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Molecular Interaction Mapping of *Paederia foetida* Phytochemicals against NF-κB p65 Highlights Their Potential in Modulating Systemic Inflammation

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Abstract

Systemic inflammation is a fundamental pathological process involved in the development of various chronic diseases, with nuclear factor kappa B (NF-κB) p65 acting as a central transcriptional regulator of inflammatory responses. *Paederia foetida* L., commonly known as Daun Kentut, has been traditionally used as an anti-inflammatory medicinal plant; however, its molecular mechanisms of action remain poorly understood. This study aimed to investigate the molecular interactions between selected *P. foetida* phytochemicals and the NF-κB p65 protein using an in silico approach. Molecular docking was performed to evaluate binding affinity and interaction profiles of major phytochemical constituents, including flavonoids, iridoid glycosides, and phenolic acids. The docking results revealed that several compounds exhibited favorable binding energies and stable interaction patterns with key amino acid residues of NF-κB p65, such as Lys221, Arg246, Ser276, and Glu279. Among the tested compounds, quercetin and asperuloside demonstrated strong binding affinity and multiple hydrogen bonds within the transcriptionally active region of NF-κB p65. These interaction profiles were comparable to those of a reference inhibitor. The findings suggest that *P. foetida* phytochemicals have the potential to modulate NF-κB–mediated inflammatory signaling at the molecular level. This study provides mechanistic support for the traditional use of *P. foetida* as an anti-inflammatory agent and highlights its potential as a source of natural compounds for the development of inflammation-modulating therapeutics.

Keywords: *Paederia foetida*; NF-κB p65; systemic inflammation; molecular docking; phytochemicals.

Abbreviations: AutoDock Vina (ADV); Cyclooxygenase-2 (COX-2); Deoxyribonucleic acid (DNA); Interleukin-6 (IL-6); Inducible nitric oxide synthase (iNOS); In silico (IS); Interleukin-1 beta (IL-1β); Molecular docking (MD); Nuclear factor kappa B (NF-κB); Nuclear factor kappa B p65 subunit (NF-κB p65); Non-steroidal anti-inflammatory drugs (NSAIDs); Protein Data Bank (PDB); Reactive oxygen species (ROS); Tumor necrosis factor alpha (TNF-α).

INTRODUCTION

Systemic inflammation is a central pathological mechanism underlying a wide range of chronic diseases, including cardiovascular disorders, metabolic syndrome, autoimmune conditions, and cancer. Persistent activation of inflammatory signaling pathways contributes to tissue damage, immune dysregulation, and disease progression (Medzhitov, 2008). Among these pathways, the nuclear factor kappa B (NF-κB) signaling cascade plays a pivotal role in regulating pro-inflammatory gene expression, making it a key therapeutic target in inflammation-related diseases (Lawrence, 2009).

The NF-κB family consists of several transcription factors, with the p65 (RelA) subunit being the most transcriptionally active and functionally significant in mediating inflammatory responses. Upon activation, NF-κB p65 translocates to the nucleus and induces the expression of cytokines, chemokines, adhesion molecules, and enzymes such as TNF-α, IL-6, COX-2, and iNOS (Hayden and Ghosh, 2014). Dysregulated activation of NF-κB p65 has been closely associated with chronic inflammatory states and systemic inflammation, highlighting the importance of identifying safe and effective modulators of this pathway (Liu et al., 2017).

Although synthetic anti-inflammatory drugs such as corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) are widely used, their long-term use is often associated with significant adverse effects, including immunosuppression, gastrointestinal damage, and cardiovascular risks (Vane and Botting, 1998). This limitation has driven increasing interest in natural products and plant-derived bioactive compounds as alternative or complementary therapeutic agents with potentially lower toxicity profiles (Newman and Cragg, 2020).

Paederia foetida L., commonly known in Indonesia as Daun Kentut, is a medicinal plant traditionally used in Southeast Asia for treating inflammatory disorders, gastrointestinal disturbances, and rheumatic pain. Phytochemical investigations have revealed that *P. foetida* contains diverse bioactive compounds, including iridoid glycosides, flavonoids, phenolic acids, and terpenoids, which are known to exhibit anti-inflammatory and antioxidant activities (Zhang et al., 2016; Handayani et al., 2020). Experimental studies have suggested that extracts of *P. foetida* can suppress inflammatory mediators and oxidative stress markers in both in vitro and in vivo models (Chen et al., 2018).

