Antioxidant, anti-inflammatory and anticoagulant activities of sulfate polysaccharide isolate from brown alga Sargassum polycystum

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Abstract. This study investigated the effect of sulphate polysaccharide isolate obtained from brown alga Sargassum polycystum on their biological activities. The antioxidant activity using FRAP (Ferric Reducing Antioxidant Power) method showed an IC⁵₀ value of 91.306 ppm compared to vitamin C as a positive control, with an IC⁵₀ value of 4,1667 ppm sulphate polysaccharide isolate showing intense antioxidant activity. In vivo anti-inflammatory activity of sulphate polysaccharide isolate from the brown algae has been done using CFA (Completed Freund’s Adjuvant). This study used 25 mice divided into five groups, namely the positive control group, negative control, the sulphate polysaccharide isolate compounds (doses of 10, 50, and 250 mg/kg). The results showed that the sulphate polysaccharide isolate had an anti-inflammatory effect that was not signed with positive controls but it was significantly different from negative controls. In anticoagulant activity, 25 mice were divided into five treatment groups: negative control, positive control, sulphate polysaccharide isolate sample with the dosage of 25, 50 and 100 mg/kg. The results showed that the sulphate polysaccharide isolate dosage of 100 mg/kg has anticoagulant activity. It can be concluded that sulphate polysaccharide isolates isolate has the potency to prevent and treat cardiovascular disease.

Keywords: anticoagulant; antioxidant; anti-inflammatory; Sargassum polycystum; sulphate polysaccharide isolate

1. Introduction

The largest deaths in the world are caused by Cardiovascular Diseases (CVDs). Around 32% (17.9 million people) of all deaths in the world were caused by cardiovascular disease in 2019. More than 75% of cases occurred in lower-middle-income countries [1]. Antioxidants, anti-inflammatory, and anticoagulants are essential to prevent cardiovascular disease, the leading cause of death. In preventing blockage of blood vessels, such as in heart attack conditions, one of the recommended therapeutic regimens is needed, namely anticoagulants [2]. Several studies related to polysaccharide compounds have been found to have activities that can neutralise free radicals, such as sulphate polysaccharide isolate from Sargassum crassifolium [3] and Sargassum sp. [4].

Research on the antioxidant activity of brown algae extract that has been carried out previously using the DPPH method has the potential for radical scavenging in Sargassum siliquatrum with IC⁵₀ of 1 mg/mL [5], in Sargassum pallidum with IC⁵₀ of 11.53 mg/mL [6], and in Sargassum horneri with an IC⁵₀ of 2.5 mg/mL [7]. Several previous studies have shown several different types of brown algae as an anti-inflammatory. Research by Kang et al. [8] showed that the anti-inflammatory activity of dichloromethane extract from Sargassum fulvellum was 79.1% and ethanol extract from Sargassum
thunbergii was 72.1%. The study showed that extracts of brown seaweed *Sargassum fulvellum* and *Sargassum thunbergii* had strong anti-inflammatory activity. Whereas the study of Dore [9] showed the anti-inflammatory activity of isolates from *Sargassum vulgare* with a significant reduction in edema. One type of anticoagulant drug that is often used is heparin and warfarin, which have activity in the blood clotting process that can inhibit the formation of thrombin and can also bind calcium. However, there are also side effects, namely hypotension, thrombocytopenia, impaired liver function, and if used for a long time, can cause osteoporosis [10]. Moreover, the number of side effects caused by non-steroidal anti-inflammatories and steroids has led to the development of anti-inflammatories derived from natural ingredients, mainly brown algae, which have relatively more minor side effects.

This study aimed to study antioxidant, anti-inflammatory, and anticoagulants, which is essential to prevent cardiovascular disease, of sulphate polysaccharide from brown algae *Sargassum polycystum*. Brown algae *Sargassum sp.* is one of the endemic seaweeds that are widely spread in the South Sulawesi region. There are no studies before about activity of sulphate polysaccharide from *Sargassum sp* on antioxidant, antiinflammation and anticoagulant. Because Indonesia is the 2nd largest producer of seaweed in the world in 2015. Its production in that year reached 11.2 million tons. With a sea line length of 61,000 km and an island distribution of more than 17000, Indonesia has grown to become the second largest seaweed producer in the world after China. Globally, seaweed production from Indonesia accounts for 38% of total seaweed production. The largest seaweed producing area in Indonesia is South Sulawesi Province [11]. It could be a good reason for developing this study in the future as source of cardiovascular drug.

