Immunomodulator Activity of 5,7-dihydroxy isoflavones and β-Sitosterol from Peronema *canescens* Jack Leaves Methanol and Ethyl Acetate Extract

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Abstract. The end of 2019 marked the beginning of a global pandemic with the emergence of the Corona Virus or Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which attacks the respiratory system and causes this viral infectious disease called COVID-19. A good immune system is needed to support health and endurance in pandemic condition. One of the plants that have the potential to be developed as an immunomodulator is the Sungkai plant (*Peronema canescens* Jack). The aim of this study was to extract the bioactive compounds of *P. canescens* Jack leaves and its immunomodulatory activity against white male mice. *P. canescens* Jack was extracted using Ethyl acetate and methanol, followed by the isolation process using Liquid Vacuum Chromatography (LVC) to obtain single compound (EI) from the ethyl acetate fraction and single compound (MI) from the methanol fraction. Immunomodulator activity was tested using mice, using doses of 50 mg/Kgbb, 100 mg/Kgbb, 150 mg/Kgbb, and 450 mg/Kgbb. The methanol extract of 450 mg/KgBb *P. canescens* Jack leaves had the largest number of active leukocytes and macrophages with a value of 64.9%. Meanwhile, at a dose of 450 mg/KgBW EI, there was an increase in leukocytes and the highest % activity of active macrophages which was almost close to the positive control (C+). Based on the comparison of the literature, the UV-Vis and FTIR spectrum patterns of isolate F3 are β-Sitosterol compounds, while F2 is a 5.7 dihydroxy isoflavone compound. Our finding that two compounds of *P. cannescens* Jack leaves extract β-Sitosterol and 5,7-dihydroxy isoflavone which have immunomodulatory activity.

Keywords: Bioactive compound · Immunomodulator · P. canescens

1 Introduction

Globally, since December 2019 till now, the world has been grappling with the terror of a new type of coronavirus known as the 2019 coronavirus disease (COVID-19) by WHO [1]. The specter of the nascent coronavirus outbreak, which has resulted in devastating

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health and economic consequences around the world, has highlighted the importance of exploring natural products of plant origin for drug development [2]. Researchers have intensified efforts to elucidate the mechanism of action, safety, and efficacy of medicinal plants to reduce the deleterious effects of deadly and highly infectious viruses [3, 4]. Medicinal plants can function as prophylaxis in the management of viral diseases [5]. Several studies are investigating the potential of natural plant products as the first line of defense as well as immune system enhancers in the battle against COVID-19 [6, 7]. Medicinal plants with positive immunomodulatory activity have the potential to attenuate the effects of COVID-19 and could offer substantial therapeutic benefits if explored properly [8]. Indonesian plant, Sungkai (Peronema *canescens* Jack) is a plant from the Lamiaceae family that is commonly found in tropical rain forest widely used by the community in part for toothache and fever reduction. In addition, sungkai leaves are also used to treat malaria. Not only the leaves, the bark of the sungkai leaves also has many benefits including as a medicine for bloody diarrhea, burns and internal wounds [9]. In addition, it is also used as a medicine for wounds caused by venomous snakes. The secondary metabolites contained in P. *canescens* leaves are flavonoids, phenolics, tannins, steroids, saponins and alkaloids [10].

Chemical compounds contained in the P. *canescens* Jack include flavonoids, which have many benefits including being an antioxidant. Previous study reported that P. *canescens* memiliki aktivitas antidiabetes dan antihiperurisemia [12, 13]. Plants that have the flavonoid group can increase the activity of the immune system, related to the corona virus outbreak which has an impact on decreasing the body's resistance, so it must be able to increase immunity by consuming drinks and foods that can increase stamina. The people of Jambi province have started consuming boiled water of sungkai leaves because it is believed to be able to maintain body stamina, increase body immunity and treat malaria. As for natural ingredients as an alternative choice to increase immunity by utilizing sungkai leaves. Plants that have activity as immunomodulators include sungkai plants [13].

