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American Journal of BioScience 2014; 2(6): 244-250 Published online December 19, 2014 (<http://www.sciencepublishinggroup.com/j/ajbio>) doi: 10.11648/j.ajbio.20140206.19 ISSN: 2330-0159 (Print); ISSN: 2330-0167 (Online) Study of the extract activities of Buas buas leaves (*Premna pubescens*) as immunostimulant on rats (*Rattus novегicus*) Martina Restuati 1, Syafruddin Ilyas 2, Salomo Hutahaeen 2, Herbert Sipahutar 1 1Biology Education Department, Faculty of Mathematic and Science, State University of Medan, Medan, Indonesia 2Biology Department, Faculty of Mathematic and Science, University of North Sumatra, Medan, Indonesia Email address: tinaunimed@gmail.com (M. Restuati), Syaf_ilyas2004@yahoo.com (Syafruddin H.), Sal_hutahaeen@yahoo.com (S. Hutahaeen), herbert_sipahutar@yahoo.com (H.

Sipahutar) To cite this article: Martina Restuati, Syafruddin Ilyas, Salomo Hutahaeen, Herbert Sipahutar. Study of the Extract Activities of Buas buas Leaves (*Premna pubescens*) as Immunostimulant on Rats (*Rattus novегicus*). American Journal of BioScience. Vol. 2, No. 6, 2014, pp. 244-250. doi: 10.11648/j.ajbio.20140206.19 Abstract: Buas buas (*Premna pubescens*) is one of the plants in Indonesia which is believed to have medicinal properties, but it is not certain.

This plant is consumed by the Malay community, one of the tribes in North Sumatra - Indonesia, as vegetables, especially during the fasting month. This paper describes the effects of ethanol extract of leaves of Buas buas as an immunostimulant in rats (*Rattus norvegicus*). The experiments were performed on 24 tail of male rats, which were three months old, weighing 140-180 g.

There were four groups of experiment ie Group A0 was given Carboxy Methyl Cellulose mice (CMC) (control group), group A1 was given 500 mg/ kg bw of Ethanol Extracts of

Buas buas . Group A2, given ethanol extracts of Buas buas 500 mg / kg bw + SRBC, and the group A3 only given Sheep Red Blood Cell (SRBC). After this experiment, apigenin levels were analyzed by High Performance Liquid Chromatography (HPLC) Agilent 1220.

Then the activity of immunostimulation is determined by measuring the leukocyte count, percentage of lymphocytes, antibody titers, the levels of immunoglobulin M (IgM), immunoglobulin G (IgG), and lysozyme with ABX Micros 60. The group A2 is seen the highest group of in the number of leukocytes, antibody titer levels, imonoglobulin G (IgG), and imonoglobulin M.

The amount of lysozyme owned group A2 is 0:04 ug / ml, whereas other treatments to obtain results that are not different relative significantly. Provision of Ethanol Extracts of Buas buas on mice can increase the leukocytes, lymphocytes, antibody titers, IgM, IgG and Lysozyme. Keywords: Premna Pubescens , Titer Antibodies, Lymphocytes, SRBC, IgG, IgM, Lysozyme 1.

Introduction The immune system can be improved with the use of traditional medicine. Body's immune system increases the resistance of cells to fight disease. Traditional medicine can be used as treatment efforts (self-medication) to support modern medicine, because the plant is effective in improving the immune system and increase antioxidant activity in humans [1].

One of the plant in Indonesia, which has not been known and used widely, which have medicinal properties, is called Buas buas (*Premna pubescens*) as shown in Figure 1. The leaves of this plant are consumed by the Malay community, one of the main tribes in Indonesia, especially North Sumatra just as fresh vegetables or cooked vegetables.

The shape of the leaves of this plant looks like Figure 2. Figures 1. Buas buas Plants (*Premna pubescens*) 245 Martina Restuati et al. : Study of the Extract Activities of Buas buas Leaves (*Premna pubescens*) as Immunostimulan on Rats (*Rattus novegicus*) Figure 2. Buas buas Leaf Some of the other *Premna* genus has been studied, that related to the content of secondary metabolites owned and usefulness.

Corymbosa *Premna* compounds containing apigenin, which is one of the derivatives of the alkaloid. Secondary metabolites as anti-hyperglycemic activity. The previous research found a significant reduction of total cholesterol, Low Density Lippoproteins (LDL) cholesterol, Very Low Density Lipoproteins (VLDL), and an increase in the High Density lipoproteins (HDL) in adult wistar rats [2]. Apigenin can inhibit, prevent and treat cancer cells.

