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Research Article

Immunomodulatory Activity of Purple Button Herb (*Borreria laevis* Lamk.) on Macrophage Phagocytosis

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Abstract

Immunomodulators operate through various mechanisms, one of which involves enhancing phagocytic activity. The purple button herb (*Borreria laevis* Lamk.) is recognized for containing secondary metabolites that may function as immunomodulators; however, its influence on phagocytosis has not been previously assessed. Consequently, a study explored the immunomodulatory effects of distinct fractions of the purple button herb on macrophage phagocytosis in male mice. Over seven days, the mice were orally administered ethanolic extract, ethyl acetate, and n-hexane fractions of the purple button herb. On the eighth day, the subjects were infected with *Staphylococcus aureus*. After euthanization, intraperitoneal fluid was collected to analyze macrophage phagocytosis activity. The study's results suggested that the ethanolic extract, ethyl acetate, and n-hexane fractions of the purple button herb tended to enhance macrophage activity, with the n-hexane fraction achieving the highest phagocytosis rate of 84.7%. The corresponding p-value > 0.05 in the LSD test indicated no statistically significant difference when compared to the positive control. In conclusion, the n-hexane fraction of the purple button herb demonstrates potential as an immunostimulant by augmenting macrophage activity.
Keywords: Immunomodulator, Macrophage Phagocytosis, Purple Button Herb.

Aktivitas Imunomodulasi Herba Kancing Ungu (*Borreria laevis* Lamk.) terhadap Fagositosis Makrofag

Abstrak

Senyawa imunomodulator punya beberapa mekanisme salah satunya melalui aktivitas fagositosis. Herba kancing ungu (*Borreria laevis* Lamk.) memiliki kandungan metabolit sekunder yang berpotensi sebagai imunomodulator, namun belum terdapat uji aktivitas fagositosis, oleh karena itu dilakuan penelitian untuk mengetahui efek imunomodulasi fraksi herba kancing ungu terhadap peningkatan aktivitas fagositosis makrofag pada mencit jantan. Selama tujuh hari, mencit jantan diberikan ekstrak etanol, fraksi etil asetat, dan n-heksana dari herba kancing ungu secara oral. Pada hari kedelapan, mencit dibuat terinfeksi bakteri *Staphylococcus aureus*. Setelah dieuthanasia, cairan dari rongga intraperitoneal dikumpulkan dan dianalisis untuk mengevaluasi aktivitas fagositosis makrofag. Hasil penelitian menunjukkan bahwa ekstrak etanol, fraksi etil asetat, dan n-heksana herba kancing ungu menunjukkan kecenderungan dalam peningkatan aktivitas makrofag, dengan aktivitas tertinggi fagositosis pada fraksi n-heksana mencapai sebesar 84,7%. Nilai p>0,05 pada uji LSD menunjukkan tidak ada perbedaan yang signifikan secara statistik jika dibandingkan dengan kontrol positif. Berdasarkan temuan studi ini, fraksi n-heksana herba kancing ungu menunjukkan potensi sebagai imunostimulan dengan meningkatkan aktivitas makrofag.
Kata Kunci: Fagositosis Makrofag, Herba Kancing Ungu, Imunomodulator.

1. Introduction

Invasion of various pathogens originating from the surrounding environment, such as bacteria, viruses, worms, fungi, parasites, or protozoa, can cause infections in the body.[1] These materials can enter the body and cause various diseases and even tissue damage. This type of pathogen is an unwanted material and must be eliminated; an example of one pathogen is the bacterium *Staphylococcus aureus*, a gram-positive pathogen that is invasive and capable of causing many diseases in animals and humans.[2]

Immunomodulation, a substance that can alter the innate and adaptive immune system, is critical in immunology. It can either boost the immune system, known as immunostimulant, or suppress it, known as immunosuppressant.[3] In this intricate system, macrophages drive immune responses and combat infection and inflammation.[4] Macrophages are key components of the innate immune system, crucial for maintaining immune balance by producing inflammatory mediators like nitric oxide (NO) and the cytokine interleukin-1 beta (IL-1β). Nitric oxide is involved in pathogenic phagocytosis, while IL-1β activates other immune cells to combat infections.[5] The assessment of macrophage phagocytosis is a standard parameter in immunomodulatory research, emphasizing their vital role. Their superior phagocytic ability sets them apart from other phagocytic cells.[6] The testing of phagocytosis activity is used to observe a bioactive compound's immunomodulatory effect on macrophage cells.[7]

