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EFFECT OF SOLVENT TYPE AND EXTRACTION TEMPERATURE ON SULFATE AND URONIC ACID CONTENT IN *Eucheuma cottonii* FUCOIDAN

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ABSTRACT

Fucoidan, a sulfated polysaccharide typically obtained from seaweed, has its composition affected by various factors, including species, habitat conditions, seasonal variations, extraction techniques, and harvest maturity. The seaweed species *Eucheuma cottonii* from the Banggai Islands is a rich source of bioactive polysaccharides, including uronic acid, laminarin, and fucoidan. Fucoidan predominantly contains fucose as its main component, sulfate, and small amounts of monosaccharides like galactose and glucose. The chemical properties of fucoidan, such as sulfate and uronic acid levels, are critical for determining its functional activity. This study investigated the influence of two extraction parameters: type of solvent (HCl, $CaCl_2$, ethanol, EDTA, H_2O) and extraction temperature (40, 50, 60, 70, and 80°C). These variations were applied to assess their effects on the sulfate and uronic acid contents of fucoidan extracted from *Eucheuma cottonii*. The results showed that the EDTA solvent was the most effective for fucoidan extraction, yielding the highest sulfate content (11.22%) at 80°C. In contrast, the HCl solvent resulted in the lowest sulfate content (4.54%). Regarding uronic acid content, the highest value (4.55%) was observed at 80°C with the HCl solvent, while the lowest (1.40%) was obtained at 40°C with the EDTA solvent. These findings highlighted the significant impact of solvent type and extraction temperature on the chemical composition of fucoidan, influencing its bioactivity and applications. This study demonstrated that EDTA was an excellent solvent for fucoidan extraction, particularly at 80°C, maximizing sulfate content and providing insights for optimizing fucoidan extraction to enhance its functional and bioactive properties.

Keywords: *Eucheuma Cottonii*, Fucoidan, Seaweed, Solvent Type, Temperature.

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INTRODUCTION

Central Sulawesi boasts significant marine resources, with abundant biological diversity, including seaweed, which thrives in coastal and marine ecosystems. Seaweed, along with phytoplankton, seagrass, and mangroves, serves as a critical primary producer in these ecosystems.[1–3] The Central Sulawesi Maritime Affairs and Fisheries Service reports that Banggai Islands Regency is the largest seaweed producer in Central Sulawesi, with an impressive production volume of 800,000 tons.[4] Despite this potential, the industrial application of certain seaweed-derived compounds, such as fucoidan, remains underutilized. Fucoidan is a sulfated polysaccharide that exhibits diverse bioactive properties, including anti-inflammatory, anticancer, and antioxidant activities.[5,6] Understanding the factors influencing fucoidan's characteristics is crucial for enhancing its functional applications. Existing studies indicate that various factors, such as species, environmental conditions, extraction techniques, and harvest maturity, influence the properties and biological activities of fucoidan. For instance, variations in the sulfate and uronic acid content of fucoidan depend significantly on the type of solvent and the extraction temperature used during the extraction process.[7,8] Studies identify fucoidan's structural diversity, highlighting its dependence on the biosynthetic pathways and environmental conditions where the seaweed grows.[9] Moreover, specific solvents, such as EDTA, enhance sulfate extraction at elevated temperatures, while other solvents, such as HCl, affect the yield and composition differently.[10] These insights form a foundation for optimizing extraction techniques to maximize fucoidan's potential benefits. Despite extensive studies on the extraction and characterization of fucoidan from brown seaweed, limited research focuses on the

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Eucheuma cottonii species, particularly in the Banggai Islands region. Most studies concentrate on brown seaweed such as *Fucus vesiculosus* and *Laminaria japonica* [11]. Additionally, while solvent type and temperature are explored individually, their combined effects on sulfate and uronic acid content in fucoidan derived from *Eucheuma cottonii* remain underreported. This limitation emphasizes the importance of conducting thorough research to assess the combined influence of these factors and determine the ideal extraction conditions for this particular seaweed species. This study addresses the identified research gap by systematically investigating the influence of solvent type and extraction temperature on the sulfate and uronic acid contents of fucoidan derived from *Eucheuma cottonii* harvested in the Banggai Islands. By comparing various solvents, including HCl, $CaCl_2$, ethanol, EDTA, and H_2O, and applying temperature variations (40–80°C), this research aims to identify optimal conditions for extracting high-quality fucoidan. The study's novelty lies in its focus on a locally abundant seaweed species and its potential for industrial application, contributing to the development of value-added products and supporting the sustainable utilization of marine resources.

