seaweed was sampled on the Menganti and

Karimunjawa coast, followed by sample preparation and observation at the Laboratory of Research Jenderal Soedirman University, Purwokerto. The method used in this research is a survey method performed using the line intercept transect. The transect line is made perpendicular to the shoreline, the distance between transects is 40 meters, and as many as five transect lines are drawn. Three repetitions of each transect were drawn on the reef substrate, each measuring a 1x1 m^2 plot. Seaweed samples of *S. polycystum* were taken using a purposive random sampling technique. The samples were then stored temporarily in the icebox for transport from the location to the lab.

The samples were stored in the freezer of the Aquatic Biology Laboratory of the Faculty of Biology Jenderal Soedirman University before preparing and observing SEM images. The variables evaluated in this study of the anatomical structure of the thallus were the thallus from the axis, leaf, and vesicle from two different places. The parameters observed were thick thalli, thick epidermis, and a thick cortex. Preparation for SEM observation was performed once on any part of the thallus. Data were obtained by calculating the average of three replicate measurements on each parameter.

2.1. Scanning Electron Microscopy

Preparation of samples for SEM: simple air drying without pretreatment technique [17] and setup of the SEM [18]. The seaweed sample was removed from the freezer and dried at room temperature for no less than 24 hours. Thallus forms of axes, vesicles, and leaves were separated from each other and then cut crosswise as thinly as possible using a sharp razor blade. The resulting pieces were glued to the holder. The prepared sample pieces were placed upright with a double-adhesive tip, observing part of the cut field. SEM tools and their peripheral devices were then turned on. A holder was glued by the sample and then introduced into the chamber. The SEM was turned on, followed by the vacuum pump. Preparations were observed, and the best image sensitivity and clarity were achieved by adjusting the picture's lens button, voltage, and magnification. The results show images along with the scale to assist in determining the size of the desired image character.

2.2. Data Analysis

Data were analyzed descriptively, comparing the anatomical structures at different thalli of *S. polycystum.* A t-test was used to compare data from Menganti and Karimunjawa beaches.

3. Results and Discussion

3.1. Anatomical Structures of *S. Polycystum*

The observations are presented in Fig. 1. Anatomical structures of *S. polycystum* were observed in the axis thallus, layers of the epidermis, cortex, and medulla. Axis thalli from the Karimunjawa (A-B) and Menganti (C-D) beaches had the same arrangement of anatomical structures.

Axis thalli consist of layers of epidermis, cortex, and medulla. Thick cells with a uniform shape formed the epidermal layer. Deeper parts are followed by a layer of the cortex that is smaller than the medulla (Fig. 1 A-D).