Corticosteroid drugs (glucocorticoids), cyclosporine, and azathioprine are immunosuppressants that suppress immune system activity. Conversely, drugs like levamisole, cimetidine, and isoprinosine are immunostimulants that enhance immune system function.[8] However, immunosuppressant therapy is associated with potential side effects that may pose challenges. Calcineurin inhibitors (cyclosporine and tacrolimus) are known to cause seizures, visual disturbances, disorders of the movement system, and decreased consciousness.[9] Immunosuppressant therapy is known to increase the risk of infection. This is because the mechanism of immunosuppressants suppresses the immune system, causing a decrease in the body's ability to fight infections and diseases that attack the body.[10]

There are several active ingredients found in herbs that are believed to impact the immune system. These ingredients include polysaccharides, flavonoids (such as baicalen, baicalin, and luteolin), monoterpenoids (like linalool), triterpenoids (such as oleanolic acid), and phenolics (including caffeic acid, vanillic acid, chlorogenic acid, ferulic acid, and coumaric acid). The role of herbs as immunomodulators can either be immunostimulatory or immunosuppressive.[11]

The content of flavonoid compounds in purple button herbs plays a role in lymphokine (interferon-γ) produced by T cells so that it will stimulate phagocytic cells to carry out phagocytosis responses. It can spur lymphocyte proliferation, increase the number of T cells, and increase secretion of Interleukin-2.[12] This result was supported by research conducted by Sidarima,[13] which found that *Borreria laevis* Lamk. plant extract significantly increases macrophage cell phagocytosis activity in mice. In contrast, *Borreria laevis* Lamk. It is thought to have immunomodulating and immunostimulant properties by triggering the proliferation of macrophage cells and lymphocytes.

The purpose of fractionation is to separate compounds based on their polarity. Polar compounds are extracted with polar solvents, while nonpolar compounds are extracted with suitable solvents.[14] The solvents used are n-hexane (a nonpolar solvent) and ethyl acetate (a semipolar solvent). Metabolic compounds that can be attracted to polar solvents include polyphenolic compounds, flavonoids, saponins, and alkaloids.[15] Based on the description above, we are interested in researching the immunomodulatory activity of the purple button herb (*Borreria laevis* Lamk.) ethanolic extract and its fractions on macrophage phagocytosis using the mouse

model.

2. Methods

2.1. Tools

Scales, maceration containers, rotary vacuum evaporators, Petri dishes, a set of vacuum liquid chromatographic tools, a set of thin-layer chromatography tools, a set of UV-Vis spectrophotometers (Shimadzu), Petri dishes, test tubes, ovens, autoclaves, stirring rods, injection syringe, oral cannula, tweezers, scalpels, a set of microscope tools, glass objects, and deglass.

2.2. Materials

The materials used were purple button herb powder, ethyl-acetate (Merck, Germany), n-hexane (Merck, Germany), chloroform (Merck, Germany), 96% ethanol (Merck, Germany), aquadest, silica gel, agar media, *Staphylococcus aureus* bacteria, Stimuno® (Dexa Medika, Indonesia), 1% BaCl2, 1% H2SO4, 0.9% NaCl, phosphate buffer saline (PBS), 4% Giemsa dye, immersion oil, and methanol.

2.3. Procedures

2.3.1. Sample Collection

The purple Button Herb (*Borreria laevis* Lamk.) used in this research was collected from Sambahule Village, Baito District, South Konawe Regency, Southeast Sulawesi.

2.3.2. Extraction Process

The purple button herb is thoroughly washed under running water. Subsequently, the simplicia is placed in an open field to undergo the drying process. The dried sample was then processed in a blender to create a fine simplicia powder.

