JURNAL MULTIDISIPLIN MADANI (MUDIMA)
Homepage: https://journal.formosapublisher.org/index.php/mudima
ISSN: 2808-5639 (Online)
Research Article

Volume 4, No 6, June (2024)	DOI: https://doi.org/10.55927/mudima.v4i6.9588	Page: 830-842

Immunomodulatory Activity of Ethanol Extract and Kabau (Archidendron Bubalinum) Leaf Fractions on the Macrophage Phagocytosis Index in Male White Rats (Rattus Novergicus)

Intan Sofia Irsa Aulia[1*], Tri Wijayanti[2], Opstaria Saptarini[3]
Universitas Setia Budi, Surakarta
Corresponding Author: Intan Sofia Irsa Aulia intansofiairsa@gmail.com

ARTICLE INFO
Keywords: Immunomodulatory Activity, Ethanol Extract

Received : 5 *April*
Revised : *15 May*
Accepted : 26 *June*

ABSTRACT
Immunomodulators are substances that have the ability to balance and restore a dysfunctional immune system, reduce the body's immune response due to excessive immune activity and stimulate leukocyte cells, especially macrophages, to form a strong immune system. The aim of this study was to determine the immunomodulatory activity of kabau leaves (Archidendron bubalinum) in male white rats in terms of the phagocytosis index, number of leukocyte cell types and spleen histopathology. This research design used 35 mice as test animals and then divided them into 6 groups, namely negative control, positive control (Stimuno dose 50 mg), kabau leaf extract 20 mg, n-hexane fraction 6.66 mg, ethyl acetate fraction 4 mg, water fraction 6.66 mg, each group consisted of 5 male rats. Immunomodulatory activity was seen from the phagocytosis index and the number of leukocyte cell types at 10 and 15 minutes. The results showed that the 6 treatments had significantly different immunostimulatory activities with a significant value of less than 0.05. The results of the phagocytosis index calculation showed that the highest value was Stimuno at 3.33, then the 6.66 mg n-hexane fraction was 2.49, the 4mg ethyl acetate fraction was 1.63, the 20 mg extract was 1.26, and the water fraction was 6.66 mg. 1.27. From these results it can be concluded that kabau (Archidendron bubalinum) leaf extracts and fractions have immunostimulant activity

INTRODUCTION

Immunomodulators are substances that have the ability to balance and restore a dysfunctional immune system, reduce the body's immune response due to excessive immune activity (Handayani., 2010) and stimulate leukocyte cells, especially macrophages, thereby forming a strong immune system (Mahadi et al., 2018). Immunomodulators typically trigger changes in the immune response involving induction, expression and enhancement or inhibition of immune response steps (Mohan et al., 2019). The health industry classifies immunomodulatory compounds into three groups, namely: immunorestorators, immunostimulators and immunosuppressants. Immunomodulatory compounds play a role in the immune system to protect the body against pathogens. One of the complex functional systems of the human body is the immune system, which acts as a protective shield to protect the body from disease (Bratawidjaja et al., 2010).

The immunomodulatory effect of kabau leaf extracts and fractions on the phagocytic activity of peritoneal macrophages of male Wistar white rats (Rattus norvegicus) was tested in vivo. The ability of phagocytosis is an inherent function of macrophage cells and is important for host protection as well as the initiation of innate and acquired immune responses. Therefore, peritoneal macrophages are often used in immunological studies to study phagocytic activity (Pavlou et al., 2017). The immunomodulatory effect of kabau leaf extract and fractions on the percentage of phagocytosis of peritoneal macrophages in vivo by calculating the parameters of peritoneal macrophage cells was carried out to show a picture of the activation function of increasing the phagocytic activity of macrophage cells in destroying foreign antigens that enter the body after being induced by extracts and leaf fractions.

Extract ethanol of kabau leaf fractions can function as an immunomodulator using the carbon clearance method. The method used to determine the type of leukocyte cells is a blood smear to determine the percentage used in this study to test immunomodulatory activity, followed by spleen histopathology to see the area of the germina center in the spleen. Carbon is used as an intravenous antigen, carbon clearance is a test for phagocytosis. The amount of carbon in the blood will decrease over time due to leukocyte phagocytosis.

Indonesia's natural wealth has various types of plant species, most of which have been used to treat various types of diseases. Making medicines from natural ingredients is increasingly developing because it takes into account the mindset of people who prefer to live back to nature (Yunitasari et al., 2016). Bioactive compounds from compounds derived from plants have carried out a lot of research regarding their immunomodulatory potential which can be widely used in immunological disorders in recent years (Amirghofran, 2012). However, not much research has been found for clinical evidence. Therefore, exploring the content of bioactive compounds that have the potential to act as immunomodulators is research that is of high value and is of great interest today and in the future (Kumar., 2011).

