

Antidiabetic Activity of Green Seaweed (*Eucheuma spinosum*) Ethanol Extract in Alloxan Induced Male White Rats (*Rattus norvegicus*)

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Abstract

Diabetes is a long-term metabolic disorder with a globally increasing prevalence, including in Indonesia. This study aims to evaluate the antidiabetic effects of the ethanol extract of *Eucheuma spinosum* on alloxan-induced rats in vivo. This study used an experimental design with 24 rats divided into six groups, namely the normal control group, positive control (metformin 9 mg/200 g body weight), negative control, and three treatment groups receiving *Eucheuma spinosum* extract at doses of 30, 60, and 120 mg/200 g body weight. The extract was obtained through the maceration method and contains active compounds such as alkaloids, flavonoids, saponins, and tannins. Blood glucose measurements were taken before and after the treatment. The yield of the extract obtained was 2.5%. The highest dose, 120 mg/200 g body weight of the rats, resulted in the most significant decrease in blood glucose levels by 39.705%. Statistical analysis using ANOVA showed a significant difference ($p \leq 0.05$) between the treatment and negative control groups. The antidiabetic activity of this extract is suspected to originate from its flavonoid and saponin content, which stimulate the regeneration of pancreatic β cells and inhibit glucose absorption in the digestive tract. Ethanol extract of *Eucheuma spinosum* is effective in lowering blood glucose levels in diabetic rats and has the potential to be developed as a natural antidiabetic agent.

Keywords: alloxan, antidiabetic, *Eucheuma spinosum*, green seaweed

Introduction

Diabetes mellitus is a chronic condition with significant epidemiological impact and stands as one of the top causes of mortality globally. The World Health Organization estimates that 346 million individuals are affected by diabetes worldwide. At the same time, the International Diabetes Federation reports that over 537 million adults are living with the disease, resulting in 6.7 million related deaths.¹ Indonesia ranks among the nations with a high diabetes burden; the 2023 Indonesian Health Survey indicates a prevalence rate of 1.7% across all age groups, corresponding to about 877,531 people. This number is projected to rise sharply, with forecasts suggesting that by 2045, there could be 111.2 million people (19.9%) aged 65–79 years affected.²

Type 2 diabetes accounts for the majority of cases worldwide, and its development is strongly linked to poor dietary patterns and insufficient physical activity.³ Standard treatment typically involves pharmaceutical drugs such as metformin.⁴ However,

Indonesia is known as a center of rich medicinal plant diversity, making herbal therapy a sustainable health solution by utilizing effective natural ingredients with minimal side effects.

Green seaweed *E. spinosum* is a natural resource with promising antidiabetic properties, as it contains bioactive substances like alkaloids, flavonoids, and saponins.⁵ Flavonoids are recognized for their protective effects on pancreatic β cells, while alkaloids and saponins help reduce blood sugar levels by blocking the α -glucosidase enzyme.⁶⁻⁸

To assess antidiabetic efficacy, diabetes is often induced in white rats (*Rattus norvegicus*) using alloxan, which selectively damages pancreatic β cells.^{9,10} Although the antidiabetic potential of *E. spinosum* has been identified in vitro, in vivo studies testing this extract's effectiveness and optimal dosage are still limited. Therefore, this study aims to evaluate the antidiabetic activity of the ethanol extract of *E. spinosum* in an alloxan-induced diabetic rat model, to fill this knowledge gap and support the development of more effective herbal therapies.

Method

Tool

The equipment used for the research includes a blender (*Capsule*), mesh no.40, analytical balance (*Advanturer Ohaus, USA*), glass jars, filter cloth, vacuum rotary evaporator (*Buchi B-491, Swiss*), water bath (*Memmert, German*), volumetric flask, graduated cylinder, watch glass, spirit lamp, funnel, filter paper (*Whatman, UK*), test tube (*Pyrex, UK*), test tube rack, tripod stand, beaker (*Pyrex, UK*), volumetric flask (*Pyrex, UK*), dropper, syringe, oral swab (*Onemed, Indonesia*), disposable syringe 3 ml and 5 ml (*Terumo, Japan*), glucometer (*EasyTouch@GCU, Taiwan*), and test strips (*EasyTouch®, Taiwan*).

