

***In Silico* Analysis of Turmeric, Tamarind, and Ginger on the Immune System**

Dowmy Rahmatika¹, Nabila Swarna Puspa¹, Ummu Mastna Zuhri², Andriani Noerlita Ningrum³, Joko Tri Wibowo^{2,3,*}

¹Biologi FMIPA Militer Universitas Pertahanan RI, Kawasan IPSC Sentul, Sukahati, Citeureup, Bogor, Jawa Barat 16810 Indonesia

²Pusat Riset Vaksin dan Obat BRIN, KST Soekarno BRIN, Jl. Raya Bogor KM.46, Cibinong, Bogor, Jawa Barat 16911, Indonesia

³STIKES Mamba'ul Ulum, Jl. Ring Road Utara KM 0.3 Tawang Sari Mojosongo Surakarta 5712, Indonesia

*Corresponding Author: jk3wbowo@gmail.com

Abstract

Immune system needs to be in optimum condition. However, autoimmune diseases occur when the immune system mistakenly attacks the body's cells, resulting in chronic conditions that require long-term treatment. Current anti-inflammatory and immunosuppressive drugs have significant side effects, highlighting the need for safer and more specific therapeutic alternatives. This study aimed to evaluate turmeric, ginger, and tamarind that have been used traditionally for the drink with benefit for immune system. Compounds from these plants were retrieved from the COCONUT 2.0 database and screened using SwissADME. Target prediction was performed with SwissTargetPrediction and STITCH 5.0, while molecular docking of AKT1, PTGS2, and TP53 was carried out using AutoDockTools-1.5.7. The results show that catechin and kaempferol strongly interact with AKT1, moderately with TP53, and weakly with PTGS2. The number of hydrogen bonds and the involvement of polar residues contributed to complex stability, particularly with AKT1. The results indicated that catechin and kaempferol in these plants might play an important role as immunomodulatory agents through an *in silico* analysis. These compounds are not the major constituents of the studied plants. Further research is needed to analyze the primary phytochemicals of these plants and validate their biological activities experimentally.

Keywords: catechin, kaempferol, immunomodulatory, *in silico*, molecular docking,

Introduction

The immune system plays a crucial role in protecting the body from pathogens through two major arms: humoral (antibody-mediated) immunity and cellular-mediated immunity (Abbas *et al.*, 2021). However, in some instances, the immune system mistakenly attacks the body's healthy cells, leading to the development of autoimmune diseases (Zampieron and Kamhi, 2012). To date, over 100 distinct autoimmune disorders have been identified, with rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, psoriasis, and type 1 diabetes among the most prevalent (Richard-Eaglin and Smallheer, 2018). The global economic burden of autoimmune disease treatment is substantial, with therapeutic costs reaching approximately \$127 billion in 2021 and projected to increase to \$149 billion by 2023 (Miller, 2023).

Current management of autoimmune diseases heavily relies on long-term administration of immunosuppressive agents, such as non-steroidal anti-inflammatory drugs (NSAIDs). Despite their effectiveness, these drugs are associated with significant adverse effects, including metabolic disturbances, organ toxicity, increased susceptibility to infections, heightened cancer risk, and lack of target specificity (Ponticelli and Glasscock, 2019). These limitations highlight the urgent need for the development of safer and more targeted anti-inflammatory and immunoregulatory therapies. Effective immunomodulation requires careful regulation of the balance between pro-inflammatory cytokines (e.g., IL-2, IL-6, IL-8, IL-1 β , IFN- γ , TNF α) and anti-inflammatory cytokines (e.g., IL-4, IL-10, TGF β), as this balance is pivotal for maintaining immune homeostasis and preventing pathological inflammation (Cicchese *et al.*, 2018).

Natural products derived from medicinal plants offer a promising alternative for modulating immune responses with potentially fewer side effects. Plants such as ginger (*Zingiber officinale*) (Mashhadi *et al.*, 2013), turmeric (*Curcuma longa*) (Lantz *et al.*, 2005), and tamarind (*Tamarindus indica*) (Sundaram *et al.*, 2015) have demonstrated significant anti-inflammatory activity in both experimental and clinical studies. Remarkably, ginger and turmeric have been clinically evaluated for the management of rheumatoid arthritis, showing favorable outcomes (Heidari-Beni *et al.*, 2020; Kou *et al.*, 2023). Moreover, these plants are widely consumed as part of traditional diets and herbal remedies (Jamu), suggesting a long history of safe human use (Estiasih *et al.*, 2025). In this study, the anti-inflammatory potential of these plants was further investigated to explore their possible therapeutic roles in autoimmune disorders through computational molecular docking analysis targeting key immune-related proteins.

