International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 17, Special Issue 1, 2025

Original Article

IMMUNOMODULATORY ACTIVITY OF ACTIVE ISOLATES OF SUNGKAI LEAF (*PERONEMA CANESCENS* JACK.): IN SILICO STUDY

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Received: 27 Sep 2024, Revised and Accepted: 20 Nov 2024

ABSTRACT

Objective: The immune system is a complex network of cells, tissues, and organs that work together to defend the body from attack by foreign organisms such as bacteria, viruses, parasites, and fungi. Some natural medicines have been known to have activity as immunomodulators. One of them is Sungkai leaf (*Peronema canescens* Jack).

Methods: In this study, in silico testing was carried out between several active isolate compounds of sungkai leaves and proteins related to the immune system, namely Interleukin-6 (IL-6), Tumor Necrosis Factor-α (TNF-α), Nuclear Factor-κB (NF-κB), Toll-Like Receptor4 (TLR4), and Interleukin-1β (IL-1β).

Results: From in silico testing of apigenin, Bis(2_ethylhexyl)_phthalate and stigmasterol compounds isolated from Sungkai leaves, it is known that Apigenin and Stigmasterol work very well on TNF-α, IL-6, NF-κB, and IL-1β proteins because they have low-affinity energy. However, the three compounds have a high enough affinity energy to bind to the TLR4 protein, so they do not have the potential as immunomodulatory compounds.

Conclusion: From these results, it can be concluded that apigenin and stigmasterol have good potential as candidate immunomodulatory compounds with an inflammatory reaction mechanism through the NF-κB signaling pathway.

Keywords: In silico, Molecular docking, TNF-alpha, IL6, *Peronema canescens* Jack, Stigmasterol

DOI: https://dx.doi.org/10.22159/ijap.2025.v17s1.10 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

The immune system is the body's essential defense against various pathogens, such as bacteria, viruses and parasites. The main function of the immune system is to recognize and eliminate foreign agents that enter the body, and maintain the balance of homeostasis [1, 2]. Under some conditions, the immune response can be impaired or hyperactivated, which can lead to various autoimmune diseases or other health disorders. Inflammatory reactions are activated by proinflammatory mediators such as Interleukin-1 (IL-1), TNF-α, IL-6, gamma-interferon (IFN-γ) [3]. As a counterbalance, the inflammatory reaction is inhibited by anti-inflammatory mediators, such as Interleukin-4 (IL-4), Interleukin-10 (IL-10), and Transforming Growth Factor Beta-1 (TGF-β-1) [4].

The immune response can be controlled with an agent called an immunomodulator. Therefore, the development of immunomodulatory agents, which can regulate or modify immune responses, is an important focus in modern pharmacology and immunology research [1, 5]. Stigmasterol, one of the phytosterols present in various plants, has been recognized to have a wide range of biological activities, including anti-inflammatory, anticancer, and immunomodulatory activities [6]. Immunomodulators, compounds that modify immune system responses, are critical in addressing a growing range of health challenges. The increasing prevalence of autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis, underscores the urgency for new therapeutic approaches. These conditions arise from dysregulated immune responses that attack the body's own tissues, leading to chronic inflammation, tissue damage, and significant morbidity. The burden of autoimmune diseases is rising globally, driven by factors such as Environmental Changes, Increased exposure to pollutants, dietary shifts, and reduced microbial diversity due to urbanization may contribute to immune dysregulation.

Moreover, immunomodulatory agents have broader applications beyond autoimmunity. They are crucial in managing chronic inflammatory diseases, such as inflammatory bowel disease and asthma, and hold potential for improving outcomes in infectious diseases and cancer immunotherapy. The ability to fine-tune immune responses, whether by dampening overactive pathways or enhancing insufficient ones, makes immunomodulators a cornerstone of modern medicine. Natural compounds, like Apigenin, offer promising leads for immunomodulatory therapies. Their ability to target key signaling pathways, such as NF-κB, while potentially having fewer side effects compared to synthetic drugs, makes them attractive candidates. Furthermore, the exploration of natural agents aligns with the increasing focus on sustainable and holistic approaches to drug.

One natural source rich in stigmasterol is Sungkai leaves (Peronema canescens Jack.), which has long been used in traditional medicine in Indonesia. Sungkai is a tropical tree species found in Southeast Asia, particularly in Indonesia and Malaysia. The plant belongs to the Lamiaceae family and is traditionally used in traditional medicine for various purposes, such as treating fever, increasing stamina, and curing skin diseases [7, 8]. This plant has known bioactivities as antimalarial, antibacterial, antidiabetic, anti-inflammatory, anticancer, and antihyperuricemia [9]. We have reported studies related to the immunomodulatory activity of Sungkai leaf extracts, fractions and isolates in vitro and in vivo with the result that Sungkai leaves have the potential to be developed as immunostimulants [10-12]. However, the molecular mechanisms underlying the immunomodulatory activity of isolates from Sungkai leaves is still not fully understood.