Material

The materials used in this study are green seaweed (*Eucheuma spinosum*) obtained from Pameungpeuk Beach, Garut Regency, 96% ethanol, alloxan monohydrate, 500 mg metformin tablets, aquadest, Mayer's reagent, Dragendorff's reagent, Liebermann-Burchard's reagent, 1% NaCl, FeCl, 2N sulfuric acid, Mg/Zn powder, 1% gelatin, and 1% Na-CMC.

Procedure

Sample Collection

Seaweed obtained from Pameungpeuk, Garut Regency. Determined at the Jatinangor Herbarium, Plant Taxonomy Laboratory, FMIPA Department, Padjadjaran University (UNPAD), Bandung. No.19/HB/11/2023.

Making Ethanol Seaweed Extract

The fresh seaweed is dried under the sun until it becomes simplicia. A total of 1 kg of this simplicia is then ground using a blender, followed by extraction through the maceration process with 10 liters of 96% ethanol for 3×24 hours. The resulting macerate is concentrated using a Rotary Evaporator set at 50°C. Afterwards, the extract is further thickened by evaporating in a water bath.

Phytochemical Screening

The thick extract and the simplicia of 500 mg underwent phytochemical screening using qualitative methods, including tests for alkaloids, flavonoids, saponins, tannins, and quinones. Steroids and triterpenoids.¹¹

Preparation of Metformin Suspension

The dose of metformin used is 500 mg/70 kg of human body weight.¹² Then the dose conversion to rats is 9 mg/200 grams of rat body weight. The metformin tablet powder is weighed at 90 mg, then dissolved and suspended with 1% Na-CMC up to 100 mL, and shaken until homogeneous.

Preparation of Alloxan Induction Solution

The dose of alloxan monohydrate for male white rats is 150 mg/kg body weight.¹³ If converted for a 200-gram rat, it would be 30 mg/200 grams of rat body weight. Dissolve 3 grams of alloxan monohydrate in 100 mL of 1% NaCl, and induce it in the rat intraperitoneally at a volume of 1 mL.

Ethical Clearance

This research has received ethical approval from the Health Research Ethics Committee of Bakti Tunas Husada University, Tasikmalaya, with No.065-01/E.01/KEPK-BTH/IV/2025.

Preparation of Test Animals Test

The number of rats was determined using the Federer formula, where each group consisted of 4 rats with 6 treatment groups. The number of rats used was 24, and they were acclimatized to the laboratory environment for 7 days at room temperature while being provided with food and water.

Testing Antidiabetic Activity of Seaweed Extract

A total of 24 animals were randomly assigned to six groups. Group 1 served as the standard control, Group 2 was the positive control receiving metformin at a dose of 9 mg per 200 grams of rat body weight, and Group 3 acted as the negative control given a 1% suspension of Na-CMC, group 4 (dose 1 seaweed extract 30 mg/200 grams body weight of rats), group 5 (dose 2 seaweed extract 60 mg/200 grams body weight of rats), group 6 (dose 3 seaweed extract 120 mg/200 grams body weight of rats). Then the rats were acclimatized for 7 days to allow them to adapt to the environment, after which their glucose levels were measured to determine the normal fasting glucose level. The rats were then induced with alloxan at a dose of 30 mg/200 grams of body weight intraperitoneally for 3 consecutive days and re-measured after 3 days of induction to determine the fasting glucose level of the diabetic rats. The level of hyperglycemia in rats is when the fasting glucose level is ≥ 200 mg/dL¹² and physiological changes such as weight loss and changes in food and water consumption.¹³ After induction, the rats were given test treatments according to their groups for 7 consecutive days, with blood glucose levels measured on the 3rd and 7th days.

Data Analysis

The data analysis was performed using SPSS software, starting with tests for normality and homogeneity. Subsequently, a *One-Way ANOVA* was applied to evaluate the differences between groups. To pinpoint which groups differed significantly, a Post Hoc LSD test was carried out as the final step in the analysis.

