

Potential Antianemia Activity of Cokelat Seaweed Extract (*Sargassum Duplicatum*) on Mencit (*Mus Musculus*) Injured With NaNO₂

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ABSTRACT

Seaweed is one of the leading commodities that is rich in nutrients and bioactive compounds that have potential for human health. The content contained in brown seaweed can be used as a therapy in increasing hemoglobin levels in anemia problems. The purpose of this study was to see the potential antianemia activity of brown seaweed extract (*Sargassum duplicatum*) against white mice (*Mus musculus*) and what dose can increase hemoglobin levels in mice. This research method is an experimental method with the Hb stick method carried out on 20 white mice, which were divided into 5 groups, namely the positive control group (blood supplement tablets), negative control group NaCMC 1%, treatment group 1 dose of 21 mg / kgBB, treatment group 2 dose 42 mg / kgBB, and treatment group 3 dosis 84 mg / kgBB. Testing the potential antianemia activity of brown seaweed was carried out on mice that had been induced with NaNo 2 and then given a dose of extract treatment. The data were analyzed statistically using paired-sample t-test analysis to analyze the effectiveness of the treatment indicated by the difference in the average before and average after treatment. The results of the study Hemoglobin (Hb) g/dl levels on day 1 to day 15 showed a very significant increase in hemoglobin where the parameters of the dose group were close to the normal condition of the positive control group (blood supplement tablets), namely group 3 dose of 84 mg/KgBB, so it can be concluded that brown seaweed extract (*Sargassum duplicatum*) in group 3 dose of 84 mg/KgBB has potential antianemia activity comparable to the positive control dose (blood supplement tablets).

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INTRODUCTION

The World Health Organization (WHO) says that most people in the tropics have anemia as many as 1.62 billion or 24.8% of all residents (Meilani *et al.*, 2023). The spread of anemia in adolescent girls is very worrying, in Asia it has reached 191 million people (Febria *et al.*, 2023). The incidence of anemia in Indonesia reached 21.7% from the results of Riskesdas in 2018,

anemia patients aged between 4-14 years were 26.4% and patients aged 15-24 years were 18.4% (Zuraida & Angraini, 2024) .

Anemia is a condition where Hemoglobin (Hb) levels in the body are lower than normal levels based on age and gender groups. Normal Hb levels in women are 12-15 gr/dl and in men are 13-17 gr/dl (Aulya *et al.*, 2022) . Women affected by anemia can be known if the hemoglobin level is less than 12 g/dl (Hastuty *et al.*, . 2021)

The group that is prone to suffering from anemia is adolescent girls because anemia can be at higher risk than adolescent boys, because adolescent girls have menstruation every month and have the wrong eating habits, this can happen because many adolescent girls want to have a slim body to maintain their appearance so they diet and eat less, but the diet they run is a diet that is not proportional to the needs of the body so that it can cause deficiencies in essential substances such as iron. Anemia in adolescents has a big impact so that it becomes the basis for action to prevent cases of iron deficiency anemia, therefore young women need to be guided with knowledge about iron deficiency anemia. Anemia can occur due to nutritional and non-nutritional factors. Deficiencies in nutritional factors are bound to protein, minerals, and vitamins while non-nutritional factors are bound to disease, namely infection. In the process of hemoglobin formation, protein plays a role in the process, when the body lacks protein for a long time the formation of hemoglobin can be disrupted so that it can cause signs of anemia. Low iron (Fe) intake is the main cause of nutritional anemia in Indonesia. Iron absorption in the body depends on the iron status in the human body (Utama *et al.*, . 2019)

Anemia is caused by a condition of decreased hemoglobin levels in the blood that transports oxygen (O_2) into the tissue and returns (CO_2) and protons into the lungs, decreased hemoglobin is caused by free radicals *Reactive Oxygen Species* (ROS) which are highly reactive so as to cause oxidative stress which results in loss of fluidity and increases lipid peroxide fragilitis in the erythrocyte membrane, causing low hemoglobin count (Kasmi ati *et al.*, . 2023)

Seaweed is one of the leading commodities rich in nutrients and potential bioactive compounds for human health (Hamidah Shindy, 2018) . The utilization of seaweed is very rich, it's just that in Indonesia the potential of seaweed is still underutilized. One of the resources that grow in the coral reef plain is brown seaweed. Types of brown seaweed are divided into three parts namely *Padina*, *Sargassum*, and *Turbinaria* (Hamidah Shindy, 2018) . Brown seaweed type (*Sargassum duplicatum*) contains secondary metabolite compounds, such as phenolics, flavonoids, tannins, sterols, terpenoids, saponins, alkaloids (Sedjati *et al.*, 2018) . Brown seaweed also contains as much as 54.3-73.8% carbohydrates, 0.3- 5.9% protein, vitamins (vitamins B1, B2, B6, B16, C, niacin) and minerals especially calcium, sodium, magnesium, potassium, iodine, and iron (Rohim *et al.*, . 2019)