Indonesia is a tropical country with a high and dense biodiversity, owing primarily to its huge tropical rainforests that cover the whole country. Indonesian forests offer a broad range of plant species that are vital for maintaining ecosystem activities, such as nutrient cycling and energy flow. The diversity of plants in Indonesia significantly contributes to soil erosion prevention and the facilitation of photosynthesis. Nonetheless, the extensive variety has not been thoroughly researched for its potential use as a medicinal plant. The sungkai leaf plant (*Paronema canescens* Jack), a member of the Lamiaceae family, is widely farmed by the community due to

its economic importance and is regularly found in jungle, forest, gardens, and yards. Furthermore, ethnobotanically, the locals of Sumatra and Borneo have traditionally have been used this plant to treat malaria, fever, hypertension, intestinal worms, and cholesterol. Sungkai plants (*Paronema canescens* Jack) contain various secondary metabolites such as Flavonoids, Alkaloids, and Terpenoids. Tarigan in 2022 succeeded in isolating the flavonoid compound, namely apigenin from the leaves of the sungkai plant and has anti-inflammatory activity using the carrageenan-induced paw edema and inflammation inhibition activity cotton pellet-induced granuloma in mice method, where the findings showed that the ethanol fraction extract of sungkai leaves has an inhibitory activity of 58.12%, which in this fraction has a major compound in the form of apigenin [2].

This study aims to explore the potential and mechanism of immunomodulatory activity of isolates as active isolates of Sungkai leaves by using molecular docking system. The molecular docking method allows the assessment of interactions between ligand and receptor molecules at the atomic level, thus providing deep insight into the mechanism of action of the compound on specific immunomodulatory protein targets [13]. Through this approach, it is hoped that this study can provide stronger scientific evidence of the potential of Sungkai leaf isolate compounds as immunomodulatory agents and open up opportunities for the development of herbal medicines based on Sungkai leaves.

MATERIALS AND METHODS

Design

The in silico method uses molecular docking between the three-dimensional structures of the five receptor proteins used, namely IL-1β, IL-6, NF-κB, TNF-α, and TLR4 with compounds isolated from sungkai leaves, namely apigenin, Bis (2-ethylhexyl) phthalate and stigmasterol.

Protein search

Proteins related to immunomodulatory reactions were obtained from the Protein Data Bank (http://www.rcsb.org/pdb/), namely IL-1β (PDB ID: 4G6M), IL-6 (PDB ID: 4CNI), NF-κB (PDB ID: 2RAM), TNF-α (PDB ID: 7JRA), and TLR4 (PDB ID: 2Z62).

Protein preparation

The protein structure preparation process involves the separation of macromolecules from solvents, ligands, or unnecessary non-standard residues, which is performed using Chimera and Autodock Tools 1.5.7 software [14].

Ligand structure optimization

Ligand structure optimization was performed by adding polar hydrogen atoms and setting grid box parameters. The grid box for IL1β was set at the center coordinate (X= 4.181, Y = 29.91, Z =-26.042) with a box size of 40 Å along the x, y, and z axes. For IL6, the grid box center coordinates are at (X = 59.97, Y =-79.53, Z = 3.99) with a box size of 47 × 47 × 29 Å to ensure that all binding sites on the IL6 protein are covered. Meanwhile, the grid box for NF-κB was set at the center coordinates (X =4.74, Y = 34.57, Z = 71) with a box size of 45 Å. For TNF-α, the binding site was selected based on the position of its crystal ligand at coordinates (X =-14.97, Y =-2.306, Z =-26.22) with a box size of 15 Å. The TLR4 binding site was selected based on the position of the oligosaccharide ligand in the TLR4 structure with a center coordinate of (X = 1.063, Y =-17.08, Z = 19.6) and a box size of 15 Å. The protein structure optimization results were then saved in PDBQT format, which is a format for storing three-dimensional structural information of proteins after the addition of polar hydrogen atoms and grid box settings, as well as for preparing proteins for the docking process.

Molecular docking with AutoDock vina

Furthermore, the docking process of Apigenin, Bis(2ethylhexyl)phthalate and stigmasterol ligands was run with AutoDock Vina, which will evaluate various ligand poses in the binding site based on energy affinity. The docking results in the form of best affinity scores and ligand poses are stored and analyzed to understand the ligand-protein interactions. The molecular docking simulations were performed using AutoDock Vina, a widely used tool for predicting ligand-receptor interactions. AutoDock Vina employs a scoring function based on an empirical free energy model, which calculates the binding affinity (in kcal/mol) by considering van der Waals interactions, hydrogen bonding, electrostatic interactions, and torsional entropy penalties. The scoring function ranks the ligand-receptor complexes, with the most negative binding energy representing the most stable interaction. To identify the most stable interactions, the docking results were analyzed by selecting poses with the lowest binding energy values. Additionally, the spatial orientation of the ligands within the binding pocket was carefully examined to ensure that critical residues involved in receptor activity were engaged [15].