These five common types of flavonoids, are myricetin , kaempferol, quercetin, luteolin and apigenin . Flavonoid apigenin is nontoxic who has the ability as an anti-tumor and chemotherapy agents, and inhibits the growth of vascular endothelial protease inhibitors [3, 4, 5].

The development of cervical cancer cells (U937) in G1 phase can be prevented by lowering the activity of Bcl-2 protein expression by apigenin, which acts as an anti-apoptotic [6]. Flavonoid apigenin is nonmutagenic, which prevents the growth of human neuroblastoma cells [7]. This demonstrates the efficacy of Buas buas leaves, which have apigenin compounds can increase of the body's defense system, because it has an activity as an immunostimulant , which can stimulate the immune system non-specific and specific when no antigen.

Immunostimulatory consists of biological and synthetic compounds that enhance the body's non-specific defense mechanisms in animals that provide the comprehensive protection [8]. The immunostimulatory activity of the leaves of Buas buas can be measured by several parameters, namely, immunoglobulin G and immunoglobulin M and some hematological parameters such as the percentage of leukocytes , leukocyte count number type, antibody titers, and lysozyme .

Lysozyme is one parameter that is measured as the impact of immunostimulant [9]. The immunostimulatory activity can be measured through serological tests and hematology [10]. The immunostimulatory agents can enhance the non-specific immune system such as the number of phagocytic cells, lysozyme and other substances are soluble in serum [11]. This issue became the basis for assessing the role of the ethanol extract of Buas buas leaves as an immunostimulant. 2. Materials and Methods 2.1.

Materials and Equipment The materials used in this research is the rat feed pellet form PC 05, water (distilled water) were provided ad libitum, wooden hulls as litter of rats, ethanol extract of leaves of Buas buas , Sheep Red Blood Cell (SRBC) antigen, Carboxy Methyl cellulose (CMC). To analyze the serum immunoglobulin is used Elisa Kit Rat IgG and IgM. To analyze Lysozyme levels in serum, is used Lysozyme Kit and HPLC Apigenin Standard.

Apigenin is analyzed by ABX Micros 60. 2.2. The Animal of Test A total of twenty-four (24) rats (*Rattus norvegicus*) wistar strain male, aged 3 months with a weight range of 100-200 g, were used as experimental. The white rats were divided into 4 groups each group had 6 tails. The gender of mice used were male.

White rats maintained with acclimatization in groups (two mice per cage) in animal

cages made of plastic with a size of 40x30x20 cm. Maintenance is carried out at room temperature (24 °C-26 °C). The food is provided in the form of a standard food pellets and water ad libitum. 2.3. Making of Ethanol Extract of Premna Pubescens Leaf The fresh Buas buas leaves as weight as 3420 grams is sorted to separate the leaves from the stalk leaves, insects and other debris. Subsequently these are washed 3 times, then drained. Leaves are already clean dried in drying cabinet to become brittle.

The leaves are dried pulverized to obtain powder. Heavy powder obtained is 1050 grams, then extracted using Soxhlet, with 70% ethanol content. Extract obtained was concentrated with a rotary evaporator and then dried and get dry ethanol extract as much as 195 grams. 2.4. Antigen Sheep Red Blood Cell (SRBC) Making SRBC is started by taking blood from the jugular vein of sheep.

Disinfected prior lamb neck with 70% alcohol before taking blood. Alsover much as 5 CC aspirated and continued to suck blood from the jugular vein of sheep by the same amount (5CC + 5CC alsover sheep blood), using a 10 CC syringe. Eristrosit is washed with a buffer solution with pH 7.4 kolomer diluent to 40CC, and weighed to obtain a balanced position.

Erythrocyte suspension was obtained with a speed of 2000 rpm for 15 min later discard supernatant there. After that, the erythrocyte sedimentation redissolved in kolomer diluent, and centrifuge process is carried back to the velocity and the same deadline. Screening process with centrifuges is done in 3 times, and the results obtained are aspirated using a pasteur pipette.

SRBC stored in a tube that is inserted in a refrigerator at a temperature of 40 0 C. 2.5. Screening Apigenin The screening Apigenin was done starting from identification of Buas buas plants, testing of the active compound content in plants, and testing the apigenin, which American Journal of BioScience 2014; used a system of High Performance Liquid (HPLC) agilent 1220 with a wavelength of rate of 0.5

ml /min, temperature 25 °C and of 10 mL. 2.6. Experiment Design The experiment used a Completely Randomized (CRD) non factorial with four treatments, given a six replications. The A0 as a control 0.5 ml of distilled water orally every day. given 250 mg /kg bw of ethanol extract buas without SRBC.