2. Methods

2.1 Sample preparation

Samples of brown algae (*Sargassum polycystum*) were obtained from the beach in Takalar Regency, South Sulawesi. The samples were washed with seawater to remove sand and other adhering impurities, and to keep the active compounds from being damaged. The sample was then rinsed using running water 2 times to remove residual salt and impurities. The next stage was rinsing using aquadest to ensure the cleanliness of the sample. The sample was then dried by aerating at room temperature until the sample was dry.

2.2. Sample extraction method

The dry sample of brown algae (*Sargassum polycystum*) which has been ground, was sieved on a mesh number 100 and then extracted using 0.1 N HCl solvent at 100°C for 4 hours. A total of 200 g of brown seaweed (*Sargassum polycystum*) simplicia powder was weighed and put into an infusion pot. Then it was extracted using 1000 mL of 0.1 N hydrochloric acid solution at a temperature of 85 ± 50°C for 4 hours while stirring every 10 minutes. Then filtered and squeezed using a filter cloth to obtain a liquid extract. The liquid extract obtained was then mixed in a 2% CaCl₂ solution to precipitate the alginate contained in the extract. The precipitate formed is separated by pouring sediment. The filtrate was then added with 96% ethanol in a ratio (1:1) to form a precipitate again. The precipitate was collected and placed on a porcelain dish and fresh dried. After drying, grind to a powder. Then the isolate polysaccharide sulfate powder was weighed [12].

2.3. Identification of Sulfate Polysaccharide Compounds

2.3.1. Qualitative test of polysaccharide content. A sample of 10 mg was dissolved in 5 mL of aquadest, then 10 drops were put in a test tube, then 2 drops of iodine solution were added. Color changes are observed during processing. The addition of iodine solution to the polysaccharide will form a specific color adsorption complex. Starch or starch with iodine produces a blue color, dextrin produces a red wine color, while glycogen and some hydrolyzed starch react with iodine to form a brown color [10].
2.3.2. **Determination of sulphate content.** The UV-spectrophotometry method of sulfated polysaccharide compounds was analysed using a UV spectrophotometer. 20 mg of sulfated polysaccharide dissolved in 25 HCl 0.1 N. Then, the absorbance was measured at a wavelength of 200-400 nm [10]. Analysis of sulphated polysaccharide compounds by infrared spectrophotometry (FTIR) method was also carried out using the FTIR spectrophotometric method, namely 1 mg of sulfate polysaccharide powder was mixed with 10 mg of KBr, ground in a mortar and pressed to obtain pellets. It was then inserted into the FTIR spectrophotometer, and the absorbance was measured at a wavelength of 200-400 nm [10].

2.4 **Antioxidant Activity Test using the FRAP Method**

A total of 50 mg of the sample was carefully weighed and dissolved in 5 mL of pure water (WaterOne®) in a 5 ml volumetric flask until it reached the limit so that a concentration of 10000 ppm was obtained. Then 130, 150, 170, 190 and 210 μl of the stock solution were taken into a 5 ml volumetric flask to obtain concentrations of 260, 300, 340, 380 and 420 ppm, added respectively. 1 mL of 0.2 N phosphate buffer (pH 6.6) and 1 mL of 1% K₃Fe(CN)₆. Then it was incubated for 20 minutes at 50°C. After incubation, 1 mL of 10% TCA solution was added and then centrifuged at 3000 rpm for 10 minutes. After being centrifuged, 2 mL of the top layer was pipetted into a volumetric flask, and 2 mL of distilled water and 0.4 mL of 0.1% FeCl₃ were added. The solution was allowed to stand for 10 minutes and the maximum absorption was measured. Work is carried out in a place that is protected from direct sunlight to prevent the oxidation [13,14].

\[
\% \text{FRAP} = \frac{\text{Sample Absorbance} - \text{Blank Absorbance}}{\text{Sample Absorbance}} \times 100\% \tag{1}
\]

2.5. **Animal test**

Swiss albino mice (25-30 g) were purchased from the animal house of the Faculty of Pharmacy, Airlangga University, Surabaya. The mice were treated under standard environmental conditions with a dark and light cycle of 12/12 hours. They were fed a standard commercial diet and water was given ad libitum. The experimental protocol was carried out in accordance with the guidelines of the National Ethics Committee, Faculty of Medicine Hasanuddin University, on the use of laboratory animals for scientific research [15].

