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Research Article

Alginate Profile, Antioxidant, and Antibacterial Activities of Brown Algae *Sargassum cristaefolium* from Pane Island, North Sumatera

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Abstract

Sargassum, an ubiquitous variant of algae in North Sumatera, has grown wildly on Pane Island with the type, namely, *Sargassum cristaefolium*. This study aimed to determine the characteristics of alginates, antioxidative ability, and antibacterial ability. Alginate was extracted via the acid pathway method from three different parts of the thallus and was then characterized physically and chemically. Antioxidant activity using the DPPH method and the total phenol with a gallic acid standard. Antibacterial activity using the well diffusion method. The characteristics of alginate include yields of 53.61% ± 2.21%, viscosity of 11.44 ± 1.54 cPs, whiteness index of 56.55 ± 1.71, acidity degrees of 9.83 ± 0.34, moisture content of 9.63% ± 0.53%, ash content of 23.17% ± 0.76%, Pb and Hg levels of 0.065 ± 0.01 ppm, 0.034 ± 0.003 ppm. The IC_{50} value of antioxidant activity being 134.408 ± 0.04 ppm, moderate category. Extract concentration of 8 mL can inhibit the *E. coli* and *E. tarda* with inhibitions of 10.60 ± 0.72, 14.03 ± 2.97 mm. Although other pathogenic bacteria can be inhibited optimally at an extract concentration of 10 mL with inhibitions of 11.03 ± 1.43, 9.07 ± 0.20, 11.20 ± 1.56, 12.80 ± 3.01 mm. Alginates isolated in *S.cristaefolium* from Pane Island met food grade standards except for its viscosity. *S. cristaefolium* from Pane Island is a moderate antioxidant. Likewise, its antibacterial ability was moderate however it was strong against aquatic gram-negative bacteria.

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1. Introduction

Due to their several structures and activities, algae are becoming popular as a source of functional foods, food additives, and nutraceuticals (Plaza *et al.*, 2008; Domínguez, 2013). They are an abundant source of an array of naturally occurring bioactive compounds, including polysaccharides, steroids, lipids, polyphenols, and pigments with potential usage (Yadav *et al.*, 2022). Some of these chemicals, including polyphenols, can be found in high concentrations in nature. Polysaccharides in brown algae can also be referred to as primary metabolites, including natural bioactive ones. In most brown algae, two types of polysaccharides are especially found. The chemical compositions of both polysaccharides have sulfated L-fucose (Camara *et al.*, 2011; de Aguiar *et al.*, 2022). As suggested by Reyes *et al.* (2020), polysaccharides composed of up to 90% of L-fucose monosaccharides are called fucoidans. Those with more than 90% fucose in their composition are called fucans. Moreover, other polysaccharides found in brown algae such as laminarin and alginate and other secondary metabolites such as carotenoids (fucoxanthin) and polyphenols (phlorotannin) have also been reported in the literature with antioxidant properties (Sellimi *et al.*, 2018; Wang *et al.*, 2019; de Aguiar *et al.*, 2022).

Bioactive compounds can regulate various metabolic processes in the human body such as free radical scavenging, inhibition or induction of gene expression, receptor activity, and enzymes (Lobo *et al.*, 2010). To address the growing customer demand for natural, nutritious, and economical products, these compounds are increasingly becoming a necessary component for many food enterprises and food industry-based startups (Bansal *et al.*, 2022). Brown algae are a source of interesting natural bioactive compounds that could be employed in the development of new industrial applications (Silva *et al.*, 2021). Phlorotannin, phytosterol, and polyphenol are prominent secondary metabolites groups that are found in brown algae. The variety of compounds within a particular group plays a vital role in many biological activities (Hakim and Patel, 2020). These active metabolites such as halogenated compounds, alcohols, aldehydes, and terpenoids are produced by macroalgae and have antibacterial, antialgal, and antifungal properties that are effective in preventing biofouling and have other uses in therapeutics (Kolanjinathan *et al.*, 2014).

The genus of *Sargassum* consist of 400 species and as many as 150 species are found in tropical, subtropical and winter waters. Several types of *Sargassum* in Indonesian waters are of the type *Sargassum binderi*, *Sargassum cinereum*, *Sargassum duplicatum* (*S. cristaefolium*), *Sargaasum plagyophyllum*, *Sargassum echinocarpum* (*S. olygocystum*), *Sargassum polycystum* (*S. microphyllum*) dan *Sargassum carssifolium* (Triastinurmiatiningsih *et al.*, 2011). *Sargassum* sp. contains alginate and iodine which are used in the food, pharmaceutical, cosmetic and textile industries (Pakidi and Suwoyo, 2017). Development of brown algae application technology *Sargassum* sp. not only in the food sector such as alginate, fodder and fertilizer, but also able to inhibit damage caused by free radicals in product such as fish oil (Winberg *et al.*, 2009). Fucoidans in brown algae are also known to have antitumor, anticancer, anticoagulant, antimetastatic, fibrinolytic, antiviral, antibacterial and antioxidant properties (Prabu *et al.*, 2013). Antioxidants in *Sargassum* sp. capable of reducing oxidation that aoccurs in fish oil emulsion at storage temperature of 50°C for 24 hours with a peroxide value of 59.1 meq/kg (Santoso *et al.*, 2004). The extract of the active compound *Sargassum fillipendula* obtained DPPH free antiradical activity of 81.281 ppm (Khotimah *et al.*, 2013). In addition, *Sargassum* sp. also has the potential of active ingredients that can inhibit the growth of bacteria and viruses (Pakidi and Suwoyo, 2017). *Sargassum* sp. methanol extract demonstrated antimicrobial activity against gram-positive and gram-negative bacteria such as *Bacillus subtilis*, *Escherichia coli*, dan *Staphylococcus aureus* (Patra *et al.*, 2008). The results of the study of antibacterial activity test on tiger shrimp pathogenic bacteria obtained that the seaweed *Sargassum* sp. can be developed for dual cultivation with tiger shrimp, because Sargassum extract is active against the two tested species of Vibrio bacteria namely *Vibrio harveyi* and *Vibrio prahaemolyticus* (Izzati, 2007). There is opportunity to increase alginate production and utilization of the bioactive compounds contained from *Sargassum* sp. in Indonesia to manage resource sustainability.

