Jurnal Biolokus: Jurnal Penelitian Pendidikan Biologi dan Biologi
Vol. 8, Issue 1, June 2025
Pages : 116 – 124

p-ISSN: 2621-3702
e-ISSN: 2621-7538

The effect of genitri (*Elaeocarpus ganitrus*) in leukocytes profile of wistar rats as a prospective immunomodulatory agent

Cicilia Novi Primiani[1,2*], Pujiati Pujiati[2], Weka Sidha Bhagawan[1], Waris[3], Gabriella Chandrakirana Krisnamurti[4]

[1]Department of Pharmacy, Faculty of Health and Science, Universitas PGRI Madiun, Jl. Setia Budi No. 85 Madiun, East Java, Indonesia
[2]Department of Biological Education, Faculty of Teacher Training and Education, Universitas PGRI Madiun, Jl. Setia Budi No. 85 Madiun, East Java, Indonesia
[3]Biology Education, Faculty of Teacher Training and Education, PGRI Argopuro Jember University, Jl. Jawa No.10, Tegal Boto Lor, Sumbersari, Sumbersari, Jember, East Java, 68121, Indonesia
[4]Biotechnology Program, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, 10150 Bang Khun Thian, Bangkok, Thailand
*corresponding author: primiani@unipma.ac.id

ABSTRACT

Immunomodulator is an agent to activate and modulate the immune response. It is one of the approaches to prevent chronic disease and maintain homeostasis. Recently, plant phytochemicals have been developed as prospective immunomodulatory agents. Genitri (*Elaeocarpus ganitrus*), a local plant cultivated in Indonesia, exhibited various biological properties and potential as an immunomodulatory agent. This study aims to identify the potency of genitri as immunomodulatory agent through leukocyte profiling. Genitri leaf (P1), seed (P2), and fruit (P3) were prepared as powder and administered to male wistar rats weighted 250-300 g twith six replications for 28 days. The total leukocytes were counted and leukocytes were being profiled following the treatment with genitri powder then compared with control treatment (P0). The immunomodulatory activity of genitri was shown by the increased leukocytes count following treatment. Based on data from the treatment group (P2), genitri seeds were able to increase leukocytes by 10595.83 cells/µl. The highest total leukocytes were found in treatment P2 with total leukocytes 10595.83 cells/µl. Based on the leukocyte profile, lymphocytes and monocytes were found to be the highest in all treatments. Treatment with genitri was able to stimulate an increase in lymphocytes and monocytes. An increase in total leukocytes given genitri is an indication of an increased immune response.

Keywords: Genitri, immunomodulator, leukocytes, phytochemicals

INTRODUCTION

Immune system is a structure complex including cells, chemicals, and that provide function protection against foreign antigens, such as bacteria, fungi, parasites, toxins, and viruses that may lead to infection. The immune system is mediated by leukocytes, consisting of lymphocytes, monocytes, neutrophils, and macrophage, also specific immune systems such as antibodies, proteins, and cytokines (Marshall et al., 2018; Navegantes et al., 2017; Sun et al., 2021). The elevating production of leukocytes, antibodies, proteins, and cytokines are the sign of immune system activation. Once the immune system is activated, antigens elimination may occur, preventing the infection or further disease (Luster et al., 2005; Sun et al., 2021).

Modulating the immune response is an approach to prevent the occurrence of chronic disease, as well as maintaining the homeostasis immune system (Strzelec et al., 2023). Developing the potential agent for immunomodulators is important to stimulate the immune response as a preventive strategy in attenuating symptoms of disease (Arscott et al., 2011; Majka et al., 2017; Zhang et al., 2015). Currently immunomodulatory drug development faces challenges in developing safe and effective drugs (Bindu et al., 2020; Shi et al., 2021). The use of immunomodulators is necessary for the body to improve the immune system. Current

immunomodulatory treatments, ranging from classic pharmacological approaches, biologic strategies, and genomic strategies, showed a prospect in treating as well as preventing various diseases (Hultquist et al., 2016; Lee et al., 2021; Shi et al., 2021). Recently, potential immunomodulatory agents are still developed (Sultana et al., 2020; Strzelec et al., 2023). One of the promising immunomodulatory agents is from plant phytoconstituents (Hooda et al., 2024; Rahman et al., 2016).

