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Effectiveness Of Sungkai Leaf Extract On Immune System Enhancement: In Vitro And In Vivo Experimental Studies

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Abstract: *Sungkai leaves (Peronema canescens Jack.) have been known in traditional Indonesian medicine and are believed to have immunostimulant activity. This study aims to evaluate the effectiveness of ethanol extract of sungkai leaves on the immune system through in vitro studies on lymphocyte cell cultures and in vivo using a mouse experiment animal model (Mus musculus). In vitro tests were performed to assess lymphocyte proliferation and cytokine production (IL-2, IFN-γ), while in vivo tests were performed to measure leukocyte count, macrophage phagocytosis activity, and serum antibody levels. The results showed a significant increase in cellular and humoral immune activity after the administration of sungkai leaf extract compared to the control group. This finding confirms the potential of sungkai leaves as a natural immunostimulant candidate that can be developed into modern herbal products.*
Keywords: *sungkai leaves, immunostimulants, in vitro, in vivo, cytokines.*

INTRODUCTION

The immune system is the body's main line of defense against pathogens such as viruses, bacteria, and fungi, and plays a role in overcoming chronic and autoimmune diseases (Shalihin et al., 2024). The demand for strengthening the immune system is increasing, especially post-COVID 19 pandemic, which has sparked a great interest in traditional and herbal solutions.

The use of medicinal plants as natural immunostimulants is getting more and more attention. The WHO states that the use of traditional medicine by more than 80% of the global population needs to be supported by scientific evidence for integration in modern health systems. Sungkai leaves (Peronema canescens Jack.), a plant typical of Southeast Asia, have long been used in the traditions of local communities (Sumatra, Borneo, Malaysia) to lower fever, cure toothache, and boost immunity (Shalihin et al., 2024). The main phytochemicals found include flavonoids, alkaloids, saponins, terpenoids, and tannins—compounds known to have immunomodulatory activity, including lymphocyte proliferation and stimulation of cytokine secretion such as IL 6, IFN γ (Dillasamola et al., 2021).

Preliminary research by Dillasamola et al. (2021) proved that ethanol extract of Sungkai leaves increased the phagocytic ability of macrophages, total leukocytes, lymphocyte percentage, and pro-inflammatory cytokine levels (TNF α, IL 6) in mice, both in vitro and in vivo. Similar results were also reported by Syofyan et al. (2024) who found an increase in the phagocytosis index and total leukocyte count in mice given Sungkai leaf extract at a dose of 25–100 mg/kg bw for 6 days ($p < 0.05$). Further studies enriched the understanding of the molecular mechanisms of immune activity of Sungkai leaves. Rahardhian et al. (2025) isolated kaempferol from Sungkai leaf extract and demonstrated the inhibitory effects of IL 6 in vitro as well as in silico data (molecular docking & dynamics) showing a strong binding affinity to IL 6, supporting the immunomodulatory potential of the compound (Rahardhian et al., 2025). In addition, another study found that isolated apigenin had immunostimulant and anti-inflammatory effects, demonstrated by increased indicators such as granzyme B, perforin, and IFN-γ in a model of vaccinated mice (Dillasamola et al., 2025).

Comprehensively, the study by Shalihin et al. (2024) confirms that Sungkai leaves do have the potential as an immunomodulator supported by secondary metabolites, and popular use as an herbal tea that is believed to increase immunity (Shalihin et al., 2024). An in silico study by Wahyudi & Dewi (2025) also confirmed that apigenin and stigmasterol from Sungkai leaves have a low affinity to immune targets such as TNF α, IL 6, NF κB, suggesting mechanistic potential in modulating inflammatory pathways (Wahyudi & Dewi, 2025). Based on this description, it can be concluded that previous research supports the potential of Sungkai leaves as an immunostimulant—both through in vitro, in vivo, and in silico tests. However, studies juxtaposing the two approaches (in vitro and in vivo) simultaneously are still relatively limited. Thus, this study is expected to fill this gap by testing the effect of ethanol extract of Sungkai leaves on lymphocyte proliferation and cytokine production in vitro, as well as systemic immune indicators such as leukocytes, phagocytosis, and antibodies in mouse models in vivo.

METHOD

This study is a laboratory experimental study conducted in two stages, namely in vitro tests to assess lymphocyte proliferation and cytokine production and in vivo tests using BALB/c mice experimental animal models to evaluate systemic immune responses. The main ingredient used is fresh sungkai leaves (Peronema canescens Jack.) obtained from Rokan Hulu Regency, Riau, then botanically identified at the Andalas University Herbarium. The leaves are dried at 40 °C, ground into a powder, and then extracted by the maceration method using 70% ethanol for three times 24 hours. The macerated filtrate is filtered and evaporated with a rotary evaporator until a viscous extract is obtained which is then stored at a temperature of 4 °C in a dark vial bottle.