Materials and Methods

Materials. Compounds were collected from the COCONUT 2.0 database (<https://coconut.naturalproducts.net/>), an open-access platform containing secondary metabolites from various organisms (Chandrasekhar *et al.*, 2025). While protein crystal structures were retrieved from the Protein Data Bank (PDB).

Ligand Preparation. Three plant species were selected as sources: *Curcuma longa*, *Zingiber officinale*, and *Tamarindus indica*. After initial data curation, compounds were screened using SwissADME to evaluate pharmacokinetic properties, including drug-likeness, gastrointestinal (GI) absorption, and water solubility (Daina *et al.*, 2017). Two compounds, catechin and kaempferol, were selected for further analysis due to their favorable ADME profiles, including no violations of Lipinski's Rule of Five, high GI absorption, and good aqueous solubility.

Target Protein Prediction. Target prediction was performed using SwissTargetPrediction (Daina *et al.*, 2019). The predicted targets were further validated using the STITCH 5.0 database under *Homo sapiens* settings to identify

potential interactions between the compounds and their target proteins.

Protein Target Preparation. Based on the prediction results, three key proteins were selected for docking simulations: AKT1 (PDB ID: 3O96), PTGS2 (PDB ID: 5F1A), and TP53 (PDB ID: 2VUK). These proteins were prepared by removing water molecules and adding polar hydrogens before docking.

Molecular Docking Preparation. Molecular docking was conducted using AutoDockTools-1.5.7. Before docking with catechin and kaempferol, grid box validation was performed by redocking each target protein with its native ligand to ensure that the selected grid coordinates accurately represented the active binding site. A grid box configuration was considered valid if the redocking results showed an RMSD value $< 2 \text{ \AA}$ compared to the native ligand conformation. After validation, docking simulations were performed using the validated grid box parameters for catechin and kaempferol. The ligand-protein complexes were evaluated based on binding energy values. Visualization and analysis of hydrogen bonds, hydrophobic interactions, and ligand orientation within the binding pocket were performed using BIOVIA Discovery Studio v24.1.0.23298.

Analysis. Docking results were analyzed based on binding energy values generated by AutoDockTools-1.5.7, with the lowest-energy conformation considered as the most stable ligand-protein complex. The interactions between each ligand and target proteins were qualitatively evaluated by analyzing non-covalent interactions, including hydrogen bonds, hydrophobic interactions, and π - π stacking, using BIOVIA Discovery Studio v24.1.0.23298 for visualization. Furthermore, binding energy values were used as indicators of binding affinity strength, with more negative values indicating stronger binding affinities (Candra and Wijaya, 2021). In general, binding affinities were categorized as very strong when binding energy $\leq -10.0 \text{ kcal/mol}$, strong between -10.0 and -7.0 kcal/mol , moderate between -7.0 and -5.0 kcal/mol , and weak when $> -5.0 \text{ kcal/mol}$. These categories were applied to interpret the binding potential and possible biological relevance of each ligand-protein complex.

Results and Discussion

The initial screening from the COCONUT 2.0 database yielded 297 compounds from *Curcuma longa*, 485 compounds from *Zingiber officinale*, and 51 compounds from *Tamarindus indica*. After SwissADME filtering based on drug-likeness, gastrointestinal absorption, and water solubility, 49, 60, and 4 compounds were retained from *C. longa*, *Z. officinale*, and *T. indica*, respectively. Catechin and kaempferol were selected as the main ligands for docking

due to their pharmacokinetic properties, including high GI absorption, good solubility, and no violations of Lipinski's rule. Target prediction using SwissTargetPrediction validated by STITCH 5.0 identified AKT1, PTGS2 (COX-2), and TP53 as key proteins involved in immune regulation and inflammatory pathways. Figure 1 shows the structure of the three proteins. Redocking with native ligands showed RMSD values <2 Å for all proteins, confirming the grid positions were valid for docking (Table 1).