Samples of purple button herbs (*Borreria laevis* Lamk) powder weighed 500 g and were then put into a matching vessel. They were soaked in 96% ethanol, stirred by a macerator magnetic stirrer, tightly closed, and left stirred for 24 hours. After that, the phyrate was filtered and taken. Furthermore, the pulp was matched for three consecutive days. The purple button herb ethanol filtrate was then concentrated with an evaporator until a thick extract was formed.

2.3.3. Fractionation

Fractionation of thick purple button herb extract is carried out by liquid-liquid extraction (LLE) using n-hexane solvent and ethyl acetate. The viscous extract was dissolved with hot water, then n-hexane was added with a composition (1:1) and let stand. After the two layers were formed, the layers were separated, and the water solvent was put back into the split funnel. Then, the ethyl acetate solvent is added in equal quantities with the same treatment. All fractions obtained are concentrated with a rotary evaporator and received a 600 µL/mL concentration in ethyl acetate and n-hexane fractions. These fractions were then used for treatment in mice.

2.3.4. Animal Preparation

The test animal used was a healthy BALB/c male mouse strain (*Mus musculus*) aged 3-4 months old, which could be observed from its behavior and body weight of 20-30 g. Male mice were adapted for seven days in the pharmacology laboratory, the pharmacy study program of Mandala Waluya University, Kendari (009/KEP/UMW/III/2023). After one week of adaptation, weighing was done to determine the dose and treatment. Test animals were grouped into five groups, each consisting of three test animals.

2.3.5. Test of immunomodulating effect of purple button herb (*Borreria laevis* Lamk.)

The mice were administered treatment orally once daily for seven days, with the administration volume determined according to their body weight in grams. Group I, as a negative control, was given Na-CMC 0.5%. Group II, as a positive control, was given a dose of Stimuno® 0.13 mg/kg body weight; group III mice were given a purple button herb extract dose of 600 µL/g body weight; group IV mice were given an ethyl acetate fraction of purple button herb dose of 600 µL/g body weight, group V mice were given n-hexane fraction of purple button herb dose

600 µl/g body weight.[16]

On the eighth day, each mouse was injected intraperitoneally with 0.5 ml of Staphylococcus aureus bacteria suspension and left for one hour. The mice were euthanized using ether and dissected with surgical scissors and sterile tweezers. Peritoneal fluid was collected with a syringe and smeared onto glass slides. The smears were fixed with methanol for 5 minutes, stained with 4% Giemsa dye, left to stand for 20 minutes, and rinsed under running water. Once dry, the preparations were examined under a microscope with immersion oil at a magnification of 10–1000×, and macrophage phagocytosis activity was assessed.[17]

$$\%\ phagocytosis\ activity: \frac{\text{Number of active macrophage cells}}{\text{Number of macrophage cells observed}} \times 100$$

Phagocytosis of macrophages was widely used as an immunological parameter to evaluate immune health/function. Assessment of phagocytosis ability/activity can be calculated by measuring phagocytosis capacity, macrophage phagocytosis activity test using phagocytosis index parameters, which were the number of latex particles that can be phagocytosed by 100 active macrophages and latex were eaten indicated that the phagocytosis process occurred.[18]

2.3.6. Statistical analysis

The collected data was analyzed statistically using the One-Way ANOVA test and the Least Significant Difference (LSD) test with SPSS 27 (IBM, USA).

3. Result

3.1. Macrophage activity

After seven consecutive days of treatment for the male mice, *Staphylococcus aureus* was injected with utmost precision, up to 0.5 ml, on the eighth day. The injection was allowed to sit for one hour, enabling the bacteria to invade and infect the mice's peritoneal cavity effectively. Following the infection, the peritoneal fluid from the infected male mice was extracted using a syringe and examined under a microscope at 1000× magnification. The results, which demonstrate the accuracy of our experiment, can be seen in Figure 1.

Figure 1 demonstrates the difference between active macrophages and naïve macrophages. Phagocytosis of macrophages was widely used as an immunological parameter to evaluate immune health/function. Assessment of phagocytosis ability/activity can be calculated.