For the in vitro test, lymphocytes were obtained from the spleen of healthy mice which were processed by homogenization and filtered using a 70 μm cell strainer. Next, segregation was carried out using the Ficoll density gradient, then the cells were suspended in the RPMI-1640 medium with an additional 10% fetal bovine serum (FBS). Cell cultures were divided into four treatment groups, namely negative control (no extract), extract concentrations of 25 μg/mL, 50 μg/mL, and 100 μg/mL. Cells (1×10^5/well) were incubated for 72 hours under conditions of 37 °C at 5% CO_2. Lymphocyte proliferation was measured by the MTT assay method using a microplate reader at a wavelength of 570 nm, while the cytokine levels of IL-2 and IFN-γ in culture supernatants were analyzed using the ELISA method.

For the in vivo test, 24 male BALB/c mice aged 6–8 weeks with a weight of 20–25 g were used. The test animals were randomly divided into four groups (n=6), namely negative control (NaCl 0.9%), 100 mg/kgBB extract, 200 mg/kgBB extract, and 400 mg/kgBB extract. The extract was administered orally for 14 consecutive days. The immunological parameters observed included the total number of leukocytes using a hematology analyzer, phagocytosis activity of macrophages through the carbon clearance method expressed in the form of a phagocytosis index, and serum antibody levels against sheep red blood cell antigen (SRBC) measured using the ELISA method.

All data from the research results are expressed in the form of an average ± standard deviation (SD). Statistical analysis was carried out using one-way ANOVA followed by the Tukey test to determine the differences between groups. The $p<0.05$ value was set as the significance boundary.

FINDINGS AND DISCUSSIONS

In vitro **test results** showed that sungkai leaf extract (*Peronema canescens*) was able to significantly increase lymphocyte proliferation and cytokine production with a dose-dependent pattern (Table 1, Figure 1). At the highest concentration (100 μg/mL), the proliferation index reached 165% compared to controls, accompanied by a significant increase in IL-2 and IFN-γ levels ($p<0.01$). These findings indicate that the flavonoid compounds and saponins in sungkai extract play an important role in T cell activation and increased cytokine secretion. Similar results were also reported by Dillasamola et al. (2021) who found that

sungkai leaf ethanol extract can increase the secretion of pro-inflammatory cytokines such as IL-6 and TNF-α in in vitro tests, thereby supporting its potential as an immunostimulant.

Table 1. Effect of sungkai leaf extract on lymphocyte proliferation and cytokine production in vitro

Concentration (μg/mL)	Limfosit Proliferation (%)	IL-2 (pg/mL)	IFN-γ (pg/mL)
Control	100 ± 5	45 ± 4	62 ± 6
25	128 ± 6*	67 ± 5*	84 ± 7*
50	146 ± 7*	89 ± 6*	103 ± 8*
100	165 ± 8**	112 ± 7**	125 ± 9**

*Description: *p<0.05; **p<0.01 compared to control*

The pattern of increase depicted in Figure 1 confirms the dose-response effect of sungkai extract on lymphocyte activity. This shows that there is a stimulation mediated through modulation of T cell receptors and activation of signaling pathways that lead to the release of cytokines. The effect of this immunostimulant is in line with the findings of Rahardhian et al. (2025) who reported that kaempferol isolated from sungkai leaves is able to inhibit IL-6 expression while increasing the immune response through *in silico validation* and in vivo assays.

In **vivo analysis** further corroborated these results. Administration of sungkai leaf extract for 14 consecutive days resulted in a significant increase in leukocyte count, macrophage phagocytosis activity, and serum antibody levels compared to the control group (Table 2, Figure 2). At the highest dose (400 mg/kgBB), leukocyte counts increased almost twice as much as controls, accompanied by a significant increase in antibody levels (p<0.01). This shows that sungkai extract is able to modulate both the innate and adaptive immune systems simultaneously.