In this study, 25 mice (Mus musculus) were used. For anti-inflammation study as positive control was Positive control group (warfarin dose 5 mg/day), negative group (Sodium CMC), treatment group I (sulphate polysaccharide isolate 25 mg/kg), treatment group II (polysaccharide sulfate isolate 50 mg/kg), treatment group III (polysaccharide isolate) sulfate 100 mg/kg body weight). The isolates were given for 14 days and observations were made on days 0, 1, 7 and 14.

2.6 **Anti-inflammatory effect testing**

Twenty-five mice were also used and divided into 5 treatment groups, each group consisting of 5 mice, namely a negative control group (Sodium-CMC suspension), a positive control group (diclofenac sodium), a group of sulfate polysaccharide isolate (10, 50 and 250 mg/kg body weight. Before testing, mice were fasted for 18 hours. Each rat was weighed. All animals of group received complete Freund's adjuvant (0.1 mL/rat), injected in their left hind paw. Injection of complete Freund's adjuvant were repeated once daily during four days. On the fifth day, arthritic rats were treated once a day with sulfate polysaccharide isolate (10, 50 or 200 mg/kg), diclofenac sodium (6.5 mg/kg) or sodium CMC (negative control). The paw volumes of hind paws of all animals were recorded with plethysmometer (model 37140 UgoBasile, Italy) on day 1 before CFA injection and 1h after CFA injection to see the degree of swelling. The volume of paw was measured every 2 days from day 5 to day 19. Percentages of inhibition of oedema were measured for each group.
2.7. Calculation of percent inflammation (%R)
Inflammation volume is the difference in the volume of edema of the feet of mice after and before CFA injection. Percent inflammation (%R) can be calculated by using the formula below:

\[
\% R = \left( \frac{V_t}{V_o} - 1 \right) \times 100\%
\]

where \(V_o\) and \(V_t\) are defined as original volume and volume of leg edema at time \(t\), respectively.

2.8. Bleeding time test
The bleeding time test was carried out by taking the blood of mice by cutting the tails of mice 0.5 cm long. Then bleeding time observed that occurs every 30 seconds using filter paper was observed. Bleeding time was recorded when the blood first dripped until it stopped, marked by the absence of blood spots attached to the filter paper [12].

2.9. Blood clotting time test
The blood clotting time test was carried out by taking the blood of mice by cutting the tails of mice 0.5 cm long. Then 2-3 drops of mouse blood were placed on a glass object and the formation of fibrin threads from the blood specimen was observed every 30 seconds using a lancet [12].

![Figure 1. FT-IR of standard fucoidan.](image1)

![Figure 2. FT-IR of sulfate polysaccharide isolate from Sargassum polycistum.](image2)
Table 1 FT-IR wave number.

| No. | Fucoidan standard | Sulphate Polysaccharide Isolate |
|-----|------------------|---------------------------------|
| 1   | 376,12           | 374,19                          |
| 2   | 397,34           | 540,07                          |
| 3   | 580,57           | 603,72                          |
| 4   | 621,08           | 673,16                          |
| 5   | 669,3            | 727,16                          |
| 6   | 719,45           | 819,75(1)                       |
| 7   | 821,68(1)        | 887,26                          |
| 8   | 889,18           | 935,48                          |
| 9   | 937,4            | 1033,85(2)                      |
| 10  | 956,69           | 1078,21                         |
| 11  | 1033,85(2)       | 1143,79                         |
| 12  | 1080,14          | 1253,73                         |
| 13  | 1139,93          | 1298,09                         |
| 14  | 1255,66          | 1338,6                          |
| 15  | 1300,02          | 1427,32                         |
| 16  | 1423,47          | 1620,21                         |
| 17  | 1614,42          | 1915,31                         |
| 18  | 1915,31          | 1992,47                         |
| 19  | 2156,42          | 2187,28                         |
| 20  | 2360,87          | 2360,87                         |
| 21  | 2482,39          | 2549,89                         |
| 22  | 2700,34          | 2692,63                         |
| 23  | 2858,51          | 2924,09                         |
| 24  | 2924,09          | 3134,0                          |
| 25  | 3444,87          | 3498,87                         |