Sargassum spp. is very abundant and widely distributed in Indonesian waters, including on the west coast of North Sumatera. One of them grows wild on Pane Island with the type, namely *Sargassum cistaefolium* (Dewinta *et al.*, 2020). However, not many local people know the potential and benefits of this brown algae. Existing studies on there are still limited to ecological, biodiversity aspects, and nutritional composition. The ability of natural bioactive compounds and the characteristics of chemical components such as alginate, antioxidants, and antibacterial activity are yet to be known. Therefore, this study aimed to determine the characteristics of alginate in *Sargassum cristaefolium* and the ability of its secondary metabolites as antioxidants and antibacterial. Through

this research, it is hoped that the potential content of *Sargassum cristaefolium* that grows wild in Pane Island can be identified so that it can be utilized by the local community and improve the regional economy.

2. Materials and Methods

2.1 Time and Place of Research

This research was conducted from July 2021 to February 2022 in Pane Island, Tapanuli Tengah, North Sumatera, Indonesia. The coordinate point of the sampling location on Pane Island is 1°56′23.59″ N, 98°29′56.85″ E.

2.2 Materials

The materials used include macroalgae, the species of *S. cristaefolium*, for sample preparation using KOH 0.7%; HCl 5%, for extraction process using HCl 1%; and Na_2CO_3 2%, NaOCl 4%, HCl 10%, Na_2CO_3 10%, isopropyl alcohol p.a 95%, HCl 37%, NaOH 5 mole, and grade p.a chemicals for alginate monomer analysis. Methanol p.a, DPPH (2,2-diphenil-2-picrylhydrazil), HCL 2N, ascorbic acid, Kjeldahl tablets, H_3BO_3 2%, bromcresol green methyl red indicator, NaOH 40%, concentrated HNO_3, $HClO_4$, $NaBH_4$, distilled water, Meyer's reagent (Merck), dragendroff's reagent (Merck), 2 mL concentrated HCl and 10 mL distilled water, Wagner's reagent (Merck), ether, $FeCl_3$, and methanol p.a 99,9%. Chloramphenicol antibiotic, isolate of bacterial pathogens (*Vibrio parahaemolyticus* (ATCC 17802), *Aeromonas salmonicida* (ATCC 33658), *Aeromonas hydrophyla* (ATCC 35654), *Edwardsiella tarda* (ATCC 15947), *Escherichia coli* (ATCC 25922), *Salmonella* sp. (ATCC 14028)), all of bacteria pathogen were collected from the stock of Fish Quarantine and Inspection Agency Regional Surabaya, and dimethyl sulfoxide (DMSO) solvent. The instruments used are a notebook; tissue paper; aluminum foil; zip-lock plastic; cool box; digital scales; oven; calico cloth; 60 mesh size sifting; glass jar; blender; measuring cup of 100 mL; measuring pumpkin of 500 mL; 2 L of beaker glass; petri dish; 2 L-, 1 L-, and 500 mL-sized Erlenmeyer; water bath shaker; measuring pipette; pipette filler; pH meter; centrifuge; hot plate stirrer; viscometer; blender; vials; mortar; petri dish; paper disk; cool box; rotary evaporator; autoclave; laminar air flow; test tube; micropipette; ruler; L rod; bunsen burner; oven; loop needle; filter paper (Whatman number 1); plastic wrap; label paper; incubator; calipers; FTIR (Fourier transform infrared spectroscopy); AAS (atomic absorption spectrophotometers); and UV-Vis spectrophotometers.

2.3 Method

The *Sargassum cristaefolium* sample was taken randomly by looking at the corresponding characteristics and the location of the point that has the most distribution in intertidal zone the waters of Pane Island at a depth of 1.5–2.0 m (Dewinta *et al.*, 2020). The sample collection uses the roaming method by walking down the coast and taking samples directly that are still attached to the substrate and then washing them using seawater to remove dirt and epiphytes. *S. cristaefolium* obtained was put in zip-lock plastic with a little sea water to keep it fresh and was labeled. When there were enough samples collected, they were all combined into the cool box and transported to the laboratory. Sample preparation for extraction alginate was wet sample *S. cristaefolium* is divided into three sample parts according to the parts on the individual macroalgae (all parts of thallus), only receptacle and vesicle thallus parts, and only stem thallus parts. The sample of *S. cristaefolium* was prepared for extraction of secondary metabolites dried in an oven with a temperature of 40°C for 8 days to avoid damage to the components of secondary metabolites contained in the sample. After drying, the samples were cut into pieces and then crushed to become dry simplicial powder.

2.4 Alginic Acid Extraction

The alginate acid extraction method refers to Subagan *et al.* (2020) with modification. *S. cristefolium* has been prepared according to the parts weighed as much as 50 g, extracted by adding a 2% Na_2CO_3 solution of 250 mL, and then heated to a temperature of 70°C for three hours, the results obtained are then filtered and squeezed with the calico cloth to produce pulp and filtrate. The filtrate was then put in a erlenmeyer and was added with 4% NaOCl, that is, as much as 50 mL for 30 minutes to unseat, the resulting filtrate color becomes ivory yellow. The alginate acid deposition process was carried out by adding little by little 10% HCl, that is, as much as 200 mL, and stirring slowly until it forms an alginate acid precipitate with a Ph of 2.8–3.2. After the precipitate is obtained, filtering was carried out to separate alginate acid and residues, and deposits were thoroughly washed with aquifers up to a pH value of 5.

2.5 Alginate Isolation

The alginate isolation method refers to Husni *et al.* (2012). Alginate acid formed was further converted to alginate. The alginate acid formed was deposited by adding 10% Na_2CO_3 until pH becomes 7, and then, the separation of alginate by pouring little by little filtrate into isopropyl alcohol in comparison 1:2. Alginate

that has been obtained is dried using an oven with a temperature of 60°C for 12 hours until the moisture content <12%. The dried sample is blended so that it becomes alginate powder and then sifted with a sieve of 60 mesh. Observation of the color description of the alginate powder refers to Basmal *et al.* (2013), namely white to yellowish brown fibers.

