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Targeting TLR4 Signaling Pathway in Degenerative Diseases: *Molecular Docking* Study of Bioactive Compounds from Ramie (*Boehmeria Nivea*) as Anti-Inflammatory and Antioxidant Agents

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ABSTRACT

Toll-like receptor 4 (TLR4) acts as an upstream regulator that links chronic inflammation and oxidative stress through activation of the NF-κB pathway as well as the production of reactive oxygen species (ROS), thereby contributing to the progression of various degenerative diseases. Ramie (*Boehmeria nivea*) kombucha is known to contain polyphenol compounds and bioactive organic acids that have the potential to act as *nutraceutical* agents, but the molecular mechanisms of their interaction with the TLR4 pathway are still not fully analyzed. This study used the *network pharmacology* and *molecular docking* approaches to evaluate the potential of bioactive compounds of hemp leaf kombucha in targeting TLR4 *in silico*. Analysis of protein networks shows that Toll-like Receptor 4 (TLR4) has a central role as the main binding protein in inflammatory pathways and oxidative stress based on the high degree of connectivity in protein–protein interaction networks. Molecular docking simulations show that all ligands are able to bind to the active site. The flavonoid compound catechin showed the most stable *binding affinity* with a value of −7.8 kcal/mol, followed by quercetin of −7.4 kcal/mol, while organic acid compounds such as D-glucuronic acid, gluconic acid, and citric acid were in the range of −4.8 to −5.9 kcal/mol. The stability of the ligand–receptor complex is confirmed by the Root Mean Square Deviation (RMSD) value ≤ 2 Å. The ligand–receptor interaction is dominated by the formation of hydrogen bonds and hydrophobic interactions on amino acid residues at the TLR4 binding site, which contributes to the stability of the complex.

INTRODUCTION

The global epidemiological transition has placed metabolic degenerative diseases, specifically Diabetes Mellitus (DM), as a major health threat with a mortality rate of 75% of the world's total deaths by 2021 (WHO, 2025). Diabetes Mellitus is not just a disruption of glucose homeostasis, but rather a trigger for a systemic degenerative cascade mediated by chronic oxidative stress (Chaudhary et al., 2023). Although pharmacological interventions are available, long-term efficacy is often limited by low bioavailability and systemic side effects (De Crescenzo et al., 2022).

Hemp (*Boehmeria nivea*) emerged as a potential candidate in bioprospecting of biological resources due to its phytochemical profile rich in phenolic compounds such as *quercetin*, *chlorogenic acid*, and *caffeic acid* (Aloo et al., 2023). The innovation in the use of ramie leaves as a fermentation substrate for kombucha offers significant novelty compared to conventional use (Wandira et al., 2025). The fermentation process by the *Symbiotic Culture of Bacteria and Yeast* (*SCOBY*) microbial consortium is predicted to be able to increase the bioavailability of active compounds through the release of glycosidic bonds and the formation

of new functional organic acids (Bahtiar, 2023). This phenomenon has the potential to increase antioxidant capacity exponentially compared to its crude extract form (Zhou *et al*., 2020).

Although hemp's antioxidant potential has been widely reported, specific molecular mechanisms in mitigating diabetes-related metabolic degeneration still leave a *wide research gap*. Most studies are still limited to phenotypic testing without dissecting multi-target interactions at the systemic level. The *Network Pharmacology* and *Molecular Docking approach* is a precision methodology for mapping the interaction of hemp kombucha active compounds with key target proteins and pathways (such as *the Toll-like Receptor 4* (TLR4) pathway in *silico*.

In particular, the role *of Toll-like Receptor 4* (TLR4) as a key receptor that bridges the inflammatory response and oxidative stress in degenerative diseases has not been widely studied in the context of hemp metabolites (Sutian *et al*., 2024). Modulation of the TLR4 pathway is known to be closely related to the activation of the endogenous antioxidant pathway Nrf2/HO-1, which serves as a cellular protective mechanism (Krieger & Takumi, 2023). However, the identification of specific compounds in hemp that are capable of acting as antagonistic ligands against TLR4 is still limited.

The approach through *molecular docking* and *network pharmacology* is a precision methodology in exploring these molecular interactions at the atomic level. *Network Pharmacology* enables the mapping of complex interactions between hemp active compounds against systemic degenerative disease protein networks. Furthermore, *molecular docking* simulations using software such as AutoDock Vina (or AutoDock 4.0 as referenced) are used to accurately identify and predict the *binding affinity* between ligands and TLR4 macromolecules based on *Gibbs free energy* scores. The stability of ligand–protein complexes was further explored through RMSD parameters to ascertain the nature of their biological interactions. In addition, pharmacokinetic profile prediction using the SwissADME platform is required to evaluate the *drug-likeness* parameters of hemp compounds as *nutraceutical candidates* (Hasan *et al.*, 2023). This method allows for the rapid and precise selection of potential compounds in predicting *binding affinity* and stability of ligand-protein complexes before the experimental *stage of in vitro* or *in vivo testing.*

To address this gap, modern computational methodologies such as *network pharmacology* and *molecular docking* offer powerful tools. Network pharmacology enables the mapping of interactions between multiple compounds and a network of disease-related proteins, shifting the paradigm from "one drug, one target" to a more holistic "multi-compound, multi-target" perspective. Following this, molecular docking allows for the atomic-level simulation and prediction of how specific compounds bind to key protein targets, providing a detailed view of potential mechanisms of action (Hasan et al., 2023).