Grid box parameters and justification

The grid box parameters were designed to encompass the entire active site of each receptor, ensuring accurate docking and avoiding potential exclusion of crucial binding regions. For NF-κB, IL-6, and TNF-α receptors, the grid box dimensions were adjusted based on crystal structure data from the Protein Data Bank (PDB) and previous literature identifying active site residues. The grid box center was positioned at the active site, and the dimensions were expanded by 5-10 Å to allow sufficient space for flexible ligand binding while preventing non-specific interactions outside the active region. This approach ensured that the docking simulations captured all potential binding conformations relevant to receptor activity. The grid size and center were optimized through preliminary docking tests, confirming that they covered critical residues without unnecessary computational overhead. Such justification highlights the protocol's precision and reliability in predicting biologically meaningful ligand-receptor interactions [15].

RESULTS

Molecular docking of isolate compound

The binding affinity of the interaction between Apigenin isolate, Bis(2-ethylhexyl) phthalate and Stigmasterol with various receptors were obtained from molecular docking using AutoDock Vina. The interaction between the three compounds with all receptors showed different interaction patterns. The binding affinity taken is the binding affinity with the most negative value. (Ananda *et al.*, 2024). The results showed that the most negative binding affinity occurred at the TNF-α receptor (table 1).

Table 1: Binding affinity (Kcal/mol) interaction of isolate and receptor

Compound	IL-1β	IL-6	NF-κB	TNF-α	TLR4
Apigenin	-7.7	-6.6	-6.9	-9.2	-4.6
Bis(2_ethylhexyl) pthalate	-6.1	-5.3	-4.7	-8.7	-2.8
Stigmasterol	-7.6	-6.7	-6.7	-8.7	-4.3

Apigenin interaction with various receptors

As shown in table 1, Apigenin showed good tethering stability with IL-1β, IL-6, NF-κB, and TNF-α, with strong and consistent affinity energies in the range of-6.6 to-9.2 kcal/mol. Interactions on IL-1β were dominated by hydrogen bonds, while those on TNF-α, IL-6, NF-κB were dominated by hydrophobic interactions, such as Pi-sigma and Alkyl. On the other hand, Apigenin showed conformational variability and weaker interaction with TLR4, with an affinity energy of-4.6 kcal/mol. Binding interaction between Apigenin structures and various receptors can be seen in fig. 1.

Interaction of Bis(2_ethylhexyl) phthalate with various receptors

Bis(2-ethylhexyl) phthalate showed good stability with TNF-α, with affinity energies between-8.5 to-8.7 kcal/mol with a predominance of hydrophobic interactions, but its interactions with other receptors were less consistent. On IL-1β, this ligand shows conformational variations with affinity energies in the range of-5.8 to-6.1 kcal/mol, with hydrophobic interactions dominating. Interactions with IL6, NF-κB, and TLR4 are weaker, with low affinity and high conformational variation, especially with TLR4. Binding interaction between Bis(2_ethylhexyl) phthalate structure and various receptors can be seen in fig. 2.