2.6 Analysis of Characteristic Alginate

Alginate characteristic analyses were carried out physically and chemically, including yield, whiteness index, viscosity, pH, moisture content, ash content, metal levels (Pb and Hg), and function group analysis.

2.6.1 Yield

Alginate yield obtained from the seaweed extraction process is measured based on the weight of alginate after drying against the dry weight of the raw material. The alginate yield was measured as follows (Rashedy *et al.*, 2021):

$$Yield\ of\ sodium\ alginate\ (\%) = \frac{weight\ of\ alginate\ (g)}{weight\ of\ seaweed\ dried\ biomass\ (g)}\ x\ 100$$

… Eq 1

2.6.2 Whiteness Index (WI)

White degree testing is done by weighing a sample weighing 1 g and then placed on a cuvette and measured with *a* chromameter CR-300 by being close to the surface of the sample of alginate powder for detection of L * a * b. The alginate whiteness index was measured as follows (Acevedo-Fani *et al.*, 2017):

$$WI = 100 - \sqrt{[(100 - L^*)^2 + (a^*)^2 + (b^*)^2]}$$ … Eq 2

2.6.3 Viscosity

Viscosity analysis refers to Rashedy *et al.* (2021), observations are made at a solid concentration of 1%–5% to find out the relationship between the concentration and viscosity of the solution. The viscosity measurement of the solution is measured using a spindle 2 RVA (Rapid Visco Analyzer) at 100 rpm at room temperature, waiting until the spindle needle stabilizes (up to six revolutions).

2.6.4 Degree of Acidity (pH)

The pH was measured according to Dharmayanti *et al.* (2021). The alginate of 3 g was weighed, was put into a beaker glass size of 300 mL, and was then added with 197 g of distilled water until the total weight was 200 g. The sample was heated while stirring using a stirrer until dissolved at a temperature of 60°C–80°C. Then, the electrodes were dipped in a sample solution that has previously been calibrated first. The pH value was obtained according to what was stated on the screen. Next, the electrodes were rinsed with distilled water.

2.6.5 Moisture Content

Moisture content analysis was carried out according to AOAC (2005), and a sample of 2 g was weighed and then put in a porcelain dish. The sample was preheated in the oven at 105°C for 24 hours and stored in a desiccator for five minutes. Finally, the sample was weighed until its value was stable. The moisture content was calculated using the following formula:

$$Water\ content\ (\%) = \frac{weight\ of\ final\ sample\ (g)}{weight\ of\ initial\ sample\ (g)}\ x\ 100\%$$

… Eq 3

2.6.6 Ash Content

Ash content analysis was carried out according to AOAC (2005), and the final sample of the water content was continued to be heated using an ignition furnace at a temperature of 600°C for 24 hours. Then, the sample is stored in a desiccator for five minutes. Finally, the sample was weighed until its value was stable. The ash content was calculated using the following formula:

$$Ash\ content\ (\%) = \frac{weight\ of\ final\ ash\ sample\ (g)}{weight\ of\ initial\ sample\ (g)}\ x\ 100\%$$

… Eq 4

2.6.7 Metal Levels Pb and Hg

The method of determining Pb and Hg levels was carried out respectively according to Masitoh *et al.* (2014) and SNI 2354.6 (2016), using AAS.

2.6.8 FTIR Analysis

A sample of alginate of 2 mg was put in a small bottle and was added with 200 mL of KBr and then stirred until homogeneous. The mixture was then placed on top of the mold and pressed for a few minutes until pellets formed. The pellet was then inserted into the sample, and its absorption was measured at wavelengths of 400–4000 nm.

2.7 Algal Extraction

The extraction used the maceration method for antioxidant and antibacterial testing (Dewinta *et al.*,

2021). Simplicia powder weighed as much as 500 g and dissolved in a solvent of 5 liters. The solvent used was methanol p.a. Extraction was carried out via the maceration process 48 hours at room temperature via the immersion process. The maceration results were filtered using filter paper and then concentrated with a rotary vacuum evaporator to form a thick extract at a temperature 44°C. Then, the filtrate was stored using a small vial wrapped in aluminum foil.

2.7.1 Phytochemical Analysis

Phytochemical testing of extracts was carried out qualitatively referring to Harbone (1987) with the group of compounds tested including alkaloids, flavonoids, triterpenoids, steroids, saponins, and tannins.

2.7.2 Analysis of Total Phenol Content

Analysis of the total phenol content was carried out based on Kang *et al.* (2010) with modification. Standard gallic acid was prepared by dissolving 5 mg of gallic acid in distilled water using a 25 mL measuring flask. Then, standards were made with concentrations of 31.25, 62.5, 125, 250, and 500 µ/mL. Testing for total phenol content was carried out by dissolving 20 mg of the extract with methanol solvents each in a 25 mL measuring flask and homogenizing with shakers. A total of 0.5 mL extract was taken and added with 50% Folin Ciocalteu reagent as much as 1 mL and allowed to rest for 5 min. Afterward, 1 mL of 5% Na_2CO_3 was added and homogenized in the dark for 1 h. The absorbance value was measured at a wavelength of 725 nm using a UV-Vis spectrophotometer. Using the gallic acid standard curve, the total phenolic content of the extract was calculated. The total phenolic content is calculated using the formula:

$$Total\ Phenolic = \frac{axV/1000}{G} \quad \ldots \text{Eq 5}$$

Description:
α = gallic acid concentration (mg/L)
V = total volume of test solution (mL)
G = sample mass (g)
1000 = conversion factor (mL)

2.7.3 Antioxidant Activity

The antioxidant activity test using the DPPH (1-diphenyl-2-picrylhydrazyl) method with a concentration of 0.1 mM refers to Shekhar and Anju (2014) with modification. Samples of crude seaweed extract were dissolved in methanol with concentrations of 50, 75, 100, 125, and 150 ppm. Ascorbic acid was used as a positive control with concentrations of 1, 2, 3, 4, and 5 ppm. Antioxidant activity testing using the DPPH method is interpreted into the parameter IC50 or inhibition concentration of 50. The antioxidant activity of each sample is expressed by the percentage of free radical inhibition calculate by the formula:

$$Inhibition\ (\%) = \frac{Blank\ absorbance - Sample\ absorbance}{Blank\ absorbance} x\ 100\% \quad \ldots \text{Eq 6}$$

The category of IC_{50} value as an antioxidant according Utami *et al.* (2017) are categorized as very strong if the concentration is <50 ppm, strong category at a concentration of 50-100 ppm, moderate category at a concentration of 101-150 ppm, and weak category at a concentration of 151-200 ppm.