Despite its traditional use and reported pharmacological activities, the molecular mechanisms underlying the anti-inflammatory effects of *P. foetida* remain insufficiently elucidated. In particular, there is a lack of systematic studies exploring how individual phytochemical constituents of *P. foetida* interact directly with key inflammatory regulators at the molecular level. Most existing studies focus on crude extracts or general biological outcomes, without identifying specific molecular targets or binding interactions (Handayani et al., 2020).

Moreover, computational approaches such as molecular docking and interaction mapping have become powerful tools in early-stage drug discovery and mechanistic studies. These methods allow for the prediction of ligand–protein interactions, binding affinities, and key amino acid residues involved in biological activity, providing valuable insights prior to experimental validation (Meng et al., 2011). However, to date, there is limited computational evidence describing the interaction between *P. foetida* phytochemicals and NF-κB p65, particularly in the context of systemic inflammation.

Therefore, this study aimed to address this research gap by conducting a molecular interaction mapping of selected *P. foetida* phytochemicals against NF-κB p65 using in silico approaches. By identifying potential binding modes and interaction profiles, this work seeks to provide a mechanistic basis for the anti-inflammatory potential of *P. foetida* at the molecular level. The findings are expected to contribute to the scientific validation of traditional medicinal plants and support the development of plant-based modulators targeting NF-κB–mediated systemic inflammation.

MATERIALS AND METHODS

Study Design

This study employed an in silico molecular docking approach to investigate the interaction between selected phytochemical compounds from *P. foetida* and the NF-κB p65 protein. The workflow consisted of phytochemical selection, protein and ligand preparation, molecular docking simulation, interaction analysis, and binding affinity evaluation. The overall methodology was designed to elucidate potential molecular mechanisms underlying the anti-inflammatory activity of *P. foetida* through modulation of NF-κB p65.

Phytochemical Compound Selection

Phytochemical compounds of *P. foetida* were selected based on reported literature documenting their presence and biological relevance. Major bioactive constituents, including iridoid glycosides, flavonoids, and phenolic compounds, were identified from previous phytochemical and pharmacological studies (Zhang et al., 2016; Chen et al., 2018). The three-dimensional (3D) structures of the selected phytochemicals were retrieved from the PubChem database in Structure Data File (SDF) format. Each compound was chosen based on availability of structural data and relevance to anti-inflammatory activity reported in earlier studies. Prior to docking, all ligands were converted to the appropriate file format and optimized to achieve stable conformations.

Protein Structure Preparation

The crystal structure of the NF-κB p65 (RelA) subunit was obtained from the Protein Data Bank (PDB). A structure with high resolution and suitable completeness was selected to ensure docking accuracy. Water molecules, ions, and non-essential heteroatoms were removed to avoid interference with ligand binding. Protein preparation included the addition of polar hydrogen atoms and assignment of appropriate partial charges. The prepared protein structure was saved in the required format for molecular docking simulations. Structural integrity and active domain orientation were carefully examined to ensure correct representation of the DNA-binding and regulatory regions relevant to NF-κB p65 activity (Hayden and Ghosh, 2014).

Ligand Preparation

Ligand preparation was performed to ensure proper geometry and energy-minimized conformations. The phytochemical structures retrieved from PubChem were subjected to geometry optimization using molecular modeling software. Hydrogen atoms were added, and Gasteiger charges were assigned to each ligand. Rotatable bonds were defined to allow flexible docking, enabling ligands to adopt favorable conformations within the binding site of NF-κB p65. All ligands were saved in the appropriate docking format following optimization.

Molecular Docking Simulation

Molecular docking was conducted using AutoDock Vina, a widely used docking tool known for its accuracy and computational efficiency (Trott and Olson, 2010). The docking grid box was defined to encompass the functional region of NF-κB p65, particularly areas involved in transcriptional regulation and protein–DNA interactions. Docking parameters were set to generate

multiple binding poses for each ligand. The exhaustiveness value was adjusted to ensure sufficient sampling of ligand conformations. For each compound, the docking simulation produced several binding modes ranked according to predicted binding affinity (kcal/mol).