3.2. Differences in macrophage activity

Measurements were conducted on male mice previously treated and induced with Staphylococcus aureus. The objective was to evaluate the level of active macrophage activity in each treatment. This was achieved by observing samples under a microscope with a magnification of 1000×.

The table presented the average percentage of macrophage phagocytosis activity for various samples: the n-hexane fraction (84.67%±2.08), the ethyl acetate fraction (63.33%±2.89), purple button herb extract (81.33%±2.52), Stimuno® (75%±10), and Na-CMC (23.5%±3.5).

The LSD test results indicated that each of the purple button herb fractions had a p-value of less than 0.05 in the negative control, signifying a significant difference from the negative control. Conversely, they

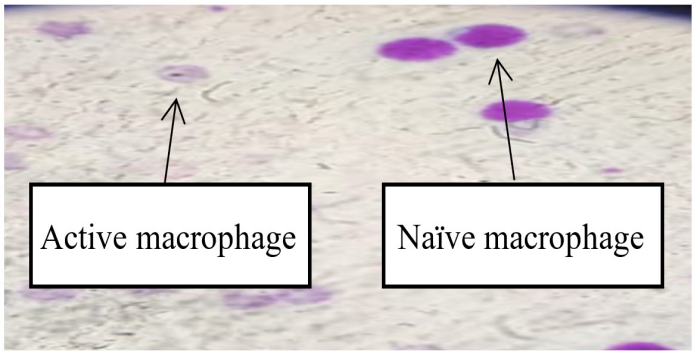

Figure 1. The difference between active and naïve macrophage in the n-hexane fraction-treated group.

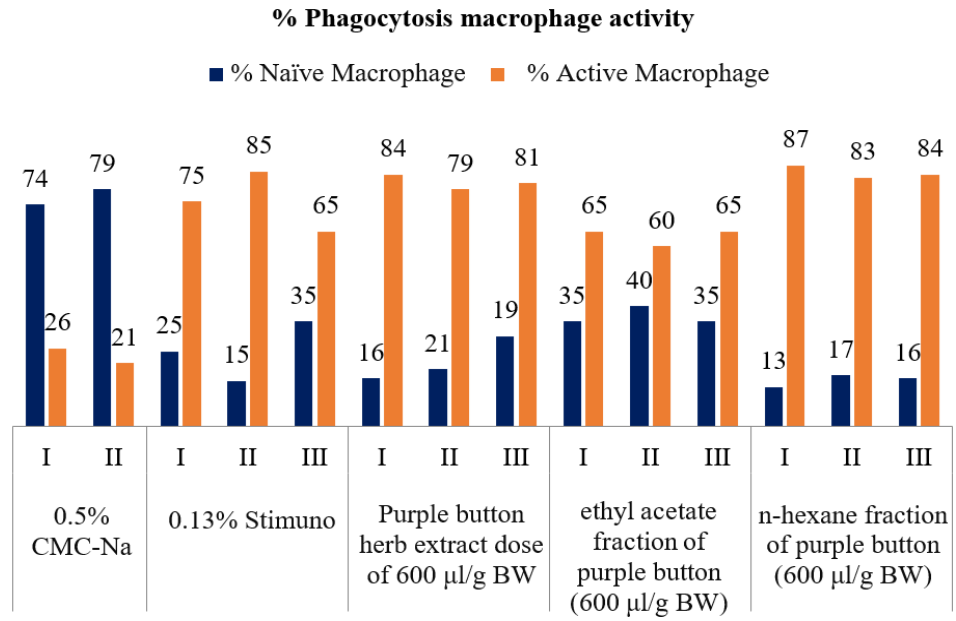

Figure 2. The percentage macrophage activity of the purple button herb (*Borreria laevis* Lamk) ethanolic extract and its ethyl acetate and n-hexane fraction.

recorded a P-value greater than 0.05 in the positive control, suggesting no significant difference in macrophage activity compared to the positive control. This promising outcome demonstrates macrophage activity in all fractions of the purple button herb. The ethyl acetate fraction and the n-hexane fraction exhibited significant phagocytosis activity, closely resembling that of the positive control, with no statistically significant differences observed. Notably, the n-hexane fraction demonstrated the highest phagocytosis activity among the fractions studied. These results indicate that the n-hexane fraction possesses the potential to function as an immunomodulator, thereby enhancing the immune system.