Histopathology is the microscopic study of cells and tissues through staining and viewing them under a microscope. Histological tissue processing functions in diagnosing diseases that involve changes in physiological function and organ deformation. According to Mescher (2016), good results can provide an overview of the shape, arrangement of cells, cell nucleus, cytoplasm, arrangement of connective tissue fibers, muscles and so on in accordance with the description of the tissue in its condition when it was still alive. Several studies have investigated the pharmacological effects, namely as an antioxidant (Riasari, 2019), kabau seeds have high antioxidant and phenol activity. The research results of Irawan et al., (2018) showed that kabau seed skin showed diabetes activity with an IC of 7.77 µg/mL. In research by Styani (2018), a study of the anti-gout potential of ethyl acetate extract from kabau fruit shells resulted in reducing uric acid with the compound allopurinol. The lack of information regarding kabau leaves as a potential anti-inflammatory drug that can be developed means it is

necessary to carry out tests using several methods that can be used to confirm the possible immunomodulatory activity of kabau leaf compounds.

METHODS

A. Plant Determination

The samples in this study were kabau leaves (Archidendron bubalinum) determined at the Testing Laboratory – UF Tawangmangu Traditional Health Services.

B. Extraction

Extraction is carried out using the maceration method. The kabau leaf powder obtained was then extracted using the maceration method using 96% ethanol in a ratio of 1:10 and macerated at room temperature. Soaked for the first 6 hours while stirring frequently, then left for 18 hours and then the macerate was filtered. The liquid extract is then separated from the solvent using a rotary evaporator, after which a fractionation process is carried out to obtain the N-hexane fraction, ethyl acetate fraction and water fraction (Indonesian Herbal Pharmacopoeia, 2017).

C. Fractionation of N-Hexane, Ethyl Acetate Fraction and Water Fraction

The dry ethanol extract of kabau leaves was added with distilled water in a ratio of 1:10 (10g:100 mL) to dissolve the extract, then placed in a separating funnel and added 100 mL of n-hexane and shaken until the extract partitioned into both filter layers for 15 minutes. After that, leave the separating funnel until the distilled water layer and n-hexane layer separate. The two layers were separated and the distilled water layer was filtered again using n-hexane twice 100 mL. The n-hexane fraction was collected and then evaporated to obtain a dry fraction. This layer is the dry fraction of n-hexane. The distilled water layer was put back into the separating funnel then 75 mL of ethyl acetate was added and shaken until the extract partitioned into both filter layers for 15 minutes. After that, leave the separating funnel until the distilled water layer and ethyl acetate layer separate. The top layer was taken as the ethyl acetate fraction and the bottom layer was taken as the water fraction. The ethyl acetate fraction was collected and then evaporated to obtain the dry ethyl acetate fraction. The distilled water layer was also evaporated to obtain a dry water fraction.

D. Test for the Content of Secondary Metabolite Compounds

1. Tannin Compounds

The tannin test was carried out by weighing 2 mL of the extract and adding sufficient ethanol, then dissolving it. 1 mL of sample solution was transferred into a test tube and 2-3 drops of 1% FeCl3 solution were added. If the sample is positive for containing tannin, it is indicated by the formation of a bluish black or green color (Achsia, et al., 2021).

2. Saponin Compounds

A sample of approximately 1 mL was put into a test tube and 5 mL of distilled water was added and shaken for 10 minutes. Then add 1-2 drops of 1 N HCl. If the foam formed can last for 10 minutes with a height of 1-3 cm, it shows that the extract is positive for containing saponin (Hidayati et al., 2021).

3. Steroid Compounds

1 mL of ethanol extract sample was mixed with 25 mL of diethyl ether and shaken vigorously. The diethyl ether layer was separated then 2-3 drops of Lieberman-Burchard reagent were added. The bluish color that is formed indicates a positive result for the presence of terpenoids while the greenish color indicates a positive result for the presence of steroids (Irawan et al., 2018).

4. Flavonoid Compounds

A total of 2 mL of sample was added with 10 drops of HCl, and the tip of a spatula of Mg powder, then the mixture was homogenized and the color change was observed, if the extract was orange then the sample contained flavonoids (Rahmawati et al., 2020).

E. Immunomodulatory Activity Assay

The immunomodulatory activity test was carried out using the carbon cleanup method. Test treatment was given orally to male mice. This treatment consisted of 6 mice in group I negative control CMC Na, group II positive control with a dose of diclofenac sodium 50 mg/kg BW, group III kabau leaf extract 20 mg/kg BW, group IV n-hexane fraction 6.66 mg/kg BW , group V ethyl acetate fraction 4 mg/kg BW, group VI water fraction 6.66 mg/kg BW. Kabau leaf extract and fractions were given once a day for 7 days. Data analysis using the one way ANOVA method.