Plant contains various phytochemicals which exhibit various biological properties. It may perform various medicinal activities, such as anti-inflammatory, antidiabetic, antivirus, anti-bacteria, antihypertensive, anticancer, and others. The phytochemicals, such as flavonoid, alkaloid, phenol, terpenoid, and tannin, are natural bioactive compounds produced as plant secondary metabolites (Agidew, 2022; Saranraj et al., 2016; Selvakumar et al., 2018). The production was a response to stimuli, such as stress, infection, nutrition, and climate change. The secondary metabolites are potential for treatment and therapy (Ali et al., 2021; Bhatti et al., 2022; Mahmoudi et al., 2024). Some medicinal plants are proven clinically and widely used in the industries for supplements, cosmetics, traditional drugs, and food. Interestingly, Indonesia is a country with high biodiversity, approximately 30.000 to 40.000 plant species while 2500 to 7500 of those species are medicinal plants (Cahyaningsih et al., 2021; Ministry of National Development Planning, 2016; Myers et al., 2000). Those are valuable for drugs, cosmetics, and applied in traditional and modern ways (Ministry of Agriculture, 2014; Ministry of Agriculture, 2015; Liu, 2022).

Genitri (*Elaeocarpus ganitrus*) is one of medicinal plants cultivated in Indonesia. Though it is rich in biological properties, the seeds are widely used as traditional jewelry. Recently genitri is widely explored in medicinal areas, later discovered for its activities as antidiabetic, anticholesterol, antioxidant, and anti-inflammatory, this is the presence of phytochemical constituents (Manoharan & Chitra, 2022; Primiani et al., 2022; Primiani et al., 2024; Tripathi et al., 2014; Vinay et al., 2021). Previous study showed antiinflammatory and antibacterial activities of genitri. Phytoconstituents of genitri fruit extracts performed inhibitory activity against COX-2 (Primiani et al., 2022). The seed extracts prevent *Staphylococcus aureus* and *Escherichia coli* infection, indicated by the inhibitory zone of the bacteria agar (Primiani et al., 2024). The activity of genitri in modulating immune response also could be an indication of the immunomodulatory activity of genitri. Based on the results of previous research, genitri seeds have antibacterial activity (Primiani et al., 2021). Though genitri has wide beneficial use, the exploration of genitri's potential benefits is still limited. This study aims to identify the immunomodulatory potency of genitri through leukocyte profile on the mice treated with genitri extracts.

METHOD

Sample collection

Genitri leaf, seed, and fruit was gathered from the forest edge of Desa Plumpung, Magetan, East Java. Leaf, seed, and fruit were identified at Plant Taxonomy Laboratory, Universitas PGRI Madiun with identification number 117/Taxo-Plant/Biology/IX.H.

Animal model

The animal study has been approved by the Research Ethics Committee Padjadjaran University with ethical clearance number 442/UN6.KEP/EC/2021.The 4-5 months of 24 male wistar rat with 250-300 g weight were obtained from LPPT Universitas Gadjah Mada. Rats were maintained for 7 days with the access to food and drink ad libitum according to the standard maintenance procedure (Afrianti et al., 2017).

Preparation of genitri powder

Make simplicia leaf, seed, and fruit 250 g each. Each part of genitri (leaf, seed, and fruit)

was prepared in powder form. Leaf, seed, and fruit were cleansed with water and dried at 40° - 60°C for 15 minutes until the water concentration reached 3%. Leaf, seed, and fruit of genitri were grinded with 60 mesh sieves until formed as a powder.

Treatment with genitri powder

Rats were grouped into four treatment groups, consisting of 8 rats in each group. The treatment groups included control (P0), rats-induced genitri leaf powder (P1), rats-induced genitri seed powder (P2), and rats-induced genitri fruit powder (P3). Rats in each group were administered with genitri powder with 0.25 - 1 g/kg weight through oral gavage. For each 1 g powder was diluted in a 6 ml aquadest (Hohakay et al., 2019; Salim et al., 2018). Each treatment was replicated 6 times during a total 28 days of treatment (Ali et al., 2021).