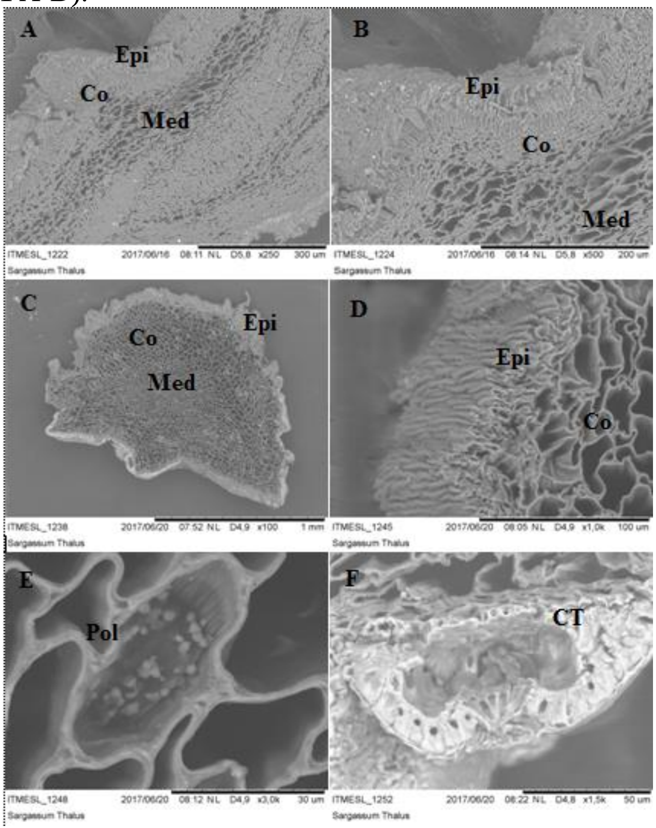

Fig. 1 Anatomical Structures of Axis Thalli from Karimunjawa (A-B) and Menganti (C-F) beaches
Notes: Epi - Epidermis, Co - Cortex, Med - Medulla, Pol - Polysaccharide, CT - Early Stage of Bud

According to [3], the epidermal layer in the axis thallus, also called the mesoderm, forms a compact peripheral that resembles a brick structure, and the cells are oriented around the axis. Layers of the cortex are more extensive than the other parts, formed by polygonal cells, and sometimes pigmented. Polysaccharides in the cortex's cell walls form the medulla (Fig. 1 E). The shapes of the medulla cells are more elongated than those of the cortex, as shown in Fig. 1 B. [16] explained that the cell walls of the cortex and medulla of the axis's thallus consist of the polysaccharide cellulose. The deeper part beyond the cortex, called the medulla, is located more towards the center to form more elongated cells and plays a role in the transport system. Fig. 1 F shows a section developing bud branch candidates. According to [19], the outer layer of the thallus forms an axis that is still meristematic and likely contains structures that will develop new shoots.

The anatomical structure in the leaf thallus has the same structure as in the axis thallus, i.e., epidermis, cortex, and medulla (Fig. 2). According to [16], the leaf thallus in cross-section provides an overview of the epidermis, cortex, and medulla. [20] adds that leaf thallus epidermal cells have many chloroplasts containing chromatophores. Therefore, the leaf thallus plays an essential role in the process of photosynthesis. Part of the cortex is within the epidermis. The medulla is composed of parenchymatic cells and mostly mediates the process of assimilation.

The result of photosynthesis is stored in the cortex and transported by the medulla to all plant parts. The vesicle thallus anatomical structure revealed the epidermis and cortex layers (Fig. 3 A-B, E-F). The cavity is the structure of the vesicle thallus. The presence of the structure is related to its buoyancy function when the plant is submerged in water in the intertidal area and stores water it is beached onto the shore during low tide [16]. [19] stated that the anatomical structure of the vesicle thallus in *the Sargassum* consists of the epidermis and cortex, composed of medulla cells that have undergone lysis in the early stage of development. The medulla cells are only found in the early stages of development; in adult plants, the cells mediate an irreplaceable function of the epidermis and cortex. Fig. 3 C-E provides information about the polysaccharides in the epidermis layer of the vesicle thallus. [21] stated that brown algae cell walls consist of at least two different layers. The innermost layer contains microfibrils that cause cells to become a rigid wall and an outer layer, including an amorphous matrix. This matrix is dominated by small fractions of the compound polysaccharides alginate and sulfate.

The alginate contents in the axis, leaf, and vesicle thallus are different. According to [22], the extracted portion of the thallus affects the seaweed alginate content. Other polysaccharides, such as cellulose, are also present. Sulfate is also found in almost all epidermis layers, including axes, leaves, and vesicles. There is a difference in the number of polysaccharides contained, which is related to each of the structural functions of the thallus.