Immunomodulators are substances that can modulate (change or affect) the body's immune system to a normal direction. Immunomodulatory products play a role in strengthening the body's immune system (immuno stimulator) or suppressing excessive immune system reactions (immuno suppressant). Apparently, there are certain plants that have the effect of increasing the body's immortalit [14]. The immune system is needed to defend against the dangers that can be caused by various substances in the environment. The immune system or the immune system can be assessed through the large number of white blood cells and leukocytes in the blood. White blood cells are an important aspect of the immune system. This research aims to isolate the secondary metabolites from the methanol and ethyl acetate extract of P. *canescens* Jack and characterized its immunomodulatory activity.

2 Material and Methods

2.1 Materials

The materials used in this study were Peronema *canescens* Jack leaves, H_2SO_4 2N, Dragendroff's reagent, Mayer's reagent, n-hexane, ethyl acetate, methanol, methanol chloroform, Dichloromethane (DCM), dimethyl sulfoxide (DMSO), Mg powder, HCL 2N, Concentrated HCL, acetic anhydride, $FeCl_3$, silica gel. Imboost, aquadest, ethanol, and Turk's solution as reagents as materials for testing the immunomodulatory activity used were mice.

2.2 Research Equipment

The equipment used in this research is the equipment for the extraction process of maceration bottles, round bottom flask, vacuum rotary evaporator. Then the equipment for the process of separating organic compounds, namely Vacuum Liquid Chromatography (KVC), Thin Layer Chromatography (TLC), chamber chromatography, tweezers, vials, hot plates, mortar and pestle. Phytochemical test equipment for dropper pipettes and drip plates. Glass equipment such as measuring cups, measuring flasks, beakers, erlenmeyer, stirring rods, porcelain dishes, test tubes, watch glasses, as well as other equipment such as analytical balances. Cutting knife, 1 ml syringe, haemasitometer, counting room, microscope, microscope camera, newsprint, digital camera as a tool for testing immunomodulatory activity.

2.3 Sample Preparation

Scalar *variables* and *physical constants* should be The sample used in this study was the leaves of Peronema *canescens* J. which were taken in Koto Baru Hiang Village, Silihat Laut District, Kerinci Regency, Jambi Province. The collected samples of Peronema *canescens* J. were cleaned. Furthermore, the sample was cut into small pieces and then dried at room temperature in an open room that was not exposed to direct sunlight. After that the sample is crushed into smaller sizes to facilitate the extraction process by means of a grinder.

2.4 Extraction and Fractiation

The extraction used is multilevel extraction. A total of 1 kg of Peronema *canescens* J. leaves were macerated in n-hexane solution for 2×24 h. The obtained maserate was then filtered using filter paper. The separated sample dregs were dried until the previous solvent had evaporated and reweighed to be extracted again with ethyl acetate for 2×24 h and then filtered using filter paper. The same was done with methanol solvent.

2.4.1 Thin Layer Chromatography (TLC)

A TLC plate measuring 1 × 5 cm is prepared with a lower value of 1 cm and an upper limit of 0.5 cm so that the eluent distance is 3.5 cm. Furthermore, the eluent is made by comparing organic solvents with graded polarity. The extract was spotted on the lower border of the plate with a capillary tube, then eluted with the mobile phase or eluent. After the movement of the developer solution reached the upper limit, the elution process was stopped. Furthermore, the shape of the stain was observed directly and under a UV lamp of 254 nm. After column chromatography was performed, all fractions were tested by TLC to see the stain components. Fractions that have the same spot stains are combined and reanalyzed by TLC.

2.4.2 Column Chromatography

Vacuum column chromatography (KVC) was performed using silica gel as a stationary phase. The sample extract was impregnated using silica gel, then added to the column containing the stationary phase. While the mobile phase used is n-hexane: ethyl acetate and ethyl acetate: ethanol with various ratios (10:0; 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9 and 0;10). The fraction obtained was accommodated in vials, the eluate was collected based on each band obtained and then evaporated. The results of column chromatography were carried out by TLC again. The eluates that have identical stain patterns are combined based on the Rf value on the chromatogram.