Histological analysis

Blood histological preparate was prepared by standard Hematoxylin-Eosin (HE) staining (Alturkistani et al., 2015). Total leukocytes were counted by microhematocrit. Total leukocytes count was applied to the formula:

$$\frac{n \times p}{v}$$

n = total cells counted
p = dilution
v = volume of four squares counted (each 0.1 mm^3)

RESULTS AND DISCUSSION

Leukocytes or white blood cells are part of the immune system which participated during body defense (Sun et al., 2021), circulated in the blood and performed inflammatory and cellular responses to the antigens (Prinyakupt & Pluempitiwiriyawej, 2015). According to the data, this study found that genitri powder raises the total leukocyte of male wistar rats (Table 1). The total leukocytes of rats-treated genitri powder were three times more than control (3870.83 cells/µl). The highest total leukocytes were exhibited by rats-induced genitri seed powder (P2) with 10595.83 cells/µl. It is one of the signs that the immune system is rising. When the leukocytes are recruited, there is a mechanism of protective and pathological immune response (Luster et al., 2005). Leukocytes play an important role in the immune response, as protection against pathological conditions. Leukocytes have the ability to phagocytose foreign objects. Leukocytes also have the ability to penetrate the walls of blood capillaries and then enter cells or tissues (Marshall et al., 2018; Sun et al., 2021).

White blood cells, or leukocytes, are components of the immune system that contribute to both the innate and humoral immune responses (Bonilla & Oettgen, 2010; Turvey & Broide, 2010). The total leukocyte count is an important indicator used to detect infection, inflammation, and immune system activation (Sun et al., 2021). When the body encounters an infection or experiences inflammation, the immune system may respond by recruiting leukocytes to the affected area. Therefore, the total leukocyte count can serve as a marker for immune response activation (Marshall et al., 2018).

Table 1. Total leukocytes count

Treatment	Total leukocytes (cell/µl)
Control (P_0)	3870.83
Leaf (P_1)	10366.66
Seed (P_2)	10595.83
Fruit (P_3)	10170.83

Some types of leukocytes, such as B cells, T cells, neutrophils, and monocytes support the regulation of immune defense and inflammation (Kelly et al., 2007). This study showed total leukocytes in all treatments were higher than control. However, the number of each leukocyte type was not similarly increased in the rat-treated genitri group (Figure 1). Treatment of genitri powder also able to maintain the condition of blood histological structure as in normal condition (Figure 2). The number of basophil of rat-reated genitri group was higher than control. However, the eosinophil of the control group was higher than all rat-treated genitri groups. The lowest eosinophil level was shown in the rat-treated genitri leaf extract (P1).

The neutrophil segment of the control group was slightly higher than rat-treated genitri seed extract (P2) and rat-treated genitri fruit (P3). It might be the sign that the neutrophil segment was not optimally stimulated by genitri extracts. It is also similarly observed in the basal neutrophil level, which is the basal neutrophil level of rat-treated genitri extracts was higher than control treatment. Thus, the genitri extracts probably do not stimulate neutrophil. Based on the data, lymphocyte and monocyte of the rat-treated genitri extracts was significantly higher than P0. Treatment of genitri extracts successfully modulates leukocyte components, dominated by segmented neutrophil, lymphocyte, and monocyte.

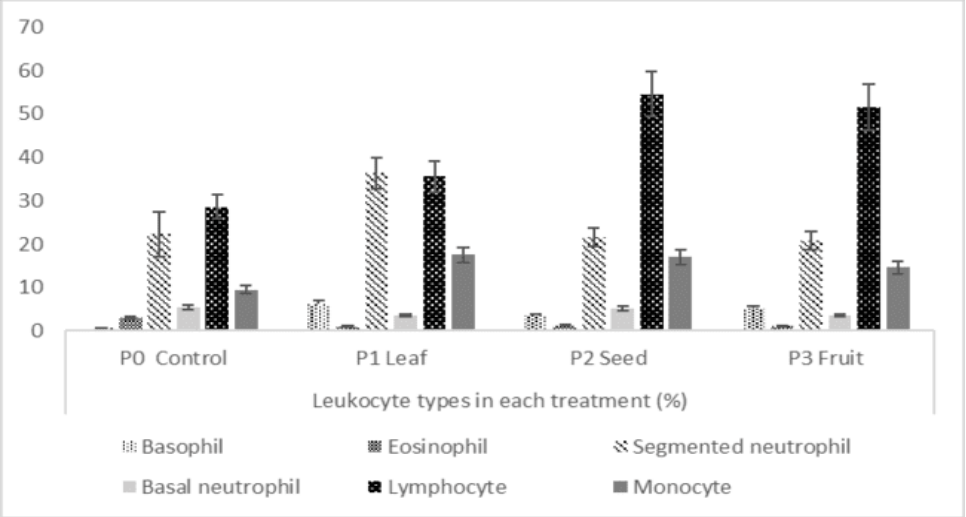

Figure 1. Types of leukocytes in each treatment.