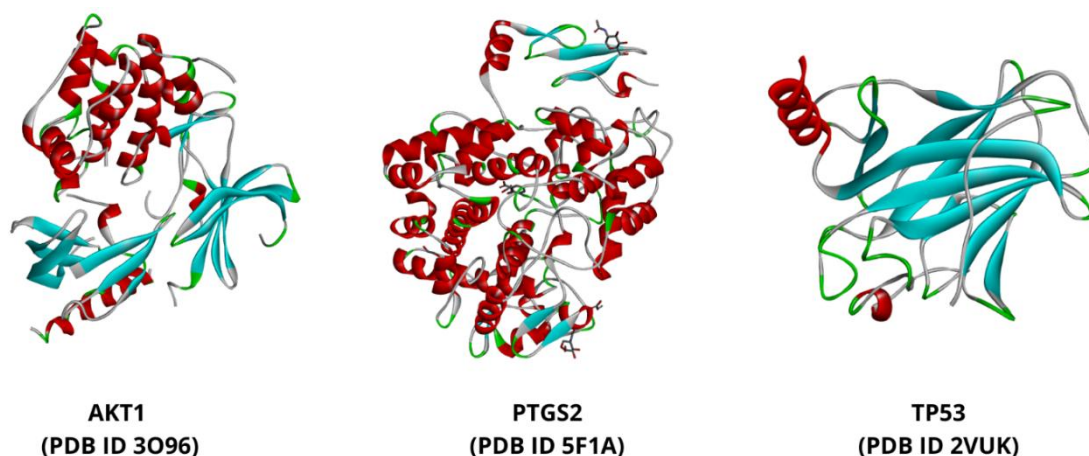


Figure 1. Structure of the three selected proteins

Table 1. Docking validation

Protein	Native Ligand	RMSD Value (Å)
AKT1	IQO	1.18
PTGS2	BOG	1.31
TP53	P83	1.02

Docking simulations revealed that catechin and kaempferol exhibited strong binding affinity toward AKT1, moderate affinity toward TP53, and weak affinity toward PTGS2 (Table 2). Both catechin and kaempferol exhibited the lowest binding energy values with AKT1 (−8.80 and −8.81 kcal/mol, respectively), indicating a strong binding affinity. For TP53, both compounds demonstrated moderate interactions, with catechin and kaempferol having binding energies of −6.89 kcal/mol and −6.59 kcal/mol, respectively. In contrast, the interactions with PTGS2 were weaker, as reflected by higher binding energy values of −4.60 kcal/mol for catechin and −3.92 kcal/mol for kaempferol. These findings suggest that AKT1 is the most favorable target for both compounds, followed by TP53, while PTGS2 is less likely to be firmly bound by these ligands.

AKT1 is a central signalling molecule in adaptive immune cell activation, regulating

metabolism, proliferation, and survival (Ai *et al.*, 2021). Both catechin and kaempferol formed three hydrogen bonds with key residues within the active pocket of AKT1. Catechin interacted with Thr211, Thr291, and Ile290, while kaempferol interacted with Ser205, Lys268, and Ile290 (Figure 2). The higher number of hydrogen bonds and the involvement of polar residues likely contribute to the strong and stable interactions, consistent with their low binding energy values (Zhou and Pang, 2018).

For TP53, catechin formed a single hydrogen bond with Thr150, whereas kaempferol established three hydrogen bonds with Thr150, Pro151, and Asp228 (Figure 4). The greater number of hydrogen bonds formed by kaempferol may partially explain the slightly lower binding energy compared to catechin. TP53 plays a role in innate immune responses by modulating macrophage activity and suppressing pro-inflammatory proteins such as NF-κB and STAT3 (Shi and Jiang, 2021). These interactions suggest that both compounds could exert immunoregulatory effects via TP53-related pathways.