2.8 Antibacterial Activity

Antibacterial activity included positive and negative control tests and seaweed extract concentration. The positive control test was carried out using the antibiotic chloramphenicol, and the negative control test was carried out using water against the pathogen bacteria (*Vibrio parahaemolyticus* (ATCC 17802), *Aeromonas salmonicida* (ATCC 33658), *Aeromonas hydrophyla* (ATCC 35654), *Edwardsiella tarda* (ATCC 15947), *Escherichia coli* (ATCC 25922), and *Salmonella* sp. (ATCC 14028)). The method used was the diffusion method according to Kirby–Bauer. In the media inoculated with the test bacteria. Sterile paper disks were placed on the agar medium and then 2, 4, 6, 8, and 10 mL of crude extract, and DMSO (dimethyl sulfoxide) solvent was dropped with concentrations of 8, 4, 6, 4, 2, and 0 mL. DMSO can dissolve almost all compounds, both polar and nonpolar, and does not have bactericidal activity so it does not interfere with the results of observations of antibacterial activity testing (Akib *et al.*, 2019). The Petri dish was wrapped and stored in an incubator at 37°C for 24 hours. Inhibition of the growth of microorganisms by antimicrobials is seen as clear areas around the disk paper by measuring the diameter of the inhibition zone. Measurement of the resistence zone diameter was carried out using a caliper and was repeated three times in each bacterial isolate tested.

2.9 Data Analysis

The data on alginate characteristics were analyzed using the SPSS program for normality and homogeneity of variance, followed by one-way

analysis of variance and Duncan's multiple range test. The statistical significance level was set at $p < 0.05$. Repetition was carried out three times. Data are expressed as mean ± standard deviation (SD). Linier regression analysis, correlation coefficient and coefficient of determination were performed to correlate the parameters of total phenolic content with % radical scavenging and gallic acid oxidation inhibitory. The data on antibacterial activities were processed descriptively and quantitatively using Excel.

3. Results and Discussion

3.1 Alginate Characteristics

Quality testing is carried out by characterizing alginate products that have been isolated from *S. cristaefolium* using several physical and chemical test parameters. Analysis of the physic quality of alginate from different parts of the thallus obtained a lowest and highest average value, among other yields of 30.60% ± 0.56%–53.61% ± 2.21%, viscosity of

Table 1. Physical characteristics of alginate *S. cristaefolium* quality in different parts

Component	Source of alginate			Standard
	Whole Individual	Receptacle and Vesicles	Stem	
Yield (%)	30.60±0.56	49.31±3.63	53.61±2.21	> 18.00*
Viscosity (cPs)	11.44±1.54	7.39±4.99	9.23±5.69	> 27.00*
Whiteness index	53.28±1.76	56.55±1.71	51.40±1.25	52.80**

Description: *) FAO (2009), **) Yunizal (2004)

Table 2. The chemical characteristics of alginate *S. cristaefolium* in different thallus parts

Component	Source of alginate			Standard
	Whole Individual	Receptacle and Vesicles	Stem	
Degree of Acidity (pH)	9.83±0.34^{b}	8.30±0.17^{a}	9.12±0.43^{b}	3.5-10
Moisture content (%)	9.63±0.53^{b}	9.17±0.06^{ab}	8.62±0.14^{a}	5-20 %
Ash content (%)	23.17±0.76^{b}	20.59±0.35^{a}	22.91±0.69^{b}	18-27 %
Pb (ppm)	0.065±0.01^{b}	0.040±0.08^{a}	0.029±0.01^{a}	< 10 ppm
Hg (ppm)	0.034±0.03^{c}	0.020±0.01^{b}	0.014±0.01^{a}	< 0.04 ppm

Description: FAO (2009)

Table 3. Functional groups of alginates on FTIR result

Wavelength (cm^{-1})	% Transmittance (%T)			Functional groups
	Whole Individual	Receptacle and Vesicles	Stem	
3500-3200	72.6	67.28	72.28	Alcohol/phenol (O-H)
1627.92-1612.49	67.73	66.84	70.45	Carbonyl group (C=O)
3066.82-2931.8	76.31	72.69	78.63	Alkane group (C-H)
1465.9-1415.75	67.5	63.48	65.97	Na in alginate isomer
1300-1000	66.93	64.77	66.73	Carboxyl Group (C-O)
848.68-844.82	64.48	63.43	65.14	Mannuronic fingerprint area

7.39 ± 4.99–11.44 ± 1.54 cPs, and whiteness index of 51.40 ± 1.25–56.55 ± 1.71 (Table 1).

Yields of alginate showed that the thallus stem tissue part of *S. cristaefolium* had the highest yield with a value of 53.61% ± 2.21% but the lowest yield on the whole tissue with a value of 30.60% ± 0.56% (Table 1). The results of the analysis can be found that there is no influence ($p>0.05$) between the difference in tissue parts and the yield value of alginate. The high yield value on the thallus part of the stem is suspected because the thallus stem is the most numerous and strong part of each *S. cristaefolium* compared to the other parts. However, all the yield produced from each part of the *S. cristaefolium* tissue and the whole individual has a value above the standard. Additionally, the proper extraction process, habitat, and lifespan of macroalgae can also affect the yield value. This is in accordance with Dharmayanti *et al.* (2019) who stated that the yield of alginate produced from seaweed is influenced by habitat (light intensity, small wave, or current and aquatic nutrients), the age of brown seaweed, and brown seaweed handling techniques after harvesting and before and the extraction process used. Similarly, according to Sinurat and Marliani (2017), the high yield value in alginate shows that the better the extraction process is carried out and can increase the economic value of alginate.