F. Determination of Carbon Content

5 grams of Chinese ink was put into an evaporator cup and evaporated in the oven at 105 ºC for 30 minutes. Drying was then continued in a desiccator until constant weight.

G. Making a Carbon Standard Curve

The dried Chinese ink was then weighed as much as 100 mg, dispersed in 100 mL of acetic acid so that the concentration was 1 mg/mL. 2, 3, 4, 5, and 6 mL of each solution were pipetted, then added with 1% acetic acid to a volume of 50 mL, to obtain carbon levels of 40, 60, 80, 100, and 120 µg/mL. 4 mL of each level was pipetted, then 25 µL of rat blood taken from the tip of the tail vein was added. After homogenization, measure the adsorbent with a UV-Vis spectrophotometer at a wavelength of 650 nm. The adsorbent plot obtained with carbon content was used to create a calibration curve. As a blank, male white rat blood and distilled water were used only.

H. Carbon Suspension Preparation

A total of 1.6 ml of Chinese ink was then put into a mortar, added to the suspension with 1% CMC-Na which was dissolved in sufficient 0.9% physiological NaCl solution and homogenized, then put into a 10 ml measuring flask, filled with a suspension of 0.9% physiological NaCl 9%, up to the cutoff mark.

I. Carbon Distance Measurement

The group that had been given oral treatment once a day for 6 consecutive days. After administering the sample suspension to each group, the tip of the rat's tail was cut and the blood was collected on a drip plate with NaEDTA added until homogeneous. 25µl of blood was taken and lysed with 4 mL of 1% acetic acid. This first blood sample was used as a blank (0 minute). Then 0.1 mL/10 gBB of carbon suspension was injected intravenously into the tail, 25µL of rat blood was taken during 0, 10 and 15 minutes after injection. Each blood was lysed with 4 mL of 1% acetic acid and its absorbance was measured at a wavelength of 650 nm using a UV-Vis spectrophotometer. The absorbance value is used to measure the level of carbon removal (k) and the phagocytosis index (α) according to the following formula: (Okta et al., 2022).

$$k = \frac{Ln\ OD1\ -\ Ln\ OD2}{t2 - t1}$$

Description:

K : Phagocytosis constant

OD1: Absorbance of blood samples at minute 0

OD2: Absorbance of blood samples at 10 and 15 minutes

t1 and t2: Time of initial and last blood draw.

$$(IF) : \frac{mouse\ constant\ Z}{phagocytosis\ constant\ of\ negative\ control\ mice}$$

Description:

IF: Phagocytosis index of each test group compared with the control group.

Mice Z: Mice that have been treated and the phagocytosis constant value determined.

J. Calculation of the Number of Types of Leukocytes

A total of 1 mL of blood was dropped on a glass object then spread evenly with another glass object to obtain a homogeneous layer of blood, then dried by airing and adding methanol,

leave for 5 minutes, then add 10 drops of Giemsa solution (diluted with distilled water), leave for 20 minutes, rinse with flowing distilled water and air dry. Count the number of lymphocyte and monocyte cells under a microscope with 100x magnification using immersion oil (Aldi et al., 2014). Each lymphocyte and monocyte cell is counted to find 100 leukocyte cells (WHO, 2003). Then the percentage of lymphocyte, monocyte and neutrophil cells was calculated.

K. Histopathology of the spleen

The experimental animals were sacrificed under anesthesia with chloroform, then the entire spleen was taken. Samples of spleen tissue that had been fixed in 10% formalin and paraffin were used for microscopic testing based on laboratory procedures.

RESULTS AND DISCUSSION

A. Plant Determination

Determination of kabau plants is carried out with the aim of determining and ensuring that the plant specifications are indeed kabau plants (Archidendron bubalinum). The determination results obtained from the Testing Laboratory – UF Tawangmangu Traditional Health Services showed that the plant was a kabau plant (Archidendron bubalinum). Samples of kabau plants (Archidendron bubalinum) were obtained from the city of Muara Dua, South Oku, South Sumatra. Then the maceration and fractionation process is carried out to produce extracts and thick fractions of kabau leaves. 96% ethanol is used as a solvent from a safety perspective and can attract all polar and non-polar substances optimally.

Based on the results of phytochemical screening to determine secondary metabolite compounds present in kabau leaf extracts and fractions, including tannins, saponins, steroids and flavonoids. The data results are seen in table 1.