4. Discussion

This study aimed to evaluate the immunomodulatory effects of the purple button (*Borreria laevis* Lamk.) herb fraction on the phagocytic activity of male mice and compare the different fractions' efficacy in modulating immune responses. *Staphylococcus aureus* is a commensal bacterium and a significant human pathogen, colonizing approximately 30% of the population. It is also a leading

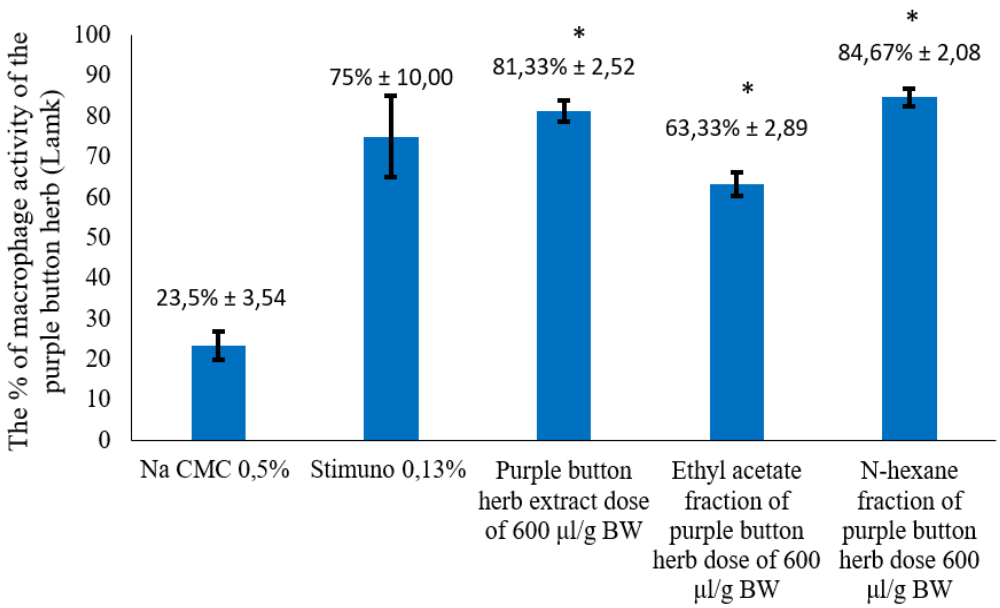

Figure 3. The average percentage of macrophage activity of the purple button herb (*Borreria laevis* Lamk.) fraction (n = 3)

cause of bacteremia, infective endocarditis (IE), osteoarticular infections, skin and soft tissue infections, pleuropulmonary infections, and device-related infections. As a gram-positive bacterium, *S. aureus* is commonly found within the human body.[19] This bacterium was used to conduct a phagocytosis test on experimental animals because it strongly binds with the Giemsa stain, making it easier to observe under a microscope. When mice are infected with this bacterium, they develop an infection. During the infection process, T lymphocytes produce lymphokines that attract macrophages to the site of infection and activate them. Activated macrophages release several essential substances such as enzymes, lysozyme, elastase, collagenase, complement, and cytokines.[20]

Furthermore, the treatment group showed that there were differences in each group; this was based on the average amount of phagocytosis of macrophage cells in purple button herbs, ethyl acetate fraction, and n-hexane fraction had differences, so based on the results that could show purple button herb extract has activity as immunomodulation by increasing the phagocytosis activity of macrophage cells by 81.3%. In comparison, the ethyl acetate fraction has activity as immunomodulation by increasing the phagocytosis activity of macrophage cells by 63.3%, and the n-hexane fraction has the highest immunomodulating activity with 84.7%.

