International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Immunomodulatory Effect of Songgak: *In-vivo* Experiment.

Lale Budi Kusuma Dewi[1], Ari Khusuma[1*], Agrijanti[1], Lina Sundayani[2], Handa Muliasari[3]

[1]Department of Medical Laboratory Technology, Ministry of Health Polytechnic Mataram, Indonesia.

[2]Department of Midwifery, Ministry of Health Polytechnic Mataram, Indonesia.

[3]Department of Pharmacy, Faculty of Medicine, University of Mataram, Indonesia.

Abstract

This research is a laboratory experimental study to test Songgak as an immunomodulator candidate. Songgak is a term in the Sasak language to refer to potions that can increase stamina. Songgak is coffee added with seven spices: pepper, Javanese chili, nutmeg, cloves, anyang, ules wood, and coriander. Previous research results show that this herb has vigorous antioxidant activity. Antioxidants can reduce oxidative stress associated with inflammation, disease, and neurodegenerative functions to protect immune cells and the immune system. Antioxidants from natural ingredients are often associated with immunomodulators as immunostimulatory and immunosuppressors. Various parameters to measure a natural product as an immunomodulator candidate include lymphocyte proliferation, cytokines IL1, IL6, and TNF-α. This study used Balb/B mice as animal models of ovalbumin-induced inflammation. The study results showed that songgak has the potential to be an immunostimulatory.

Keywords: Songgak, Immunomodulator, Antioxidant, Cytokines

Full length article **Corresponding author's* e-mail: khusumaari@gmail.com

1. Introduction

Songgak is a term in the Sasak to refer to ingredients that can increase stamina. Songgak coffee is coffee that is added with seven types of pepper (Piper nigrum L.), coriander (Coriandrum sativum L.), Javanese chili (Piper retrofractum Vahl), the flesh of nutmeg (Myristica fructus courtex), cloves (Syzygium aromaticum), Anyang and lilit or ules wood fruit (Helicteres isora). Songgak spices have potential as antioxidants, hypolipidemic and hypocholesterolaemia effects, antidiabetic, antibacterial, anticancer, anti-obesity, hepatoprotective [1, 2, 3, 4]. In previous research, the antioxidant activity of songgak was tested using the DPPH method, measuring the total phenolics and flavonoids of songgak. The results of strong antioxidant activity with IC50 of 63.78 ± 4.29, total phenolic Songgak of 26.85 ± 4.29 mgGAE/g, and total flavonoid content of 6.99 ± 0.29 mgRE/gram [5]. Antioxidants reduce oxidative stress associated with atherosclerosis, cancer, aging, inflammation, and disease, as well as neurodegenerative functions to protect immune cells in the immune system [6]. The immune system is directly related to human health because it prevents attacks by foreign objects into the body. Deregulation of the immune response is strongly associated with various inflammatory or degenerative disorders including chronic and acute infections [7]. The research results on various natural ingredients containing antioxidants as immunomodulator candidates are Sternberg taxon's from Turkey [8], durian seeds and flour [9], and 25 types of vegetables, herbs, and spices from Indonesia. *Immunomodulators* are substances or compounds that tend to precisely or non-specifically modulate the function and activity of the immune system [6]. Immunomodulation involves immunostimulant and immunosuppression of certain cellular and/or humoral immune responses [10]. Immunostimulants or immunostimulatory can increase the immune system's ability to fight infection and disease by increasing the activity of immune system components [11]. *Immunosuppressors* are substances used to suppress the activity and function of the immune system. Immunostimulatory treat infectious, inflammatory, or malignant diseases and treat immunodeficiency cases. Immunosuppressors are applied for the treatment of autoimmune diseases such as SLE, rheumatoid arthritis, myasthenia gravis, and post-organ transplantation treatment.

Various parameters were used to test the immunomodulatory effect in vivo and in vitro. Lymphocyte proliferation is widely used in immune function research due to its high sensitivity [6]. The immunomodulatory ability of various essential oils in vitro was also carried out by measuring inflammatory parameters, including ROS, PMNs (macrophages), proliferation of T-cells, B cells, IL-2, and the cytokine TNF-α [7]. This research was conducted to test the songgak herb as a candidate immunomodulator by measuring lymphocyte proliferation, IL1, IL6, and the cytokine TNF-α. This study aims to examine the in vivo immunomodulatory effects of Songgak.