This study aims to target the TLR4 signaling pathway using hemp bioactive metabolites through *a full in silico study*. The main focus of this research is to analyze the potential of hemp as an anti-inflammatory and antioxidant agent that works by inhibiting TLR4 activation, so that it can support the activation of the Nrf2/HO-1 protection pathway. The results of this study are expected to provide a strong molecular blueprint for the development of functional products based on *Boehmeria nivea* in mitigating metabolic degenerative processes.

RESEARCH METHODS

Identification of bioactive compounds

Identification of hemp leaf metabolite profiles (*Boehmeria nivea*) is carried out through the integration of LC-HRMS analysis data *(Liquid Chromatography High-Resolution Mass Spectrometry)* and a comprehensive literature study. Active compounds found such as *quercetin, Glucuronic acid, Gluconic acid, D-saccharic acid-1,4-lactone (DSL), Citric acid, Lactic acid, Malic acid, Succinic acid, Tartaric acid,* and *Catechin* used as a ligand. The three-dimensional (3D) structure of the compounds is downloaded from the PubChem database in .SDF format, then converted to .PDB format using OpenBabel software. Optimization of ligand structure is carried out by adding polar hydrogen atoms and a charge through *Discover Studio.*

Table 1. List of bioactive compounds of flax leaf kombucha (*Boehmeria nivea*) along with their chemical classification and biological activity

No.	Compounds	Molecular Formula	Classes	Activities
1	Quercetin	$C_{15}H_{10}O_7$	Organic acid	Antioxidant
2	Glucuronic acid	$C_6H_{10}O_7$	Organic acid	Antioxidant
3	Gluconic acid	$C_6H_{12}O_7$	Organic acid	Antioxidant
4	D-saccharic acid-1,4-lactone (DSL)	$C_6H_8O_6$	Lactone	Antioxidant and anti-inflammatory
5	Citric acid	$C_6H_8O_7$	Organic acid	Anti-inflammatory
6	Lactic acid	$C_3H_6O_3$	Organic acid	Antioxidant
7	Malic acid	$C_4H_6O_5$	Organic acid	Antioxidants & oxidative stress
8	Succinic acid	$C_4H_6O_4$	Organic acid	Antioxidant
9	Tartaric acid	$C_4H_6O_6$	Organic acid	Antioxidant
10	Catechin	$C_{15}H_{14}O_6$	Flavonoids (polyphenol)	Antioxidant, anti-inflammatory, neuroprotective

Protein Target Prediction and Protein-Protein Interaction (PPI) Analysis

The target proteins and bioactive compounds of Hemp were predicted using SwissTargetPrediction based on similarity in molecular structure (*probability score* > 0). Simultaneously, the GeneCards database is used to identify genes/proteins associated with degenerative diseases, specifically those that play a role in anti-inflammatory and antioxidant mechanisms. The target data of the compound and the target disease were then compared using the Venny 2.1 tool to obtain slices of genes (*overlapping genes*). The sliced genes were further analyzed through the STRING database to see the interaction between proteins (*Protein-Protein Interaction*). Visualization and analysis of the network topology was carried out using

Cytoscape 3.9.1 with *the degree centrality* parameter to determine the key protein (*hub protein*). Based on the analysis of the network, *Toll-like Receptor 4 (TLR4)* (PDB ID: 2Z64) was designated as the primary target macromolecule due to its central role in bridging inflammatory pathways and oxidative stress.

Macromolecular Preparation and Method Validation

Macromolecular preparation is carried out using Discovery Studio by removing water molecules as well as natural ligands bound to proteins. The accuracy of the method is determined based on *the value of the Root-Mean-Square Deviation* (RMSD). The *results of Molecular Docking* are declared valid and accurate if the RMSD value is less than 2.0 Å, which indicates that the simulation parameters are able to replicate the orientation of the ligand biologically.

Molecular Docking

Molecular Docking was performed using AutoDock Vina on pyrex to evaluate the *binding affinity* between hemp metabolites and TLR4. The tethering area (*grid box*) is *maximized* at the coordinates of the active site of the receptor. The simulation results are expressed in *binding affinity* in kcal/mol, where a more negative value indicates a more stable bond strength. Visualizations of molecular interactions, including hydrogen bonds and hydrophobic interactions, were analyzed using *the Discovery Studio Visualizer.*

RESULTS AND DISCUSSION

Molecular Docking Results on TLR4

Analysis *Molecular Docking* showed that all of the major ligands of hemp leaf kombucha (quercetin, catechin, D-glucuronic acid, citric acid, and gluconic acid) were able to spontaneously interact with the TLR4 binding domain, indicated by the negative affinity binding value. Lower bond-free energy values indicate a more thermodynamically stable ligand–receptor complex.