The bioactive compounds in *Sargassum duplicatum* have the potential as a source of nutrasetical, pharmaceutical and functional food (Sedjati *et al.*, 2018) . Bioactive compounds are compounds contained in plants, animals or humans that are able to provide positive physiological effects beyond the basic nutritional value of food ingredients. Typically, bioactive compounds are absorbed from the gastrointestinal tract into the circulatory system,

and carried to the organs. Bioactivities of brown seaweed (*Sargassum duplicatum*) on health are as antioxidant, antibacterial, antiviral, antidiabetic, antitumor, anticancer, neuroprotective, antiobesity, cholinesterase inhibitor, anticoagulant, immunomodulator, fibrinolytic, analgesic, anti-inflammatory and gastric-protective (Sedjati *et al.*, . 2018)

Antioxidants are compounds that can remove, clean and resist the effects of free radicals. Antioxidants can stabilize free radicals by fulfilling the weakness of electrons possessed by free radicals, and can inhibit the occurrence of chain reactions from the preparation of free radicals. (Pratiwi *et al.*, 2023) . Oxidative stress can be overcome by being stabilized and neutralized by antioxidant compounds thereby reducing the risk of disease and damage to body cells (Chinensis *et al.*,2024) . Based on previous research, the results of literature review showed that *Eucheuma* sp seaweed is one of the foodstuffs that contains several intermediate compounds needed in hemoglobin synthesis such as iron, protein and vitamin B complex, . *Eucheuma* sp is also a seaweed that can stabilize the number of red blood cells, white blood cells, and hemoglobin. In addition, seaweed serves to reduce the side effects of inhibited production of blood cell-producing cells. In addition, seaweed is the main source of iodine and high in fiber, making it effective for increasing hemoglobin levels in pregnant women. Based on the description above, researchers are interested in examining the potential antianemia activity of brown seaweed extract (*Sargassum duplicatum*) against white mice (*Mus musculus*) and what dose can increase hemoglobin levels in mice.

METHODS

Tools and Materials

Tools and used are scales, trays, blenders (cosmos), glass jars, stirrers, beakers, measuring cups, masks, aluminum foil, filter paper, funnels, sieves, waterbaths, plastic cups, pipettes, label paper, cannulas, Hb test meters (Easy Touch GCHb), hot plates and cages. The materials used were male mice (*Mus musculus*), brown seaweed extract (*Sargassum duplicatum*), methanol, methanol p.a CMC Na 1%, NaNO₂, distilled water and Hb strips.

Research Procedure

Sample Preparation of Brown Seaweed (*Sargassum Duplicatum*)

Fresh brown seaweed (*Sargassum duplicatum*) was obtained from Saupapir Beach, Sorong City, Southwest Papua Province, then carried out a wet sorting process followed by washing using running water to clean the dirt that was still left on the sample. Furthermore, the samples were aerated and then chopped into small pieces that were useful to help the drying process, drying was carried out using an oven at 50 ° C for 360 minutes (Orilda *et al.*,2021) . After the sample dried, the sample was pulverized using a blender and filtered with a sieve.

Preparation of Brown Seaweed Extract (*Sargassum duplicatum*)

The method used to extract simplisia is by cold extraction or maceration method. The simplisia powder was put into a jar container as much as 267 grams then soaked with matanol solvent in a ratio (1: 5 b / v) until the sample was submerged, the maceration process was carried out for 3x24 hours and remaceration was carried out 1x24 hours, maceration and remaceration were stirred every 24 hours. After the maceration and re-maseration process is

complete, then the filtering process is carried out with filter paper which functions to separate the filtrate (liquid extract) and residue (substances left behind) (Nadialista Kurniawan, 2021). The resulting filtrate or liquid extract is then evaporated using a waterbath at 40°C to obtain a thick extract that still contains organic salts (Lantah *et al.*, . 2017)

The evaporated thick extract is then subjected to a salt removal process by decantation technique, namely by mixing the thick extract with methanol pro analysis then the salt will precipitate. Repeat until the white color that shows the presence of salt in the solvent disappears. The extract was evaporated again using a *waterbath* (Lantah *et al.*, .2017)

Sodium Nitrite Preparation

NaNO₂ powder was weighed as much as 62.5 mg and dissolved with distilled water until it reached 25 mL. Effective pathological anemia treatment is, LD50 = ½ x 5 mg = 2.5 mg. NaNO₂ solution was made in a dose of 2.5 mg/mL for treatment in pathological anemia (Pradawahyuningtyas *et al.*., 2020)

Animal Test Treatment

The test animals used were 20 male mice aged 2-3 months with a standard weight of 20-30 grams. The test animals were acclimatized for 7 days, after which they were weighed and divided into 5 groups, namely the positive control group of blood supplement tablets, the negative control group of 1% NaCMC, the treatment group of 21 mg/kgBB dose, the treatment group of 42 mg/kgBB dose, and the treatment group of 84 mg/kgBB dose of brown seaweed extract.