Result

The yield results of the green seaweed extract (*E. spinosum*) obtained can be seen in Table 1. The results of the seaweed extraction (Table 1) show that the seaweed extract has a poor rendement value because it is $\leq 10\%$. Because the extract is considered good if the rendement obtained is more than 10% according to the Indonesian herbal pharmacopoeia.¹⁴

Table 1. Seaweed Extraction Results

No	Parameter	Result
1.	Weight of the seaweed simplicia powder	1000 gram
2.	Weight of concentrated seaweed extract	25 gram
3.	Rendemen of seaweed extract	2,5 %

The phytochemical analysis of the simplicia and the green seaweed extract (*E. spinosum*) identified various compounds. From these results, it can be concluded that the *E. spinosum* extract contains metabolites including flavonoids, alkaloids, saponins, and tannins, as shown in Table 2.

Table 2. Phytochemical Screening Results

No	Identification	Simplisia	Ekstrakt
1.	Saponin	(+)	(+)
2.	Tanin	(+)	(+)
3.	Flavonoid	(+)	(+)
4.	Kuion	(-)	(-)
5.	Alkaloid	(+)	(+)
6.	Triterpenoid dan Steroid	(-)	(-)
7.	Monoterpenoid dan Sesquiterpenoid	(-)	(-)

note : (+) = identified, (-) = not identified

The average increase in blood glucose levels of mice after 3 days of alloxan induction can be seen in Figure 1.

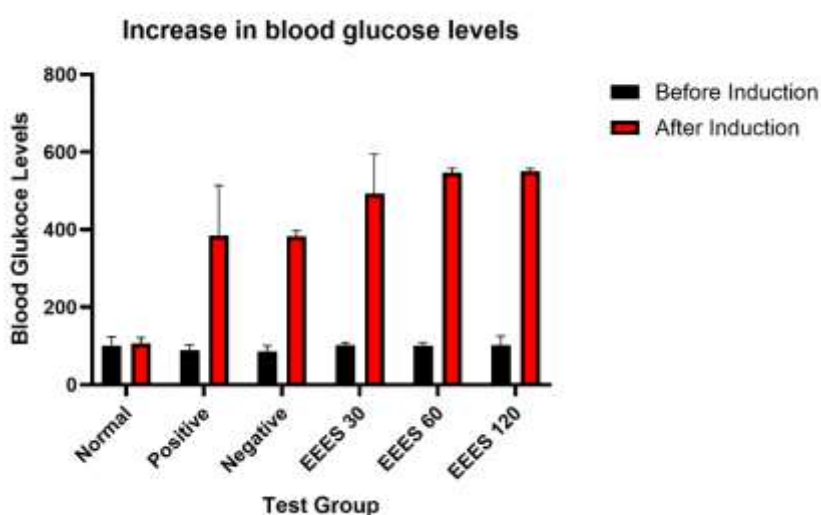


Figure 1. The average blood glucose level increases

Figure 2 illustrates the reduction in blood glucose levels in rats following each treatment, while Table 4 presents the average decrease in blood glucose among the rats.