The viscosity value of alginate produced from whole *S. cristaefolium* has the highest levels of 11.44 ± 1.54 cPs compared to other parts of the tissue. The lowest viscosity levels are produced in the tissue's receptacle and vesicles of 7.39 ± 4.99 cPs. Although the stem has formed polysaccharides, the viscosity level is higher than the receptacle and vesicles. Overall, the viscosity of the alginate produced is quite low when compared to the standard. The results of the analysis found that there was no influence ($p>0.05$) between the difference in thallus tissue parts and the viscosity levels of alginate. The highest viscosity levels were produced in alginate, allegedly because, in whole *S. cristaefolium*, the content of mannuronic salts and guluronic combines well to form polysaccharides so that many gels can be formed. The lowest viscosity level was suspected because this part of the tissue had high guluronate salt content, so it was easily ionized with water. According to Rashedy *et al.* (2021), low viscosity levels due to the tip of seaweed still formed thick guluronate salt where the compound is easily ionized and reacts with water, whereas the base is not in the form of salt anymore but a polysaccharide that when mixed with water will form a gel. The gel is not ionized, and its nonelectrolyte polysaccharide properties cause its viscosity to be high. Additionally, low viscosity levels are thought to result from the amount of solvent used too much, thus shortening the chain of alginate polymer produced. This result is not much different from Aristya *et al.* (2017) who stated that *Sargassum* sp. seaweed, which produces an alginate viscosity value of 5–10 cP. This is in accordance with Nurkhanifah and Husni (2020), in that the shorter the chain of alginate polymers, the smaller the molecular weight. The more solvents that can extract seaweed, it will eventually multiply the long chain of alginate cut into short chains that can lower the viscosity of alginate (Husni *et al.*, 2012).

The whiteness index obtained from L*a*b is 53.28 ± 1.76; 56.55 ± 1.71; 51.40 ± 1.25 so the highest value for degrees of discharge is generated from the receptacle and vesicle tissue parts *S. cristaefolium*. The results of the analysis can be found that there is no influence ($p>0.05$) between the difference in plant tissue parts and the white degree value of alginate. In the stem, the whiteness index value is in accordance with the standard, whereas in other parts, it is slightly higher than the standard. The whiteness index affected by the alginate bleaching agent is NaOCl. The whiteness index produced from *S.cristaefolium* is higher than the research by Herdianto and Husni (2019) using *S. muticum* produced whiteness index from 32.30 ± 4.79 to 42.20 ± 10.85. According Herdianto and Husni (2019), a higher the whiteness index indicates a paler alginate color, the paler color, the better quality of the alginate. Research results from Maharani *et al.* (2017) also explained that the alginate extraction path method between the alginic acid and calcium alginate method in *S. fluitans* has a higher degree of whiteness (73.43 ± 3.09 dan 75.30 ± 4.83). This shows that the extraction method is also one of the factors that affect the degree of whiteness. In addition to other factors, according to Finotelli *et al.* (2008), bleaching with NaOCl can give the alginate a pale-yellow color. NaOCl can cause oxidation of the brown color of alginate. Fucoxanthin compounds and other pigments found in alginate can determine the color.

The alginate acidity from the whole thallus of 9.83 ± 0.34 was the highest pH value in the study, whereas that from receptacle and vesicle tissue of 8.3 ± 0.17 was the lowest pH value (Table 2). In general, the pH value of alginate ranges from 9 to 10. Alginate produced from intact individual parts has the highest moisture content, that is, 9.63% ± 0.53%, whereas the thallus part of the stem has the lowest moisture content, that is, 8.62% ± 0.14%. Overall, the water content obtained is quite good and follows alginate quality standards. Differences in moisture

content occur because each material can absorb water differently. This can be seen from the results of the various analysis showing a noticeable influence of the use of *S. cristaefolium* parts against the moisture content ($p<0.05$) and the interaction between the tissue parts used. According to Zailanie *et al.* (2001), the high content of alginate moisture made from the thallus part of all tissues where the result is also not much different from the receptacle and vesicle parts are suspected because in the receptacle and vesicle are still formed guluronate salts that have a free water content bound with hydrogen bonds. While at the base, the moisture content is low because the base has formed guluronic acid and mannuronic acid, which have hydrophilic properties that bind water, increasing the amount of water trapped in guluronic acid and mannuronic acid. According to Dharmayanti *et al.* (2019), the moisture content in alginate is between 5-20%, while the moisture allowed by the FCC (2004) is <15%. JECFA also stated that the moisture cotent of food additives of alginate is maximum at 15% (FAO, 2009). Moisture content of the alginate produced in part thallus of *S. cristaefolium* are 8.62% ± 0.14% – 9.63% ± 0.53% met the standard from FAO and FCC. The moisture content greatly affects the quality of a material. Water contained in an ingredient can affect its taste, texture, shelf life, and appearance. High water content can make fungi and bacteria easy to grow, which causes the material to be easily damaged and cannot be stored for longer (Halim *et al*., 2011; Tambunan *et al*., 2013).

The highest ash levels are produced in the intact part of *S. cristaefolium* which is 23.17% ± 0.76% while the receptacle and vesicle have the lowest ash content with a content of 20.59% ± 0.35%. This level still meets the quality standards of the food chemical codex The results of various analyses showed a significant effect of the use of *S. cristaefolium* tissue parts on the ash content ($p < 0.05$). Ash levels in alginate still meet the quality requirements of alginate, this is influenced by the living habitat of *S. cristaefolium*. Alginate ash levels in this study were almost the same as alginate ash levels in Srikandi *et al.* (2013) and Gazali *et al.* (2018) by 23.31%–30%. Alginate ash levels are expressed as a percentage of the weight of the ash against the weight of the dry sample. *Sargassum* grows by attaching to the rock and absorbing minerals from the rock through the entire talus surface, so many minerals are absorbed. The number of minerals absorbed will affect the ash levels in seaweed which makes the ash level in seaweed high (Gazali *et al*., 2018).