Blood collection is done through the tail of the mice using Hb sticks to measure the initial level of Hemoglobin. Good mice Hb blood collection is the second blood collection in order to get more accurate results. After that, induction is carried out on mice using NaNO₂. Checking Hb levels before and after induction, then given a dose of brown seaweed extract in each treatment group with a positive control dose (60 mg Fe tablets), negative control group (1% NaCMC), group 1 dose of 21 mg / kgBB, group 2 dose of 42 mg / kgBB and group 3 dose of 84 mg / kgBB. Administration of the suspension is done orally until day 14 and checking hemoglobin levels on day 15 to see the antianemia activity of brown seaweed extract (*sargassum duplicatum*) in increasing hemoglobin levels in white mice.

Data Analysis

Data analysis in the form of Hemoglobin (Hb) level examination was analyzed using *Analysis of Variance* (Anova). The results of the data obtained were carried out *Shapiro Wilk* normality test and *Levene's* homogeneity test. then the data were analyzed statistically using *paired-sample t-test* analysis to analyze the effectiveness of the treatment shown by the difference in the average before and average after treatment. After obtaining a significant value from the *One Way ANOVA test* results, it is then continued with the *LSD (Least Significant Difference)* test (Keswara *et al.*, 2019).

RESULTS AND DISCUSSION

The samples used were brown seaweed (*Sargassum duplicatum*) taken from Saupapir beach area, West Sorong District, Sorong City, Southwest Papua Province. Samples that have been taken then carried out wet and dry sorting process to clean dirt. Then the samples of brown

seaweed (*Sargassum duplicatum*) were aerated by hanging on a rope as a dryer, then the samples were chopped and dried in a *dry oven* for 7 days at a temperature of 50°C-55°C until constant weight. Then the sample was extracted for 3 days with methanol solvent as much as 1,335 mL, then the sample was filtered and evaporated with a waterbath for 7 days to produce a thick extract. The thick extract of the evaporation results then carried out the process of separating the salt content by mixing the thick extract with methanol pro analysis then the salt will precipitate. Repeat until the white color that indicates the presence of salt in the solvent disappears, then the extract is evaporated again with a waterbath until it becomes a thick extract. The thick extract obtained was 47 grams and the yield was 17.6%.

Brown seaweed (*Sargassum duplicatum*) is rich in iron and folic acid, two essential nutrients that play a role in hemoglobin production and red blood cell formation. It also has the potential to stabilize the number of red blood cells, white blood cells, and hemoglobin. In addition, brown seaweed can help reduce the side effects of impaired production of blood-forming cells, thereby maintaining the balance of the hematological system. Dried seaweed contains about 0.5-3.5 mg of iron per 100 grams. In addition, the bioavailability (ability of the body to absorb) of iron in seaweed is also higher, about 2-10% compared to vegetables. This is because the content of phytic acid that can interfere with iron absorption in seaweed is very low (Untari Anggeni *et al.*, . 2024)

According to (Sedjati *et al.*, 2018) brown seaweed type (*Sargassum duplicatum*) contains secondary metabolite compounds, such as phenolics, flavonoids, tannins, sterols, terpenoids, saponins, and alkaloids. Bioactivity compounds in brown seaweed (*Sargassum duplicatum*) against health are as antioxidants, antibacterial, antiviral, antidiabetic, antitumor and anticancer.

Anemia criteria in mice (*Mus musculus*) as test animals are determined based on the value of hemoglobin (Hb) in the blood, namely mild anemia (10-11 g/dl), moderate anemia (8.2-10 g/dl), and severe anemia (<8 g/dl) This category is used to assess the severity of anemia in test animals.

According to (Yusrin *et al.*, 2023) , hemoglobin formation occurs in the bone marrow through a maturation process, where iron is released from hem and transported by transferrin plasma to the bone marrow for the formation of new red blood cells within 5-9 days. This is the same as the theory of Guyton & Hall (2019) which states that the process of hemoglobin formation takes about 7-10 days to become perfect and ready to be circulated throughout the body with red blood cells because hemoglobin is in red blood cells, so its life time is the same as the life span of red blood cells which is about 120 days. So in this study it takes 14 days or two weeks to see changes in hemoglobin levels in mice.

Based on the results of the study, the test animals only experienced mild anemia, so the hemoglobin (Hb) level will be increased to normal levels with a predetermined dose of extract and will see the potential antianemia activity of brown seaweed extract (*Sargassum duplicatum*) against test animals.