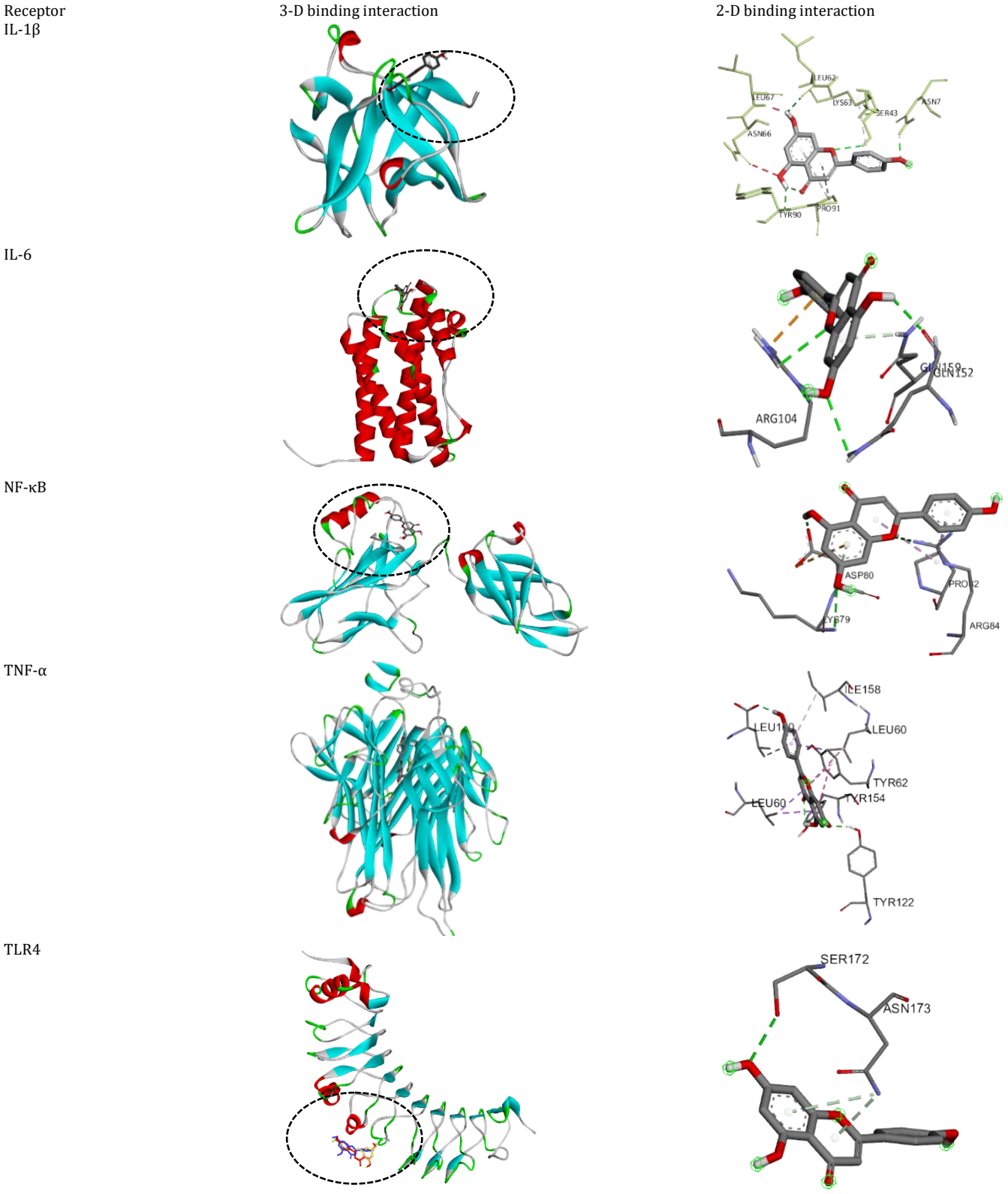


Fig. 1: Binding interaction of apigenin with various receptors

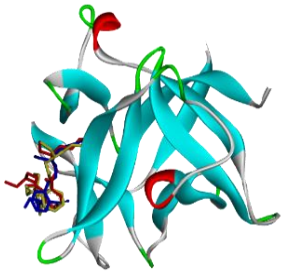
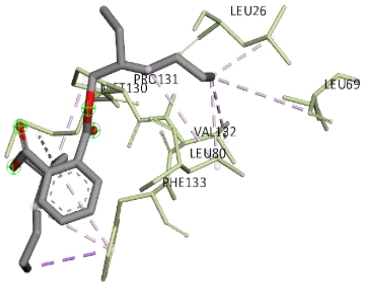
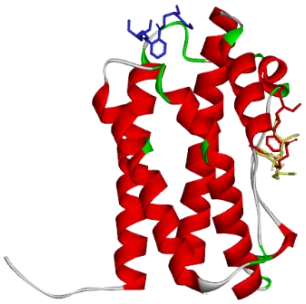
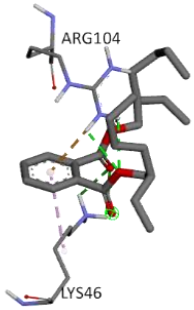
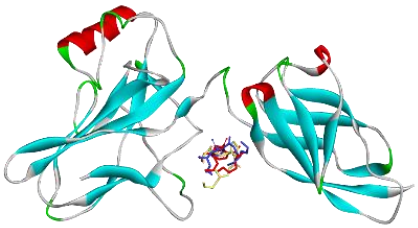
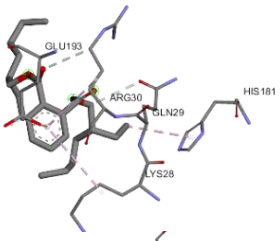
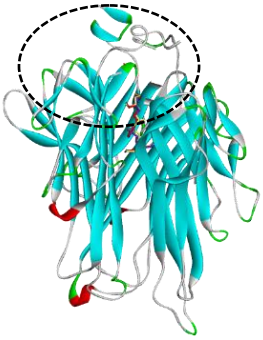
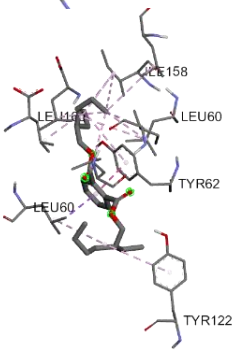
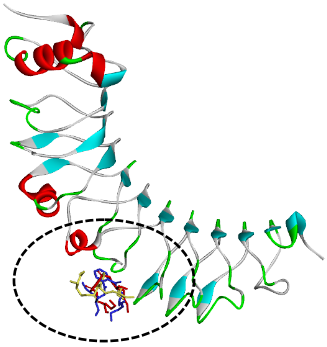
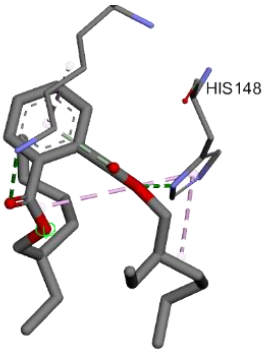
Receptor	3-D binding interaction	2-D binding interaction
IL-1β		
IL-6		
NF-κB		
TNF-α		
TLR4		