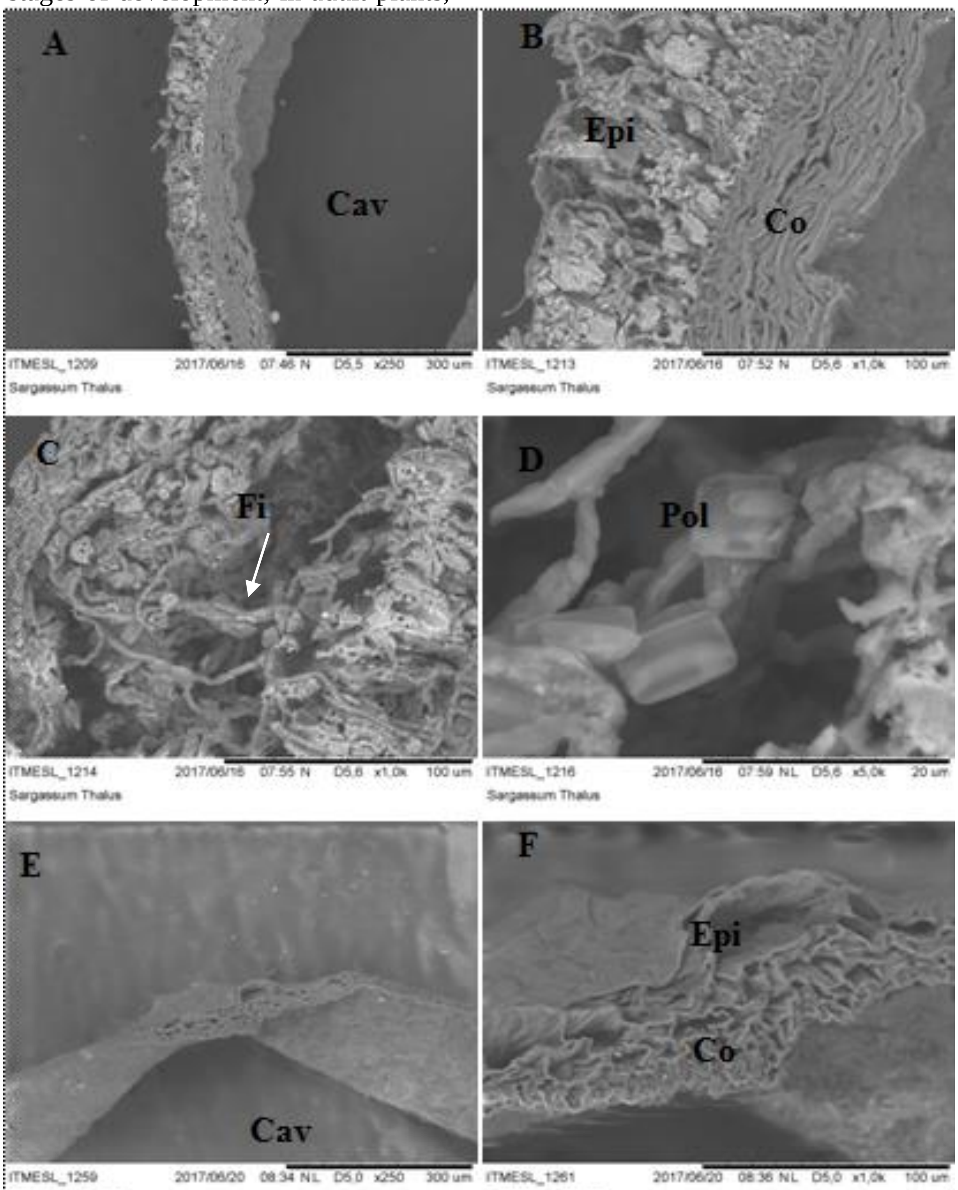

Fig. 2 The anatomical structures of the leaf thalli from Karimunjawa (A-B) and Menganti (C-D) beaches
Notes: Epi - Epidermis, Co - Cortex, Med – Medulla