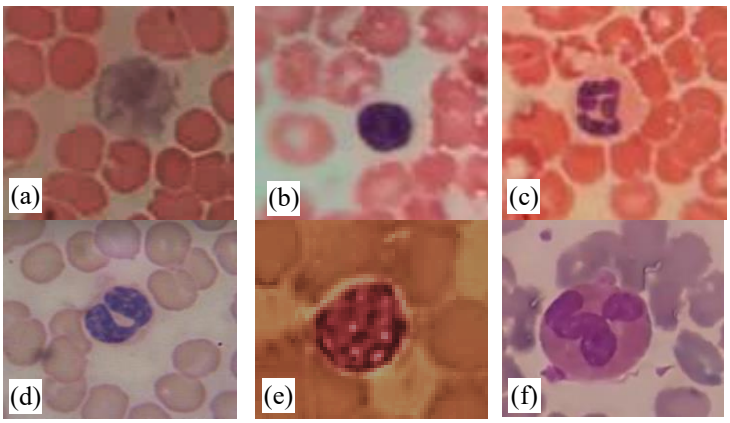

Figure 2. Types of leukocytes observed in preparat. (a) Monocyte, (b) Lymphocyte, (c) Segmented Neutrophil, (d) Basalis Neutrophil, (e) Basophil, (f) Eosinophil.

Neutrophils are polymorphonuclear cells (PMNs) that are key components of the nonspecific immune system, responding rapidly to antigens. Lymphocytes are mononuclear cells that play a role in the immune system. There are two types of lymphocytes, namely B lymphocytes and T lymphocytes (Luster et al. 2005). Neutrophils, monocytes, and macrophages are essential components of the innate immune system, working to eliminate antigens through phagocytosis. This mechanism differs from that of basophils, mast cells, and eosinophils, which act by releasing proinflammatory mediators to target antigens (Borriello et al. 2017; Karasuyama et al., 2011). According to the results of this study, giving

genitri (leaf, seeds, and fruit) can increase the number of leucocytes. Neutrophil were found in the greatest number (Figure 1).

Treatment with genitri seed extract (P2) has been shown to increase the total leukocyte count and the percentage of lymphocytes. Lymphocytes play a critical role in the immune system, producing antibodies, possessing memory functions for antigens, regulating immune response, mediating cells for virus-infected and tumor cells (Mešťanová & Varga, 2016; Orakpoghenor et al., 2019). Previous study reported that plants exhibit phytochemicals which are able to stimulate the lymphocytes production (Mukherjee et al., 2014; Sriwanthana, et al., 2007). It is also observed in the P2 of this study, showing the stimulation of total leukocytes and leading to a stimulated immune system.

Genitri has phytoconstituents with complex mechanisms that are able to stimulate the immune system (Shukla et al., 2014; Shukla et al. 2022). As a medicinal plant, the bioactive compounds of genitri exhibited various biological activities, including antibacterial, anti-inflammatory, antioxidant, and immunomodulator (Brindha, 2016; Primiani et al., 2021; Primiani et al., 2022; Shukla et al., 2022). The LCMS analysis of genitri revealed the flavonoid in average 1.9 - 2.58% of the total 72 detected bioactive compounds (Primiani et al. 2021). Previous studies mentioned the potency of flavonoids as immunomodulatory agents (Brindha, 2016; Peluso et al., 2015). The mechanism of genitri as anti-inflammatory acts on the production of interleukin IL1β, IL 6 β, IL8 β, and TNFα in the blood cells (Prabowo et al., 2021; Zaragozá et al., 2020). It also prevents the inflammation via COX-1 and COX-2 by suppressing prostaglandin production, lessening the inflammatory cells production (Ribeiro et al., 2015a; 2015b; Qu et al., 2021). Genitri seeds contain terpenoids, dominated by elaeocarpucin which composed 0.7 - 1.45% of compounds. It potentiates to inhibit inflammation allergic reaction by preventing mast cells degranulation (Kim et al., 2018; Primiani et al., 2021). The mechanism of terpenoids able to decrease PGE2, TNFα, NO. IL6 dan IL β secretion from macrophage (Sarkar & Bhattacharjee, 2019).