Fig. 2: Binding interaction of Bis (2 ethylhexyl) phthalate with various receptors

Stigmasterol interaction with various receptors

Stigmasterol also showed good stability with TNF-α, with affinity energy consistent in the range of-8.6 to-8.7 kcal/mol with predominance of hydrophobic interactions. On IL1β, this ligand tethers at the same cleft with conformational variations with affinity energies of-7.3 kcal/mol to-7.6 kcal/mol suggesting flexibility in tethering. Interactions with IL6 and NF-κB were in the affinity range of-6.2 to-6.7 kcal/mol, indicating multiple binding modes. However, the interaction with TLR4 is relatively weak, with low-affinity energies in the range of-4 kcal/mol to-4.3 kcal/mol and almost identical conformations. The binding

interaction between Stigmasterol structures and various receptors can be seen in fig. 3.

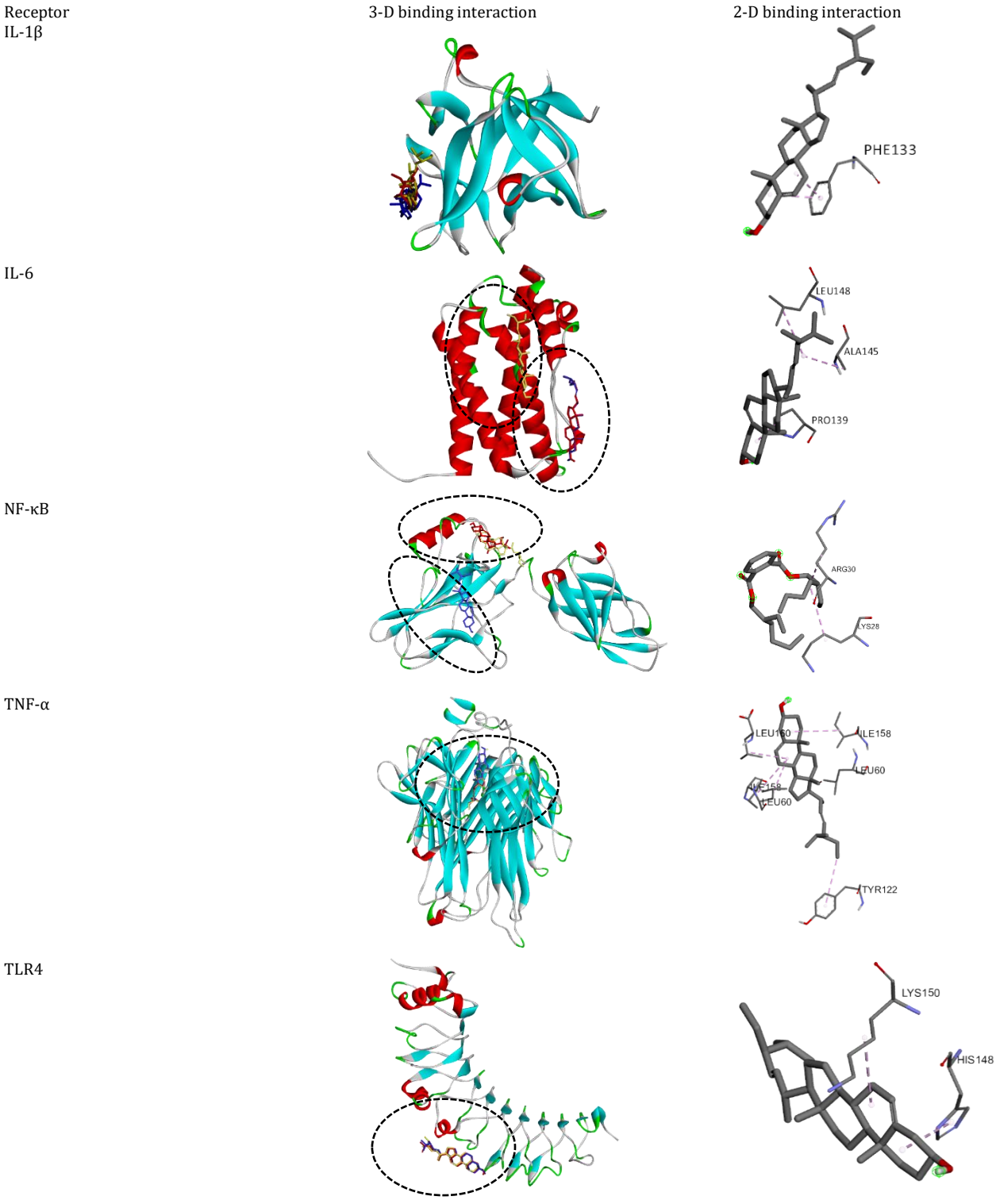


Fig. 3: Binding interaction of stigmasterol with various receptors

DISCUSSION

An immunomodulator is a substance that can modify or regulate one or more immune functions. It can either stimulate or suppress the immune system, depending on the need. Immunomodulators are often used to enhance the body's natural immune response against diseases or to dampen an overactive immune system, such as in autoimmune disorders [16, 17]. They include a variety of agents, such as cytokines, vaccines, monoclonal antibodies, and synthetic drugs. Cytokine secretion is a macrophage response to inflammatory stimuli [18, 19]. Macrophages are activated by foreign particles and serve as a major source of various cytokines and growth factors. However, if the inflammatory response is not well controlled, it will lead to chronic inflammation causing further tissue damage. Macrophages manage inflammation by releasing several inflammatory mediators, including nitric oxide (NO), tumor necrosis

factor-alpha (TNF-α), interleukin-6 (IL-6), prostaglandins, and interleukin-1β (IL-1β). In addition, Toll-like receptor 4 (TLR4) is involved in regulating the innate immune response by detecting and modulating molecular signals that recognize bacterial-microbial interactions. Controlling macrophage activity or secretion may facilitate the repair of tissue damage that occurs during inflammation [20-22].

Prior studies have reported moderate binding affinities for Apigenin with NF-κB, primarily through hydrogen bonding and hydrophobic interactions at key active site residues. In this study, Apigenin demonstrated stronger binding affinities, possibly due to enhanced interactions with additional residues or unique binding conformations identified in the docking simulations. This suggests an improved potential