The results of the analysis of Pb and Hg lead levels in alginate still meet the standard requirements. The highest Pb levels in alginate were obtained in the intact tissue with levels of 0.065 ± 0.01 ppm and the lowest Pb levels in the stem with levels of 0.029 ± 0.001 ppm. The Hg level in the sample was obtained in the intact tissue section at 0.034 ± 0.0036 ppm, and the lowest Hg level was 0.014 ± 0.014 ppm in the stem. The results of the various analyses showed a noticeable influence of the use of *S. cristaefolium* parts on the levels of heavy metals Pb and Hg ($p<0.05$). Pb and Hg levels are still in accordance with the quality standards of alginate products. According to Nurkhanifah and Husni (2020), seaweed has mineral-rich materials such as Na, K, Ca, and Mg. The Pb and Hg metal levels of alginate produced from *S. cristaefolium* have low levels. This means that the living habitat environment of *S. cristaefolium* in the waters of Pane Island is not polluted by heavy metals Pb and Hg, so it is feasible and safe to use as a raw material for making alginate. According to Dewinta *et al.* (2022), *Sargassum* sp. is capable of absorbing heavy metals. It is known to be effective in removing metal ions and polar organic compounds. In seaweed cells, there is a large shallow area, as a place where the rapid and reversible binding of ions occurs. *S. cristaefolium* is a brown marine alga that has a high adsorption ability because its cell wall contains polysaccharides.

3.2 Alginate Function Group

The resulting alginate powder can be analyzed by its function group to prove that the resulting product is alginate. Analysis of the function group is carried out using an infrared spectrophotometer. The infrared spectrum is used to determine the presence of several chemical bonds in organic compounds. Alginate produced from three different parts of *the S. cristaefolium* tissues has the same functional group. The alginate spectrum shows a typical uptake indicating a hydroxyl group (–OH) at wavelength 3500–3200 cm^{-1} (Figure 1) and a carbonyl group (C=O) at wavelength 1627.92–1612.49 cm^{-1} and 3066.82–2931.8 cm^{-1} indicating an alkane group (Table 3). Wavelength 1465.9–1415.75 cm^{-1} indicates Na in the alginate isomer; the carboxyl group (C–O) is at wavelength 1300–1000 cm^{-1}. The mannuronic fingerprint region at wave number 848.68–844.82 cm^{-1}. Alginate constituent function groups, namely, hydroxyl function groups (OH), carbonyl function groups (C=O), and carboxyl function groups (C–O). The results obtained from the analysis of the function group in this study have similarities with the research of Jayanudin *et al.* (2014) and Nurkhanifah and Husni (2020).