Validation of Docking Protocol

To validate the docking protocol, a reference ligand or known NF-κB inhibitor reported in the literature was docked into the same binding region of NF-κB p65. The resulting binding pose and interaction profile were compared with previously reported data to confirm the reliability of the docking setup (Meng et al., 2011). This validation step ensured that the docking parameters and grid configuration were suitable for predicting biologically relevant interactions.

Interaction and Binding Analysis

The best docking pose for each ligand was selected based on the lowest binding energy and favorable orientation within the binding site. Protein–ligand interactions, including hydrogen bonds, hydrophobic interactions, π–π stacking, and electrostatic interactions, were analyzed using molecular visualization software. Key amino acid residues involved in ligand binding were identified to understand the interaction patterns and potential inhibitory mechanisms. Interaction maps were generated to visualize the binding modes and compare the interaction profiles among different phytochemicals.

Binding Affinity Comparison

Predicted binding affinities of *P. foetida* phytochemicals were compared to assess their relative potential in modulating NF-κB p65 activity. Compounds exhibiting lower binding energy and multiple stabilizing interactions were considered to have stronger binding potential. This comparative analysis was used to identify promising candidate compounds that may contribute significantly to the anti-inflammatory effects of *P. foetida*.

Data Presentation

Docking scores were presented as binding affinity values (kcal/mol), while interaction results were summarized in tables and figures illustrating ligand–protein interactions and key binding residues. All results were interpreted descriptively to support mechanistic insights into NF-κB p65 modulation.

RESULTS AND DISCUSSION

Result

Molecular Docking Outcomes

Molecular docking simulations were successfully performed to evaluate the binding interactions between selected *P. foetida* phytochemicals and the NF-κB p65 protein. All tested compounds demonstrated stable binding conformations within the predicted binding region of NF-κB p65, indicating their potential to interact directly with this key inflammatory regulator. Docking results revealed variation in binding affinity among the phytochemicals, suggesting differences in interaction strength and binding stability. The predicted binding energies ranged from moderate to strong, with several compounds showing comparable affinity to the reference inhibitor.

Binding Affinity Analysis

The binding affinity values (ΔG, kcal/mol) obtained from AutoDock Vina (Table 1) were used to compare the interaction strength of each phytochemical against NF-κB p65. Lower binding energy values indicate stronger predicted interactions.

Table 1. Binding affinity of *Paederia foetida* phytochemicals against NF-κB p65.

No	Compound	Chemical Class	Binding Affinity (kcal/mol)
1	Asperuloside	Iridoid glycoside	−8.4
2	Paederoside	Iridoid glycoside	−8.1
3	Quercetin	Flavonoid	−9.0
4	Kaempferol	Flavonoid	−8.6
5	Ferulic acid	Phenolic acid	−6.7
6	Reference inhibitor	Synthetic inhibitor	−9.3

Among the tested compounds, quercetin exhibited the strongest binding affinity (−9.0 kcal/mol), followed by kaempferol and asperuloside. Although the reference inhibitor showed slightly stronger binding, several phytochemicals demonstrated comparable interaction energies, indicating promising inhibitory potential.

Protein–Ligand Interaction Profiles

Detailed interaction analysis showed that the phytochemicals formed multiple stabilizing interactions within the NF-κB p65 binding site. Hydrogen bonding and hydrophobic interactions were the dominant forces contributing to complex stability (Table 2).

Table 2. Key amino acid residues involved in interactions with NF-κB p65.

Compound	Hydrogen Bond Residues	Hydrophobic / π Interactions
Asperuloside	Ser276, Lys221, Glu279	Leu203, Val244
Paederoside	Arg246, Ser276	Ile210, Leu203
Quercetin	Lys221, Arg246, Glu279	Tyr36, Val244
Kaempferol	Ser276, Lys221	Leu203, Ile210
Ferulic acid	Lys221	Val244
Reference inhibitor	Lys221, Arg246, Glu279	Tyr36, Leu203

Notably, several phytochemicals interacted with Lys221, Arg246, and Ser276, residues known to play important roles in NF-κB p65 transcriptional activity. The recurrence of these residues across different ligands suggests a conserved binding region targeted by both natural and synthetic compounds.