The purple button herb (*Borreria laevis* Lamk.) is known to contain secondary metabolites, specifically flavonoids.[21] These flavonoids have the potential to counteract the lymphokines produced by T cells, thereby enhancing phagocyte responses during phagocytosis.[22] Flavonoids have been shown to enhance the production of IL-2 and promote lymphocyte proliferation. This increase in lymphocyte proliferation affects $CD4^+$ cells, which subsequently activates Th1 cells. The activation of Th1 cells leads to the production of specific macrophage-activating factors (SMAFs), one of which is IFN-γ. IFN-γ plays a crucial role in activating macrophages, increasing their phagocytic activity. This enhancement enables macrophages to kill bacteria more effectively. Additionally, flavonoids can stimulate natural killer (NK) cells, further boosting the production of IFN-γ. As a key macrophage-activating cytokine, IFN-γ activates macrophages and increases their phagocytic activity. Activated macrophages and neutrophils produce proteolytic enzymes in their phagolysosomes, such as elastase and cathepsin G, which are instrumental in destroying bacteria.[23]

5. Conclusion

Based on the research results, it can be concluded that the ethanolic extract of purple button herb 600 µl/g BW has an immunomodulating effect on increasing the phagocytosis activity of macrophages in male mice with an activity of 81.3%. In comparison, the ethyl acetate fraction of 600 µl / g BW has an immunomodulating effect on increasing the phagocytosis activity of macrophages with an activity of 63.3%, followed by 600 µl / g BW n-hexane fraction had the highest immunomodulating effect on increased phagocytosis activity of macrophages with an activity of 84.7% in mice that infected by *S. aureus*. Therefore, the purple button herb can be further developed for its potency as an immunomodulator by triggering macrophage activity against bacterium infection.

References

1. Purkon DB, Nuraisyah ZAN, Namina DR, Roseno MH, Haerussana AENM, Fadhlillah FM, et al. Immunomodulatory activity of Ananas comosus (L.) Merr. flesh and stem fruit juice on mice with carbon clearance and Staphylococcus aures-induced methods. Journal of Research in Pharmacy. 2023;27(6): 2497-510.
2. Rengganis I and Baratawidjaja KG. Imunologi dasar. Badan Penerbit Fakultas Kedokteran Universitas Indonesia; 2018.
3. Amalia L and Hiola F. Analisis gejala klinis dan peningkatan kekebalan tubuh untuk mencegah penyakit covid-19. Jambura Journal of Health Sciences and Research. 2020; 2(2): 71-76.