Table 1. Results of Phytochemical Screening of Ethanol Extract of Kabau Leaves (Archidendron Bubalinum)

Compound	Result	Information
Tannin	+	The solution is blackish green
Saponin	+	The solution there is foam
Steroids	+	The solution is blackish green
Flavonoids	+	The solution is orange colored

Description: there is (+) and there is not (-)

Table 2. Results of Phytochemical Screening of Kabau (Archidendron Bubalinum) Leaf Fractions

Fraction	Compound class			
	Tannin	Saponin	Steroids	Flavonoids
N-hexane	+	+	+	+
Ethyl acetate	+	+	+	+
Water	+	-	-	+

Description: there is (+) and there is not (-)

Standard curve between carbon content in blood and absorbance value using a UV-Vis spectrophotometer to see the phagocytosis effect of the extract concentrate and kabau leaf fractions. This standard curve was created to show the linear relationship between absorbent and carbon levels in rat blood. The aim of making a standard curve was to see the linear relationship between carbon levels in rat blood and sorbent as measured

using a UV-Vis spectrophotometer. Carbon cleaning method (carbon clearance) is a spectrophotometric measurement of the rate of elimination of carbon particles from rat blood. This method can represent phagocytic activity (Linsentia, 2011). The carbon standard curve is found with two regression equations: y = 0.0039x + 0.4045, with R = 0.999. The relapse condition value can show a direct correlation between carbon convergence in rat blood and absorbance.

Based on the average value of carbon absorbance in rat blood which was seen using a UV spectrophotometer at a wavelength of 636 nm, a decrease in the absorbance value obtained in all groups given the preparation was seen when compared with the negative control group. The lower the carbon value, the lower the carbon concentration remaining in the rat's blood. Carbon contained in the body will stimulate the formation of a non-specific immune system in the form of phagocyte cells. Phagocyte cells will be activated due to non-specific immune system stimulation and will quickly recognize the type of foreign antigen that enters the body and then destroy and clean the antigen (carbon) in the blood circulation (Zilhadia et al., 2012)

Table 4. Results of Phagocytosis Constants and Indexes in the Treatment Group

Treatment	Average (KF)	Average±standard deviation (IF)
Normal Control	0,011	1,18 ± 0,59b
Negative Control	0,010	1 ± 0ac
Positive Control	0,032	3,33 ± 0,87b
Extract 20 mg	0,012	1,26 ± 0,70b
N-Hexane Fraction	0,027	2,49 ± 0,98ab
Ethyl Acetate Fraction	0,016	1,63 ± 0,45b
Water Fractio	0,012	1,27 ± 0,77b

Description : Numbers Followed by Different Letters in the Same Column Indicate Significantly Different Test Results (P<0.05)

a = Significantly Different From Normal Controls

B = Significantly Different From Negative Control

C = Significantly Different From The Positive Control

The phagocytosis constant value is calculated to determine one of the limits of phagocytosis. The higher the phagocytosis value, the more carbon is lost, which means the phagocytic cell completes the phagocytosis cycle more quickly. Normal control is 0.011; extract of 0.012; N-hexane fraction of 0.027; The ethyl acetate fraction was 0.016 and the water fraction was 0.012, for the positive control with an average constant of 0.032, for the negative control 0.010. This shows that each dose variation group experienced an increase in phagocytic activity. Table 4 displays a comparison of the results of the average, standard deviation, and statistical analysis of phagocytosis constants for each group.

Based on Table 4, all test groups had higher phagocytosis constant values than the negative control group. The test group with the n-hexane fraction had a higher phagocytosis constant value than the other test groups. The higher the phagocytosis constant value, the higher the carbon removal speed. From the data obtained, there was an increase in the phagocytosis constant value from varying doses of kabau leaf extract and fractions, indicating immunostimulatory activity. Research on testing the immunomodulatory effects of skin extracts kabau that the higher the average

phagocytosis constant, the faster the elimination of carbon ink, the highest value of the phagocytosis constant for kabau peel extract is 1.1682 from research by Okta (2022).

Based on the SPSS test results, the phagocytosis constant data showed that the results were normally distributed and homogeneous ($p > 0.05$), the test was continued using One Way Anova which showed that the results contained significant differences, so to confirm the differences, a Post Hoc Tukey test was carried out which showed that there were 3 subset groups. namely the first column is negative control, normal control, water fraction, extract and ethyl acetate fraction, in the second column, normal control, water fraction, extract, ethyl acetate fraction and n-hexane fraction and the third column is positive control and n-hexane fraction.