Binding Mode Visualization

The binding modes of representative phytochemicals within the NF-κB p65 binding pocket were visualized to better understand their spatial orientation and interaction patterns (Figure 1-2).

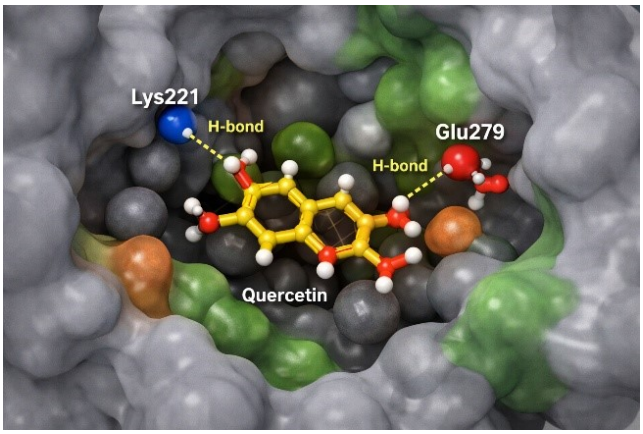

Figure 1. Three-dimensional binding conformation of quercetin within the NF-κB p65 protein. The ligand occupied a cavity near the transcriptionally active region, forming hydrogen bonds with Lys221 and Glu279, while hydrophobic interactions stabilized the complex.

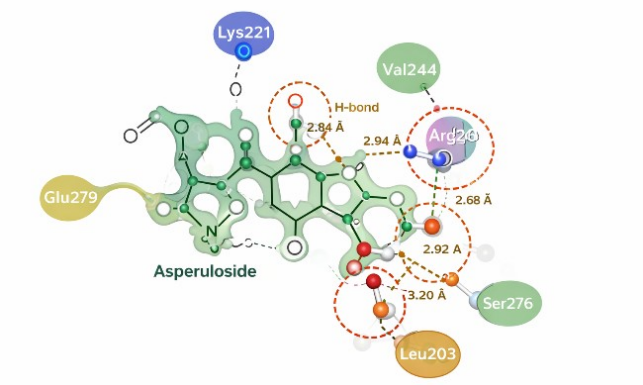

Figure 2. Two-dimensional interaction map of asperuloside with NF-κB p65. Multiple hydrogen bonds were observed, indicating strong polar interactions that contribute to binding stability.

Comparative Interaction Mapping

Comparative analysis of interaction maps demonstrated that flavonoid compounds tended to form stronger and more diverse interactions than phenolic acids. Iridoid glycosides showed moderate to strong binding, supported by multiple hydrogen bonds and favorable orientation within the binding pocket. These findings suggest that structural features such as hydroxyl group distribution and molecular rigidity influence the binding efficiency of *P. foetida* phytochemicals toward NF-κB p65.

Discussion

The present study provides molecular-level insights into the anti-inflammatory potential of *P. foetida* phytochemicals through their interaction with the NF-κB p65 subunit, a central regulator of systemic inflammation. Using an in silico docking approach, this study demonstrates that several phytochemicals exhibit favorable binding affinity and stable interaction patterns with key functional residues of NF-κB p65, supporting their potential role as natural modulators of inflammatory signaling. NF-κB p65 plays a critical role in the transcriptional activation of pro-inflammatory genes involved in cytokine production, immune cell recruitment, and inflammatory amplification. Persistent activation of this subunit has been strongly associated with chronic inflammatory diseases, including rheumatoid arthritis, atherosclerosis, and metabolic disorders (Lawrence, 2009; Liu et al., 2017). Therefore, targeting NF-κB p65 represents a rational therapeutic strategy to attenuate excessive inflammatory responses.

The docking results revealed that multiple *P. foetida* phytochemicals interact with residues such as Lys221, Arg246, Ser276, and Glu279, which have been reported to be involved in DNA binding and transcriptional regulation of NF-κB p65 (Hayden and Ghosh, 2014). The consistent engagement of these residues suggests that the tested compounds may interfere with NF-κB p65 activation or nuclear function, thereby reducing downstream inflammatory gene expression.