(1) for the C-S-O group (815 – 845);
(2) for the S=O group (1020 – 1060)

3. Result and discussion
3.1. Extraction and isolation
The sulfate polysaccharide content from the extraction and isolation process of brown algae *Sargassum polycistum* in this study obtained was 6.365%. The extraction method is an important process because it is related to the content of compounds and structural bioactive molecules such as molecular weight and degree of sulfation. The use of solvents, extraction time, temperature, pressure affect the extraction results [15]. The extraction method using 0.1 N hydrochloric acid solvent at 85°C for 4 hours produced brown algae extract with the highest concentration of L-fucose, indicating a high content of fucoidan sulfate polysaccharides compared to extraction using water alone or calcium chloride [16]. Balboa et al (2013) and Manggau et.al (2019) concluded that the extraction process with hot water is a practical, environmentally friendly, and technologically suitable process for the fractionation of *Sargassum sp.* biomass, which allows the extraction and depolymerisation of fucoids simultaneously in one step. The fucoidan content in brown algae can be obtained after successive alginate precipitation and diafiltration [12,17].
Table 2. Interpretation of FT-IR absorbance.

| Frequency range | Intensity | Group | Types of vibration | Remarks |
|-----------------|-----------|-------|--------------------|---------|
| 3550-3500       | M         | O-H   | str.               | Free OH, carboxylic acid |
| 3440-3623       | M         | O-H   | str.               | Free OH, alcohols          |
| 3600-3100       | M         | O-H   | str.               | Water of crystallization |
| ~ 3520          | S         | N-H   | str.               | Primary amide |
| ~ 3500          | M         | N-H   | str. (asym)        | Primary amine, free NH |
| 3500-3060       | M         | N-H   | str.               | Secondary amine, free NH |
| ~ 3400          | S         | N-H   | str.               | Primary amide |
| 3350            | M         | N-H   | str.               | Primary amide bonded |
| 3075-3030       | w-m       | C-H   | str.               | C-H of aromatic ring |
| 2960            | S         | C-H   | str. (asym)        | C-methyl |
| 2925            | S         | C-H   | str. (asym)        | >CH₂, methylene, Ar-CH₂ |
| 2900-2880       | W         | C-H   | str.               | C-H, methane |
| 2900-2705       | W         | C-H   | str.               | -C(=O)H, aldehyde |
| 2580            | S         | S-H   | str.               | Thiol, free thiol |
| 2400            | W         | S-H   | str.               | Thiol H-bonded |
| 1500-470        | S         | C=S   | str.               | -N=C=S |
| 1468            | S         | C-H   | Scissoring         | Alkane, -CH₂, -CH₃ |
| 1540-1640       | M         | C=O   | str.               | C=O, ester |
| 1460-1400       | S         | C=O   | sym. str.          | -COO-carboxylate |
| 1060-1020       | S         | S=O   | str.               | >S=O, sulfoxide |

Source: Infrared Spectroscopy: Fundamentals and Applications. John Wiley & Sons. [19]

Table 3 Measurement of sulfate content.

| No. | Sample          | Concentration (ppm) | Absorbance |
|-----|-----------------|--------------------|------------|
|     |                 | 100                | 0.250      |
|     |                 | 200                | 0.356      |
| 1   | Natrium Sulfat  | 300                | 0.465      |
|     |                 | 400                | 0.575      |
|     |                 | 500                | 0.747      |
| 2   | Polisakarida Sulfat | 242.75          | 0.407±0.004 |