2. Materials and Methods

This research has passed the ethical test permit No: LB.01.03/6/235/2023 by Poltekkes Kemenkes Mataram. This study aimed to measure IL1, IL6, TNFα, and lymphocyte proliferation in experimental animals. The in-vivo inflammation model used in this study is the ovalbumin model. IL6, together with TNFα and IL-1β, is a general marker of inflammation; its levels can be increased in several inflammatory diseases.

ovalbumin model is the most common mouse allergic airway inflammation/inflammation model [12]. IL-6 and TNF are important inflammatory mediators in the pathogenesis of asthma [13]. IL1β regulates IL6, so an increase in IL6 also marks an increase in IL1β activity [14].

There were 30 experimental animals given ovalbumin on the first day and eighth days, and they were divided into five treatment groups with six replications in each group. Group 1, as the control group, was given distilled water; group 2 was given cyclosporine at a dose of 6.5 mg/KgBW; group 3 was given levamisole at a dose of 2.5 mg/KgBW; group 4 was given songgak at a concentration of 8%. Moreover, group 4 was given a 12% stomp. Experimental animal treatment was carried out at the Nutrition Laboratory, Gajah Mada University; measurements of IL1, IL6, and TNFα were carried out at the Biotechnology Laboratory, and measurements of lymphocyte proliferation were carried out at the FKKMK Parasitology Laboratory at Gajah Mada University.

3. Results and Discussions

The average results of IL1β, IL6, and TNFα measurements show almost the same values. The results of IL1β, IL6, and TNFα measurements were carried out using the ANOVA statistical test. The test results showed no effect of various treatment groups on IL1, IL6, and TNFα levels. Ovalbumin administration can increase IL1β, IL6 and TNFα [14] [15]. In this study, songgak was tested as an anti-inflammatory. There was no difference in IL1β, IL6, and TNFα values between group 1 and groups 4 and 5, so it can be concluded that songgak does not have an anti-inflammatory effect.Analysis of clinical features in asthma shows that the group of people with more severe asthma is influenced by age and obesity. The association with age and obesity raises the possibility that systemic inflammation is joint in obesity because increased production of IL1β, IL6, or TNFα by adipose tissue can impact the airway function of asthmatics [14]. In this study, the body weight of the experimental animals was also measured during treatment. Body weight was measured four times before acclimatization: on the first day, the 8th day, and the 14th day. The average increase in body weight of the experimental animals in each group is presented in graphical form in Figure 1. OD value of ech group treatment if given in figure 2.

There was an increase in body weight in all experimental animals. In groups 2, 3, 4, and 5, the body weight of the experimental animals at the beginning and end was the same. In group 1, the body weight gain was the least compared to groups 2, 3, 4, and 5. The average body weight of the experimental animals did not differ, indicating clinical conditions that were not different per the study's results. IL1β, IL6, and TNFα tests were not significantly different. Significant in each group. Lymphocyte proliferation and differentiation play an essential role in the stages of the immune response. Lymph is the largest immune organ in the human body, which contains many lymphocytes. T cells are mainly responsible for cellular immunity, and B cells are responsible for humoral immunity. The relative proportion of lymphocytes in the lymph is usually within a specific range under normal circumstances, but this changes with exogenous stimulation. When antigens stimulate the host, lymphocytes are immediately activated and begin proliferating and differentiating [16]. Data on lymphocyte proliferation OD values were tested for normality using the Shapiro-Wilk statistical test. The results of the Shapiro-Wilk test showed that the data groups that were not normally distributed were group 1 (0.008), group 2 (0.001), and group 3 (0.013). The data was not normally distributed, so the test was continued with a non-parametric statistical test, namely the Kruskal Walis test. The test results showed a probability value of $0.001<0.05$, so it was concluded that there was an effect of giving Songgak herb as an immunostimulant.

The OD value of lymphocyte proliferation in group 2 was lower than in group 1. Group 1 was a group of experimental animals induced by ovalbumin and given distilled water. Group 2 was the group given the immunosuppressant cyclosporine. The administration of cyclosporine immunosuppressant decreased lymphocyte proliferation, with the lowest OD value indicating that the stimulation of lymphocyte cell proliferation was inhibited. Cyclosporine is an immunosuppressive agent that can attenuate T-cell activation by interfering with activated T-cell calcineurin-nuclear factor (NFAT) signal transduction. Cyclosporine can decrease NFAT-dependent IL-2 gene expression, directly affecting T cell expansion and function (17). Levamisole is an immunostimulant. Levamisole can increase the lymphocyte proliferation response [18]. Group 3 was the group given levamisole. The effect of levamisole as an immunostimulator increased the lymphocyte proliferation response compared to group 1. The OD value in group 3 (0.117) is no different from the OD value in group 5 (0.124), indicating the presence of lymphocytes. The proliferation response in group 3 was the same as in group 5, which was given coffee—Songgak with a concentration of 12%. The highest OD value was in group 4 by administering songgak at a concentration of 8%, indicating that the maximum dose of songgak coffee was used to stimulate lymphocyte proliferation. Lymphocyte proliferation can be affected by exposure to flavonoids [19]. The results of the 2021 research show that Songgak coffee contains 6.99 mgRE/gram of flavonoids in the liquid extract. As a single ingredient, the immunomodulatory effect of Songgak coffee has also been widely reported, including Coriandrum sativum L as an anti-inflammatory and immunostimulant [20] and Piper retrofractum Vahl as an immunostimulant [20]. Songgak is an example of non-pharmacological therapy that can improve the body's immune system [1].