2.4.3 Isolated Characterization

Characterization of the isolated carried out using UV-Vis (1). Spectrophotometer. 2 mL of isolate was placed in a cuvette and observed for its spectrum at a wavelength of 200–800 nm to identify the absorbance value of the active compound at the maximum wavelength. (2). FTIR spectrophotometer. 0.2 g of KBr pellet was added with 1 drop of isolate, dried and then identified by FTIR spectrophotometer at a wave number of 4000-400.

2.5 Immunomodulator Activity

2.5.1 Leukocytes Number

Mus musculus used was 8 weeks old with an average weight of 30 g. The treatment was carried out as many as 18 male M. musculus with the age of 7–8 weeks, and body weight ranging from 30 g. Mice were divided into 6 groups, with each group consisting of three Mus musculus. The six groups are: Group I: Positive control (C+) Boost force 0.7 mg/KgBW, group II: Negative control (C-) was given Aquadest, group III: was given extract with a dose of 50 mg/KgBW, group IV: Extract of with a dose of 150 mg/KgBW, group V: was given extract with a dose of 450 mg/KgBW, group VI: Isolate with a dose of 0.7 mg/KgBW for each MetOH and EtAce fraction. Treatment with one gavage, during the day, with a span of 24 h. After that, the leukocyte count was carried out by taking the blood of the mice from the tail. Blood Sampling. In the study the number of white blood cells (leukocytes) is needed blood that is not too much. A small amount

of blood can be obtained through an incision at the tail end, this is usually done for blood differential. Blood sampling steps, counting booth and cover glass are prepared in a clean condition. Blood is taken by cutting the tail, use a disinfectant to clean the part where the blood will be drawn. Blood that comes out of the wound is sucked with a white stone haemocytometer pipette to the 0.5 mark. Work fast, try not to let the blood clot in the pipette. Blood is diluted in a pipette using Turk's solution to the 11 mark, the suspension is shaken until homogeneous, thus the blood has been diluted 100 times. The blood solution is left in this Turk's solution for 5 min. The mixed solution (blood + Turk) was pipetted with a pasteur pipette then the tip of the pipette was touched at an angle of 300 to the surface of the counting chamber by touching the cover glass, then covered with a cover glass. Place it on a flat surface and let the counting chamber fill slowly with its own capillary power. The counting chamber is left for 2–3 min so that the leukocytes can settle.Then viewed under a microscope, the leukocyte grains that are in the 4 large boxes on the edge are counted.Observations using a 40X magnification microscope.

2.5.2 Macrophage Cell Phagocytosis Test

Bacterial Suspension Preparation. Test Bacteria on an inclined agar medium were taken with sterile wire and then suspended into a tube containing 2 mL of 0.9% NaCl solution until the turbidity was the same as the standard turbidity of Mc. Farland. Bacterial turbidity was measured according to the standard Mc Farlan 0.5 using a 20 D spectrophotometer at a wavelength of 625 nm. Mc Farlan's solution was made with a composition of 0.05 mL 1% BaCl2 and 9.95 mL H_2SO_4.

Phagocytosis Test. The treatment of the test animals was carried out once a day for 7 days orally according to the volume of administration. On the eighth day, each mouse was infected with 0.5 mL of SA bacterial suspension intraperitoneally, left for one hour. The mice were anesthetized with ether and then the abdomen was dissected using surgical scissors and sterile tweezers. If a small amount of peritoneal fluid is found in the abdomen, 1–2 mL of sterile Phosphate buffered saline (PBS) solution of pH 7.8 is added as much as 1–2 mL, shaken slowly and then taken peritoneal fluid with a 1 cc syringe. Peritoneal fluid was stained on an object glass and fixed with methanol for 5 min, then stained with 10% Giemsa stain, allowed to stand for 20 min, rinsed with running water. After the preparation is dry, it is viewed under a microscope using immersion oil with a magnification (10x–1000x). Calculating Phagocytic Activity. The immunostimulant activity was determined by calculating the phagocytic activity of the peritoneal macrophage cells of mice. The value of phagocytic activity is the percentage of macrophage cells that actively carry out the phagocytosis process among 100 macrophage cell:

$$\%\ \text{Activity} = \frac{number\ of\ active\ macrophages}{the\ number\ of\ macrophages\ observed} x\ 100\%$$