These results indicate that the n-hexane fraction shows that kabau leaves have the effect of increasing phagocytosis, the higher the dose of extract used causes an increase in the phagocytosis constant. The negative control is the group with the lowest value because it was not treated at all, then the water fraction also has the lowest value of all the test treatments due to the first possibility, which could be due to damage to the raw material, the absence of chemical compounds present during the second fractionation process because it is not homogeneous. and the blood and solvent are not completely mixed. After determining the phagocytosis constant value, the phagocytosis index value can be calculated. The phagocytosis index was calculated by comparing the phagocytosis constant value of the negative control group. The phagocytosis constant is directly correlated with the phagocytosis index, which means that the more values the phagocytosis constant and phagocytosis index have, the faster the phagocytosis cycle is completed by the phagocyte cell to remove carbon from the circulatory system, so it can be concluded that these results can play a role as immunostimulant.

Macrophage phagocytosis is widely used as an immunological parameter to evaluate health or from mouse blood. The highest results were obtained from the positive control test variant with a value of 33.3 and the n-hexane fraction with a dose of 6.66 mg/kg mice, namely with a value of 2.49. Phagocytosis index with strong immune activity. The normal group, extract group, and water fraction had a phagocytosis index with moderate immune activity, then the positive 0.9mg/kgBB group showed a phagocytosis index with strong immune activity followed by the n-hexane fraction group and the ethyl acetate fraction in the statistical test, still in the same group. subset.

Based on the SPSS test results, the phagocytosis index data showed that the results were normally distributed and homogeneous ($p > 0.05$), the test was continued using One Way Anova which showed that there were significant differences in the results, so to confirm the differences a Post Hoc Tukey test was carried out which showed that there were 3 groups different subsets, namely normal control, extract, water fraction and ethyl acetate fraction, the second group of extract, water fraction, ethyl acetate fraction and n-hexane control and the third subset group, namely positive control and n-hexane fraction. These results indicate that kabau leaves have the effect of increasing phagocytosis. The positive control has the highest value due to the first possible factor, namely that it has been tested pre-clinically and clinically, the quality of the raw materials is guaranteed, for other test groups with less than optimal results it could be due to the possibility of treatment errors, namely not being homogeneous and not completely mixed between blood and the solvent.

This shows that the n-hexane fraction of kabau leaves is effective as an immunostimulant based on the results of calculating the phagocytosis constant, phagocytosis index and statistical data processing using the SPSS method. The results of these calculations state that the effective dose variant is the n-hexane fraction, this is because this dose has a phagocytosis constant value with comparable strength as the positive control, apart from that the phagocytosis index (IF) value is > 1.5 (indicating strong immune activity) and the SPSS value obtained $P > 0.05$ (indicating that the calculation results are significant).

Table 5. Results of Mean and SD Percentage of Leukocyte Cell Types

Treatment	Mean ± SD Percentage of Leukocyte Types (%)			
	Stem neutrophils	Segment neutrophils	lymphocytes	Monocytes
Normal Control	11,8 ± 1,92[b]	76 ± 5,78[b]	62,8 ± 3,42[b]	13,8 ± 1,30[b]
Negative Control	4,2 ± 1,64[ac]	62,6 ± 2,30[ac]	56,6 ± 6,87[c]	22,4 ± 2,88[ac]
Positive Control	13 ± 1[b]	77,8 ± 3,03[b]	78,4 ± 6,98[b]	13,6 ± 1,34[b]
Extract 20 mg/kg BW	13 ± 2,91[b]	76 ± 3,16[b]	62,8 ± 3,42[c]	12,6 ± I,51[b]
N-hexane fraction	12,6 ± 2,50[b]	73,6 ± 1,94[b]	78 ± 5,61[b]	14,2 ± 1,92[b]
Ethyl Acetate Fraction	12,6 ± 2,50[b]	73,6 ± 1,94[b]	68,2 ± 6,79[b]	14,2 ± 1,92[b]
Water Fraction	13 ± 1[b]	77,8 ± 3,003[b]	56,6 ± 6,87[c]	13,6 ± 1,34[b]

Description: Numbers Followed by Different Letters in the Same Column Indicate Significantly Different Test Results (P<0.05)

A = Significantly Different from Normal Controls

B = Significantly Different from Negative Control

C = Significantly Different from the Positive Control

The percentage results of the number of leukocyte cell types using the blood smear method after staining with Giemsa, blood smear analysis is carried out to count various types of leukocyte cells, including eosinophil cells, stem neutrophil cells, segment neutrophil cells, lymphocytes and monocytes. The number of eosinophil cells was not counted in this study because its concentration is very low (approximately 2-4 percent of circulating leukocytes) and increases as a result of allergic reactions (Ashton, 2010; Armstrong and Glenn, 2019). In contrast, basophils cannot be seen because these cells dissolve in base and Giemsa dye (Aldi et al., 2014). The immune response to parasites and worms also results in an increase in eosinophil and basophil cells (Eberle & Voehringer, 2016).