EXPERIMENTAL

Preparation for Seaweed

The seaweed type *Eucheuma cottonii* was obtained from the waters of the Banggai Islands. The *Eucheuma cottonii* seaweed sample was cleaned with clean water to remove dirt and then dried in the sun. Once dried, the seaweed was ground using a blender and sieved with a 60-mesh screen to obtain fine seaweed powder, which was subsequently used for the fucoidan extraction process. The extraction process diagram is presented in Fig.-1.

Seaweed Fucoidan Extraction Using Type of Solvent and Extraction Temperature

The extraction process was carried out using 0.1 N HCl solvent at room temperature for 6 h. A solid-to-liquid ratio of 1:30 (w/v) was applied for the Eucheuma cottonii seaweed powder and HCl solvent. The resulting suspension was subjected to continuous stirring using a magnetic stirrer at a speed of 3000 rpm for 6 hours. Subsequently, the mixture was filtered through A50-grade filter paper. The resulting filtrate was neutralized with 0.5 M NaOH, followed by the addition of 4M $ZnCl_2$ solution (1:10) while stirring for 30 min at room temperature. The solution was then centrifuged at 8000 rpm for 15 min. The obtained filtrate was diluted by adding 0.5 M KCl_2 and 5% cetylpyridinium chloride (CPC) until a precipitate formed. The mixture was centrifuged at 8000 rpm for 15 minutes, after which the resulting precipitate was collected, redissolved in distilled water, and subjected to a second centrifugation at 3000 rpm for 30 minutes. The resulting supernatant was then mixed with ethanol at a ratio of 1:2 to facilitate precipitation. The formed precipitate was separated using filter paper, then dried with a freeze dryer. As a result, the crude fucoidan extract from seaweed was obtained. The same procedure was applied using ethanol, EDTA, $CaCl_2$, and distilled water as solvents. Crude fucoidan obtained from each solvent was then used for fucoidan characterization, including sulfate content ($BaCl_2$-Gelatin method) and uronic acid content analysis (Blumenkrantz-Asboe-Hansen method). The next stage of extraction was carried out using various extraction temperatures, namely 40°C, 50°C, 60°C, 70°C, and 80°C. The observed parameters were the same as those used for the solvent type method: sulfate content and fucoidan uronic acid content.

Analysis of Sulfate Concentration

The $BaCl_2$-gelatin method (Dodgson and Price) was employed to analyze fucoidan sulfate levels. A uniform solution was obtained by dissolving 0.5 g of gelatin in 100 mL of distilled water under continuous stirring on a hot plate maintained at 60–70°C. Subsequently, 0.5 g of $BaCl_2$ was incorporated into the solution, which was then stored at 4°C for 24 hours. For sample preparation, 6 mg of fucoidan was dissolved in 2 mL of 3.5 N HCl with stirring until a homogeneous mixture was achieved. The mixture was then centrifuged, ensuring that only the supernatant was collected. A commercial fucoidan solution underwent the same procedure as the samples. Standard solutions of K_2SO_4 were prepared at concentrations of 200, 400, 600, 800, and 1,000 ppm. In a cuvette, 200 µL of the sample solution, 600 µL of 3% TCA, and 300 µL of $BaCl_2$-gelatin were sequentially combined. The mixture was manually stirred and allowed to stand undisturbed for 15 minutes. Absorbance was recorded using a UV-VIS spectrophotometer at a wavelength of 360 nm. The same procedure was applied to the standard K_2SO_4 and commercial fucoidan solutions.