The A2 group was given of ethanol extract of leaves of Buas buas + and the A3 group was given 0.1 ml SRBC. day 8 and day 15. It refers to the blood obtained from all test animals at the day neck decapitation. Serum was separated for several parameters. 2.7. Evaluation of Immunostimulants Activity Blood samples of mice obtained by

decapitation all mice that had been given each treatment Blood collected in the tube which has anticoagulant (EDTA), and then analyzed Micros 60. Hemagglutination method is antibody titers.

Serum lysozyme activity was measured procedure factory (Sigma Cat Number L7651). of lysozyme based on the lysis of *Micrococcus* bacteria suspension in accordance with developed by [12], namely through the following mg /ml . *Micrococcus lysodeiktycus* (Sigma) 66 mM PBS (pH 6.2). 50 mL of serum was bacterial suspension. Decrease of absorbance and 4.5

min for 3 minutes on a spectrophotometer wavelength of 450 nM. One unit of lysozyme defined as a decrease in absorbance Determination of IgG and IgM is performed dilution treatment, which is done through Linke d Immunosorbent Assay (ELISA). 3. Result 3.1. Results of Buas Buas Plant Identification of Bioactive Compounds Table 1.

The result of phytochemical screening of ethanol pubescens leaves Secondary Metabolites Test Alkaloid Flavonoid Saponin Steroid Based on the identification of these plants, plants include Verbenaceae , the type of Premna Blume . The test results showed that the American Journal of BioScience 2014; 2(6): 244-250 Liquid Chromatography of 337 nm, the flow and injection volume Randomized Design treatments, and each was control group was given The A1 group was of leaves of Buas given 250 mg/ kg bw + 0.1 ml of SRBC SRBC.

SRBC given on blood samples were day 31 st , through the for measurement of Activity decapitation neck in treatment on the day 31 st . has been given an analyzed by using ABX used to test the measured by following the L7651). Measurement *Micrococcus lysodeiktycus* with the method following ways: 0.15 (Sigma) was dissolved in was added to 1 ml of absorbance recorded at 0.5

spectrophotometer with a lysozyme activity is of 0.001L/min. performed with serum methods Enzyme- Identification and Testing ethanol extract of Premna Test result + + + + + plants, the Buas buas Premna pubescens the identification of secondary metabolites of the Premna extract was positive as alkaloids, steroids , as shown in Table 1.

Apigenin assays contained Premna pubescens leaves, indicates owned (Figures 4) almost the apigenin in Figure 3. The content are 0.2845 mcg/10 ml; 28,45 mcg/ml; Figure 3. Chromatogram Figure 4. Chromatogram apigenin on 3.2. Test Results of Immunostimulatory Pubescens Leaf Ethanol Extract Table 2. Effect of ethanol extract of Premna leaves against hematocrit and blood hemoglobin Treatment Hematokrit (%) A0

36.50±5.84 A1 40.95±4.09 A2 35.75±6.19 A3 42.93±2.84 A preliminary description of *Premna pubescens* leaves is shown by the average percentage which had an average of the amount of 42.93% (exceeding male rats is 32-40 %). Hematocrit shows, that the state of the viscosity higher than the other three treatments.

lower hematocrit percentage compared the concentration of hemoglobin 246 *Premna pubescens* leaf ethanol alkaloids, flavonoids, saponins, and in the ethanol extract of the indicates that the pattern of curves the same as the standard raw content of apigenin were analyzed mcg/ml; dan 35,56 mcg/ml. Chromatogram raw apigenin standards ethanol extract of *Premna pubescens*.

Immunostimulatory Activity of *Premna* Extract *Premna* immunostimulatory *pubescens* hemoglobin mice Mean ± SD (%) Hemoglobin (gm/dl) 36.50±5.84 11.82±2.93 40.95±4.09 13.60±0.95 35.75±6.19 13.13±1.26 42.93±2.84 14.23±0.50 of the effect of ethanol extract of hematocrit and hemoglobin , percentage of hematocrit on the A3, the highest percentages in the (exceeding normal hematocrit values in Hematocrit value in the treatment A3 viscosity of blood concentrations treatments. A2 treatment had compared with the A3.

