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Research Article

Antiinflammatory Activity Test Through Method Denaturation of Bovine Serum Albumin (BSA) Protein Extract and Fraction of *Eucheuma cottonii*

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Abstract. Inflammation is the body's protective response to harmful stimuli, but chronic inflammation can trigger various degenerative diseases, so safer anti-inflammatory alternatives from natural ingredients are needed. This study aims to evaluate the anti-inflammatory activity of *Eucheuma cottonii* from several regions using the Bovine Serum Albumin (BSA) protein denaturation inhibition method and to examine the effect of fraction polarity on the resulting activity. Samples were extracted by the remaceration method using 70% ethanol, then the most active extract was fractionated through liquid-liquid partitioning using n-hexane, ethyl acetate, and water. Anti-inflammatory activity was tested in vitro based on the percentage of inhibition and IC50 values measured with a UV-Vis spectrophotometer at a wavelength of 660 nm, using diclofenac sodium as a positive control. The results showed that extract C had the highest activity with an inhibition percentage of 79.92% ± 0.26%, while the ethyl acetate fraction of the most active extract showed the lowest IC_{50} value (146.53 µg/mL ± 4.10%) compared to the n-hexane and water fractions. Statistical analysis showed a significant difference in anti-inflammatory activity between extracts and fractions ($p < 0.05$), which indicated that the sample origin and the polarity level of the fractions affected the anti-inflammatory activity of *Eucheuma cottonii*.

Keywords: Anti-inflammatory; BSA; *Eucheuma cottonii*; Extract; Fraction.

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1. Introduction

Inflammation is the body's protective mechanism against harmful stimuli, but if it persists chronically, it can contribute to various degenerative diseases. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac sodium are effective in reducing inflammation, but long-term use can potentially cause side effects, making the development of safer anti-inflammatory agents from natural sources crucial[1].

*Eucheuma cottonii*is a seaweed with high economic value in Indonesia that is not only used as a source of carrageenan but also contains secondary metabolites such as saponins, terpenoids, and steroids that have potential anti-inflammatory properties. Its content and biological activity are influenced by environmental factors and growing region, as well as the polarity of the compound. Therefore, fractionation is necessary to evaluate the contribution of bioactivity more specifically[2].

This study aims to assess the anti-inflammatory activity of extracts and fractions of Eucheuma cottonii from several regions in Indonesia using the Bovine Serum Albumin (BSA) protein denaturation inhibition method, while also examining the influence of geographical origin and polarity of the fraction on the resulting activity as a basis for its use as a source of natural anti-inflammatory.

2. Method

Tools and materials

The research materials included *Eucheuma cottonii* from four different regions, 70% ethanol, n-hexane, ethyl acetate, distilled water, Bovine Serum Albumin (BSA), sodium diclofenac, and Tris Buffer Saline (TBS). The equipment used included a UV-Vis spectrophotometer, a rotary evaporator, a water bath, an analytical balance, and laboratory glassware.

Sample Collection and Preparation

Eucheuma cottonii samples were obtained from four different regions in Indonesia, namely Lampung, Banten, and South Sulawesi (regular and saccate species), collected by the National Research and Innovation Agency (BRIN) and then coded A (Lampung), B (Banten), C (South Sulawesi – regular species), and D (South Sulawesi – saccate species). All samples were determined and identified for species at the Diponegoro University Laboratory, Semarang. The identified samples were washed, dried in the sun, and then ground to obtain a simple powder.

Preparation of Eucheuma cottonii extract and fraction

Extraction was carried out using the remaceration method using 70% ethanol solvent with a material to solvent ratio of 1:5. A total of 50 g of simplex powder was mixed with 70% ethanol. Maceration was carried out for 3 x 24 hours with solvent replacement every cycle. All filtrates were combined and evaporated using a rotary evaporator until a thick extract was obtained, then the yield was calculated[3]

The extract with the highest anti-inflammatory activity was then fractionated using the liquid–liquid partition method. Fractionation was carried out using n-hexane, ethyl acetate, and water as solvents. All extracts and fractions were subjected to phytochemical screening to identify secondary metabolites, including alkaloids, flavonoids, saponins, tannins, phenolics, steroids, and triterpenoids. Qualitative tests used specific reagents based on color changes or the formation of precipitates. The results were confirmed by Thin Layer Chromatography (TLC) using silica gel GF254 as the stationary phase and the appropriate mobile phase. Spots were observed under UV 254 nm and 366 nm, then the Rf value was determined.