2.6 Data Analysis

Immunomodulator test data obtained in the form of leukocyte cell counts can be analyzed using one-way analysis of variance (ANOVA) and followed by Tukey's test using statistical software SPSS 20.1.

3 Result and Discussion

A total of 1003 g of Sungkai leaf samples that have been cleaned and chopped, were extracted using the maceration method in stages. The chopping process is carried out to increase the surface area so that the extraction process can be more perfect. The solvent used was distillation solvent, namely n-hexane, ethyl acetate and methanol. The maceration results were separated using a glass funnel and evaporated using a rotary evaporator. Based on the results of phytochemical screening (Table 1), it can be seen that the best extract is methanol extract with a yield value of 8.454% (Table 2).

3.1 Isolation Bioactive Compound

The compound isolation process was carried out using the chromatographic method. The initial stages of isolation of the methanol extract of Sungkai Leaf were using the column chromatography method which was enforced using a stative. The initial separation was carried out using liquid vacuum chromatography (LVC) using a vacuum pump to speed

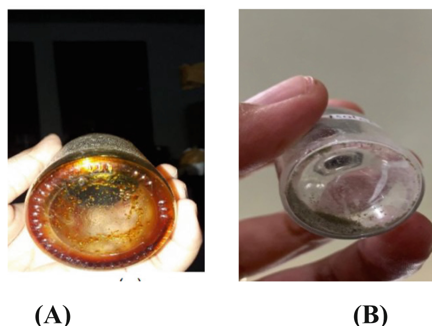
Table 1. % yield extract

Extract	Mass (gram)	Yield (%)
n-hexana	13	1,2961
Ethyl acetate	40,7	3,090
Methanol	84,7	8,454

Table 2. Phytochemical extract screening

Phytochemical	**Reagent**	Result	
		B	**C**
Alkaloids	Dragendorf	-	-
Flavonoids	Mg + HCl	+	+
Phenolic	$FeCl_3$ 1%	+	+
Saponins	Foam Test	-	+
Triterpenoids	Lieberman bourchard	-	-
Steroids	Lieberman bourchard	+	-

n-hexane (A); Ethyl acetate (B); Methanol



(A) (B)

Fig. 1. Isolate sediment on the bottom of the vial (A). MetOH, (B) EtAce

Table 3. Isolate phytochemical screening

Phytochemical Analysis	Reagent	Result	
		A	B
Alkaloids	Pereaksi dragendorf	-	-
Flavonoids	Mg + HCl	+	-
Phenolic	$FeCl_3$ 1%	-	-
Saponins	Foam test	-	-
Triterpenoids	Lieberman bourchard	-	-
Steroids	Liebermann-Burchard	-	+

A: MetOH Isolate; B: EtAce Isolate

up the separation process. Separation of the methanol fraction using Liquid Vacuum Chromatography produced 36 vials, then the solvent was evaporated. From each vial, thin layer chromatography (TLC) was performed. The TLC results which have the same stain pattern are then combined to form 3 fractions, then isolated the single compound (Fig. 1 and Table 3).

3.2 Isolate Characterization

Characterization using UV-Vis spectrophotometer was carried out to determine the basic framework of the isolated compounds. The result of the UV-Vis spectrum of the Methanol and Ethyl Acetate isolate can be seen in Fig. 2.