Table 1. Results of IL1 measurements in experimental animals

	R1	R2	R3	R4	R5	R6	Average	SD
KLP 1	1.375	1.308	1.353	1.000	1.225	1.254	1.254	± 0.136
KLP 2	1.310	1.357	1.555	1.454	1.407	1.443	1.421	± 0.085
KLP 3	1.595	1.334	1.483	1.368	1.476	1.364	1.437	± 0.099
KLP 4	1.490	1.473	1.470	1.393	1.404	1.358	1.431	± 0.053
KLP 5	1.377	1.487	1.548	1.168	0.989	1.188	1.293	± 0.214

Table 2. Results of IL6 measurements in experimental animals

	R1	R2	R3	R4	R5	R6	Average	SD
KLP 1	1.128	0.630	1.191	1.382	1.239	1.151	1.120	± 0.256
KLP 2	1.167	1.271	1.291	1.409	1.366	1.323	1.304	± 0.084
KLP 3	1.336	0.877	1.294	1.267	1.236	1.043	1.175	± 0.178
KLP 4	1.165	1.071	1.115	1.067	1.172	1.131	1.120	± 0.045
KLP 5	1.177	1.156	1.346	1.143	1.086	1.165	1.179	± 0.088

Table 3. TNFα measurement results in experimental animals

	R1	R2	R3	R4	R5	R6	Average	SD
KLP 1	1.123	1.194	1.314	1.261	1.089	1.087	1.178	± 0.095
KLP 2	1.071	1.169	1.383	1.192	1.182	1.141	1.190	± 0.104
KLP 3	0.913	1.149	1.261	1.174	1.258	1.148	1.150	± 0.127
KLP 4	1.185	1.234	1.162	1.051	1.164	1.063	1.143	± 0.072
KLP 5	1.132	1.242	1.352	1.156	0.908	1.008	1.133	± 0.159

Table 4. Optical Density (OD) values in measuring lymphocyte proliferation in experimental mice

	R1	R2	R3	R4	R5	R6	Average	SD
KLP 1	0.220	0.083	0.112	0.079	0.104	0.091	0.115	± 0.053
KLP 2	0.070	0.068	0.063	0.070	0.067	0.116	0.076	± 0.020
KLP 3	0.103	0.089	0.098	0.098	0.122	0.192	0.117	± 0.038
KLP 4	0.291	0.289	0.373	0.376	0.216	0.341	0.314	± 0.061
KLP 5	0.068	0.100	0.212	0.093	0.082	0.189	0.124	± 0.061