Anti-inflammatory Activity Test

The anti-inflammatory test of *Eucheuma cottonii* extracts and fractions was carried out in vitro by inhibiting Bovine Serum Albumin (BSA) protein denaturation, with the extract tested at a concentration of 300 ppm and the fractions at a range of 100–300 ppm. The test solution was reacted with BSA and Tris Buffer Saline (TBS), incubated at room temperature, heated at 72 °C for 5 minutes, then the absorbance was measured using a UV–Vis spectrophotometer at 660 nm (Tiarawati et al., 2025). Diclofenac sodium was used as a positive control and TBS as a negative control, while the anti-inflammatory activity was expressed as the percentage of protein denaturation inhibition for determining the IC_{50} value of the fraction [4].

Data Analysis

The research data were obtained from absorbance measurements of the extract and fractions of n-hexane, ethyl acetate, and water using a UV-Vis spectrophotometer, which were then used to calculate the percentage of anti-inflammatory activity. The percentage of protein denaturation inhibition was calculated based on the formula

$$\% \text{ inhibition } x100\% \frac{\boldsymbol{Negative\ control\ absorbance - Absorbance\ of\ the\ test\ solution}}{\boldsymbol{Negative\ contrl\ absorbance}}$$

[4]

Next, the IC_{50} value was determined through a linear regression equation (y = bx + a). The obtained IC_{50} data were analyzed using SPSS version 23.0 through homogeneity and normality tests; if they met the assumptions, the analysis was continued with a One Way ANOVA parametric test to determine any differences and the effect of anti-inflammatory activity, so that the results obtained had statistical validity that could be scientifically accounted for.

3. Results and Discussion

The extraction results of *Eucheuma cottonii* from various regions using 70% ethanol solvent showed differences in yield values. The yield results from the remaceration process for each Eucheuma cottonii sample are listed in Table 1.

Table 1. Results of Calculation of *Eucheuma cottonii* Extract Yield.

Sample	Yield (%)
Extract A	11.14%
Extract B	9.36%
Extract C	46%
Extract D	9.06%

Information
Extract A: Lampung
Extract B: Banten
Extract C: South Sulawesi (Ordinary Type)
Extract D: South Sulawesi (Sakul Type)

These yield variations indicate differences in the chemical composition of the material, influenced by the region of origin and the type of *Eucheuma cottonii*. Extract C yielded the highest yield (46%), likely related to its polysaccharide content, particularly carrageenan, and the dominance of other solvent-soluble metabolites. Differences between the common and saccule types within the same region also indicate that morphological characteristics and internal chemical composition play a role in determining the efficiency of the extraction process.

Phytochemical screening showed that all *Eucheuma cottonii* extracts were positive for saponins, steroids, and triterpenoids, but negative for flavonoids, phenolics, and tannins. The absence of phenolics and tannins, both qualitatively and by TLC, indicates a low level of free phenolics in red algae, which are generally bound to cell walls or associated with polysaccharides, while the negative flavonoid results are consistent with the characteristics of marine macroalgae. Thus, the anti-inflammatory activity of Eucheuma cottonii is thought to be primarily influenced by saponins, steroids, and triterpenoids, where an inhibition percentage of >20% indicates high anti-inflammatory activity[5]. The results of the inhibition percentage of Eucheuma cottonii extract can be seen in Figure 1