From the results of characterization using UV-Vis spectrophotometer isolates methanol (MI) has one maximum absorption peak was obtained at a wavelength of 329 nm. The position of the hydroxyl group on the flavonoid core was determined by the addition of shear reagent. Based on a comparison of the literature, the UV-Vis spectrum of MI almost had a similar maximum absorption with the UV-Vis spectrum of flavonoid compounds of the isoflavone group, namely 5.7 dihydroxy isoflavones with a maximum absorption of 329 nm [14]. This shows that MI isolates have the same basic compound

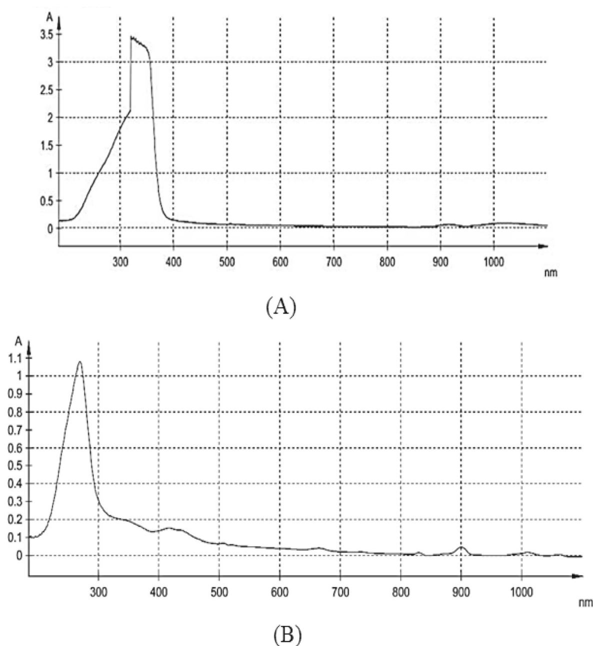


Fig. 2. UV-Vis spectrum of Isolate, (A). MetOH Isolate, (B). EtAce Isolate.

framework. While the UV-Vis spectrum of EtAce Isolate (EI) showed maximum absorption at a wavelength of 267 nm (Abs = 1.0609). UV-Vis spectral data of EI showed an electron transition $\rightarrow \pi^*$ which indicated the presence of a C = C chromophore which is a non-conjugated double bond. Based on a comparison of the literature, the UV-Vis spectrum of EI almost has a similar pattern with theUV-Vis spectrum of steroid group compounds, namely –Sitosterol [16].

3.3 IR-Spectrum

Characterization of compounds using FT-IR spectrophotometer to identified the functional groups contained in single compound based on the specific wave numbers. The IR spectrum of MetOH showed wide absorption and weak intensity. In the area of 3356.98 cm^{-1} which indicated the presence of OH groups bound to aliphatic and aromatic groups caused by intramolecular hydrogen bond vibrations. Based on the results of the FT-IR spectrophotometer characterization of isolate F2, it was suspected that isolate F2 was a compound of the flavonoid group. The allegation was strengthened by the literature's FT-IR spectrum which isolated flavonoid compounds from the isoflavone group, namely 5,7 dihydroxy isoflavones. The comparison of the FT-IR spectrum data can be seen in Table 3. Based on the results of phytochemical tests and the characterization of F2 isolates with infrared and UV-Vis spectrophotometers, it can be concluded that the F2 isolates contain flavonoid compounds of the isoflavone group, namely 5.7 dihydroxy

isoflavones with hydroxy groups on ring A, namely C-5 and C-7 atoms. The alleged structure of 5.7 dihydroxy isoflavones can be seen in Fig. 4.