Interactions with PTGS2 showed weaker binding affinities. Catechin formed three hydrogen bonds with Arg184, Arg185, and Glu490, while kaempferol formed two hydrogen bonds with Leu183 and Glu179 (Figure 3). The smaller number of hydrogen bonds for kaempferol and the involvement of residues with less polar characteristics may account for the

weaker interactions. PTGS2 is a key enzyme in prostaglandin biosynthesis that mediates inflammatory processes (Li *et al.*, 2025; Roulis *et al.*, 2020). The weak interactions imply that catechin and kaempferol are unlikely to exert their anti-inflammatory effects via direct inhibition of PTGS2.

Table 2. Docking output

Ligand	Lowest Binding Energy (kcal/mol)		
	AKT1	PTGS2	TP53
Catechin	-8.80	-4.60	-6.89
Kaempferol	-8.81	-3.92	-6.59
Binding Affinity Category	Strong	Weak	Moderate

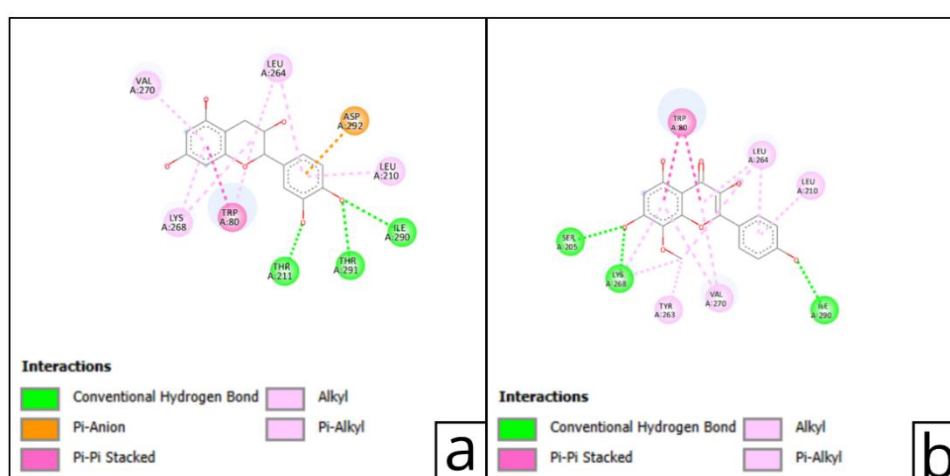


Figure 2. Interaction between AKT1 with (a) Catechin and (b) Kaempferol

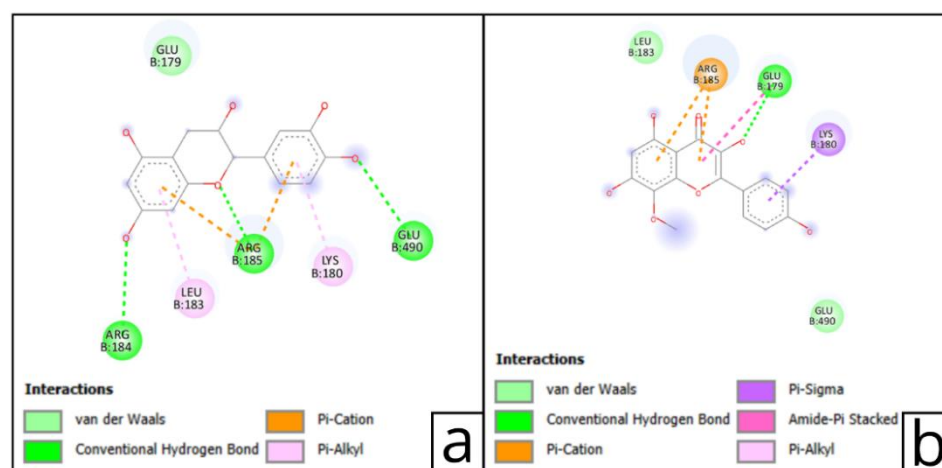


Figure 3. Interaction between PTGS2 with (a) Catechin and (b) Kaempferol

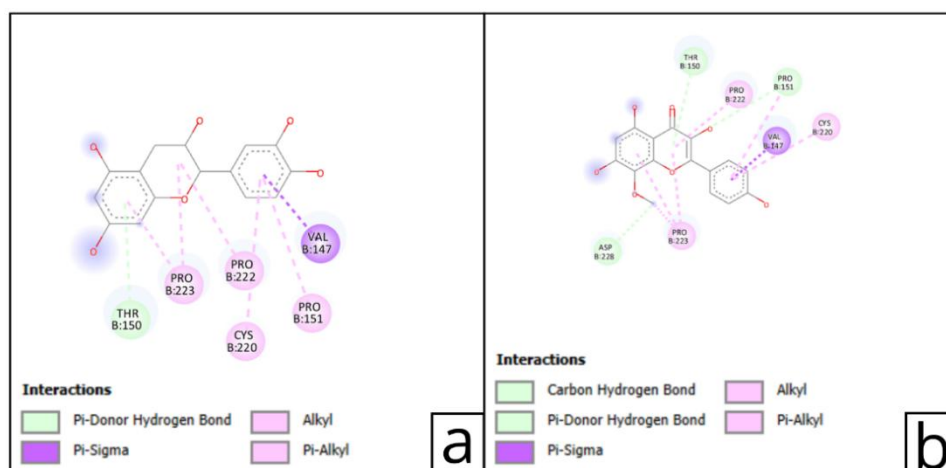


Figure 4. Interaction between TP53 with (a) Catechin and (b) Kaempferol

Our study findings correspond with results from previous studies, especially concerning the interactions between kaempferol and AKT1. Numerous network pharmacology studies have indicated that kaempferol demonstrates significant binding affinity for AKT1, showing binding energies in the -6.5 kcal/mol range (Liu *et al.*, 2021). In one study, it was demonstrated that kaempferol docks with AKT1 through key hydrogen bonds involving ASN204, SER205, and VAL271/272, along with hydrophobic and π - π stacking interactions, consistent with our observed interactions at residues Ser205, Lys268, and Ile290 (Anqi and Shijun, 2023). This supports the hypothesis that AKT1 is a physiologically relevant target for kaempferol's anti-inflammatory effects. Concerning TP53, previous docking studies show that kaempferol can form multiple hydrogen bonds with residues such as PHE113, GLN144, ARG110, and ASN131, resulting in binding affinities of -6.1 kcal/mol (Geng *et al.*, 2023). Our results for TP53 (-6.59 kcal/mol for kaempferol and -6.89 kcal/mol for catechin) fall within this moderate range, confirming that similar non-covalent interactions may contribute to the immunomodulatory potential via TP53 pathways. Comparatively, interactions between kaempferol and PTGS2 have been shown in another study to exhibit stronger affinities (-7.2 kcal/mol) (Zhang *et al.