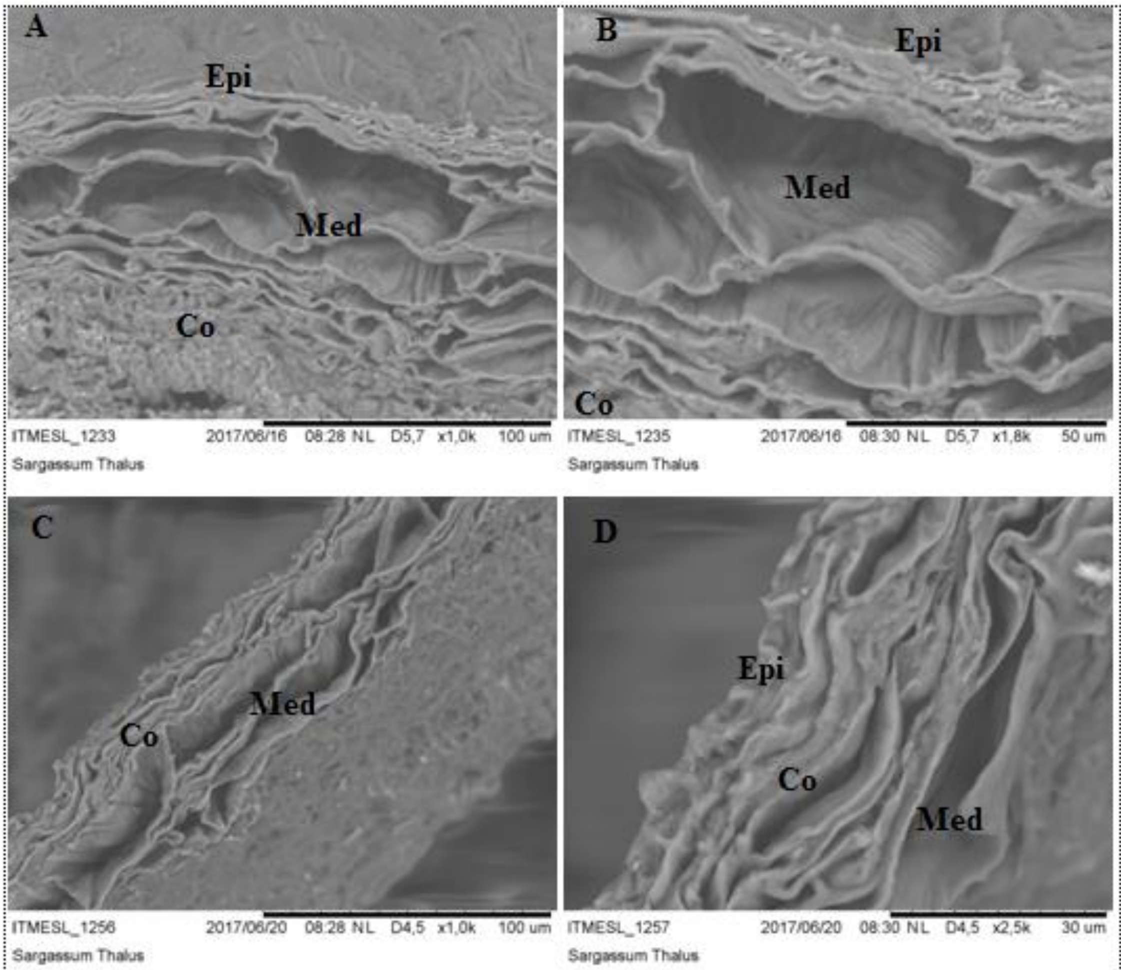

Fig. 3 The anatomical structure of the vesicle thallus from the Karimunjawa (A-D) and Menganti (E-F) beaches
Notes: Epi - Epidermis, Co - Cortex, Cav - Cavity, Pol - Polysaccharide, Fi - Fibrils

According to [23], the thallus, located at the end of the leaf, performs photosynthesis due to its more frequent exposure to sunlight. Axis thalli at the base have a lower water content because they are older and thicker; thus, alginates are more contained in this section. [24] stated that the axis thallus functions as a reserve, hoarding food substances. [25] also explained that the base thallus in *Kappaphycus alvarezii* contains the hormone cytokinin. Thus, base thallus plays a role in cell division and functions as a place to store photosynthesis results. [26] added that the base also has space to grow narrow, as other parts cover its functions. The light absorption is not optimal; therefore, the base thallus plays a role in the assimilation system.