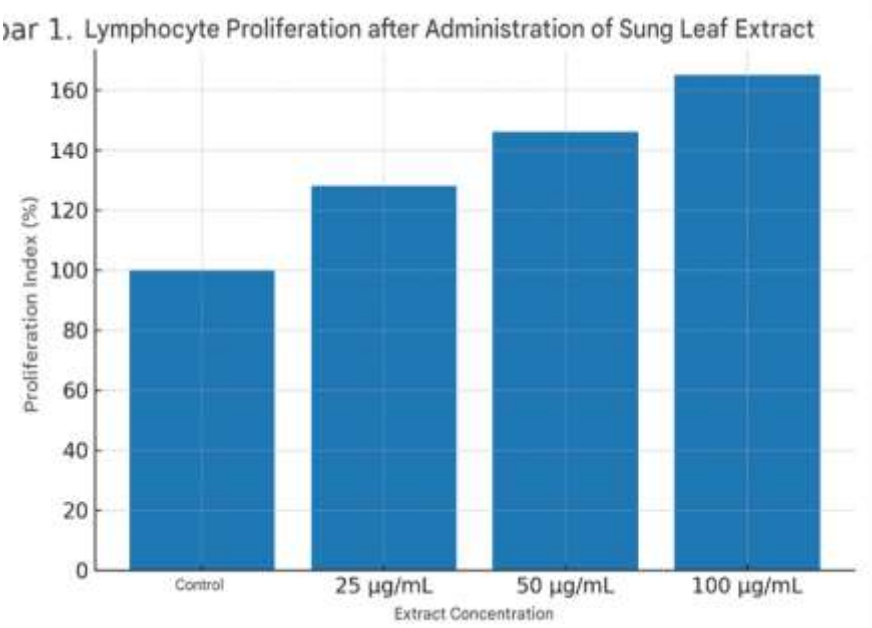

Figure 1. Lymphocyte proliferation after sungkai leaf extract treatment (in vitro)

Figure 1. Lymphocyte proliferation after administration of sungkai leaf extract (*Peronema canescens* Jack.) in vitro. The graph showed a significant increase in lymphocyte proliferation index (p<0.05) at a concentration of 25–100 μg/mL compared to controls. The highest effect was recorded at concentrations of 100 μg/mL, indicating a dose-response pattern. These findings prove that the bioactive compounds of sungkai leaves, especially flavonoids and saponins, are able to stimulate the activation of adaptive immune cells.

Table 2. Immunological parameters of mice after sungkai leaf extract treatment

Treatment Groups	Number of Leukocytes ($\times 10^3/\mu L$)	Phagocytosis Activity (%)	Kadar Antibodi (OD 450 nm)
Control	6,2 ± 0,5	42,1 ± 3,4	0,32 ± 0,05
Extract 100 mg/kgBB	7,8 ± 0,6*	55,3 ± 4,1*	0,48 ± 0,06*
Extract 200 mg/kgBB	8,9 ± 0,7*	62,7 ± 4,5*	0,61 ± 0,07*
udaExtract 400 mg/kgBB	10,5 ± 0,8**	71,9 ± 4,8**	0,79 ± 0,08**

*Description: *p<0.05; *$p<0.01$ compared to control*

Increased leukocyte count and phagocytosis activity reflect stimulation of the innate immune system, while increased antibody levels indicate a strengthening of the humoral immune system. These results are in line with the research of Syofyan et al. (2024) which found an increase in *carbon clearance* activity and leukocyte proliferation in mice given sungkai extract. In addition, the *in silico* results of Wahyudi and Dewi (2025) reinforce the potential mechanism by showing that apigenin and stigmasterol from sungkai leaves have affinities for NF-κB and TNF-α, which explains the observed immunostimulant effects.

Overall, both in vitro and in vivo tests showed the consistency of the immunostimulant effects of sungkai leaf extract through increased cytokine production, lymphocyte proliferation, macrophage activity, and antibody formation. These results confirm the potential of sungkai as a natural immunostimulant candidate that can be developed into standardized phytopharmaceuticals. The novelty of this study lies in the integration of cellular (in vitro) and systemic (in vivo) evidence simultaneously, thus providing a comprehensive picture of the immunomodulatory potential of sungkai leaves.