The IR spectrum of EI showed absorption at a wave number of 3381.13 cm^{-1} with a wide intensity which is a characteristic of absorption for the -OH functional group. This is reinforced by absorption at a wave number of 1022.32 cm^{-1} as the stretching vibration of the C-O bond. Absorption at wave number 2939.10 cm^{-1} with sharp intensity indicates absorption for the aliphatic C-H functional group. Absorption at wave number 1631.44 cm^{-1} indicates the presence of a $C = C$ functional group. The appearance of absorption at wave numbers 1449.42 cm^{-1} and 1362.82 cm^{-1} indicates the presence of aliphatic C-H bond stretching vibrations (alkanes) indicating the presence of methyl (CH_3) and methylene (CH2) groups. At the peak of the aliphatic C-H stretch in the area (2939.10 cm^{-1} and the $C = C$ group (1631.44 cm^{-1}) from the characterization results on isolate EI indicated the presence of a steroid compound skeleton in the isolate and the C-O stretch (1022.32 cm^{-1}) also supports that the isolate is a steroid compound because it has a hydroxyl group (Fig. 3, Tables 4 and 5).

Based on the results of the FTIR analysis of EI isolates, absorption at various wave numbers has similarities with the FTIR spectrum data of -Sitosterol [16, 18] and then compared with the absorption of pure -Sitosterol compounds research. A comparison of FTIR spectrum data can be seen in Table 6.

The allegation that the Ethyl Acetate Isolate is β-Sitosterol was strengthened by the obtained isolate form which was in the form of a white powder. The -sitosterol compound has characteristics such as white color, distinctive odor, hydrophobic nature, soluble in

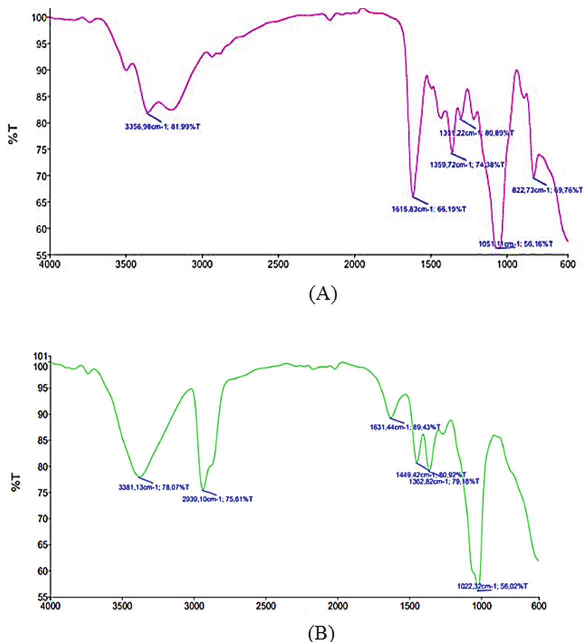


Fig. 3. IR Spectrum of Isolate, (A). Methanol, (B). Ethyl acetate

Table 4. IR-Comparison of the MetOH isolate

Wave Number (cm-1)			Functional Group
MetOH Isolate	5,7 dihidroksi isoflavon [17]	Asih, 2009	
3356	3286	3000–3450	OH
1615	1662	1600–1900	C = C Alkena
1359	1448	1300–1400	C-H Alifatik
1051	1031	800–1300	C-O-C Eter
822	779	700–900	C-H Aromatik

Table 5. IR-Comparison of EtAce Isolate

Wave Number (cm^{-1})			Functional Group
Isolate	References [16]	References [18]	
3381,13	3439,68	3427,85	O-H
2939,10	2923,93 2852,92	2959,64 2852,27	C-H alifatik
1631,44	1736,80	1641,91	C = C
1449,42 1362,82	1463,81 1377,24	1464,59 1382,09	C-H (pada CH_2) C-H (pada CH_3)
1022,32	1011,10	1052,35	C-O alkohol

ethanol and chloroform, and insoluble in water [19]. The results of the phytochemical screening of isolates with Liebermann-Burchard reagent produced a green solution indicating that EI was positive as a steroid. Thus, it is suspected that the EI isolated from the ethyl acetate extract of *Peronema canescens* Jack is a steroid derivative compound, namely β-Sitosterol which has the IUPAC name stigmast-5-en-3β-ol, $C_{29}H_{50}O$. The structure of -Sitosterol is presented in Fig. 4.