Observation Results of Antianemia Activity of Brown Seaweed Extract (*Sargassum Duplicatum*) Average Increase in Hemoglobin Level.

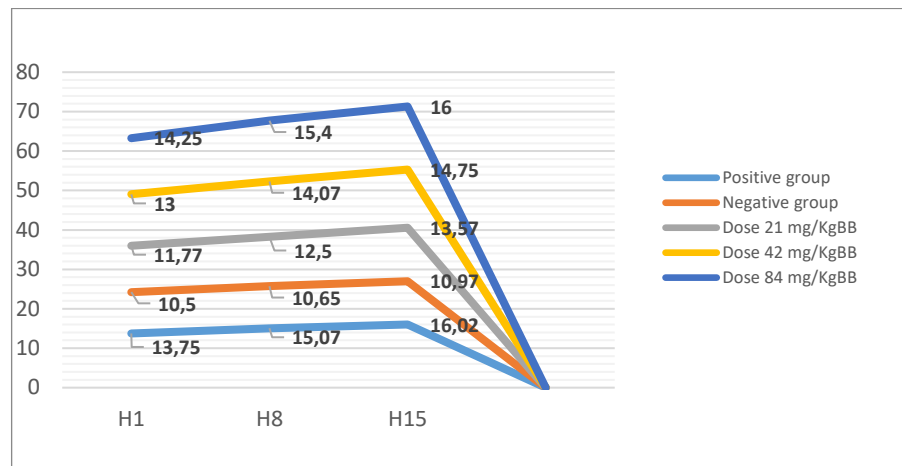


Figure 1. Hemoglobin level increase graph (day 1 to day 15)

Observations were made after test animals that had experienced mild anemia were given treatment, namely positive control (blood supplement tablets), negative control (Na-CMC 1%) brown seaweed extract (*Sargassum duplicatum*) with three different dose groups, namely group 1 light dose of 21 mg / kgBB, group 2 medium dose 42 mg / kgBB, and group 3 high dose 84 mg / kgBB. The condition of the test animals will be observed for 15 days starting from H1, H8 to H15. Hemoglobin (Hb) g/dl levels on day 15 showed a very significant increase in hemoglobin where the dose 1, dose 2 and dose 3 groups had increased hemoglobin levels, but the dose group parameters were close to the normal condition of the positive control group (blood tablet tambahan is group 3 dose 84 mg / kgBB, so it can be concluded that there is potential antianemia activity in brown seaweed extract (*Sargassum duplicatum*).

The data obtained in this study were statistically analyzed with the *one way anova* test in the normality and homogeneity test showed that the data were normally distributed because the significant value in all groups was ($p > 0.05$).

Table 1. Differences in Hemoglobin Levels in Each Group Between *Pre-test* and *Post-test*

Group	n (20)	<i>Pre-test</i>	<i>Post-test</i>	Difference	<i>p-value</i>
K+	4	16,175 ± 2,053	12,325 ± 0,943	3,850 ± 1,9122	0,014
K-	4	13,325 ± 1,292	10,450 ± 0,614	2,875 ± 1,668	0,021
F1	4	13,625 ± 1,057	12,150 ± 0,444	1,475 ± 1,247	0,049
F2	4	14,400 ± 2,110	12,725 ± 2,404	1,675 ± 4,992	0,003
F3	4	16,875 ± 2,269	13,875 ± 1,700	3,000 ± 1,390	0,001

In table 1, the paired sample t test shows that all treatment groups have a *p-value* < 0.05, which means that there is an effect of giving brown seaweed extract before treatment and after treatment.

The results of statistical tests in the LSD (*Least Significant Difference*) test showed that the extract with group 1 dose of 21 mg/KgBB and group 2 dose of 42 mg/KgBB was not effective in overcoming anemia in mice, because the results obtained were significantly different from the results of positive control (blood supplement tablets). However, the extract

at a dose of 84 mg/KgBB proved effective in overcoming anemia because it has good antianemia activity and is equivalent to positive control. So it can be said that the 84 mg/KgBB dose contains active compounds that can act as antianemia.

CONCLUSIONS

Based on the results of the research that has been done, it can be concluded that the methanol extract of brown seaweed (*Sargasum duplicatum*) is able to increase hemoglobin levels in mice (*Mus musculus*) that have been induced with sodium nitrite. The extract dose of 84 mg/KgBB has the same potential activity as antianemia as the positive control dose (blood supplement tablets). To improve the accuracy of the research results, it is recommended that future researchers use more adequate tools and meet the Research Standards on Hemoglobin (Hb) levels to improve the accuracy and validity of the results of research on test animals and it is recommended to conduct further research on brown seaweed extract (*Sargasum duplicatum*) on other pharmacological activities so that other benefits can be known as potential treatments.

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