Likewise, hemoglobin testing, treatment A3 has the 247 Martina Restuati et al. : Study of the Extract Activities of Buas buas Leaves (*Premna pubescens*) as Immunostimulan on Rats (*Rattus novvegicus*) highest concentration compared with other treatments hasil pengujian shown in Table 2.

Description: A0: as a control; A1: given the ethanol extract of *Premna pubescens* leaves, A2: given the ethanol extract of *Premna pubescens* leaves and SRBC, A3: given SRBC. Testing of the effect of *Premna pubescens* leaves ethanol extract against each concentration of blood cells namely erythrocytes, leukocytes , and platelets showed that blood concentrations of each treatment showed normal concentrations, ie 6.20-7.64 x 10⁶/mm³.

The white rat erythrocytes concentration pattern has the same pattern as the percentage of hematocrit . The treatment A3 has the highest concentration of erythrocytes after A1 while A0 have the lowest concentration of erythrocytes . The difference in erythrocyte concentrations obtained in all four treatment have not been able to explain in detail the effect of ethanol extract of *Premna pubescens* leaves against erythrocyte concentrations. This is caused by differences in the four concentrations of erythrocytes is still quite normal and not significantly different.

Leukocyte concentration test results showed the highest concentration of leukocytes is

the treatment A3 ($9.88 \times 10^3/\text{mm}^3$), while the lowest concentration is in treatment A0 ($5.50 \times 10^3/\text{mm}^3$). Treatment A2 are also added leukocyte antigen SRBC resulted in lower concentrations than treatment A3 yaitu $7.52 \times 10^3/\text{mm}^3$.

The test results are indicated in Table 3. Table 3. Effect of ethanol extract of *Premna pubescens* leaves immunostimulatory against erythrocytes, leukocytes, and platelets
Treatment Mean \pm SD Eritrosit ($\times 10^6/\text{mm}^3$) Leukosit ($\times 10^3/\text{mm}^3$) Trombosit ($\times 10^5/\text{mm}^3$) A0 6.58 ± 1.80 5.50 ± 1.48 6.00 ± 2.12 A1 7.41 ± 0.36 9.82 ± 1.36 7.35 ± 1.05 A2 6.69 ± 0.26 7.52 ± 1.82 10.13 ± 1.61 A3 7.78 ± 0.23 9.88 ± 2.31 8.85 ± 0.50 Description: A0: as a control; A1: given the ethanol extract of leaves *Premna pubescens*, A2: given the ethanol extract of leaves *Premna pubescens* and SRBC, A3: given SRBC. 3.3.

Test Count the Number of Leukocytes and Lymphocytes The highest number of leukocytes contained in the A2 treatment, ie the treatment given ethanol extracts of Buas buas and SRBC. While the lowest is the number of leukocytes contained in the A1 treatment, ie given only ethanol extract of Buas buas. The value of group A0, as a control group, is relatively the same as the value of A2.

On the treatment given SRBC, namely the A2 group and the A3 group, it seems that the A3 has a number of leukocytes were slightly lower than the A2 group. The results of the calculations are shown in Table 4. Table 4. Effect of Ethanol Extracts of Buas buas to the count number of leukocytes and lymphocytes. Group Mean \pm SD Leukosit ($\times 10^3/\text{mm}^3$) ($\times 10^3/\text{mm}^3$) Limfosit (%) A0 9.82 ± 1.36 76.47 ± 3.10 A1 5.50 ± 1.48 71.90 ± 7.00 A2 9.88 ± 2.31 74.94 ± 9.66 A3 7.52 ± 1.82 61.98 ± 9.41 Description: SD: Standard Deviation; A0: as a control; A1: given the ethanol extract of leaves *Premna pubescens*, A2: given the ethanol extract of leaves *Premna pubescens* and antigens of sheep red blood cells, A3: given antigen of sheep red blood cells. 3.4. Antibody Titer Test Table 5.

Effect of Ethanol Extracts of Buas buas to the count number and types of leukocytes. Treatment Mean of antibody Titer (HI) \pm SD A0 1.00 ± 0.89 A1 1.67 ± 0.52 A2 7.17 ± 0.75 A3 6.67 ± 1.51 Test performed with antibody titers of hemagglutination test that is based on the agglutination of red blood cells. The test results showed antibody titers showed the A2 treatment are the highest values (7:17) than the other three treatments.