ACKNOWLEDGMENT

We sincerely acknowledge the financial assistance from the National Innovation Research Agency (BRIN) RIIM Programme and Education Fund Management Institution (LPDP) No. B1468/II.7/FR/10/2022.

CONCLUSION

Treatment with genitri extracts was able to increase the total leukocytes count. The highest leukocytes count observed on the treatment with genitri seed extracts (P2) which successfully increase the total leukocytes to 10595.83 cell/µL. Segmented neutrophil is the most abundant leukocyte observed in all treatments, followed by lymphocytes and monocytes with the percentage 21.6%, 54.5%, and 17%, respectively.

REFERENCES

Afrianti, R., Ramadheni, P., & Irsanti, P N. (2017). Uji aktivitas estrogenik ekstrak etanol jintan hitam (*Nigella sativa* L.) terhadap perkembangan uterus tikus putih betina. *Scientia, 7*(1), 49–55.

Agidew, M. G. (2022). Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. *Bulletin of the National Research Centre, 46*(87), 1-22. https://doi.org/10.1186/s42269-022-00770-8

Ali, S. A., Singh, G., & Datusalia, A. K. (2021). Potential therapeutic applications of phytoconstituents as immunomodulators: Pre-clinical and clinical evidences. *Phytotherapy Research: PTR, 35*(7), 3702–3731. https://doi.org/10.1002/ptr.7068

Alturkistani, H. A., Tashkandi, F. M., & Mohammedsaleh, Z. M. (2015). Histological stains: A literature review and case study. *Global Journal of Health Science, 8*(3), 72-9. https://doi.org/10.5539/gjhs.v8n3p72

Arscott, W. T., Soltys, J., Knight, J., & Mao-Draayer, Y. (2011). Interferon β-1b directly modulates human neural stem/progenitor cell fate. *Brain Research, 1413*, 1–8. https://doi.org/10.1016/j.brainres.2011.07.037

Bhatti, M. Z., Ismail, H., & Khan Kayani, W. (2022). *Plant Secondary Metabolites: Therapeutic Potential and Pharmacological Properties*. IntechOpen. https://doi.org/10.5772/intechopen.103698

Bindu, S., Mazumder, S., & Bandyopadhyay, U. (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochemical Pharmacology, 180*(1), 114147. https://doi.org/10.1016/j.bcp.2020.114147

Bonilla, F. A., & Oettgen, H. C. (2010). Adaptive immunity. *Journal of Allergy and Clinical Immunology, 125*(2), S33-S40.

Borriello, F., Iannone, R., & Marone, G. (2017). Histamine Release from Mast Cells and Basophils. *Handbook of Experimental Pharmacology, 241*, 121–139. https://doi.org/10.1007/164_2017_18

Brindha, P. (2016). Role of phytochemicals as immunomodulatory agents: A review. *International Journal of Green Pharmacy (IJGP), 10*(1), 1-18. https://doi.org/10.22377/ijgp.v10i1.600

Cahyaningsih, R., Magos Brehm, J., & Maxted, N. (2021). Gap analysis of Indonesian priority medicinal plant species as part of their conservation planning. *Global Ecology and Conservation, 26*, e01459. https://doi.org/10.1016/j.gecco.2021.e01459

Hooda, P., Malik, R., Bhatia, S., Al-Harrasi, A., Najmi, A., Zoghebi, K., Halawi, M. A., Makeen, H. A., & Mohan, S. (2024). Phytoimmunomodulators: A review of natural modulators for complex immune system. *Heliyon, 10*(1), e23790. https://doi.org/10.1016/j.heliyon.2023.e23790

Hohakay, J. J., Pontoh, J., & Yudistira, A. (2019). Pengaruh metode pengeringan terhadap kadar flavonoid daun sesewanua (clerodendron squamatum vahl.). *Pharmacon*, *8*(3), 748-757. https://doi.org/10.35799/pha.8.2019.29401

Hultquist, Judd F., Schumann, K., Woo, Jonathan M., Manganaro, L., McGregor, Michael J., Doudna, J., Simon, V., Krogan, Nevan J., & Marson, A. (2016). A Cas9 Ribonucleoprotein Platform for Functional Genetic Studies of HIV-Host Interactions in Primary Human T Cells. *Cell Reports, 17*(5), 1438–1452. https://doi.org/10.1016/j.celrep.2016.09.080