All types of leukocyte cells, except monocytes, were significantly affected by administration of kabau leaf extract and fractions to male white rats for seven days (P<0.05), according to the results of one-way ANOVA. This means that the number of various types of leukocytes in the negative control group was significantly lower than in the test group.

The increase in the number of lymphocytes, monocytes and neutrophils in the peripheral blood of mice after carbon injection indicates an increase in phagocytic activity. This is comparable to the increase in the constant value and phagocytosis index in the carbon cleanup method. According to Baratawidjaja and Rengganis (2018), the specific and non-specific immune system such as neutrophils, basophils, eosinophils, macrophages and lymphoid cells (such as B, T and NK lymphocytes) function to protect the body against incoming antigens by increasing phagocytic activity quickly and efficient. According to Pringguotomo et al., (2002), when acute inflammation occurs, the cells that react the most are neutrophil cells. These cells will move to

the area of infection or injury within the first 24 hours as an initial phase. At the same time, monocyte cells will migrate but in smaller numbers and at a slower speed (Bonardo et al., 2015). Likewise with lymphocytes, these cells will enter the area of inflammation within 24-48 hours (Ackermann, 2017).

The number of lymphocytes in the negative control group was not significantly different from the number of lymphocytes in the water fraction, extract, normal control and ethyl acetate fraction but was significantly different from the number of lymphocytes in the n-hexane fraction and positive control groups (appendix 33). Subsequent statistical tests on the number of monocytes showed that the number of monocytes of the control extract, water fraction, n-hexane fraction, ethyl acetate fraction, normal control and positive control were included in the same subset, which means there was no real difference between the data. However, it was significantly different in the negative control group (appendix 34).

Lymphocytes are a type of leukocyte cell that has a very important role in the immune system. These cells will respond to foreign objects (antigens) that enter the body through the humoral and cellular immunity systems (Koolman & Rohm, 2001). T helper (Th) cells with CD4+ marker molecules will activate T lymphocyte cells and induce a cellular immune response. Meanwhile, cytotoxic T cells (Tc) with CD8+ markers play an important role in activating and directing other immune cells to eliminate incoming foreign objects. Helper T cells also have an important role in the proliferation of cytotoxic T cells and increasing the phagocytic activity of macrophages. The number of CD4+ lymphocyte cells will increase due to the presence of bioactive compounds that are immunomodulatory (Baeke et al., 2010; Takahasyi et al., 2014; Yan., 2014).

The flavonoid mechanism can activate NK cells to stimulate the production of interferon γ. IFN-γ produced by various cells of the immune system is the main MAC cytokine (Macrophage Activating Cytokine) and plays a major role in non-specific cellular immunity. IFN-γ is a cytokine that can activate macrophages, so that macrophages experience increased phagocytic activity quickly and efficiently in removing antigens (Baratawidjaja, 2010). Flavonoids also work by increasing the activity of IL-12 and lymphocyte cell proliferation. CD4+ cells will activate Th-1 cells by influencing the proliferation of lymphocyte cells. Once Th-1 cells are activated, these cells will influence IFN-γ so that they can activate macrophages and increase their phagocytic activity in killing antigens quickly and more efficiently. The results of the research that has been carried out show that extracts and fractions of kabau leaves can increase phagocytic activity and the average percentage of rat leukocyte cell types.

The spleen is the largest lymphoid organ which has an important role in the immune system. The spleen also plays a role in erythrocyte filtration, cleaning old and damaged erythrocyte cells, and is a reservoir of blood platelets. Based on this, the spleen is an important organ that plays a role in antigen defense in the blood and individual hematopoiesis (Brendolan et al., 2007; Hanadhita et al., 2018). Examination of the rat spleen is carried out microscopically or by tissue histology. When examining the spleen, it will be known that changes have occurred in the histology of the spleen, especially in the area of the white pulp, the area of the germina center and the diameter of the white pulp. Changes that occur in the spleen organ preparation will be observed histopathologically, an increase in the immune system can be determined by looking at the increase in the number of lymphoid cells in the spleen.

White pulp is found in the spleen which is a collection of lymph nodes. The white pulp functions as a site for antibody production and maturation of T and B lymphocytes and macrophages. Lymphocyte cells that play a role in specific immunity gather and proliferate in the germinal center. Several parameters to determine whether there is an increase in immune system activity are characterized by changes in the diameter of the white pulp and germinal center (Rousdy et al., 2017). There are several compounds

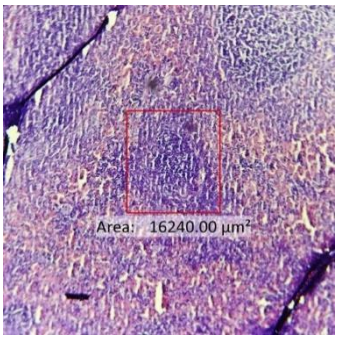
that can increase the activity of the immunomodulatory immune system and play a role in increasing the body's resistance, one of which is chemical compounds from kabau leaves.