3.2. Differences in the Anatomical Structures of S. Polycystum from the Menganti and Karimunjawa Beaches

The thicknesses of the thallus, epidermis, and cortex in different *S. polycystum* thallus forms are different from each other (Table 1). Overall, this variation is the same whether the *S. polycystum* thallus is from Menganti or Karimunjawa. Axis thalli from Menganti and Karimunjawa beaches are thickest at the epidermis, and cortex, followed by the leaf and vesicle thalli. The size differences are quite distinct between the two places, which is likely due to the age factor of seaweed. According to [27], the length of time spent planting seaweed can determine the seaweed quality. In this case, fewer polysaccharides are produced. The presence of polysaccharides causes the thickening of characteristic seaweed anatomical structures; thus, older seaweed can have characteristic anatomical structures that are thicker than younger seaweed.

Analysis using the t-test indicates that the axis thallus has thick thalli and thick cortex, with t values > t table (2.776), 12.597, and 5.559, respectively. These results indicate that the anatomical structure-derived thalli at the Menganti and Karimunjawa beaches are different at their thick thalli and epidermis. The thick epidermis in the axis thallus, thick thallus, thick epidermis, and thick cortex in the leaf and vesicle thalli from the Menganti and Karimunjawa beaches are not different from each other, as indicated by the t value < t table (Table 2).

Table 1 Average thickness measurement results in thick epidermis, thick cortex, and thick thallus in various *S. polycystum* thallus forms from the Menganti and Karimunjawa beaches

No.	Part of Thallus	Distinctive Characteristic	Menganti Beach (μm)	Karimunjawa Beach (μm)
1.	Axis	Thickness of epidermis	46.67	79

Continuation of Table 1

		Thickness of cortex	383.33	133.33
		Thickness of thallus	1230	530.67
2.	Leaf	Thickness of epidermis	4.67	24.33
		Thickness of cortex	26.67	92.63
		Thickness of thallus	35.83	155
3.	Vesicle	Thickness of epidermis	4	51.73
		Thickness of cortex	29	58.9
		Thickness of thallus	36.83	116

Table 2 Comparative analysis of the anatomical structures of *S. polycystum* in the Menganti and Karimunjawa beaches

Thallus form	**Characteristics**	**Location**	**Mean ± StD**	**T_{value}**	**$t_{tab\ .05}$**	**Sig (p)**
Axis	Thickness of epidermis	Menganti Beach	46.67 ± 5.77	-2.525	2.776	0.065
		Karimunjawa Beach	79 ± 21.41			
	Thickness of cortex	Menganti Beach	383.33 ± 76.38	5.559		0.000
		Karimunjawa Beach	133.33 ± 15.28			
	Thickness of thallus	Menganti Beach	1230 ± 95.39	12.597		0.000
		Karimunjawa Beach	530.67 ± 12.1			
Leaf	Thickness of epidermis	Menganti Beach	4.67 ± 1.15	-17.789		0.000
		Karimunjawa Beach	24.33 ± 1.53			
	Thickness of cortex	Menganti Beach	26.67 ± 2.52	-12.494		0.000
		Karimunjawa Beach	92.63 ± 8.79			
	Thickness of thallus	Menganti Beach	35.83 ± 6.31	-20.553		0.000
		Karimunjawa Beach	155 ± 7.81			
Vesicle	Thickness of epidermis	Menganti Beach	4 ± 1	-22.086		0.000
		Karimunjawa Beach	51.73 ± 3.61			
	Thickness of cortex	Menganti Beach	29 ± 6	-5.217		0.001
		Karimunjawa Beach	58.9 ± 7.91			
	Thickness of thallus	Menganti Beach	36.83 ± 9.84	-9.489		0.000
		Karimunjawa Beach	116 ± 10.58			

[28] stated that anatomical observations showed apparent variations in the size, shape, and number of cells in the genus *Sargassum* spp. These anatomical variations would then support their morphological variation. Morphological differences arise as a result of adaptations of seaweed to environmental factors. [5] explained that some morphological characteristics, such as the shape, size, and color of the thallus, positively correlate with environmental factors, such as pH, temperature, and salinity, meaning that the presence of growth factors significantly affects the variations in seaweed morphology and anatomy. According to [29], morphology is strongly influenced by seaweed physiological tolerance to adapt to the environment, such as temperature, pH, and salinity. [30] added that chemical and physical factors can affect seaweed growth. Biological factors can include waves, and the current can dissolve chemical elements such as nutrients.