Karasuyama, H., Obata, K., Wada, T., Tsujimura, Y., & Mukai, K. (2011). Newly appreciated roles for basophils in allergy and protective immunity. *Allergy, 66*(9), 1133–1141. https://doi.org/10.1111/j.1398-9995.2011.02613.x

Kelly, M., Hwang, J.M., Kubes, P. (2007). Modulating leukocyte recruitment in inflammation. *Journal of Allergy and Clinical Immunology, 120*(1), 3–10. https://doi.org/10.1016/j.jaci.2007.05.017

Kim, M.-J., Kim, Y.-Y., Choi, Y.-A., Baek, M.-C., Lee, B., Park, P.-H., Shin, T.-Y., Kwon, T. K., Khang, D., & Kim, S.-H. (2018). Elaeocarpusin Inhibits Mast Cell-Mediated Allergic Inflammation. *Frontiers in Pharmacology, 9*(591), 1-9. https://doi.org/10.3389/fphar.2018.00591

Lee, Z.-Y., Tam, J. K. C., & Tran, T. (2021). Corticosteroid use in respiratory viral infections — friend or foe?. *Current Opinion in Physiology*, *22*, 100450. https://doi.org/10.1016/j.cophys.2021.06.004

Liu, J.-K. (2022). Natural products in cosmetics. *Natural Products and Bioprospecting, 12*(40), 1-43. https://doi.org/10.1007/s13659-022-00363-y

Luster, A. D., Alon, R., & von Andrian, U. H. (2005). Immune cell migration in inflammation: present and future therapeutic targets. *Nature Immunology, 6*(12), 1182–1190. https://doi.org/10.1038/ni1275

Mahmoudi, F., Hadi, M., Azadeh Montaseri, Mohamed Khosroshahi, L., & Behzad Baradaran. (2024). Microbial natural compounds and secondary metabolites as Immunomodulators: A review. *International Journal of Biological Macromolecules, 278*(3), 134778–134778. https://doi.org/10.1016/j.ijbiomac.2024.134778

Majka, M., Sułkowski, M., Badyra, B., & Musiałek, P. (2017). Concise Review: Mesenchymal Stem Cells in Cardiovascular Regeneration: Emerging Research Directions and Clinical Applications. *STEM CELLS Translational Medicine, 6*(10), 1859–1867. https://doi.org/10.1002/sctm.16-0484

Manoharan, K., & Chitra, P. C. (2022). Hypoglycemic and Hypolipidemic Activities of Ethanolic Extract of Elaeocarpus Tectorius (Lour.) Poir. Leaves in Streptozotocin- Nicotinamide Induced Diabetic Rats. *Biomedical and Pharmacology Journal, 15*(2), 1167–1178. https://doi.org/10.13005/bpj/2453

Marshall, J.S., Warrington, R., Watson, W., & Kim, H.L. (2018). An introduction to immunology and immunopathology. *Allergy Asthma Clin Immunol, 14*(49), 5-14. https://doi.org/10.1186/s13223-018-0278-1

Mešťanová, V., & Varga, I. (2016). Morphological view on the evolution of the immunity and lymphoid organs of vertebrates, focused on thymus. *Biologia, 71*(10). https://doi.org/10.1515/biolog-2016-0137

Ministry of Agriculture. (2014). *Renstra Kementrian Pertanian Pertanian Tahun 2015 - 2019 (Strategic Plan of Ministry of Agriculture 2015-2019).* Ministry of Agriculture of Republic of Indonesia

Ministry of Agriculture. (2015). *Agricultural Statistics.* Ministry of Agriculture Republic of Indonesia

Ministry of National Development Planning. (2016). *Indonesian Biodiversity Strategy and Action Plan 2015-2020*. Indonesian Government

Mukherjee, P., Nema, N., Bhadra, S., Mukherjee, D., Braga, F., & Matsabisa, M. (2014). Immunomodulatory leads from medicinal plants. *Indian Journal of Traditional Knowledge, 13*(2), 235–256.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature, 403*(6772), 853–858.