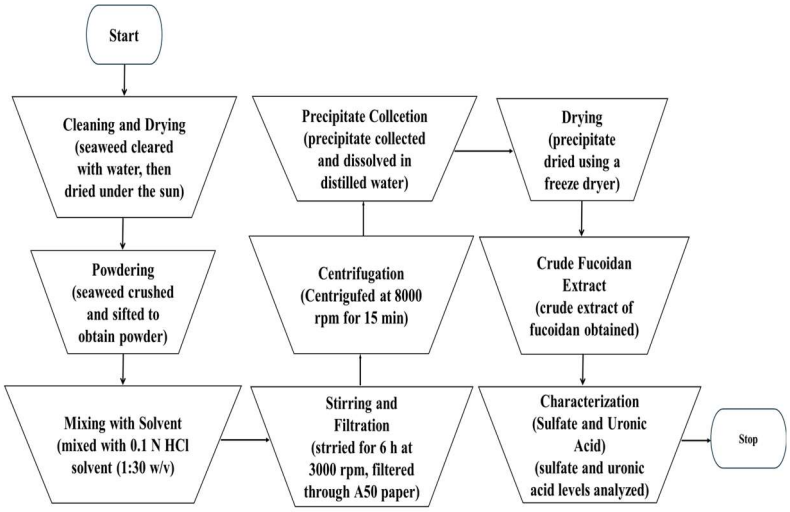

Fig.-1: Process Diagram of Seaweed Fucoidan Extraction

Analysis of Uronic Acid Concentration

A 0.4 mL sample containing 20 µg fucoidan extract was mixed with 2.4 mL of $Na.B_2O_7$. 10 mL H_2O 0.125 in H_2SO_4 p.a, then cooled with ice cubes. The samples were mixed using a vortex and then incubated in a water bath at 100°C for 5 min. After being cooled to room temperature, 20 µL of carbazole was added, followed by shaking. The absorbance was then measured using a UV-VIS spectrophotometer at a wavelength of 520 nm. Glucuronic acid was used as the standard, with standard series solutions prepared at concentrations of 2, 4, 6, 8, and 10 ppm. Uronic acid levels were calculated based on a standard curve obtained using a linear regression equation.

RESULTS AND DISCUSSION

Crude Fucoidan Content of Seaweed as Influenced by Different Solvent Types

The analysis results indicated that fucoidan extraction with EDTA solvent yielded the highest sulfate content at 11.22%, whereas the lowest sulfate content of 4.54% was obtained using HCl solvent. Gajić *et al.*[12] and Lomartire *et al.*[13] stated that calcium salts had been able to precipitate the alginate components contained in seaweed, allowing for the proper isolation of alginate compounds. Meanwhile, the ethanol solvent used in fucoidan extraction had yielded lower sulfate levels compared to the EDTA solvent but higher than the acid solvent, because ethanol had been able to remove fat and protein components as well as pigment compounds in seaweed. This had caused fucoidan not to dissolve during extraction, resulting in a relatively high sulfate content of 8.07%. The sulfate content of each extract, based on the type of solvent, was presented in Fig.-2.

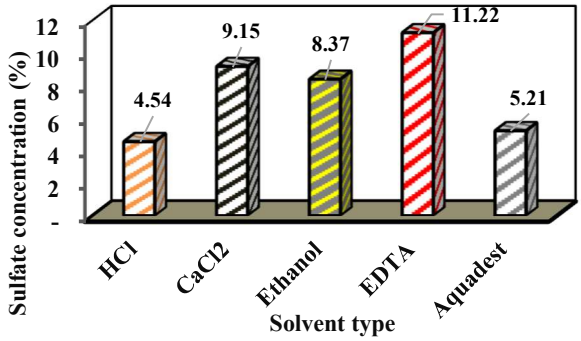

Fig.-2: Effect of Solvent Type on Sulfate Concentration of seaweed Fucoidan

Effect of Extraction Temperature on Crude Fucoidan Uronic Acid Content in Seaweed

Uronic acid was an indication of the presence of alginic acid, which was a compound that made up the alginate polymer. Based on the analysis results obtained, in all treatments, uronic acid was still present. This showed that the fucoidan obtained still contained other polysaccharide contaminants. In brown

seaweed, alginate was the most dominant polysaccharide, so further stages needed to be carried out to obtain purer fucoidan.[14] The research results showed that the highest uronic acid content was obtained through extraction treatment using HCl at a temperature of 85°C, namely 2.68%, as shown in Fig.-3. Meanwhile, the lowest uronic acid content was obtained using HCl without heating, namely 0.36%. The possibility of alginic acid extraction was influenced by temperature or heating. The high content of uronic acid indicated a high level of alginic acid contained in it.