for Apigenin as an NF-κB inhibitor, which could enhance its immunomodulatory efficacy. Similarly, Stigmasterol's binding affinities with IL-6 and TNF-α in earlier research were generally found to be lower compared to other sterols. However, this study revealed more robust interactions, including multiple hydrophobic contacts and π-π stacking interactions, which were less emphasized in previous analyses. These findings indicate that Stigmasterol may exert stronger inhibitory effects on pro-inflammatory pathways than previously anticipated.

In this study, the prediction of activity and the search for the mechanism of action of the isolate structure obtained from sungkai leaf isolation as an immunomodulator were carried out. From the data obtained, it is known that Apigenin provides good interaction with IL-1β, IL-6, NF-κB and TNF-α receptors because it has low affinity energy. The more negative the binding affinity value, the higher the stability between the ligand and the receptor, indicating a stronger interaction [23]. This means that Apigenin influences IL-1β, IL-6, NF-κB and TNF-α receptors in the occurrence of inflammatory processes and has the potential to be developed as an immunomodulatory compound with an inflammatory reaction mechanism through the NF-κB signaling pathway. In contrast, the interaction of Apigenin with TLR4 provides a weak interaction with high conformational variation, so it can be concluded that the signaling process of Apigenin's inflammatory reaction is not through TLR4. Exploration of Additional Pathways While NF-κB is central to inflammatory responses, pathways such as MAPK (Mitogen-Activated Protein Kinases), JAK-STAT (Janus Kinase-Signal Transducer and Activator of Transcription), and PI3K-Akt (Phosphoinositide 3-Kinases-Protein Kinase B) also play significant roles in immune modulation and inflammation. TLR4 Interaction Insights Despite the weak interaction of Apigenin with TLR4, its potential to indirectly modulate Toll-like receptor signaling through other mediators or cofactors could be worth examining. Beyond IL-1β, IL-6, and TNF-α, other cytokines like IL-10, IFN-γ, and chemokines could provide insight into Apigenin's immunomodulatory effects, especially in the context of chronic inflammation or autoimmune conditions. Given that bioflavonoids like Apigenin can influence gene expression, exploring its role in epigenetic modifications (e. g., histone acetylation, DNA methylation) related to inflammation could provide a novel perspective on its immunomodulatory properties. The interplay between NF-κB and other pathways like JAK-STAT or MAPK might reveal synergistic or antagonistic effects influenced by Apigenin.