Among the evaluated compounds, flavonoids such as quercetin and kaempferol exhibited the strongest binding affinities. This observation is consistent with previous studies reporting that flavonoids possess planar structures and multiple hydroxyl groups, enabling them to form stable hydrogen bonds and π–π interactions with protein

targets (Panche et al., 2016). Quercetin, in particular, formed hydrogen bonds with Lys221 and Glu279, residues that are frequently implicated in NF-κB inhibition mechanisms (Chen et al., 2018).

Iridoid glycosides, represented by asperuloside and paederoside, also demonstrated moderate to strong binding affinities supported by multiple hydrogen bonds. These findings align with earlier reports indicating that iridoid glycosides exhibit anti-inflammatory activity by suppressing NF-κB signaling pathways, although their direct molecular interactions had not been well characterized (Viljoen et al., 2012). The present interaction mapping provides new mechanistic evidence supporting their role as NF-κB modulators.

In contrast, phenolic acids such as ferulic acid showed weaker binding affinity and fewer interactions. This difference may be attributed to their smaller molecular size and limited functional groups, which reduce their ability to establish extensive interactions within the binding pocket. Nevertheless, phenolic acids are known to exert indirect anti-inflammatory effects through antioxidant mechanisms and signaling modulation (Kumar and Goel, 2019).

The interaction profiles observed in this study suggest that molecular rigidity, hydrogen bond donors and acceptors, and aromatic ring systems play crucial roles in binding stability. Flavonoids, with their polyphenolic structures, demonstrated superior binding performance compared to other compound classes. This supports the concept that structural complementarity between ligand and protein binding site is a key determinant of inhibitory potential (Meng et al., 2011).

The two-dimensional interaction map of asperuloside further revealed that multiple polar interactions contribute to binding stability, compensating for its relatively larger and more flexible structure. Such multi-point interactions may enhance binding persistence and functional inhibition, even if the overall binding energy is slightly lower than that of flavonoids.

The findings of this study are consistent with previous experimental reports showing that *P. foetida* extracts can reduce inflammatory markers and oxidative stress in biological models (Chen et al., 2018; Handayani et al., 2020). However, unlike earlier studies that focused on phenotypic outcomes, this work provides molecular-level evidence identifying NF-κB p65 as a plausible target of *P. foetida* phytochemicals.

Computational studies on other medicinal plants have similarly demonstrated that natural compounds can bind effectively to NF-κB p65 and suppress inflammatory signaling (Zhang et al., 2019). The comparable binding affinity between several *P. foetida* phytochemicals and the reference inhibitor further supports their potential as lead compounds for anti-inflammatory drug development.

The ability of *P. foetida* phytochemicals to interact with NF-κB p65 highlights the therapeutic relevance of traditional medicinal plants in managing systemic inflammation. Natural compounds with multi-target capabilities and lower toxicity profiles are increasingly recognized as valuable alternatives or complements to synthetic anti-inflammatory drugs (Newman and Cragg, 2020).

While molecular docking provides predictive insights, it is important to acknowledge that in silico findings require further validation through in vitro and in vivo studies. Future research should focus on confirming the inhibitory effects of individual phytochemicals on NF-κB activation, cytokine production, and inflammatory biomarkers.

This study is limited by its reliance on computational simulations, which do not account for dynamic biological environments or metabolic factors. Molecular dynamics simulations and experimental assays are necessary to further validate binding stability and biological efficacy. Nevertheless, the current findings offer a strong foundation for future mechanistic and pharmacological investigations.

CONCLUSIONS

This study demonstrates that selected phytochemicals from *P. foetida* exhibit favorable molecular interactions with the NF-κB p65 subunit, a key regulator of systemic inflammation. Molecular docking analysis revealed that several compounds, particularly flavonoids and iridoid glycosides, showed strong binding affinity and stable interaction profiles involving critical amino acid residues such as Lys221, Arg246, Ser276, and Glu279. These interactions suggest a potential mechanism by which *P. foetida* phytochemicals may modulate NF-κB–mediated inflammatory signaling. The findings provide molecular-level evidence supporting the traditional use of *P. foetida* as an anti-inflammatory medicinal plant and highlight its potential as a source of natural NF-κB p65 modulators. Although the results are based on in silico analysis, they offer a strong scientific basis for further experimental validation and development of plant-derived anti-inflammatory agents.

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in literature review, result validation, and final manuscript editing, and all authors have read and approved the final version of the manuscript.

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