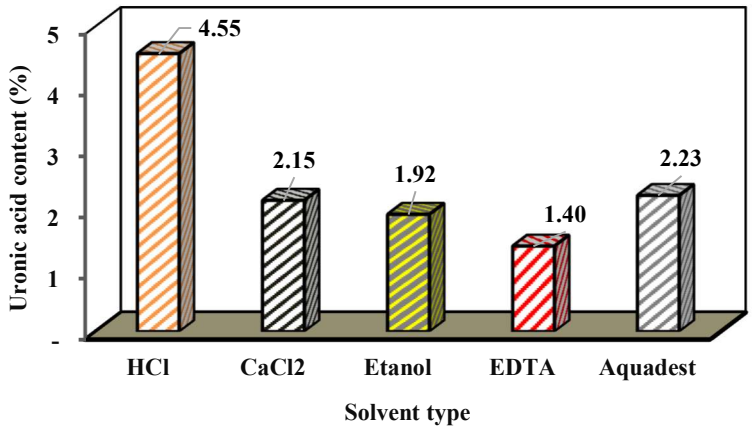

Fig.-3: Effect of Solvent Type on Uronic Acid Content of Fucoidan Seaweed

The color of the extract from distilled water and 2% $CaCl_2$ was browner, indicating that the fucoidan polymer was more dominant. $CaCl_2$ solution was used to precipitate alginate and increase the purity of fucoidan, but this solution could reduce the yield. Overall, when viewed from the total carbohydrate and sulfate content, extraction using distilled water and $CaCl_2$ solvents was 2% higher compared to using acid solvents. On the other hand, the uronic acid content in the extraction method using distilled water and 2% $CaCl_2$ was lower compared to using EDTA solvent, while the use of HCl solvent produced the highest uronic acid content. According to Bojorges *et al.*[15] the use of strong acids such as hydrochloric acid in the extraction of fucoidan caused degradation of the polymer chain, producing uronic acid that precipitated with other impurities. In the extraction of fucoidan using distilled water and methanol, the results obtained showed lower total sugar and uronic acid content compared to extraction using acid. Therefore, to obtain sulfate and total carbohydrate content, it was better to extract fucoidan using distilled water.

Effect of Extraction Temperature on Sulfate Content of Fucoidan in Seaweed

The research conducted by Dobrinˇci´c *et al.*[16] indicated that the solvent type used in the extraction process was significantly affected by temperature, particularly in the formation of fucoidan polymer structures. The use of low temperatures with acidic solvents tended to accelerate the breaking of ulhate ester bonds in fucoidan polymers with alginic acid structures, compared to using non-acidic solvents such as ethanol and calcium chloride. The effect of extraction temperature on the uronic acid content of fucoidan seaweed was presented in Fig.-4.

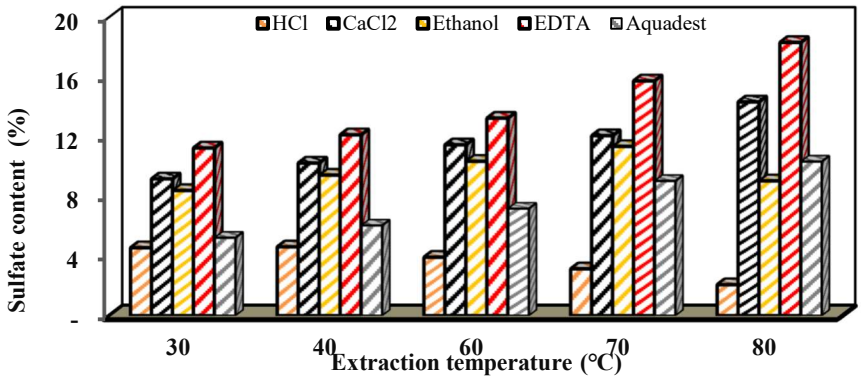

Fig.-4: Effect of Extraction Temperature on Sulfate Content of Fucoidan in Seaweed

The use of EDTA solvents for all extraction plants produced the highest sulfate levels compared to other solvents, namely $CaCl_2$, HCl, ethanol, and aquadest. Aquadest solvent provided low yields for all extraction temperatures, ranging from 2.01% to 4.35%. This was because H_2O molecules were unable to break the sulfate esters in the structure of fucoidan polymers, even with increasing extraction temperatures. Compared to ethanol solvents, which were polar, aquadest also gave lower yields than EDTA and $CaCl_2$ solvents. According to the results of research conducted by Zhang *et al.*[17]It was stated that the type of solvent used in the extraction process was greatly influenced by temperature factors, especially in the formation of fucoidan polymer structures. The use of low temperatures with acidic solvents tended to accelerate the breaking of sulfate ester bonds in fucoidan polymers with alginic acid structures, compared to using other solvents.