Navegantes, K. C., de Souza Gomes, R., Pereira, P. A. T., Czaikoski, P. G., Azevedo, C. H. M., & Monteiro, M. C. (2017). Immune modulation of some autoimmune diseases: the critical role of macrophages and neutrophils in the innate and adaptive immunity. *Journal of Translational Medicine, 15*(1), 36. https://doi.org/10.1186/s12967-017-1141-8

Orakpoghenor, O., Avazi, D. O., Markus, T. P., & Olaolu, O. S. (2019). Lymphocytes: a brief review. *SCIRES Lit, 3*(1), 005-8.

Peluso, I., Miglio, C., Morabito, G., Ioannone, F., & Serafini, M. (2014). Flavonoids and Immune Function in Human: A Systematic Review. *Critical Reviews in Food Science and Nutrition, 55*(3), 383–395. https://doi.org/10.1080/10408398.2012.656770

Prabowo, N. A., Nurudhin, A., & Buanantri, A. C. (2021, January). *The Effect of Moringa Oleifera Leaf Extract on Mean Platelet Volume and Neutrophil-to-Lymphocyte Ratio in Autoimmune Patients.* 4th

International Conference on Sustainable Innovation 2020–Health Science and Nursing (ICoSIHSN 2020) (625-629). Atlantis Press.

Primiani, C. N., Pujiati, P., & Setiawan, M. A. (2021). Phytochemical constituents and antimicrobial activity of Elaeocarpus sphaericus Schum seed extract. *Tropical Journal of Natural Product Research, 5*(10), 1775-1781.

Primiani, C. N., Sari, D. R., Krisnamurti, G. C., Pujiati, P., & Setiawan, M. A. (2022). Anti-Inflammatory Potentials of Elaeocarpus sphaericus Schum Fruit Compounds by Molecular Docking Approach. *Tropical Journal of Natural Product Research, 6*(10), 1663-1669.

Primiani, C. N., Bhagawan, W. S., Pujiati, P., & Sari, D. R. T. (2024). Phytochemical screening, in vitro and in silico antibacterial investigation of Elaeocarpus ganitrus extract. *Jurnal Biota, 10*(1), 1–14. https://doi.org/10.19109/biota.v10i1.20038

Prinyakupt, J., & Pluempitiwiriyawej, C. (2015). Segmentation of white blood cells and comparison of cell morphology by linear and naïve Bayes classifiers. *BioMedical Engineering OnLine, 14*(63), 1-19. https://doi.org/10.1186/s12938-015-0037-1

Qu, T., Gao, Y., Li, A., Li, Z., & Qin, X. (2021). Systems biology analysis of the effect and mechanism of total flavonoids of Astragali Radix against cyclophosphamide-induced leucopenia in mice. *Journal of Pharmaceutical and Biomedical Analysis, 205,* 114357–114357. https://doi.org/10.1016/j.jpba.2021.114357

Rahman, H., Aldi, Y., & Mayanti, E. (2017). Aktifitas Imunomodulator Dan Jumlah Sel Leukosit Dari Ekstrak Kulit Buah Naga Merah (Hylocereus lemairei (Hook.) Britton & Rose) Pada Mencit Putih Jantan. *Jurnal Farmasi Higea, 8*(1), 44–58. https://doi.org/10.52689/higea.v8i1.137

Ribeiro, D., Freitas, M., Tomé, S. M., Silva, A. M., Laufer, S., Lima, J. L., & Fernandes, E. (2015a). Flavonoids inhibit COX-1 and COX-2 enzymes and cytokine/chemokine production in human whole blood. *Inflammation, 38*, 858-870.

Ribeiro, D., Freitas, M., Lima, J. L., & Fernandes, E. (2015b). Proinflammatory pathways: the modulation by flavonoids. *Medicinal research reviews, 35*(5), 877-936.