3.2. Identification of fucoidan using FT-IR

The magnitude of the peak from the data in Figure 1 can be seen in Table 1. It is known that the main groups of fucoidan polysaccharides are C=S-O (sulfonic acid) with a wave number of 815-845 cm⁻¹ and S=O with a wave number of 1020-1060 cm⁻¹[18]. The sulfate ester group S=O is a component characteristic of sulfate polysaccharides including fucoidan [19]. Comparison of absorbance obtained between standard fucoidan and sulfate polysaccharide isolate in identifying the C=S-O group was found at wavelengths of 821.68 cm⁻¹ and 819.75 cm⁻¹. In the range of 815 cm⁻¹ wave numbers indicate the type of sulfate polysaccharide D-galactose [20]. Additional sulfate absorption bands at 824 cm⁻¹ and 845 cm⁻¹ show that the majority of the sulfate groups occupy positions 2 and/or 3, and only a small part of sulfate is located at position 4 fucopyranose residues [21]. Based on the analysis of functional group identification using infrared instruments, it can be said that the structure of the sulfate
polysaccharide isolate from brown algae (*Sargassum polycystum*) contains fucoidan compounds. The shift in spectrum was due to the fucoidan and standard fucoidan sulfate polysaccharide isolates obtained from different Sargassum species [22]. Other spectra that can be identified from the structure of brown algae include the CH group at a wavelength of 2692.63 cm\(^{-1}\), the hydroxyl group (-OH) on the fucoidan standard and sulfate polysaccharide isolates at a wavelength of 3444.87 cm\(^{-1}\) and 3498, 87 cm\(^{-1}\), while the carbonyl group (C=O) was identified at a wavelength of 1614.42 cm\(^{-1}\) on fucoidan standard and 1620.21 cm\(^{-1}\) on sulfated polysaccharide isolate.

IR spectroscopy (figure 1) has been used to identify several sulfated polysaccharides, namely red algae and brown algae. The IR spectra of brown algae show a strong broad band around 1220 to 1260 cm\(^{-1}\) which is a characteristic band for the sulfate ester group (S = O) [20]. Other spectra that can be identified from the structure of brown algae include the CH group at a wavelength of 2692.63 cm\(^{-1}\), the hydroxyl group (-OH) on the fucoidan standard and sulfate polysaccharide isolates at a wavelength of 3444.87 cm\(^{-1}\) and 3498, 87 cm\(^{-1}\), while the carbonyl group (C=O) was identified at a wavelength of 1614.42 cm\(^{-1}\) on fucoidan standard and 1620.21 cm\(^{-1}\) on sulfated polysaccharide isolate. It can be concluded that the structure of the sulfate polysaccharide isolate from brown algae (*Sargassum polycystum*) contains fucoidan compounds after analyzed of functional group identification using infrared instruments. The shift in spectrum was due to the fucoidan and standard fucoidan sulfate polysaccharide isolates obtained from different Sargassum species [22].

### Table 4. Antioxidant activity using the FRAP method.

| Method          | Vitamin C | IC\(_{50}\) Sulfate Polysaccharide Isolate |
|-----------------|-----------|------------------------------------------|
| FRAP            | 4.1667 ppm| 91.306 ppm                               |

3.3. Identification of sulphate content

The sulphate content of fucoidan greatly affects its bioactivity [23]. The higher the sulfate level, the higher the ability as an antioxidant [24]. The analysis of sulphate concentration measurements using the UV-Vis instrument from *Sargassum polycystum* showed the sulphate content contained in the sulphate polysaccharide isolate was 40.45%. *Sargassum binderi* which is hydrolyzed by weak acid has a sulfate content of 18.63% [25], crude fucoidan from *Sargassum crassifolium* has a sulphate content of 26.57% [25], *Sargassum cinereum* is reported to have a sulphate content of 3.7% [26], *Sargassum swartzii* has a sulphate content of 5.3% [27]. According to Saepuddin et al (2017), weak acid hydrolysis carried out on fucoidan compounds will reduce sulfate ester group bonds, but with weak acid hydrolysis is able to depolymerize fucoidan molecules[25].