Then the antibody titer in the treatment A3 is 6.67. While on A0 and A1 showed antibody titer levels were relatively similar, as shown in Table 5. Description: SD: Standard Deviation; A0: as a control; A1: given the ethanol extract of leaves *Premna pubescens*, A2: given the ethanol extract of leaves *Premna pubescens* and antigens of sheep red blood cells, A3: given antigen of sheep red blood cells. 3.5.

Measurement of Immunoglobulin G dan Immunoglobulin M The measurement results showed that the highest levels of IgM are those of A2, which is 3.96 ± 1.05 ng/ml, whereas IgM levels were lowest in the control treatment was 0.88 ± 0.28 ng/ml. When compared with the treatment added American Journal of BioScience 2014; 2(6): 244-250 248 SRBC, namely A2 and A3, both have higher IgM levels than treatment that is added SRBC ie groups A0 and A1 group, as shown in Table 6. Table 6.

Effect of ethanol extract of Buas buas to count number and types of leukocytes
Treatment Mean \pm SD Immunoglobulin M (ng/ml) Immunoglobulin G (ng/ml) A0 0.88 ± 0.28 4.52 ± 1.30 A1 1.74 ± 0.57 8.70 ± 0.83 A2 3.96 ± 1.05 9.48 ± 5.90 A3 2.20 ± 1.05 8.96 ± 3.61 Description: SD: Standard Deviation; A0: as a control; A1: given the ethanol extract of Premna pubescens leaves, A2: given the ethanol extract of Premna pubescens leaves and antigens of sheep red blood cells, A3: given antigen of sheep red blood cells.

On testing IgG levels, treatment A2 have the highest levels of IgG (9.48 ± 5.90 ng/ml), while A0 has the lowest levels of IgG 4.52 ± 1.30 ng/ml. On A1 treatment have relatively similar levels of IgG with A3 is 8.70 ± 0.83 ng/ml and 8.96 ± 3.61 ng/ml. Based on the difference in the number of levels obtained, IgM levels lower than IgG. 3.6.

Measurement of Lisozim Levels On lysozyme assay , the three treatments (A 0, A 1, A 3) have an average value unchanged at 0.03 ng/ml. While on treatment A 2 have higher levels ie 0.04 ± 0.01 ng/ml as shown in Table 7. Table 7. Effect of ethanol extract of Buas buas against the percentage of white rat lysozyme Treatment Mean of Lisozim value (μ g/ml) \pm SD A0 0.03 ± 0.02 A1 0.03 ± 0.01 A2 0.04 ± 0.01 A3 0.03 ± 0.02 Description: SD: Standard Deviation; A0: as a control; A1: given the ethanol extract of Premna pubescens leaves, A2: given the ethanol extract of Premna pubescens leaves and antigens of sheep red blood cells, A3: given antigen of sheep red blood cells. 4.

Discussion Leukocytes are activated cells of the immune system that can respond to antigens that enter the body. On this study, Sheep Red Blood Cell (SRBC) was used as antigen due to the combination with skin proteins qualify as antigens when used to obtain the contact hypersensitivity reaction in mice. It is in base line with Kannan, Singh, kumar, Jegatheswari and Subburayalu (2007).

On testing leukocytes known that the highest concentration of leukocytes is at A2 ($9.88 \pm 2.31 \times 10^3$ /mm³), while the lowest concentration is on the A1 ($5.50 \pm 1.48 \times 10^3$ /mm³). The height of the leukocyte concentration in A2 due to the addition of SRBC antigens so that the number of leukocytes increased to fight the antigen into the body of white mice.

In addition, the treatment A2 is also added immunostimulatory substance that the ethanol extract of Buas buas mechanism that stimulates the immune system to produce more in the number of leukocytes. The number of leukocytes in the treatment added ethanol extracts of Buas buas and SRBC (A 2) is almost the same as the control treatment (A 0).

It is clear that when rats fed ethanol extracts of Buas buas for 30 days, added antigen SRBC, condition endurance of mice is similar to the control condition is not added ethanol extracts of Buas buas and antigen SRBC. Overall, each of the number of leukocytes in the four treatment has a normal number of leukocytes ie 4.19-9.73 X10³/mm³. It is in line with the findings of [13]. Antigen given in treatment A2 and A3, can multiply intracellularly, making it difficult to reach antibody.

To combat these intracellular antigens required cellular immune response, which is a function of lymphocytes, especially T lymphocytes. The helper T cells will recognize microorganisms or antigen via MHC class II found on the cell surface of macrophages. This signal is triggered lymphocytes to produce various types of lymphokines which can help the macrophages destroy microorganisms, that is in line with findings of [14]. The results show that the number of lymphocytes in the treatment A0, is the highest among the three other treatments ie 76.47 ± 3:10%, while the percentage of lymphocytes that at least is at A3 treatment.