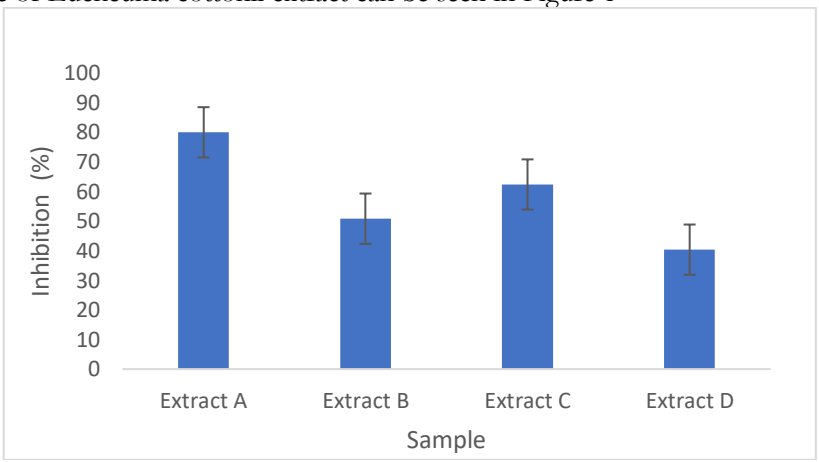

Figure 1. Results of the percentage of inhibition of *Eucheuma cottonii* extract.

Differences in the percentage inhibition of *Eucheuma cottonii* extracts from various regions confirm the influence of geographical factors and environmental conditions on the biosynthesis of secondary metabolites and sulfated polysaccharides. Extract C showed the highest inhibition in the BSA protein denaturation test (79.92% ± 0.26%), followed by D, B, and A, in line with the variation in chemical profiles and the presence of alkaloids, saponins, and triterpenoids that synergize with sulfated polysaccharides, especially carrageenan, in modulating inflammation. Water conditions such as salinity and light stability in South Sulawesi compared to fluctuations in Banten and Lampung also affect the quality of the metabolites produced.

So the combination of environmental factors and chemical composition makes extract C the most prospective as a source of natural anti-inflammatory drugs[6]. The percentage inhibition data of *Eucheuma cottonii* extract from the BSA protein denaturation test were analyzed using SPSS version 23. The results of the normality test showed that the data were normally distributed (Sig. > 0.05), but the homogeneity test indicated that the variance between groups was not homogeneous, so the Kruskal–Wallis nonparametric test was used. The analysis showed a significant difference between treatment groups ($p = 0.03 < 0.05$). To determine the specific differences between pairs of groups, a further Mann–Whitney test was performed, with the results listed in Table 2.

Table 2. Decision Table of SPSS Test Results % Inhibition of *Eucheuma cottonii* Extract.

	Extract A	**Extract B**	**Extract C**	**Extract D**
Extract A		BS	BS	BS
Extract B	BS		BS	BS
Extract C	BS	BS		BS
Extract D	BS	BS	BS	

Information :
BS : Significantly Different
Extract A: Lampung
Extract B: Banten
Extract C: South Sulawesi (Ordinary Type)
Extract D: South Sulawesi (Sakul Type)

Statistical analysis showed that the anti-inflammatory activity of *Eucheuma cottonii* extracts from the four locations differed significantly, with all pairs of extracts showing significant differences, so that no two locations produced equivalent activity. This confirms that regional origin and ecological conditions influence the formation of secondary metabolites and sulfated polysaccharides, resulting in different chemical profiles and biological potentials. Extract C showed the highest activity and was selected as a candidate for fractionation and IC_{50} determination.

The highest yield was obtained in the water fraction at 45.72. The high yield of the water fraction indicates that most of the compounds in the *Eucheuma cottonii* fraction are polar and more easily soluble in water solvents. This is in accordance with these characteristics because it is possible that the compound components contained in the *Eucheuma cottonii* water fraction are more attracted to polar solvents[7]

Phytochemical screening of n-hexane, ethyl acetate, and water fractions of *Eucheuma cottonii* showed the distribution of secondary metabolites according to solvent polarity, with steroids and triterpenoids in the n-hexane–ethyl acetate fraction and saponins in the water fraction, while flavonoids, phenolics, and tannins were not detected. TLC results confirmed these findings through the appearance of steroid and triterpenoid spots after Liebermann–Burchard with higher Rf values in the n-hexane fraction, as well as saponin spots with low Rf values in the water fraction after anisaldehyde–sulfuric acid, while the absence of spots at UV 254 and 366 nm indicated that the compounds were not dominant or were in bound form. All fractions were analyzed for IC_{50} value as a parameter of anti-inflammatory potential based on the ability to inhibit 50% of BSA protein denaturation, where a lower IC_{50} value indicates stronger anti-inflammatory activity. The IC_{50} results of the ethyl acetate, n-hexane, and water fractions show differences in potential between fractions, as can be seen in Figure 2.