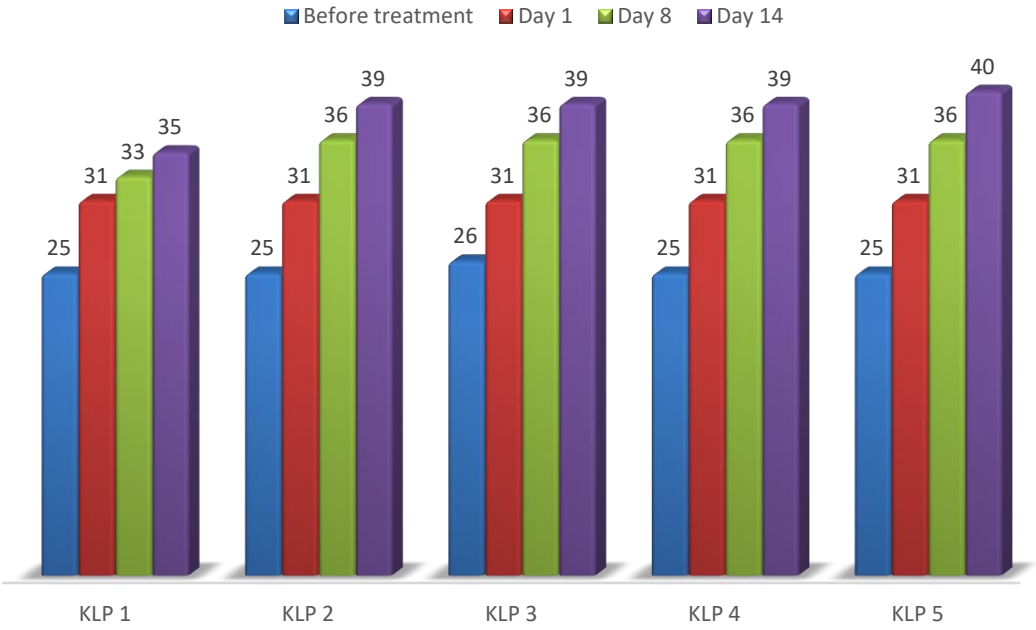

Figure 1: Body weight of experimental mice

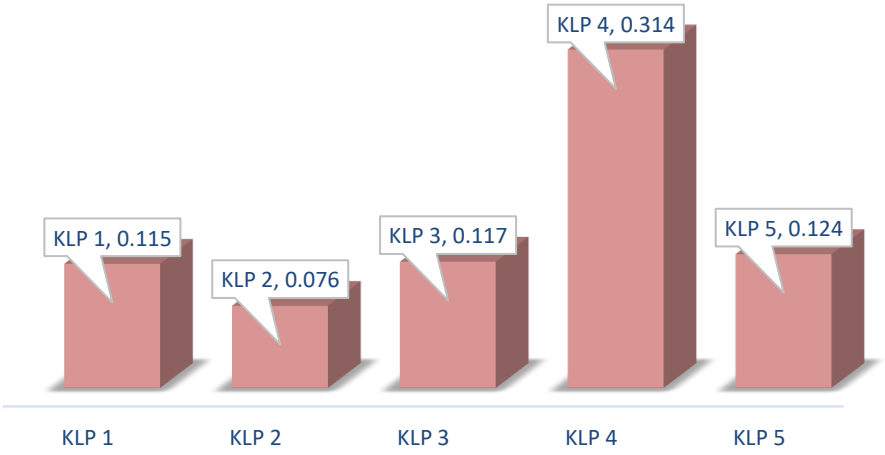

Figure 2: OD Value of each group treatment

4. Conclusions

This study strengthens our belief that songgak is a safe and valuable immunomodulator for the immune system. The immunomodulatory test via lymphocyte proliferation statistically has an effect. The highest proliferative activity was obtained at a concentration of 8%. This research has proven songgak ability as an immunomodulator. gest consuming songgak once a day according to the dosage, which can improve the body's immune system.

References

[1] Z. Zarai, E. Boujelbene, N.B. Salem, Y. Gargouri, and A. Sayari. (2013). Antioxidant and antimicrobial activities of various solvent extracts, piperine and piperic acid from Piper nigrum. LWT - Food Science and Technology. 50(2): 634–641. doi: 10.1016 /j.lwt.2012.07.036.

[2] K.J. Kim, M.S. Lee, K. Jo, and J.K. Hwang. (2011). Piperidine alkaloids from Piper retrofractum Vahl. protect against high-fat diet-induced obesity by regulating lipid metabolism and activating AMP-activated protein kinase. Biochemistry and Biophysics Research Communications. 411(1): 219–225. doi: 10.1016/j.bbrc.2011.06.153.

[3] D.F. Cortés-Rojas, C.R.F. de Souza, and W.P. Oliveira. (2014). Clove (Syzygium aromaticum): A precious spice. Asian Pac.ific Journal of Tropical Biomedicne. 4(2): 90–96. doi: 10.1016/S2221-1691(14)60215-X.

[4] P. Jaiswal, P. Kumar, V.K. Singh, and D.K. Singh. (2015). Biological effects of Myristica fragrans.

Annual Reviews of Biomedical Sciences. 11: 21–29. doi: 10.5016/1806-8774.2009v11p21.

[5] L.B.K. Dewi, A. Khusuma, H. Muliasari, and H. Widodo. (2021). Antioxidant Properties Of 'Songgak', A Sasakness Traditional Herbal Tea In Lombok, Indonesia in PROGRAM & ABSTRACT BOOK The 4thInternational Conference on Bioscience and Biotechnology. (ICBB2021), 4: 108.

[6] N. Safriani, F.Z. Rungkat, N.D. Yuliana, and E. Prangdimurti. (2021). Immunomodulatory and Antioxidant Activities of Select Indonesian Vegetables, Herbs, and Spices on Human Lymphocytes. International Journal of Food Science. 1-12. doi: 10.1155/2021/6340476.