Salim, A. N., Sumardianto, S., & Amalia, U. (2018). Efektivitas serbuk simplisia biji pepaya sebagai antibakteri pada udang putih (Penaeus merguensis) selama penyimpanan dingin. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *21*(2), 188-198. https://doi.org/10.17844/jphpi.v21i2.22836

Saranraj, P., Sivasakthi, S., & Deepa, M. S. (2016). Phytochemistry of pharmacologically important medicinal plants – A Review. *International Journal of Current Research in Chemistry and Pharmaceutical Sciences, 3*(11), 56–66. https://doi.org/10.22192/ijcrcps.2016.03.11.009

Sarkar, A., & Bhattacharjee, S. (2019). *Terpenoids in Treatment of Immunological Disease*. CRC Press Ebooks. https://doi.org/10.1201/9781351026703-6

Selvakumar, P. M., Rajkumar S, R. J., & MSA, M. N. (2018). Phytochemicals as a potential source for anti-microbial, anti-oxidant and wound healing - a review. *MOJ Bioorganic & Organic Chemistry, 2*(2), 61-70. https://doi.org/10.15406/mojboc.2018.02.0058

Shi, F., Xue, R., Zhou, X., Shen, P., Wang, S., & Yang, Y. (2021). Telitacicept as a BLyS/APRIL dual inhibitor for autoimmune disease. *Immunopharmacology and Immunotoxicology, 43*(6), 666–673. https://doi.org/10.1080/08923973.2021.1973493

Shukla, S., Bajpai, V. K., & Kim, M. (2014). Plants as potential sources of natural immunomodulators. *Reviews in Environmental Science and Bio/Technology, 13*(1), 17–33. https://doi.org/10.1007/s11157-012-9303-x.

Shukla, M. K., Singh, S. K., Pandey, S., Gupta, P. K., Choudhary, A., Jindal, D. K., Dua, K., & Kumar, D. (2022). Potential Immunomodulatory Activities of Plant Products. *South African Journal of Botany*, *149,* 937-943 https://doi.org/10.1016/j.sajb.2022.04.055

Sriwanthana, B., Treesangsri, W., Boriboontrakul, B., Niumsakul, S., & Chavalittumrong, P. (2007). In vitro effects of Thai medicinal plants on human lymphocyte activity. *Songklanakarin Journal of Science and Technology, 29* (Suppl. 1), 17-28.

Strzelec, M., Detka, J., Patrycja Mieszczak, Małgorzata Katarzyna Sobocińska, & Majka, M. (2023). Immunomodulation—a general review of the current state-of-the-art and new therapeutic strategies for targeting the immune system. *Frontiers in Immunology, 14,* 1127704. https://doi.org/10.3389/fimmu.2023.1127704

Sultana, S., & Biswadev Bishayi. (2019). Potential anti-arthritic and anti-inflammatory effects of TNF-α processing inhibitor-1 (TAPI-1): A new approach to the treatment of S. aureus arthritis. *Immunobiology, 225*(2), 151887–151887. https://doi.org/10.1016/j.imbio.2019.11.022

Sun, H., Huo, Y., & Fan, Z. (2021). Editorial: Imaging and Mechanism of Leukocyte Recruitment and Function in Inflammation and Infections. *Frontiers in Cell and Developmental Biology, 9*(690003), 1-5. https://doi.org/10.3389/fcell.2021.690003

Tripathi, Y. C., Shukla, P., & Tewari, D. (2014). Phytochemical Evaluation and Antihyperglycemic Effects Of Elaeocarpus Ganitrus Roxb (Rudraksha) In Streptozotocin Induced Diabetes. *International Journal of Pharmacy and Pharmaceutical Sciences, 7*(1), 280–283.

Turvey, S. E., & Broide, D. H. (2010). Innate immunity. *Journal of Allergy and Clinical Immunology, 125*(2), S24-S32.

Vinay, S. P., Udayabhanu, Sumedha, H. N., Shashank, M., Nagaraju, G., & Chandrasekhar, N. (2021). In-vitro antibacterial, antioxidant and cytotoxic potential of gold nanoparticles synthesized using novel Elaeocarpus ganitrus seeds extract. *Journal of Science: Advanced Materials and Devices, 6*(1), 127–133. https://doi.org/10.1016/j.jsamd.2020.09.008

Zaragozá, C., Villaescusa, L., Monserrat, J., Zaragozá, F., & Álvarez-Mon, M. (2020). Potential Therapeutic Anti-Inflammatory and Immunomodulatory Effects of Dihydroflavones, Flavones, and Flavonols. *Molecules, 25*(4), 1017. https://doi.org/10.3390/molecules25041017

Zhang, X.-H., Tee, L. Y., Wang, X.-G., Huang, Q.-S., & Yang, S.-H. (2015). Off-target Effects in CRISPR/Cas9-mediated Genome Engineering. *Molecular Therapy - Nucleic Acids, 4*(1), e264. https://doi.org/10.1038/mtna.2015.37