3.3 Phytochemical Constituents of S. Cristaefolium

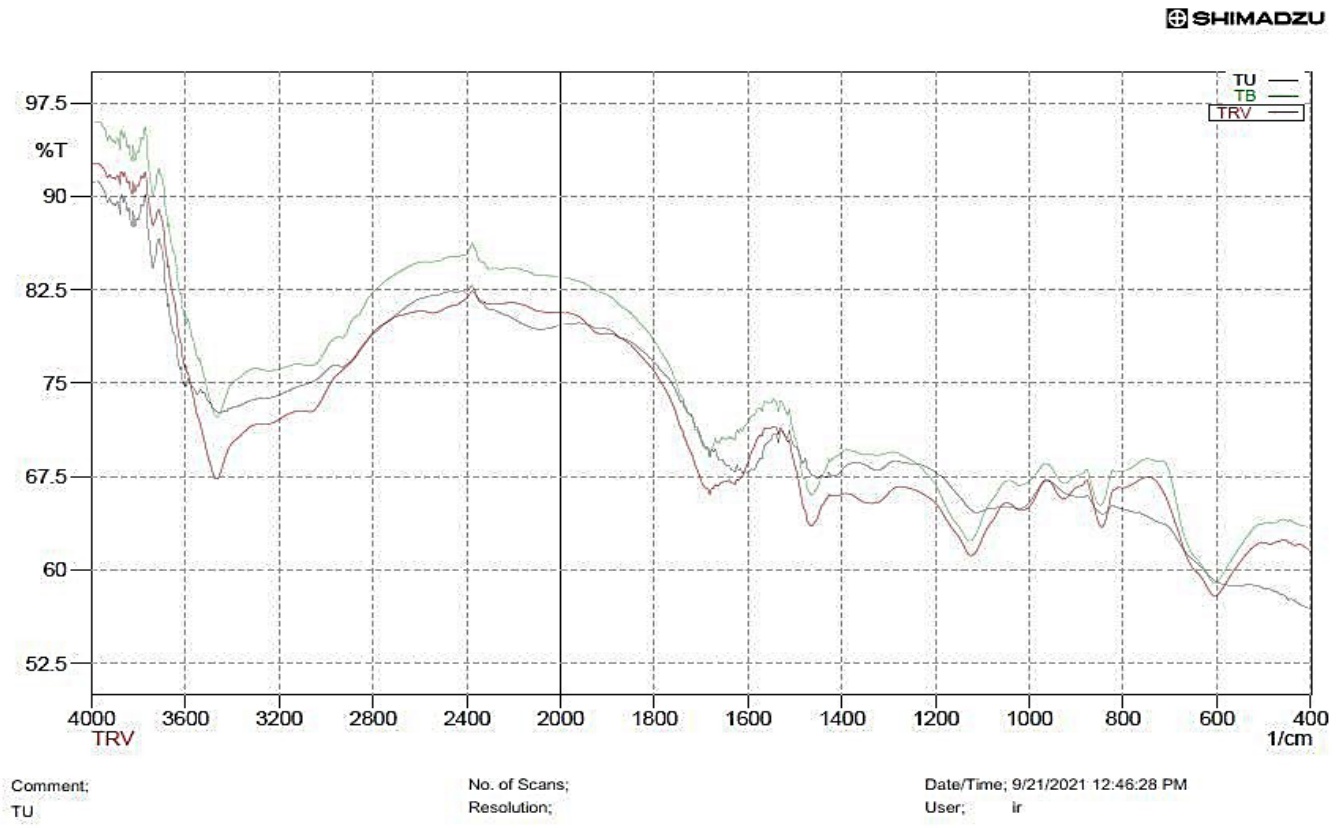

Figure 1. FTIR results of alginate per part of *S. cristaefolium*

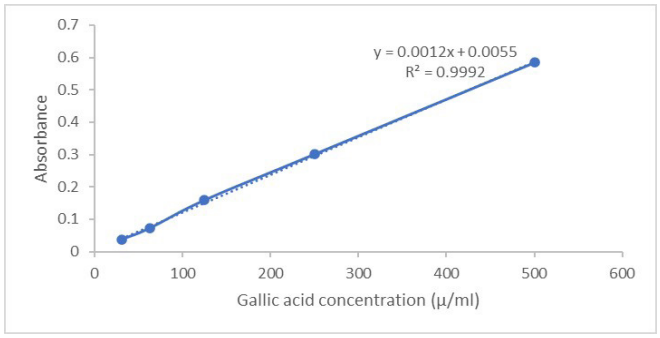

Figure 2. Relationship of absorption with gallic acid concentration

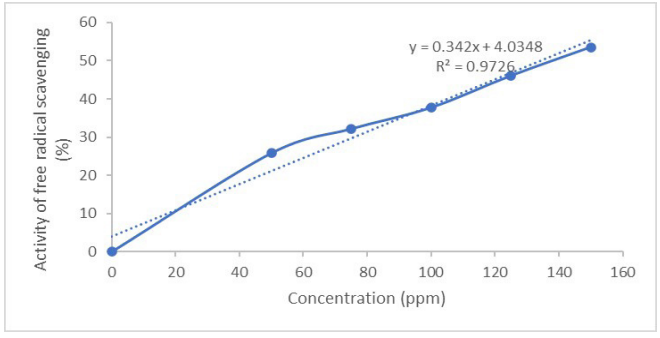

Figure 3. Total phenolic content of *S. cristaefolium* extract

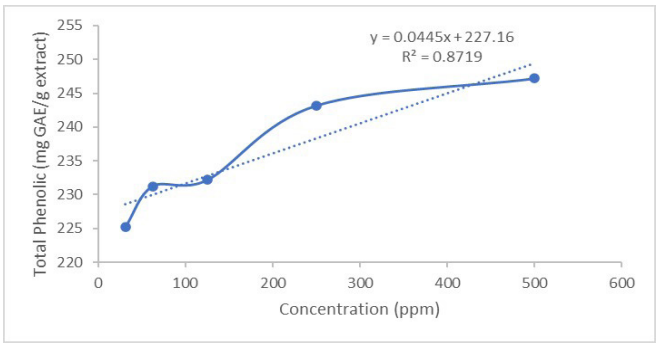

Figure 4. Antioxidant activity of *S. cristaefolium* extract

Phytochemical constituents show distinct patterns of chemical compositions in constituents of the extracts. This analysis showed the most abundant compounds in *S. cristaefolium* were glycosides, flavonoids, alkaloids, triterpenes, and sterols. Saponins and tannins were absent in this seaweed. The phytochemical constituent showed that the crude methanol extract of *S. cristaefolium* could act as an antioxidant and antibacterial (Table 4). Nofal *et al.* (2022) reported that flavonoids are bioactive compounds that exited the Sargassaceae family, these flavonoids indicate that seaweed can be used in medicine and agriculture as an alternative source

of natural antimicrobial. Flavonoids are effective antioxidants and have lately been of great importance in treating diseases because of their possible beneficial effects on human health (Subathraa and Poonguzhali, 2013). Based on Dominguez (2013), the main sterols in macroalgae are cholesterol, fucosterol, isofucosterol, clionasterol. Fucosterol is present in high amounts in a brown seaweed, exhibited considerable antioxidant activity in the 2,2-dipenyl-1-picrylhydrazyl (DPPH) assay (Ito *et al*., 2018). Fernando *et al*. (2019) have reported that fucosterol from brown seaweed *S. binderi* increased the level of enzymatic antioxidant in the nucleus. Triterpenes compounds are toxic and contain halogens, especially chlorine compounds, besides that triterpenes also contain nitrogen in the form of amides or indole which have antibacterial and antifungal activity (Radiena and Dompeipen, 2019). Alkaloid compounds have the ability to inhibit bacterial growth by changing the genetic balance in DNA acids so that the bacterial DNA is damaged which encourages bacterial cells to lyse (Siregar *et al*., 2012).

Table 4. Phytochemical constituents of *S. cristaefolium*

Constituents	Extract *S. cristaefolium* in Methanol
Saponins	-
Tannins	-
Glycosides	+
Flavonoids	+
Alkaloids	+
Triterpenes and Steroids	+

Description: (+) detected, (-) not detected

3.4 Total Phenolic Content

The total phenolic content of *S.cristaefolium* extract increased the value of the sample concentration, which was increasing. The total phenol content value at a concentration of 500 µ/mL was 2.47 ± 0.63 g GAE/100 g extract (Figure 2). This result is slightly higher than the total phenol in *S. muticum* of 2.30 ± 17.1 g/100 g dry weight (Farvin and Jacobsen, 2013); *S. binderi* of 0.03 g GAE/100 g extract (Boonchum *et al*., 2011). The value of total phenol in the methanol extract of *S. cristaefolium* will have a positive correlation with the value of antioxidant activity (Figure 3) which can be detected from the phytochemical analysis. The level of total phenol content is influenced by intrinsic factors (species and type of sample, sample age, and sampling location) and extrinsic factors (climate, temperature, salinity, depth, tidal zone, and tidal cycle) (Lann *et al*., 2012). The total phenolic content was supported by the results of the extract's phytochemical tests, which indicated the presence of phenolic compounds in the flavonoid. Flavonoids have a more complex phenol group with higher degree of hydroxylation, the presence of this hydroxyl group will cause antioxidant activity (Martono *et al*., 2016).