[7] I.E. Orhan, M.A. Mesaik, A. Jabeen, and Y. Kan. (2016). Immunomodulatory properties of various natural compounds and essential oils through modulation of human cellular immune response. Industrial Crops and Products. 81: 117–122. doi: 10.1016/j.indcrop.2015.11.088.

[8] A.C. Ağca, A.N.Y. Ekici, S.Y. Sarıaltın, T. Çoban, G.S. İşcan, and B.S. Yılmaz. (2021). Antioxidant, anti-inflammatory and antidiabetic activity of two Sternbergia taxons from Turkey. South African Journal of Botany. 136: 105–109. doi: 10.1016/j.sajb.2020.04.002.

[9] N. Charoenphun and W.K. Klangbud. (2022). Antioxidant and anti-inflammatory activities of durian (Durio zibethinus Murr.) pulp, seed and peel flour. PeerJ. 10: 1–15. doi: 10.7717/peerj.12933.

[10] M.R. Khazdair, Z. Gholamnezhad, R. Rezaee, and M.H. Boskabady. (2021). A qualitative and quantitative comparison of Crocus sativus and Nigella sativa immunomodulatory effects. Biomedicine and Pharmacotherapy. 140: 111774. doi: 10.1016/j.biopha.2021.111774.

[11] M. Martinus, T. Agustin, A.S. Dachlan, and E.H. Effendi. (2019). Penggunaan Imunostimulan Dalam Bidang Dermatovenereologi. Indonesian Journal of Dermatology and Veteronology. 46(2): 111–115. doi: 10.33820/mdvi.v46i2.65.

[12] M. Rincon and C.G. Irvin. Role of IL-6 in asthma and other inflammatory pulmonary diseases. International Journal of Biological Sciences. 8(9): 1281–1290. doi: 10.7150/ijbs.4874.

[13] E.O. Gubernatorova, O.A. Namakanova, E.A. Gorshkova, A.D. Medvedovskaya, S.A. Nedospasov, and M.S. Drutskaya. (2021). Novel Anti-Cytokine Strategies for Prevention and Treatment of Respiratory Allergic Diseases. Frontiers in Immunology. 12: 1–9 doi:10.3389/fimmu.2021. 601842.

[14] B.N. Lambrecht, H. Hammad, and J.V. Fahy. (2019). The Cytokines of Asthma. Immunity. 50(4): 975–991. doi: 10.1016/j.immuni.2019.03.018.

[15] A. Xin, Y. Cheng, S.A. Alharbi, and C. Zhang, "Flavokawain mitigates ovalbumin (OVA)-induced asthma by regulating NLRP3 inflammasome and inflammatory markers: In-vitro and in-vivo models. Arabian Journal of Chemistry. 16(8): 104971. doi: 10.1016/j.arabjc.2023.104971.

[16] Y. Yiqi, W. Xiaowan, Y.U. Wanlin, H. Lihua, and W. U. Yunshan. (2022). Regulating effects of triptriolide on the proliferation and apoptosis of splenic lymphocyte in vivo and in vitro. Journal of Holistic Integrative Pharmacy. 3(2): 95–105. doi: 10.1016/ S2707-3688(23)00051-1.

[17] Dobutr T, Kantamala W, Phimwapi S, et al. (2022). The effects of edible bird's nest on T-lymphocyte proliferation, secondary lymphoid organs, and interleukin-2 production. Journal of Functional Foods. 90: 104977. doi: 10.10 16/j.jff.2022.104977.

[18] T.A. Sujono, A. Nurrochmad, E. Lukitaningsih, and A.E. Nugroho. (2022). Aktivitas Imunomodulator Ekstrak Petroleum Eter Umbi Bengkoang (*Pachyrhizus erosus*) pada Mencit Balb/c yang Diinduksi Vaksin Hepatitis B. Journal of Pharmaceutical Science and Clinical Research. 7(2): 162. doi: 10.20961/jpscr.v7i2.56212.

[19] F. Ioannone, C. Miglio, A. Raguzzini, and M. Serafini. (2013). Flavonoids and immune function. Woodhead Publishing Limited. doi: 10.1533/97 80857095749.3.379.

[20] S.D. Kothalawala *et al.*, (2020). Immunomodulatory Activity of a Traditional Sri Lankan Concoction of Coriandrum sativum L. And Coscinium fenestratum G. Evidence-based Complementary and Alternative Medicine. 2020. doi: 10.1155/2020/9715060.

[21] H. Heriyanto, B.A. Nugraha, and E. Hariadi. (2022). Red Ginger Stew Lowers Blood Pressure in Elderly With Hypertension In Bengkulu City. Proceeding B-ICON. 1(1): 217–225. doi:10.33088/ bicon.v1i1.38.