The spleen's immune response is to increase the proliferation activity of lymphocyte cells in the white pulp, resulting in an increase in the diameter of the white pulp. The spleen is involved in both humoral and cellular immune responses through its role in spleen multiplication, maturation, and storage. Proliferation in this study almost occurred in all treatment groups. In the negative control group there was less proliferation compared to the other treatment groups. Lymphocyte proliferation is a marker of the activation phase of the body's immune response. This lymphocyte proliferation takes the form of increased production of lymphoblasts which then become lymphocytes. The activity of the spleen in producing lymphocyte cells during an immune response can result in enlargement of the spleen. This enlargement of the spleen can be caused by an increase in the body's immune response. An increase in the immune response can occur due to infection or after immunization or circulatory disorders or tumors.

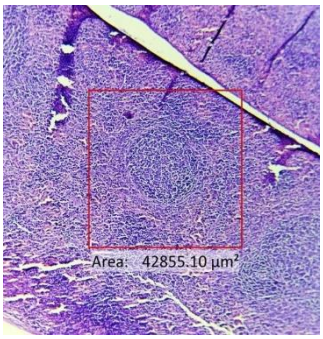
In mice treated with extracts and fractions of kabau leaves, hyperplasia of lymphoid follicles occurred and they were in a reactive condition. The increase in follicular area indicates the presence of B cells that are ready to carry out activities in the mouse's immune system. The main active substance that can improve the mouse's immune system is the flavonoids contained in kabau leaves. Apart from that, flavonoids also act as antioxidants which can prevent oxidative stress in mice so that the mice's health condition improves (Mansour et al., 2002).

In the spleen, lymphocytes recognize non-selfantigen fragments presented by macrophages, dendritic cells and phagocytes. Presentation of non-self antigen fragments is followed by secretion of IL-12 and IL18 which will stimulate T cells to produce interferon-γ (INF-γ) which activates NK (natural killer) and CD8+ cells. 13 One component of the spleen is white pulp (alba). The alba pulp structurally resembles a lymph node. In the white pulp there is a zone of T lymphocytes and B cells (follicles) and allows the emergence of a specific immune response against antigens that protect the body from bacterial, viral and fungal infections carried in the blood (Anggarasari et al., 2014).

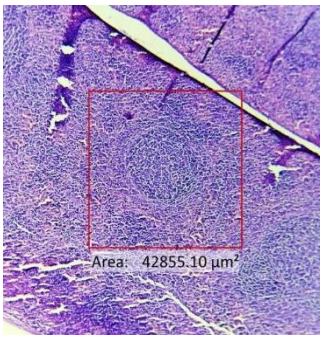
Examination of the spleen is carried out microscopically or by tissue histology. The image below shows several results from testing the negative group and the positive control group for diclofenac sodium. It is also known that the histology results show differences in terms of the area of the white pulp, the diameter of the white pulp, and the area of the germinal center of the spleen.



A



B



C

Figure 1. Histology of Spleen White Pulp Area in Negative Group (A) and Positive Group (B) N-Hexane Fraction (C)

Table 6. Results of Average White Pulp Area, White Pulp Diameter and Germinal Center Area of Rat Spleen in Each Treatment Group

Treatment	Spleen White Pulp Area (µm2)	Spleen White Pulp Diameter (µm2)	Germina Center area (µm2)
Normal Control	38361,33 ± 3128,294b	171,46 ± 2,4544b	138,77 ± 1551,816b
Negative Control	26892 ± 4075,144b	147,126 ± 10,808ac	10173 ± 2440,64b
Positive Control	41217,67 ± 4589,075b	174,983 ± 1,3606b	14122,33 ± 1278,017b
Extract 20 mg/kg	40856,33 ± 3986,722b	174,213 ± 2,083b	14084,33 ± 1716,349b

N-hexane fraction	50270,67 ± 3235,94ab	184,063 ± 7,5702b	21845,33± 956,672abc
Ethyl Acetat fraction	47970± 3562,86b	181,013± 0,88861b	16598,67± 1098,248b
Water Fraction	43507,33 ± 5166,99b	177,1633 ± 1,641b	15881,33 ± 3031,293b

Description: Numbers Followed by Different Letters in the Same Column Indicate Significantly Different Test Results (P<0.05)

A = Significantly Different from Normal Controls

B = Significantly Different from Negative Control

C = Significantly Different from the Positive Control

The results of statistical tests on the area of white pulp showed that there was a significant difference between treatments because (P<0.05). Where all treatments were significantly different from the negative control and normal control. In the statistical test, the diameter of the spleen white pulp also showed a significant difference between treatments because (P<0.05). The results of the treatment were significantly different from the negative control. The statistical test for the area of the germinal center of the spleen showed a significant difference between treatments (P <0.05). In the n-hexane fraction treatment, it showed a significant difference, namely having a wider germinal center area compared to the positive control. Analysis of this data shows that secondary metabolite compounds from extracts and fractions of kabau leaves have an influence on increasing the area of the germinal center.