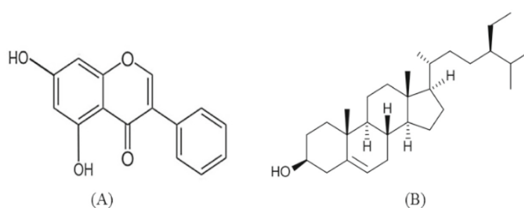


Fig. 4. Isolate compound, (A). 5,7 dihidroksi isoflavon, (B) β-Sitosterol

3.4 Immunomodulator Activites

The immunomodulatory activity test was carried out using hite male mice (*mus musculus*) swiss webster strain weighing 20–30 g. Before testing, mice were acclimatized for 7 days. In this study, the immune system-enhancing drug was also used, namely the Immunos drug as a comparison drug. Immunos (POM SD 021 502 661) is a drug commonly used by the public as an immune system enhancer. The treatment was carried out using the gavage method on M. musculus according to the treatment group at random. The treatment was carried out with 1 gavage during the day. The results of observations of leukocytes under a microscope are as follows [19].

Based on the results shown in Table 6 and Fig. 5, it can be seen that the activity of leukocytes is directly proportional to the increase in the dosage of the preparation. The activity of leukocytes increased with increasing dosage of the preparation. The principle of leukocyte cell calculation is that the blood is diluted using turk's solution and then it will release cells other than leukocytes, thus facilitating the calculation of leukocytes in the counting chamber. Blood was diluted 10 times and then counted in four leukocyte boxes.

This shows that the treatment of sungkai leaf extract has an effect on immunity. Where leukocytes are cells that make up blood components, so with an increased content of white blood cells can help the body fight various infectious diseases, as part of the immune system. The administration of sungkai leaf extract was more effective than the administration of imonos drug as a comparison drug. Immunos as a single drug, while the sungkai extract contains several active substances, namely, peronemin, sitosterol, isopropanol, phytol, dipterpenoid, flavonoid so it is possible that these elements help in increasing the number of leukocytes. In the Immunity test, the most effective dose in helping the immune system with a dose of sungkai extract was 450mg/KgBW, tended to increase the leukocyte count by 36% (Table 7).

Table 6. Average % of Leucocyte number

Sample	**Group**	**Doses**	**Average ± SEM**
Positive Control	C (+)	0.7 mg/KgBW	$8.8^{d} \pm 0.56$
Negative Control	C (-)	50 mg/KgBW	$5.7^{a} \pm 0.06$
Methanol Extract	ME1	50 mg/KgBW	$5.6^{a} \pm 0.20$
	ME2	150 mg/KgBW	$7.1^{b} \pm 0.23$
	ME3	450 mg/KgBW	$8{,}4^{cd} \pm 0{,}47$
Ethyl Acetat Extract	EE1	50 mg/KgBW	$6.67^{ab} \pm 0.14$
	EE2	150 mg/KgBW	$7.50^{b} \pm 0.26$
	EE3	450 mg/KgBW	$9.10^{c} \pm 0.15$
Methanol Isolate	MI	0.7 mg/KgBW	$7.7^{b} \pm 0.45$
Ethyl Acetate Isolate	EI	0.7 mg/KgBW	$6.33^{ab} \pm 0.28$

[a,b,c,d] Superscripts with different lowercase letters on the same line showed a significant difference ($P < 0.05$).