The low percentage of lymphocytes in A3 caused by the administration of SRBC antigens, are not added immunostimulatory substances such as in the commission of A2. So when the antigen is given without adding immunostimulatory substances, the body is not stimulated to produce more antibodies. On the A2 treatment, administration of antigen followed by the addition of immunostimulatory substances will increase the production of immunoglobulin and approaching the normal value is 76- 98%, in line with [13].

On antibody titer testing, measurements on changes in the number of antibodies in an immune response in the body that the highest antibody titers found in A2 treatment, which is treatment given immunostimulatory substances such as apigenin in ethanol extracts of Buas buas and antigen SRBC. Given antigen triggers the body's immune system to produce antibodies.

In addition, the treatment is added antigen (A 2 and A3), A2 has a higher antibody titers value than A3 is also caused by the presence of immunostimulatory substances that help increase the production of antibodies, thereby reducing the duration of the inflammatory reaction. It is in line the 249 Martina Restuati et al. : Study of the Extract

Activities of Buas buas Leaves (*Premna pubescens*) as Immunostimulan on Rats (*Rattus novegicus*) statement of [15], that the increase of humoral response due to SRBC showed an increase in the responsiveness of macrophages, and B and T lymphocytes in antibody synthesis.

This is also consistent with the results obtained in the percentage of lymphocytes in the treatment of A2 ($74.94 \pm 9.66\%$) higher than A3 ($61.98 \pm 9.66\%$). Immunoglobulin M is used as a parameter in this study because it is the first time that antibodies present in the immune response to the antigen and the primary antibody in blood group naturally in accordance with the opinion of [16]. The results show that the highest levels of IgM is in A2 ($1:05 \pm 3.96$ ng/ml) is the treatment given immunostimulatory substance ethanol extracts of Buas buas and SRBC.

While only added SRBC treatment and no immunostimulatory substance (A 3) have lower levels of IgM ($2:20 \pm 1:05$ ng/ml). High levels of IgM in A2 shows that the immunostimulatory substances such as apigenin in ethanol extracts of Buas buas can increase the production of IgM. Similarly, immunoglobulin G, is used as a parameter of this study, because the percentage of immunoglobulin G has at most percentage is about 75% of the total immunoglobulins . This is in line with the opinions of [16]. The test results also show that A2 has the highest levels of IgG is $9:48 \pm 5.90$ ng/ml.

The lowest level of IgG and IgM was on the treatment A0, ie 0.88 ± 0.28 ng/ml and $4:52 \pm 1:30$ ng/ml. In [14] is explained that the appearance of antibodies in the form of immunoglobulins in the blood due to the differentiation of B lymphocytes , antibodies that bind to the antigen forming antigen-antibody complexes can activate complement and lead to the destruction of the antigen.

In order to differentiate B lymphocytes, and make antibodies, its' needed the help of T lymphocytes by certain signals through MHC and signals released by macrophages stimulates the production of antibodies. The percentage of lymphocytes in A2 treatment also showed a higher percentage of lymphocytes than A3 treatent. Other parameters are used to determine the effect of immunostimulatory substances in the body of the rat is lysozyme .

It is used in line with the statement of [11], that lysozyme can enhance the body's non-specific defense system. From these experiments it is known that the treatment A2 has the highest levels of lysozyme ($0:04$ ug/ml), while the other treatments showed similar values ie $0:03$ ug/ml. Lysozyme protects several places in the body which is a potential place for food for bacterial growth.

In the blood, lysozyme provides protection, with a more powerful method to that used by the immune system. Immunostimulatory bind specific receptors on the surface membrane of phagocytes and lymphocytes, which activate these cells to produce several enzymes including lysozyme to destroy pathogens are like the opinion of [17].

On this basis, it can be stated that the ethanol extract of Buas buas is to be as antibacterial as reported by [18]. Acknowledgement The author is very grateful to Hamonangan Tambunan, Lecturer of Electrical Engineering Education Department, Faculty of Engineering, State University of Medan. He gives a lot of relief to the author. References [1] Lie, T., et. all., 2010. Flavonoid, Phenol and Polysaccharide Contents of *Echinacea purpurea* L.

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Study of the extract activities of *Buas buas* leaves (*Premna pubescens*) as immunostimulant on rats (*Rattus novvegicus*)

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