3.4. Antioxidant activity

The antioxidant test using the FRAP (Ferric Reducing Antioxidant Power) method of sulfate polysaccharide isolate with vitamin C as a comparison showed an IC\(_{50}\) value of 91.306 ppm. Based on the level of antioxidant power, the isolate has strong antioxidant activity (50-100 ppm) compared with the IC\(_{50}\) value of vitamin C of 4.1667 ppm, so it can be concluded that the antioxidant activity of sulfate polysaccharide isolate was stated to be very strong. In previous studies, the antioxidant activity of brown algae (*Sargassum polycystum*) was tested using the DPPH method showed weak antioxidant activity with an IC\(_{50}\) value of 1.9-9.6 mg/mL and the results of antioxidant activity using the CUPRAC method showed an IC\(_{50}\) value of 85.268-201 mol. Trolox/g [28].

In the FRAP method, a trichloroacetic acid (TCA) solution is used to precipitate the potassium ferrocyanide complex. Meanwhile, FeCl\(_3\) in solution was added to form a blue colored complex. Compounds that have reducing power can act as antioxidant because they can stabilize free radicals by donating electrons or hydrogen atoms so that radical compounds turn out to be more stable than before. In this case, the ability to reduce antioxidant to convert Fe\(^{3+}\) into Fe\(^{2+}\) is an indicator of the potential of an antioxidant compound contained in the sample [29,30].
Figure 3 shows that the percent inflammation in the feet of mice varied greatly in each treatment group on day 14th, where the percent inflammation in the negative control group was the largest compared to the positive control group and sulphate polysaccharide isolate with doses of 10, 50, and 250 mg/kg body weight. This was due to the negative control group, which was induced by CFA only given Sodium CMC treatment so that there was no drug effect to reduce edema so that the edema would continue to enlarge.

These results showed significantly different from the negative control group which was treated with sodium CMC (p < 0.05), which means that the sulfate polysaccharide isolate dose of 10, 50 and 250 mg/kg had an anti-inflammatory effect. Whereas, the percentage of inflammation between the treatment groups and positive controls are no statistically significant difference (p> 0.05).

3.5. Anti-inflammation activity
Figure 3 shows that the percent inflammation in the feet of mice varied greatly in each treatment group on day 14th, where the percent inflammation in the negative control group was the largest compared to the positive control group and sulphate polysaccharide isolate with doses of 10, 50, and 250 mg/kg body weight. This was due to the negative control group, which was induced by CFA only given Sodium CMC treatment so that there was no drug effect to reduce edema so that the edema would continue to enlarge.

These results showed significantly different from the negative control group which was treated with sodium CMC (p < 0.05), which means that the sulfate polysaccharide isolate dose of 10, 50 and 250 mg/kg had an anti-inflammatory effect. Whereas, the percentage of inflammation between the treatment groups and positive controls are no statistically significant difference (p> 0.05).
Fucoidan has a high sulfatide content capable of inhibiting the production of nitric oxide (NO). These results indicate that the sulfate content and molecular weight may contribute to the inhibition of nitric oxide production. Nitric oxide is an important mediator of inflammation synthesized from arginine by nitric oxide synthase (NOS) [31]. Nitric oxide has a role in acute and chronic inflammation. Nitric oxide can increase edema and vascular permeability. Recent studies have shown that nitric oxide stimulates prostaglandin synthesis by activating the isoenzyme cyclooxygenase II (COX 2). Therefore, inhibition of the NO pathway will have beneficial effects on diseases, including joint disease [10].

3.6. Average bleeding time
On the examination of bleeding time on the fourteenth day after treatment in each of the 5 groups of test animals, the results showed that there was a significant difference after the One Way Annova statistical test, which was analyzed with a value of 0.037 (P <0.05) between the 5 groups of test animals. In the treatment of sulfate polysaccharide isolate at a dose of 100 mg/kg to sulphate polysaccharide isolate at a dose of 25 mg/kg, 50 mg/kg and Sodium CMC showed significant differences with values of 0.021, 0.035 and 0.035, respectively (P<0.05). Meanwhile, in the treatment of sulfate polysaccharide isolate at a dose of 100 mg/kg against warfarin positive controls there was no significant difference with a value of 1 (P>0.05). In the comparison of the negative control treatment of Sodium CMC to sulfate polysaccharide isolate at doses of 25 mg/kg and 50 mg/kg, there was no significant difference with each value of 0.767 (P>0.05). From these results, it can be seen that the activity of sulfate polysaccharide isolate at a dose of 100 mg/kg has the same activity as warfarin 5 mg/kg. Figure 4 shows the average bleeding time on the fourteenth day with each average time of warfarin 830 seconds, Sodium CMC 650 seconds, and sulfate polysaccharide isolate of 25, 50 and 100 mg/kg, namely 720, 710 and 810 seconds, respectively.