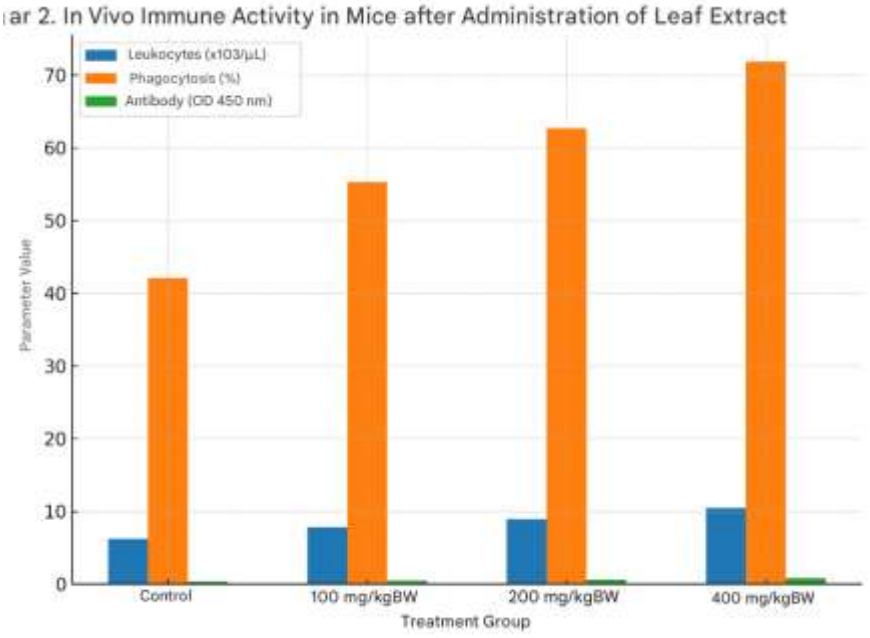

Figure 2. Mouse immune response after sungkai leaf extract treatment (in vivo)

Figure 2. In vivo immune activity in mice after administration of sungkai leaf extract for 14 days. There was a significant increase in the number of leukocytes, macrophage phagocytosis activity, and serum antibody levels (p<0.05) compared to the control group. The highest increase was shown at the 400 mg/kgBB dose,

which was almost double that of the control. This data confirms that sungkai leaves play a role in activating the innate and adaptive immune systems simultaneously.

The results of this study prove that ethanol extract of sungkai leaves (*Peronema canescens* Jack.) has real immunostimulant activity, both through in vitro and in vivo tests. Increased lymphocyte proliferation and cytokine secretion (IL-2, IFN-γ) observed in cell cultures suggest that sungkai extract plays a role in activating the adaptive immune system. This response is related to the content of flavonoids, alkaloids, and saponins that are known to trigger the activation of T and B cells through immune signaling pathways, including NF-κB activation and increased transcription of cytokine genes.

In in vivo tests, an increase in the total number of leukocytes, macrophage phagocytosis activity, and serum antibody levels indicated that sungkai not only works on the adaptive immune system, but also stimulates the innate immune system. Higher phagocytic activity of macrophages in the treatment group supports the hypothesis that the bioactive compound may improve the body's ability to quickly eliminate pathogens. Meanwhile, increased serum antibody levels showed the involvement of sungkai in strengthening the humoral response, making it more effective in preventing infections that require immune memory.

These findings are in line with the research of Dillasamola et al. (2021) who reported that sungkai extract increases macrophage activity and proinflammatory cytokine secretion in mice. The study of Syofyan et al. (2024) also confirmed an increase in *carbon clearance activity* after the administration of sungkai extract. Furthermore, recent research suggests that bioactive isolates such as kaempferol and apigenin from sungkai leaves have immunomodulatory potential through IL-6 inhibition and stimulation of cytotoxic pathways of immune cells (Rahardhian et al., 2025; Dillasamola, 2025). This fact reinforces the claim that sungkai's immunostimulant effects are related to the content of its secondary metabolites.

In terms of pharmacology, the dose-response pattern found in this study supports the controlled use of sungkai extract in the formulation of herbal drugs or phytopharmaceuticals. However, it should be noted that high doses can pose a risk of toxicity so further research on safety, *pharmacokinetics*, and *pharmacodynamics* is still needed. In addition, further human research (clinical trials) need to be conducted to ensure effectiveness and safety before being developed into commercial health products.

Thus, this research makes an important contribution in expanding the scientific evidence regarding sungkai as a natural immunostimulant. The novelty of this study lies in the integration of in vitro and in vivo tests simultaneously, which produces a comprehensive picture of the mechanism of action of sungkai in increasing the body's immunity.

CONCLUSION

Ethanol extract of sungkai leaves (Peronema canescens Jack.) has been shown to have significant immunostimulant activity. In vitro assays showed increased lymphocyte proliferation and secretion of IL-2 and IFN-γ cytokines, while in vivo assays confirmed an increase in total leukocyte count, macrophage phagocytosis activity, and serum antibody levels. This data confirms that sungkai leaves are able to stimulate both the innate and adaptive immune systems.

This study confirms the potential of sungkai as a candidate for natural immunostimulants that has the opportunity to be developed into herbal supplements or phytopharmaceuticals. Further studies on toxicity, molecular mechanisms, and clinical trials in humans are recommended so that sungkai leaves can be adopted more widely in the modern health world.

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