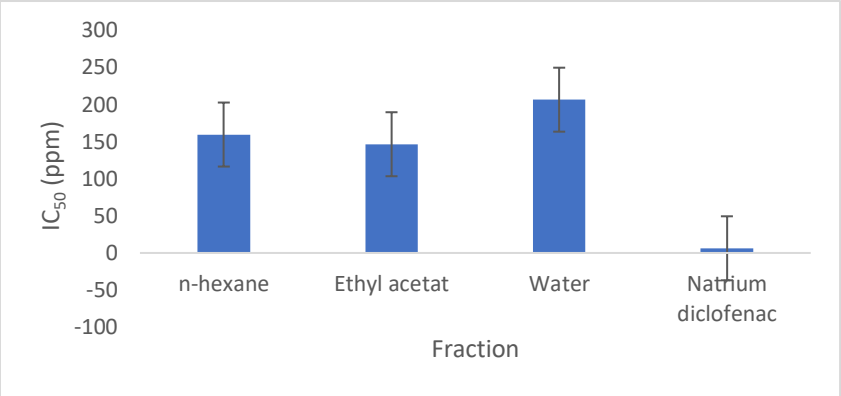

Figure 2. Results of determining the IC50 value of *Eucheuma cottonii.*

Extract C as the most active extract was fractionated liquid-liquid into n-hexane, ethyl acetate, and water fractions, with IC_{50} values of 159.57 ± 1.83; 146.53 ± 4.1; and 206.56 ± 8.70 µg/mL, respectively, while diclofenac sodium showed the lowest IC_{50} (6.29 ± 0.05 µg/mL). The ethyl acetate fraction was the most active in inhibiting BSA protein denaturation, indicating the dominance of semipolar compounds, while the n-hexane and water fractions showed lower activity even though the water fraction was rich in sulfated polysaccharides. Statistical analysis showed that the data were normally distributed but not homogeneous, so the Kruskal–Wallis test showed significant differences between groups ($p = 0.03 < 0.05$) and was continued with the Mann–Whitney test. in table 3.

Table 3. Decision Table of IC50 Test Results of *Eucheuma cottonii.*

	n-Hexane Fraction	Ethyl Acetate Fraction	Water Fraction	Na diclofenac
n-Hexane Fraction		BS	BS	BS
Ethyl Acetate Fraction	BS		BS	BS
Water Fraction	BS	BS		BS
Nadic	BS	BS	BS	

Information :

BS : Significantly Different

The results of statistical analysis showed that each fraction had significantly different anti-inflammatory activity, confirming the role of solvent polarity in determining the biological character of the fraction. Nonpolar (n-hexane), semipolar (ethyl acetate), and polar (water) solvents extract compounds with different chemical properties, resulting in a unique composition that affects the interaction with BSA proteins through hydrophobic mechanisms and hydrogen bond formation. Thus, solvent selection is a key factor in determining the anti-inflammatory potential of *Eucheuma cottonii* fractions.

4. Conclusion

Extract C had the highest inhibition percentage (79.92%) ± 0.26%, followed by extract D (62.31%) ± 0.80%, extract B (50.77%) ± 0.58, and extract A (40.31%) ± 0.22 based on the BSA protein denaturation method. The Ethyl Acetate fraction is the fraction with the best anti-inflammatory activity, which is indicated by the smallest IC50 value compared to the n-Hexane and water fractions. With an IC50 value of ethyl acetate having an IC_{50} value of 146.53 µg/mL ± 4.1%, the n-hexane fraction of 159.57 µg/mL ± 1.83%, and the water fraction of 206.56 µg/mL ± 8.70%, while the positive control of sodium diclofenac has the lowest IC_{50} value of 6.29 µg/mL ± 0.05%. There were statistically significant differences in anti-inflammatory activity between 70% ethanol extracts of *Eucheuma cottonii* from various regions and between fractions with different polarities.

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Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request. The data are not publicly available due to research documentation and institutional data management policies.

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Conflicts of Interest: The authors declare no conflict of interest

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