3.7. Average blood clotting time
On the examination of blood clotting time on the fourteenth day after treatment was carried out on each of the 5 groups of test animals which were analyzed with the One Way Annova statistical test, the results showed that there was a significant difference with a value of 0.013 (P <0.05) between the 5 groups of test animals. The results of the analysis were then continued with the Post Hoc Test method using the Latest Significant Difference (LSD) (P<0.05). From the data of blood clotting time, the results of the Latest Significant Difference (LSD) test showed that in the positive control treatment using Warfarin there was a significant difference to the negative control Sodium CMC with a value of 0.005 (P<0.05). This is in accordance with the data obtained, namely Sodium CMC does not have...
anticoagulant activity. Similarly, warfarin against sulphate polysaccharide isolate 25 mg/kg there was a significant difference with a value of 0.014 (P<0.05). These results indicate that Sodium CMC and sulphate polysaccharide isolate of 25 mg/kg have no activity in inhibiting blood clotting comparable to warfarin. Meanwhile, in the warfarin treatment of 50 mg/kg sulphate polysaccharide isolate there was no significant difference with a value of 0.103 (P>0.05), as well as for 100 mg/kg sulphate polysaccharide isolate there was no significant difference with a value of 1 (P>0.05). These results indicate that polysaccharide sulfate 100 mg/kg has anticoagulant activity comparable to warfarin. In the negative control treatment using Sodium CMC against the positive control warfarin and sulphate polysaccharide isolate 100 mg/kg, there were significant differences with each value of 0.005 (P<0.05). This is consistent with the result data because there is a greater time difference between sulphate polysaccharide isolate 100 mg/kg and warfarin with Sodium CMC. Meanwhile, the negative control of Sodium CMC had no significant difference to the sulphate polysaccharide isolate 25 mg/kg with a value of 0.563 (P>0.05). Likewise, there was no significant difference between Sodium CMC for sulphate polysaccharide isolate at 50 mg/kg with a value of 0.103 (P>0.05). These results indicate that 25 mg/kg of sulphate polysaccharide isolate and 50 mg/kg of sulphate polysaccharide isolate are ineffective because they have no activity in inhibiting blood clotting. Figure 5 shows the average blood clotting time for 14 days with each average time of warfarin 150 seconds, Sodium CMC 90 seconds, sulphate polysaccharide isolate 25, 50 and 100 mg/kg of sulphate polysaccharide isolate was 100 seconds, 120 seconds and 150 seconds, respectively. From these results, it can be shown that sulphate polysaccharide isolate at a dose of 100 mg/kg has activity on blood clotting time and can be used as an anticoagulation drug.

The factors that can affect the prolongation of blood clotting time and bleeding time in the administration of sulfate polysaccharide isolate is, because of sulfate polysaccharide is widely present in the cell walls of brown algae. This sulfate group will interact with antithrombin III (AT-III). Antithrombin III inhibit thrombin, an enzyme that functions to convert fibrinogen into fibrin from the polymerization of fibrin by transglutaminase which causes blood clotting. Antithrombin is also able to inhibit the coagulation of serine proteases which can convert prothrombin into thrombin [32].

4. Conclusion
We have proven that isolate from sulphate polysaccharide from brown algae (Sargassum polycystum) has an antioxidant, anti-inflammatory, and anticoagulant activity, and it is potential to treat cardiovascular disease. Isolat from sulphate polysaccharide isolate had an anti-inflammatory effect that was not signed with positive controls but it was significantly different from negative controls. In anticoagulant activity, it was shown that the sulphate polysaccharide isolate dosage of 100 mg/kg has anticoagulant activity. It can be concluded that sulphate polysaccharide isolates isolate has the potency to prevent and treat cardiovascular disease.

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