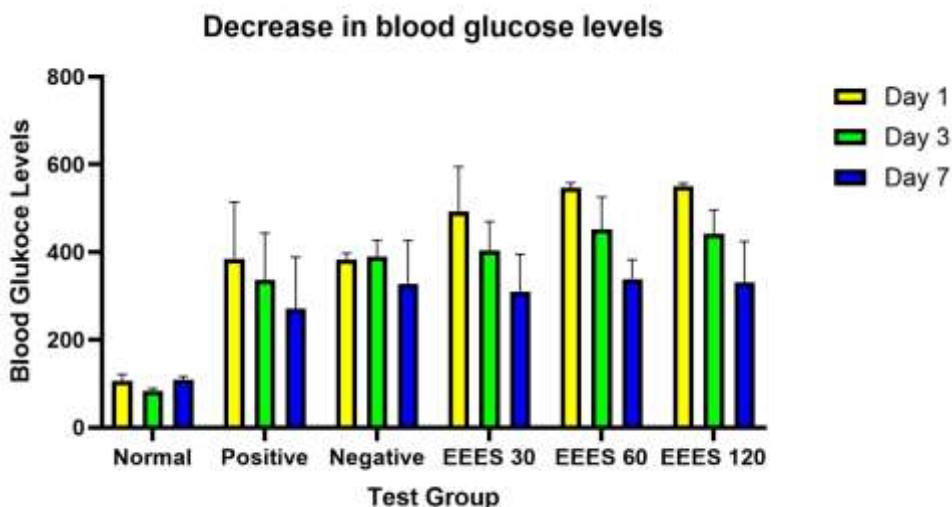


Figure 2. Decrease in blood glucose levels diagram

Figure 2 illustrates that the treatment control group showed a more pronounced decrease in the rats' blood glucose levels for 7 days. Since the green seaweed extract *E. spinosum* effectively reduces blood glucose, it can be considered to possess antidiabetic properties.

Table 3. Average decrease in blood glucose levels

Group	Day 1	Day 3	Day 7
Normal	106,25 ±15,17	83,25 ±6,18	109,25 ±7,54
Positive	384,75 ±129,82	337,5 ±106,2	271,25 ±109,39
Negative	382,75 ±14,43	389,75 ±37,57	336 ±87,89
EEES 30	493 ±102,38	404 ±66,07	309,25 ±86,465
EEES 60	546,75 ±11,32	452,25 ±73,81	338,75 ±43,666
EEES 120	550,25 ±7,41	442 ±54,68	331,75 ±93,021

Note: (EEES: Ethanol Extract of *E. spinosum*/mg in 200 g BW of rat)

Table 3 shows a notable reduction in glucose levels in both the dose and positive control groups, demonstrating the seaweed extract's antidiabetic potential in lowering blood glucose in hyperglycemic rats. Additionally, the percentage reduction in blood glucose for each group is presented in Table 4.

Table 4. Percentage Decrease in Blood Glucose Levels on Day 7 After Administration of Extract

%Decrease	Normal	Positive	Negative	EEES 30	EEES 60	EEES 120
	2,82%	29,49%	12,21%	37,277%	38,04%	39,70%

Note: (EEES: Ethanol Extract of *E. spinosum*/mg in 200 g BW of rat)

The results of the Normality and Homogeneity tests show that the data follow a normal distribution and are homogeneous. All treatment groups obtained p-values of ≥ 0.05 in the normality test, indicating normality. Furthermore, the homogeneity test

confirmed that the data are homogeneous, with a p-value of 0.138 as detailed in Table 5.

Table 5. Test of Homogeneity of Variances: Glucose Level of Rats

Test of Homogeneity of Variances			
Glucose level days-7			
Levene Statistic	df1	df2	Sig.
1.937	5	18	.138

Then, it can be seen from the inter-group comparison data using the *one-way ANOVA* test to determine whether significant differences exist in each group. Each group has significant differences because the significance value is $p < 0.05$, which is 0.000. This means that the green seaweed extract (*E. spinosum*) is effective as an antidiabetic in rats, as shown in Table 6.

Table 6. Test of One-Way ANOVA Variances Glucose Level of Rats

ANOVA					
Glukose Level Days-7					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	284180.708	5	56836.142	46.995	.000
Within Groups	21769.250	18	1209.403		
Total	305949.958	23			

The post hoc LSD analysis was conducted to determine which treatment group had the greatest significant impact and to evaluate the effectiveness of the control groups in lowering blood glucose levels in rats on the seventh day. Results indicated that the first dose group did not differ significantly from the positive control, with a p-value of 0.536. In contrast, the second and third dose groups showed significant differences compared to the positive control, with the third dose group demonstrating the most pronounced significance at a p-value of 0.001, as presented in Table 7.

Therefore, based on the post hoc LSD findings, the extract administered at 120 mg per 200 grams of rat body weight proved to be the most effective in reducing blood glucose levels in alloxan-induced rats.