The immunomodulatory potential of Apigenin and Stigmasterol extends beyond their interactions with NF-κB, IL-6, and TNF-α. For instance, Apigenin has been reported to modulate the MAPK pathway by inhibiting ERK1/2 and p38 activation, which are involved in inflammatory cytokine production. Similarly, Stigmasterol may interact with the JAK-STAT pathway, potentially downregulating pro-inflammatory mediators like IL-6 and IFN-γ. These interactions highlight the compounds' ability to regulate multiple pathways, contributing to a broader anti-inflammatory and immunoregulatory profile. When compared with known immunomodulators, Apigenin and Stigmasterol offer distinct advantages. For example, Apigenin, a flavonoid, exhibits potent anti-inflammatory effects with minimal cytotoxicity, comparable to curcumin, another plant-derived immunomodulator. Meanwhile, Stigmasterol, a phytosterol, has been shown to modulate lipid metabolism and inflammation, making it similar to beta-sitosterol but with unique structural properties that may enhance bioactivity. Unlike synthetic immunosuppressants, such as corticosteroids, these natural compounds are associated with fewer side effects, providing a safer alternative for long-term use [24].

Notably, this study uncovers unique binding patterns, such as specific interactions with residues critical for protein activation, which were not reported earlier. These insights emphasize the potential of Apigenin and Stigmasterol as promising candidates for immunomodulatory applications, offering stronger or more targeted effects compared to earlier evaluations. This comparative analysis enriches the understanding of these compounds' mechanisms and underscores the significance of the current study in advancing the field of therapeutic development targeting inflammatory pathways.

Virtual screening isrecognized as an effective method for the discovery of compounds and help towards lead optimization in structure-based drug discovery. Molecular docking studies helps to recognize prospective lead candidates and fewer compounds need to be experimentally screened. Besides recognizing small molecules which are likely to bind well to the protein target, docking studies also explain the binding interactions [25].

Bis(2-ethylhexyl) phthalate provides good interaction with TNF-α by providing low-affinity energy with hydrophobic bonding interaction. But in other receptors, Bis(2 ethylhexyl) phthalate interaction has weak affinity and is less consistent. This means that Bis(2-ethylhexyl) phthalate does not have the potential to be developed as an immunomodulatory compound. Just like Apigenin, Stigmasterol also provides good interaction with IL-1β, IL-6, NF-κB and TNF-α receptors because it has low-affinity energy. This means that Stigmasterol also influences IL-1β, IL-6, NF-κB and TNF-α receptors in the occurrence of inflammatory processes and has the potential to be developed as an immunomodulatory compound with an inflammatory reaction mechanism through the NF-κB signaling pathway. However, its interaction with TRL4 provides low affinity because it has high-affinity energy. TLR4 activation has been associated with inflammation, oxidative stress, and endothelial dysfunction, which are central processes in the pathogenesis of cardiovascular disease. HMGB1-LPS complex uses TLR4, HMGB1-Pam_3 CSK_4 complex uses TLR2. The RAGE-HMGB1 interaction is stabilised by heparin sulphate which readily forms a complex with RAGE on the cell surface before binding to HMGB1. Members of the S100 protein family also interact with RAGE triggering immune responses in collaboration with TLR4 and activation of p38 MAPK, NF-κB and downstream signalling molecules. Although there is evidence that the S100A8/A9 complex also interacts with TLR4 directly via MD2, it remains to be investigated whether glycans expressed on TLR4 also mediate the binding between S100A8/A9 and TLR4. Moreover, *in vitro* analyses show that RAGE has a higher affinity with S100A8/A9 than TLR4, where the former interaction is associated with inflammation-mediated carcinogenesis and the latter with autoimmune disorders and infections. The direct interaction of RAGE with LPS molecules was also determined through competition assays with another RAGE ligand, AGE-BSA and resulted in comparable immune reactions as seen with TLR4 binding *in vitro* and *in vivo.* Limitations of In Silico Studies Lack of Empirical Validation, In silico studies rely on computational models to predict interactions and mechanisms, such as Apigenin's effects on NF-κB and TLR4 pathways. While these models provide valuable initial insights, they cannot replicate the complexity of biological systems. Experimental validation through *in vitro* and *in vivo* studies is essential to confirm the accuracy of these predictions. Potential Discrepancies in Predicted vs. Actual Biological Activity, Computational tools often depend on algorithms, databases, and assumptions that might oversimplify or misrepresent biological dynamic.

The interaction of Apigenin with TLR4, described as weak with high conformational variation, illustrates a limitation of static docking models, which do not fully account for the dynamic nature of proteins and ligands in a cellular environment. Advanced methods like molecular dynamics simulations can help, but they still lack the ability to model all relevant interactions accurately. Pathway simulations in silico often focus on specific signaling cascades (e. g.,

NF-κB) without considering cross-talk with other pathways or the influence of cell-specific contexts. This limitation can lead to an incomplete understanding of the compound's broader effects. In silico studies cannot predict how Apigenin is absorbed, distributed, metabolized, and excreted in living organisms, which are crucial factors for its therapeutic potential. Experimental data in this area are necessary to bridge the gap between computational findings and clinical application. Acknowledging these limitations provides transparency and demonstrates a commitment to robust scientific inquiry. It ensures that conclusions drawn from computational studies are interpreted with appropriate caution.