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that the drying loss obtained by kabau leaves is 5.33%, they contain secondary metabolite compounds of tannin, saponin, steroids and flavonoids. The characterization results showed that the water content was 7.5%, the ash content was 0.75% and the total ash content was 0.65%. Kabau leaf extracts and fractions (Archidendron bubalinum) have immunomodulatory activity as an immunostimulant which is characterized by increasing the phagocytosis index in male white rats (Rattus norvegicus). There was no damage to the cells but there was an expansion of the spleen area which was marked on the histopathological picture seen from the area of the germina center, the area of the white pulp and the diameter of the white pulp in the spleen of the male white rat (Rattus norvegicus.

ACKNOWLEDGEMENT

Infinite thanks to Dr. apt.Tri Wijayanti, S.Farm., M.P.H as supervisor one and Mrs. Dr. apt. Opstaria Saptarini, M.Si. as the second supervising lecturer who has provided guidance, insight, direction and time for the author so that the author can complete the writing of this journal.

Conflict of Interest

In writing this journal the author states that there is no conflict of interest in writing this journal, a conflict between carrying out tasks and personal interests which influences the assessment and results

REFERENCES

Baeke, F., Korf, H., Overbergh, L., van Etten, E., Verstuyf, A., Gysemans, C., & Mathieu, C. (2010). Human T lymphocytes are direct targets of 1,25-dihydroxyvitamin D3 in the immune system. *Journal of Steroid Biochemistry and Molecular Biology*, *121*(1–2), 221–227. https://doi.org/10.1016/j.jsbmb.2010.03.037

Bratawidjaja, K. G. and Rengganis, I. 2018. Basic Immunology. Jakarta: Faculty of Medicine, University of Indonesia

Bratawidjaja, Kamen Garna. 2010. Basic Immunology 11th edition (2nd printing). Jakarta: Faculty of Medicine, University of Indonesia.

Handayani, G. N. 2010. "Immunomodulators" AL-FIKR 14(1): 150-66.

Indonesian Herbal Pharmacopoeia. 2017. Edition II. Ministry of Health of the Republic of Indonesia

Irawan, C., Rochaeni, H., Sulistiawaty, L., & Roziafanto, AN (2018). Phytochemical screening, LC-MS study and antidiabetic potential of methanol extract of Archidendron bubalinum (Jack) IC Nielson (Julang Jaling) seed shells from Lampung, Indonesia. Journal of Pharmacognosy, 10(6s).Kumar S. et al. 2011. A review on immunostimulatory plants. Journal og Interactive Medicine; 9(2): 117

Linsentia, N., A. 2011. Immunomodulatory activity of basil leaf extract on male mice using carbon clearance and neutrophilma adhesion methods. Sanata Dharma University. Yogyakarta.

Mohan, K., Ravichandran, S., Muralisankar, T., Uthayakumar, V., Chandirasekar, R., Rajeevgandhi, C., Karthick Rajan, D., & Seedevi, P. 2018. Extraction and characterization of chitin from sea snail Conus inscriptus (Reeve, 1843). International Journal of Biological Macromolecules 126: 555–560

Republic of Indonesia Ministry of Health. 2000. General Standard Parameters for Medicinal Plant Extracts. Republic of Indonesia Ministry of Health, vol. 1. p. 10–11.

Riasari, H., Fitriansyah, SN, Hartati, R., & Anggadiredja, K. (2019). Comparison of Extraction Methods, Antioxidant Activity, Total Phenols in Kabau Seeds and Shells (Archidendron bubalinum (Jack) IC Nielsen) from Lampung and South Sumatra. Journal of Pharmacognosy, 11(6).

Yunitasari, D., Alifiar, I., & Priatna, M. (2016). Test of the Activity of Ethanol Extract of Jengkol Leaves (Pithecellobium lobatum Benth) on Healing Incision Wounds in Male Wistar White Rats. Journal of Scientific and Practical Pharmacy, 2(1), 30–35.

Zilhadia, Wiraswati Y. Testing the Immunomodulatory Effect of Gambier Catechin (Uncaria gambier Roxb.) Using Invitro Carbon Cleaning Parameters. J Indonesian Natural Materials 8(3), pp 181–186. 2012.