Table 7. Differences in Blood Glucose Level Reductions Between Groups from the Post Hoc LSD test results

Group	Normal	Positive	Negative	EEES 30	EEES 60	EEES 120
Normal		0,000	0,000	0,000	0,000	0,000
Positive	0,000		0,000	0,536*	0,010	0,001
Negative	0,000	0,000		0,001	0,000	0,000
EEES 30	0,000	0,536*	0,001		0,003	0,000
EEES 60	0,000	0,010	0,000	0,003		0,347*
EEES 120	0,000	0,001	0,000	0,000	0,347*	

Note: * = $p \geq 0,05$ (Having insignificant differences)

Discussion

The ethanol extract derived from the green seaweed *E. spinosum* contains essential bioactive compounds such as alkaloids, flavonoids, saponins, and tannins, which play a vital role in its antidiabetic properties. This study's results demonstrate that the extract's administration significantly decreased blood glucose levels in alloxan-

induced diabetic rats. The most substantial glucose reduction, 39.70%, was achieved at 120 mg per 200 grams of rat body weight. This decrease was more pronounced than those observed at doses of 30 and 60 mg/200 grams and closely matched the glucose-lowering effect of metformin used as a positive control. These findings align with earlier research indicating that *Eucheuma* seaweed extract exhibits a significant hypoglycemic effect.¹⁵

Mechanistically, the alkaloids in the extract act as antioxidants that enhance glucose transport and inhibit the enzyme 6-phosphatase in gluconeogenesis, thereby reducing endogenous glucose production. Flavonoids function as free radical scavengers that reduce oxidative stress and stimulate the regeneration of pancreatic β cells, which is important for optimal insulin production. Saponins inhibit the enzyme α -glucosidase, lowering glucose absorption from the intestine, while tannins increase insulin sensitivity and reduce diabetes-related inflammation.¹⁶ These findings are consistent with research on *E. cottonii*, which demonstrates antidiabetic potential through α -glucosidase enzyme inhibition and moderate antioxidant activity.¹⁷ This is supported by prior research demonstrating that flavonoids play a key role in antidiabetic mechanisms, as they effectively reduce blood.¹⁸

Furthermore, studies have demonstrated that *E. cottonii* extract can promote weight gain and reduce blood glucose levels in rats with type 1 diabetes, suggesting it has beneficial protective and metabolic effects.¹⁹ These studies reinforce the potential of seaweed as a multifunctional natural antidiabetic agent.

The limitations of this study include the use of crude extracts without the development of more applicable pharmaceutical formulations, such as tablets or capsules, as well as the lack of long-term toxicity evaluation and pharmacokinetic studies. In addition, the specific molecular mechanisms of the active compounds in the extract have not been detailed. Therefore, further research is highly recommended to isolate bioactive compounds, develop appropriate formulations, and conduct safety and efficacy tests on more complex animal models and human clinical trials.^{15,17}

Overall, the findings of this research provide strong evidence that the ethanol extract of *E. spinosum* effectively lowers blood glucose levels through multifactorial mechanisms involving antioxidant activity, pancreatic β -cell regeneration, and inhibition of glucose digestive enzymes. Further development is expected to yield a safe, effective, and affordable herbal therapy for the control of diabetes mellitus, especially in countries rich in natural resources like Indonesia, and the extract is made in pharmaceutical forms that are more applicable for diabetes mellitus patients, such as tablets, syrups, capsules, suspensions, emulsions, etc.

Conclusion

Administering the ethanol extract of green seaweed (*E. spinosum*) led to a significant decrease in blood glucose levels ($p > 0.05$) in alloxan-induced male white rats, particularly when compared to the negative control group. The most potent effect was observed with a dose of 120 mg per 200 grams of rat body weight, achieving a 39.70% reduction. This efficacy is likely attributable to the antidiabetic properties of the alkaloids, flavonoids, saponins, and tannins present in the extract. These findings provide a foundation for developing herbal treatments for diabetes; however, further studies are necessary to confirm their effectiveness and safety for human use.

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