3.5 Antioxidant Activity

The results showed that the higher the sample concentration, the more the DPPH free radical scavenging activity increased (Figure 4). The IC_{50} value of the DPPH antioxidant activity of *S.cristaefolium* extract was 134.408 ± 0.04 ppm. Based on its IC_{50} value, the methanol extract of *S. cristaefolium* has the potential as an antioxidant in the moderate category (concentration 101–150 ppm) (Utami *et al*., 2017). The results of the antioxidant activity correlated with the total phenolic content, which was obtained where the value was quite

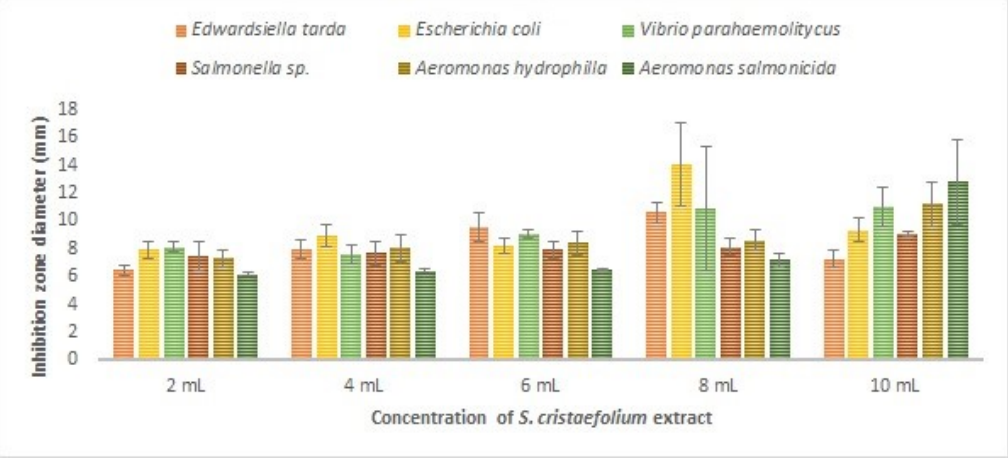

Figure 5. Antibacterial activity of *S. cristaefolium* extract against pathogenis bacteria.

low. Based on Yangthong *et al.* (2009) that *Sargassum* sp. which has the highest total phenolic content has the lowest IC_{50}. Sarini *et al.* (2014) also explained that a low IC_{50} value indicates a strong ability of the extract to act as a hydrogen atom donor. Phenolic compounds in the form of flavonoids can act as antioxidants, the activity of flavonoids is very dependent on the number and location of the -OH group which in this case plays a role in neutralizing free radicals (Apak, 2007). The ability of flavonoids to suppress free radicals is also related to their ability to donate electrons (Nur *et al*., 2019).

3.6 Antibacterial Activity

Antibacterial activity testing using pathogenic bacteria *Vibrio parahaemolyticus*, *Aeromonas salmonicida*, *Aeromonas hydrophyla*, *Edwardsiella tarda*, *Escherichia coli*, and *Salmonella* sp. The results of the antibacterial activity test of the *S. cristaefolium* extract showed an inhibition zone on the six test bacteria, which were gram-negative aquatic pathogenic bacteria. The effectiveness of the highest concentration of *S. cristaefolium* extract against the inhibition zone was shown for each pathogenic bacterium. *E. tarda* and *E. coli* growth were effectively inhibited at an extract concentration of 8 mL with the highest inhibition zones of 10.60 ± 0.72 mm dan 14.03 ± 2.97 mm, respectively. Conversely, for *V. parahaemolyticus*, *Salmonella* sp., *A. hydrophilla*, and *A. salmonicida* bacteria, the inhibition zone was most effectively inhibited at an extract concentration of 10 mL with respective values of 11.03 ± 1.43 mm, 9.07 ± 0.20 mm, 11.20 ± 1.56mm, and 12.80 ± 3.01 mm (Figure 5). Based on the category inhibition zone diameter according to Surjowardojo *et al.* (2015), the inhibition of *E. tarda* and *A. salmonicida* was in the moderate category, but against *E. coli*, *V. parahaemolyticus*, *Salmonella* sp., and *A. hydrophilla* was in strong category. This value is smaller than the research results obtained from Nofal *et al.* (2022) using methanol extract from *S. muticum*, which significantly inhibited the growth of *S. typhi*, *E. coli*, *Staphylococcus aureus*, and *Bacillus subtilis* (25.66, 24.33, 22.33, and 19.66 mm, respectively). However, the difference in antimicrobial activity may be due to the prevalence among these organisms of multiple antibacterial substances, the ability of the extraction protocol to recover the active metabolites, and the methods of assay. Antibacterial activity in *S. cristaefolium* extract is due to the presence of phenol group compounds, namely steroids (Table 4). Steroids are able to inhibit bacterial growth by inhibiting the mechanism of bacterial protein synthesis (Siregar *et al*., 2012). Triterpenes also contain nitrogen in the form of amides or indole which have antibacterial activity (Radiena and Dompeipen, 2019).

4. Conclusion

In conclusion, alginate isolated from three different thallus parts of *Sargassum cristaefolium* from Pane Island has in accordance with the food grade standards except for the viscosity parameter. The physical characteristics of alginate in this study did not significantly affect the thallus part. Meanwhile, for the chemical characteristics of alginate, all the parameters have a significant effect on the different thallus parts. Then for the analysis of the FTIR functional group, it can be ascertained that the sample contains an alginate polymer. *S. cristaefolium* from Pane Island has the potential as an antioxidant in the moderate category. Likewise, with its ability as an antibacterial, *S. cristaefolium* extract has an inhibition zone effectiveness in the moderate and strong category against aquatic gram-negative bacteria. More research is needed to establish the nutritional value of macroalga or other types of *Sargassum* on Pane Island and the entire coastline in Central Tapanuli Tengah that can contribute to fisheries sector and human.

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Authors' Contributions

AFD devised the main conceptual ideas for this research, did an experiment in a laboratory, make an analysis for data results, and draft the manuscript. IES, K, SA, and AF collected samples and data, discussed the results, and contributed to the final manuscript. MS helps translate analysis results into English.

Conflict of Interest

The authors declare that they have no competing interests.

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