4. Li N, Li S, Wang Y, Wang Q, Zhou J, Liu J, et.al. Compositional analysis and immunomodulatory activity of blue pigment fraction (BPF) from Laba garlic. Food Chemistry. 2023;406, 134976.
5. Ulvia R. Pengaruh Ekstrak Air Dan Fraksi Herba Sambiloto [Andrographis paniculata (Burm. F) Nees] Terhadap Respon Imun Melalui Produksi Nitrit Oksida Dan Sitokin Il-1beta Pada Sel RAW 264.7 (Disertasi). Yogyakarta: Universitas Gadjah Mada; 2022.
6. Istini dan Santoso B. Pengaruh Penambahan Serum dan lama Waktu Inkubasi Lateks terhadap Aktivitas Fagositosis Makrofag Tikus Sprague Dawley (SD) dalam Menunjang Kegiatan Penelitian. Indonesian Journal of Laboratory. 2018;1(1): 16-22.
7. Kirana IDAA, Bodhi W, Lebang JS, and Fatimawali F. Uji Aktivitas Fagositosis Makrofag Dari Ekstrak Kulit Buah Jeruk Nipis (Citrus aurantifolia) Sebagai Imunomodulator. Jurnal Kesehatan Tambusai. 2023; 4(3): 3021-3027.
8. Martinus AT, Dachlan AS, and Halim E. Penggunaan Imunostimulan Dalam Bidang Dermatovenerologi. Media Dermato Venereologica Indonesiana. 2019;46(2): 111–5.
9. Zahrah Z and Cahyani YD. Pelayanan Kefarmasian Pada Pasien Imunosupresif Sebagai Tindakan Preventif di Masa Pandemi COVID-19. Farmaka. 2020;18(2) : 122-31.
10. Rilansia NPA. Efektivitas dan Keamanan Terapi Imunosupresan pada Pasien Penyakit Autoimun Systemic Lupus Erythematosus dengan Manifestasi Klinis pada Mukokutaneus, Muskoleskeletal, Ginjal, Dan Neuropsychiatric (Disertasi). Bali: Universitas Mahasaraswati Denpasar; 2023.
11. Shahbazi S and Bolhassani A. Immunostimulants: types and functions. Journal of Medical Microbiology and Infectious Diseases. 2016;4(3): 45–51.
12. Asep M, Meilawati L, and Hanafi M. Karakterisasi Ekstrak Etanol Herba Katumpangan Air (Peperomia pellucida L. Kunth). Jurnal Biopropal Industri. 2014;6(2): 53–61.
13. Sidarima JIS. Skrining Fitokimia Dan Penentuan Kadar Polifenol Total, Flavonoid Total, Saporan Total, Dan Tannin Total Herba Kancing Ungu (Borreria laevis Lamk.). (Skripsi). Kendari: Universitas Mandala Waluya; 2020.
14. Uthia R, Arifin H, and Efrianti F. Pengaruh hasil fraksinasi ekstrak daun kemangi (Ocimum sanctum L.) terhadap aktivitas susunan saraf pusat pada mencit putih jantan. Farmasi Higea. 2017; 9(1): 85–95.
15. Kasminah. Antivitas Antioksidan Rumpur Laut (Halymenia durvillaei) dengan Pelarut Non Polar, Semi Polar dan Polar. (Skripsi). Surabaya: Universitas Airlangga; 2016.
16. Nugroho AE, Andrie M, Warditiani NK, Siswanto E, Pramono S, and Lukitaningsih E. Antidiabetic and antihiperlipidemic effect of Andrographis paniculata (Burm. f.) Nees and andrographolide in high-fructose-fat-fed rats. Indian journal of pharmacology. 2012; 44(3): 377-81.
17. Rauf A, Haeria H, Anas D D. Imunostimulan Fraksi Daun Katuk (Sauropus androgynus L. Merr.) terhadap Aktivitas dan Kapasitas Fagositosis Makrofag pada Mencit Jantan (Mus musculus). Jurnal farmasi UIN Alauddin Makassar. 2017;4(1): 9-15.
18. Handayani N. Uji aktivitas fagositosis makrofag ekstrak etanol daun suji (Dracaena angustifolia (medik.) Roxb.) Secara in vitro. Jurnal Farmasi Medica/ Pharmacy Medical Journal (PMJ). 2018;1(1).
19. Tong SY, Davis JS, Eichenberger E, Holland TL, and Fowler Jr VG. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical microbiology reviews. 2015;28(3): 603-61.
20. Weiss G and Schaible UE. Macrophage defense mechanisms against intracellular bacteria. Immunological Reviews. 2015; 264(1): 182–203.

21. Yuliastri, W. O., Mahmudah, R. A., Fauziah, R., Ridwan, B. A., & Salsyafirah, W. (2023). Formulasi Sediaan Lip Balm Kombinasi Ekstrak Etanol 96% Herba Kancing Ungu (Borreria laevis Lamk.) dan Ekstrak Etanol 96% Kulit Buah Manggis (Garcinia mangostana Linn.) Sebagai Antioksidan. Jurnal Mandala Pharmacon Indonesia, 9(2), 352-363.
22. Hosseinzade A, Sadeghi O, Naghdipour Biregani A, Soukhtehzari S, Brandt GS, and Esmaillzadeh A. Immunomodulatory effects of flavonoids: possible induction of T CD4+ regulatory cells through suppression of mTOR pathway signaling activity. Frontiers Immunology. 2019; 10:51.
23. Mendes LF, Gaspar VM, Conde TA, Mano JF, and Duarte IF. Flavonoid-mediated immunomodulation of human macrophages involves key metabolites and metabolic pathways. Scientific Reports. 2019; 9(1):14906.