To strengthen the reliability of the docking findings, molecular dynamics (MD) simulations can be employed. MD simulations analyze the dynamic behavior and stability of ligand-receptor complexes under physiological conditions. For instance, simulations of Apigenin and Stigmasterol bound to NF-κB, IL-6, and TNF-α over a 100 ns time frame would help evaluate key stability parameters such as Root mean Square Deviation (RMSD), Root mean Square Fluctuation (RMSF), and hydrogen bond retention. Consistent binding interactions and minimal fluctuations would confirm stable complexes, supporting the docking predictions. Furthermore, advanced techniques such as free energy calculations can refine binding affinity predictions, providing quantitative insights into ligand-receptor stability by incorporating solvent and entropic contributions [24, 26].

The computational predictions can be validated through *in vitro* and biochemical assays, including Surface Plasmon Resonance (SPR) and Isothermal Titration Calorimetry (ITC) to confirm binding affinities and thermodynamics of ligand-receptor interactions; ELISA Assays to measure the suppression of pro-inflammatory cytokines (e. g., IL-6, TNF-α) after treatment with Apigenin and Stigmasterol; Western Blotting to assess the inhibition of NF-κB pathway activation in stimulated immune cells; MTT Assay to ensure the non-cytotoxic effects of the active compounds on human cell lines. These experimental approaches complement computational findings, providing a robust framework to evaluate the immunomodulatory potential of Sungkai leaf isolates [24, 26].

In silico studies provide a foundation for understanding molecular interactions but have inherent limitations that must be acknowledged. One major limitation is the absence of pharmacokinetic (ADME) analysis, as these studies do not account for the absorption, distribution, metabolism, and excretion of compounds, which are critical for determining bioavailability and therapeutic potential. Additionally, the focus on specific targets, such as NF-κB, IL-6, and TNF-α, may overlook off-target effects, raising the possibility of unintended interactions with other biological pathways. Another limitation is the simplified and controlled computational environment, which cannot fully replicate the complexity of physiological systems, including protein-protein interactions, immune responses, and enzymatic degradation [26, 27].

To address these gaps, future studies should include *in vivo* experiments to validate the immunomodulatory effects of Apigenin and Stigmasterol in animal models of inflammation, focusing on cytokine regulation and immune signaling pathways. Pharmacokinetic and toxicological analyses should be performed to evaluate the compounds' bioavailability, metabolic stability, and safety profiles. High-throughput screening of structurally similar compounds can help identify novel candidates with enhanced immunomodulatory activity. Proteome-wide docking studies could provide insights into potential off-target interactions, while omics-based approaches, such as transcriptomics and proteomics, may reveal broader cellular effects and underlying mechanisms. Combinatorial studies exploring the synergy between these isolates and established anti-inflammatory drugs can further enhance therapeutic applications. These directions will strengthen the evidence for the immunomodulatory potential of Sungkai leaf isolates and support their development as therapeutic agents [26, 27].

Antioxidants in food are of interest for four major reasons: they can protect the food itself against oxidative damage, they can exert antioxidant effects in the human gastrointestinal tract, they can be absorbed and exert antioxidant effects in other body tissues, and they may be used in plant extracts, or as pure compounds, as therapeutic agents, and tyrosin. Ficusin, bergaptene, stigmasterol, psoralen, taraxasterol, beta-sitosterol, rutin, sapogenin, Calotropenyl acetate, lepeolacetate and oleanolic acid sistosterol are present in the leaf [28].

Based on the in silico study conducted, it is known that Apigenin and Stigmasterol have the opportunity to be developed as immunomodulatory compounds, but comprehensive research is still needed to better understand their mechanism of action and ensure the effectiveness and safety of their use in the development of immunomodulatory drugs in the future.

CONCLUSION

Based on the molecular docking test that has been conducted, Apigenin and Stigmasterol has the opportunity to be developed as an immunomodulatory compound. However, Bis (2 ethylhexyl) phthalate does not have the potential to be developed as an immunomodulatory compound.

ACKNOWLEDGMENT

The author would like to thank the Faculty of Pharmacy, Andalas University through the DIKTIRISTEK KEMDIKBUDRISTEK DRTPM Fund for the fiscal year 2024 with Contract Number 041/E5/PG.02.00. PL/2024 signed on June 11, 2024.

AUTHORS CONTRIBUTIONS

Dwisari Dillasamola: Conceptualization, Supervision, Resources, Writing–Original Draft, Funding acquisition; Yufri Aldi: Writing–Original Draft, Writing–Review and Editing; Fatma Sri Wahyuni: Writing–Original Draft, Writing–Review and Editing; Setyanto Tri Wahyudi: Methodology, Writing–Original Draft; Irene Puspa Dewi: Methodology, Writing–Original Draft.

CONFLICT OF INTERESTS

Declared none

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