Table 7. Average % of Active Macrophage Phagocytic Activity

Sample	Group	Doses	Average ± SEM
Positive Control	C (+)	0.7 mg/KgBW	$62.3^{bc} \pm 1.81$
Negative Control	C (-)	50 mg/KgBW	$45.5^{a} \pm 0.17$
Methanol Extract	ME1	50 mg/KgBW	$46.4^{a} \pm 3.24$
	ME2	150 mg/KgBW	$51.9^{ab} \pm 0.61$
	ME3	450 mg/KgBW	$64.9^{c} \pm 5.70$
Ethyl Acetat Extract	EE1	50 mg/KgBW	$43.3^{bc} \pm 1.45$
	EE2	150 mg/KgBW	$44.3^{bc} \pm 0.38$
	EE3	450 mg/KgBW	$53,33^{cd} \pm 2.85$
Methanol Isolate	MI	0.7 mg/KgBW	$50,4^{ab} \pm 1.81$
Ethyl Acetate Isolate	EI	0.7 mg/KgBW	$38.0^{ab} \pm 1.52$

[a,b,c,d] Superscripts with different lowercase letters on the same line showed a significant difference ($P < 0.05$).

The immunomodulator activity was conducted to determine the immunomodulatory effect of the methanol extract of sungkai leaves on the increasing phagocytic activity of male mice macrophages. Each group of test animals was treated with a dose of extract for 7 consecutive days orally once a day with the aim of stimulating the immune system of each group of test animals. Until the eighth day each mouse in each group was infected with 0.5 mL of *S. aureus* bacterial suspension intraperitoneally [20]. The results of observations of active macrophage cells under a microscope are presented in Fig. 5.

Immunomodulatory testing was carried out by calculating the value of the phagocytic activity of peritoneal macrophages of mice. The value of phagocytic activity can be calculated from macrophages that actively carry out phagocytosis among 100 cell numbers expressed in percent and can be seen in Table 7. Based on the results shown in Table 7, it can be seen that the phagocytic activity of macrophages is directly proportional to the increase in the dosage of the preparation. The phagocytic activity increased with increasing dosage of the preparation. The highest percent increase in macrophage cell phagocytic activity was found in the dose group of 450 mg/kgBW, which was 64.9% which was higher than the positive control group of 62.3% and the dose group of 50 mg/kgBW, 150 mg/kgBW, and Isolate. F2 0.7 mg/kgBW which, respectively, had

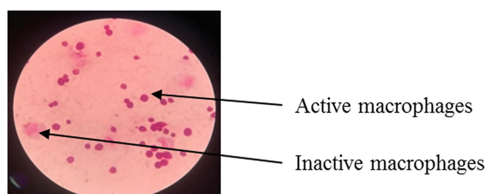


Fig. 5. Observation of macrophages cells

a phagocytic activity of 46.4%, 51.9% and 50.4%, respectively. Meanwhile, the lowest percentage of phagocytic activation was found in the negative control group, namely, 45.5%.

The administration of *Staphylococcus aureus* infection in this study triggered infection in mice. At the time of the infection process, T-lymphocytes will produce a number of lymphokines that attract macrophages to where they are needed and activate them. Active macrophages will release several important substances, namely: enzymes, lysozyme, elastase, collagenase, complement and cytokines [20]. Cytokines secreted by macrophages include; Interleukin (IL)-1, IL-6, IL-8, IL-12, IL-15 and TNFα. Under normal conditions, the process is slow so that the number of macrophage cells that are actively carrying out the phagocytosis process is also small. The increase in phagocytic activity of macrophage cells in male mice indicates that the ethanolic extract of the sponge Melophlus sarasinorum contains chemical compounds that can increase the phagocytic activity of macrophages (immunostimulants) [20].

4 Conclusion

The level of immunomodulatory activity of sungkai leaf methanol extract and F2 isolate gave an immunomodulatory effect which was characterized by a large number of leukocytes and the large number of active macrophages. The treatment of EtAce extract at a dose of 50 mg/Kgbb, 150 mg mg/Kgbb and EI at a dose of 0.7 mg/Kgbb was not significantly different from C- due to the dose used was still low so it did not show any immunomodulatory effect. While the extract treatment at a dose of 450 mg/Kgbb was not significantly different from the C+ treatment which showed an immunomodulatory effect on the muscles, which was characterized by an increase in the number of leukocytes and macrophage phagocytic activity compared to the negative control treatment.

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