*, 2022). Our results on weaker binding energies with PTGS2 (-3.92 kcal/mol for kaempferol, -4.60 kcal/mol for catechin) suggest that PTGS2 is likely not the primary mediator of their activity in our system. This distinction underscores that while kaempferol may extensively interact with PTGS2 in some studies, its binding to AKT1 appears to be more robust and potentially more biologically relevant in the

context of immune regulation. These comparative findings enhance the credibility of our results and suggest that the observed interactions with AKT1 and TP53 are consistent with the known binding behavior of flavonoids like kaempferol, reinforcing the hypothesis that these pathways may mediate their immunomodulatory effects.

Conclusions

This study highlights the potential of ginger, turmeric, and tamarind that have been used in traditional medicine contain valuable compound such as catechin and kaempferol. Their interactions with key immune-related proteins suggest possible roles in modulating pathways relevant to inflammatory and autoimmune conditions. These findings provide a rationale for further research into the pharmacological potential of bioactive compounds from ginger, turmeric, and tamarind, particularly focusing on identifying and validating the main phytochemicals responsible for these effects. Future studies integrating *in silico*, *in vitro*, and *in vivo* approaches could support the development of safer, plant-based therapeutic alternatives for immune-related disorders.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of generative AI

During the preparation of this work the authors used Grammarly and ChatGPT in order to check the English grammar. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Author contribution

D.R.: Investigation, Formal Analysis, Visualization, Writing – Original Draft; N.S.P.: Supervision, Validation, Writing – Review & Editing; U.M.Z.: Data Curation, Validation, Writing – Review & Editing; A.N.M: Project Administration, Writing – Review & Editing. J.T.W.: Conceptualization, Methodology, Supervision, Writing – Review & Editing.

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