Coastal water conditions in Menganti, directly opposite the Indian Ocean, caused strong winds, high waves, and currents, while the marine waters of Karimunjawa are subject to the quieter Java current [5, 31]. Nutrients carried by the strong waves at Menganti Beach rapidly bring nutrients from the open seas to the coast; as a result, the nutrients are evenly distributed in the region and improve seaweed growth. In contrast, in the waters of Karimunjawa, because of the quieter waves, the movement of nutrients in this area is likely to be slower, resulting in less optimal seaweed growth. Water salinity conditions that change can affect the development of seaweed. According to [12], seaweed will experience slow growth when the salinity is too low or high in the range required for seaweed. The optimum salinity range for the development of seaweed is between 28-38 ‰. Differences in salinity can also affect the physiological and biochemical mechanisms that can lead to changes in anatomical structure. According to [5], Menganti Beach has a salinity of 28 ‰, while Karimunjawa Beach – 33.6 ‰. The salinity is still in the range corresponding to the requirements of living seaweed. The degree of acidity of the water also influences the growth of seaweed. The pH value determines the carbon molecules that can be used for photosynthesis in seaweed. The pH suitable for the development of seaweed is 7. If the pH is less than 7 (more acidic), carbon molecules are available in the form of carbonic acid. If the pH value is in the range of 6-10, carbon molecules that exist will be functional in the form of bicarbonate ions, and if the pH is more than 10, carbon will be available in the form of carbonate ions [32]. Such ions would be difficult to absorb by organisms and endanger macroalgae survival, as they can affect physiological processes and lead to changes in anatomy and morphology. [5] mentions that Menganti Beach has a pH of 7, while Karimunjawa has a pH of 6. Another factor supporting the growth of seaweed is the temperature. Temperature is a physical parameter that can directly or indirectly affect the development of marine organisms. Weather plays a role in aquatic biota metabolic rates through related changes in molecular activity [33]. Water temperature affects seaweed physiological functions, such as photosynthesis, respiration, metabolism, growth, and reproduction [24]. According to [34], the ideal growth temperature *of S. polycystum* is 23°C [35], while the temperature range of *Sargassum* growth is 18-24°C. The research results of [5] mention that Karimunjawa Beach ranges between 30-31°C, while Menganti Beach ranges between 29-30°C. So this study shows that *Sargassum* can grow in warm tropical waters

4. Conclusion

The anatomical structure of thallus *Sargassum polycystum* from Menganti and Karimunjawa beaches shows that the thalli of the axes and leaves consist of the epidermis, cortex, and medulla. Vesicles consist of epidermis and cortex layers. From the structure of the epidermis, the presence of polysaccharides can be identified. Axes and vesicles with a thicker epidermis contain more alginate. The thickness of the epidermis from Menganti Beach was higher than that from Karimunjawa Beach. In the thallus axes and vesicles, the epidermal layer is thicker than the leaf thallus, which is assumed to contain more alginate. The cortex is the part between the meristoderm and medulla, composed of parenchymatous cells and serves as a store of food reserves. The medulla is in the middle of the axis of the transverse slice of the stem and leaf shape thallus with thickened walls and elongated shape. Medulla plays a role in the water transport system and metabolites.

SEM with a resolving power more significant than a light microscope will obtain a more transparent and more detailed picture of the anatomical structure of the talus axes, leaves, and vesicles. The preparation method uses the Simple Air-Drying preparation technique without pretreatment. The parameters observed were the thickness of the thallus, the thickness of the epidermis, and the cortex's thickness. The thickness of the epidermis layer of thallus from Menganti Beach is higher than that of Karimunjawa Beach. On-axis and vesicles, the epidermal layer is thicker than the leaf thallus, which is assumed to contain more alginate. The anatomical structure of the thallus, which shows the presence of polysaccharides, is a scientific novelty to conclude that the thallus contains alginate so that it can be explored as a sustainable alginate resource.

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