Effect of Extraction Temperature on Uronic Acid Fucoidan in Seaweed

According to the results of research conducted by Zayed and Ulber[18]It was stated that the type of solvent used in the extraction process was greatly influenced by temperature factors, especially in the formation of fucoidan polymer structures. The use of low temperatures with acidic solvents tended to accelerate the breaking of sulfate ester bonds in fucoidan polymers with alginic acid structures, compared to using non-acidic solvents such as ethanol and calcium chloride. The effect of extraction temperature on the uronic acid content of fucoidan seaweed was presented in Fig.-5.

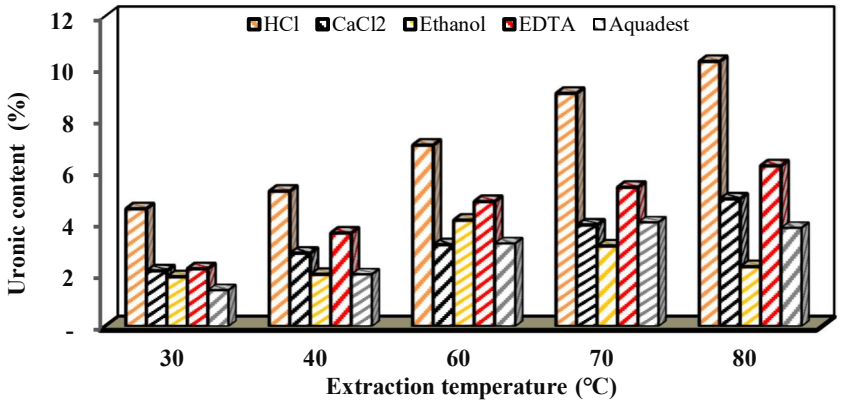

Fig.-5: Effect of Extraction Temperature on Uronic Content Fucoidan in Seaweed

It was observed that as the extraction temperature increased, the uronic acid levels in fucoidan and Eucheuma cottonii seaweed also increased. The highest increase occurred in the use of HCl solvents, followed by aquadest, calcium chloride, ethanol, and EDTA. On the other hand, at low temperatures, an increase in uronic acid levels was also observed with the use of HCl solvents. The use of hydrochloric acid solvents increased the uronic acid content of fucoidan seaweed because the acidic nature could hydrolyze the sulfate ester bonds of the fucoidan polymer structure, causing the uronic acid group to detach from the ester bond. Meanwhile, the use of polar solvents such as ethanol and water reduced their ability to hydrolyze the fucoidan sulfate ester bonds.[19,20]

CONCLUSION

The study on the sulfate and uronic acid contents of crude fucoidan derived from *Eucheuma cottonii* seaweed of the Banggai Islands explored the effects of solvent type and extraction temperature. Fucoidan, a valuable bioactive compound found in seaweed, was influenced by extraction conditions that determined its purity and composition. In this research, solvents such as EDTA, HCl, $CaCl_2$, ethanol, and water were evaluated at various temperatures. The results showed that EDTA solvent produced the highest sulfate levels, while hydrochloric acid solvent yielded the highest uronic acid levels, highlighting their distinct effectiveness in targeting specific components. Furthermore, extraction at 80°C consistently resulted in the highest sulfate content across all solvents, whereas lower temperatures, such as 40°C, led to the lowest sulfate and uronic acid levels. These findings indicated that optimizing solvent selection and extraction temperature was crucial for enhancing fucoidan extraction efficiency. Future studies were recommended to focus on refining extraction protocols, investigating the structural properties of extracted fucoidan, and

exploring its potential applications in pharmaceuticals and functional foods.

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CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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