Among the compounds tested, catechin showed the lowest *binding affinity* value of −7.8 kcal/mol, followed by quercetin (−7.4 kcal/mol), while D-glucuronic acid, gluconic acid, and citric acid were in the range of −4.8 to −5.9 kcal/mol. This places flavonoids as ligands with the highest affinity to TLR4 compared to organic acids, consistent with the conjugated aromatic structure and abundance of hydroxyl groups that support the formation of

The validation of docking results is confirmed through the RMSD value, where the best value of RMSD is ≤ 2 Å which is used as an indicator of ligand stability in binding. Quercetin was chosen as a reference complex for visual analysis because it has a lower RMSD than Catechin, thus providing a more optimal representation of ligands at the active TLR4 site.

Residual Interaction Patterns of TLR4 with Hemp Bioactive Compounds

The results of 3D and 2D visualization showed that TLR4 with quercetin was dominated by hydrogen bond interactions as well as hydrophobic interactions at active binding sites. Hydrogen bonds are formed at a donor–acceptor distance of about 2.8 Å, which is within the ideal range for strong, directional interactions, while distances above 3.5 Å more reflect weak dipole–dipole interactions. In addition to hydrogen bonds, the presence of flavonoid aromatic rings allows the formation of stacking π–π and π–cation with aromatic or charged residues on the surface of TLR4, which contributes to the addition of noncovalent bond energy and strengthens the stability of the complex (Sutian *et al*., 2024). Organic acids such as citric acid

and D-glucuronic acid tend to interact through hydrogen bonding and electrostatic forces between negatively charged carboxylic groups and positively charged residues in the binding pocket, but with lower interaction densities resulting in smaller total affinity than flavonoids.

Maps of complex hydrophobicity indicate that quercetin's aromatic ring is oriented to relatively hydrophobic pockets, while hydroxyl groups point to hydrophilic surfaces and polar residues, thus allowing the formation of extensive hydrogen bond networks. This configuration supports the interpretation that quercetin and catechin have the potential to act as competitive or allosteric antagonists against TLR4 by stabilizing the inactive conformation of receptors.

Relevance to TLR4 Signaling Mechanisms in Degenerative Diseases

Analysis of protein–protein networks (STRING–Cytoscape) showed that TLR4 acts as a protein hub with a high degree of connectivity and clustering coefficient in oxidative and inflammatory stress modules. Functionally, TLR4 is in the upstream position of the inflammatory response, the activation of MyD88 and TRIF then activates NF-κB

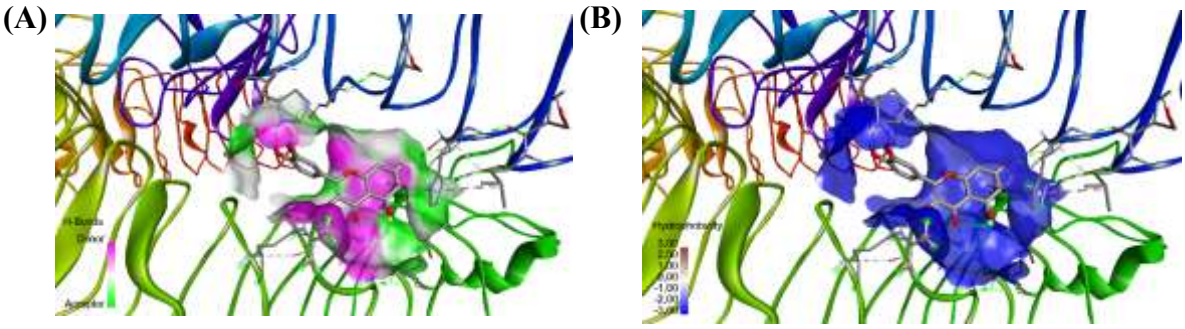

Figure 1. (A) Ligand–protein binding interactions that include hydrogen bonds interactions, on the active site of the receptor. (B) Map of hydrophobicity properties on target protein binding with values reaching −3.00.

and MAPK, as well as inducing the expression of iNOS, COX2, IL6, and TNF-α, TLR4 also links inflammatory signals to ROS production through activation of NADPH oxidase (NOX2/4) and mitochondrial ROS via ECSIT and regulates cell fate through apoptosis, pyroptosis, and ferroptosis (Cheol *et al.,* 2021). Strong binding of flavonoids of hemp to TLR4 active sites as predicted by docking has the potential to inhibit TLR4–MyD88–NF-κB activation as well as reduce mitochondrial NOX and ROS activation (Sutian *et al*., 2024). Conceptually, it is relevant to the pathogenesis of degenerative diseases (including neurodegenerative), in which chronic TLR4-based inflammation and oxidative stress contribute to the progression of neuronal and tissue damage. In addition, flax leaves and their fermented products are reported to be rich in polyphenols (quercetin, chlorogenic acid, caffeic acid) with significant antioxidant and anti-inflammatory activity. Fermentation of kombucha increases the total phenolic content, antioxidant capacity, and formation of bioactive organic metabolites. Kombucha from polyphenol-rich ingredients is also reported to be able to lower ROS and inflammatory mediators such as nitric oxide and pro-inflammatory cytokines (Alessandra *et al*., 2023).

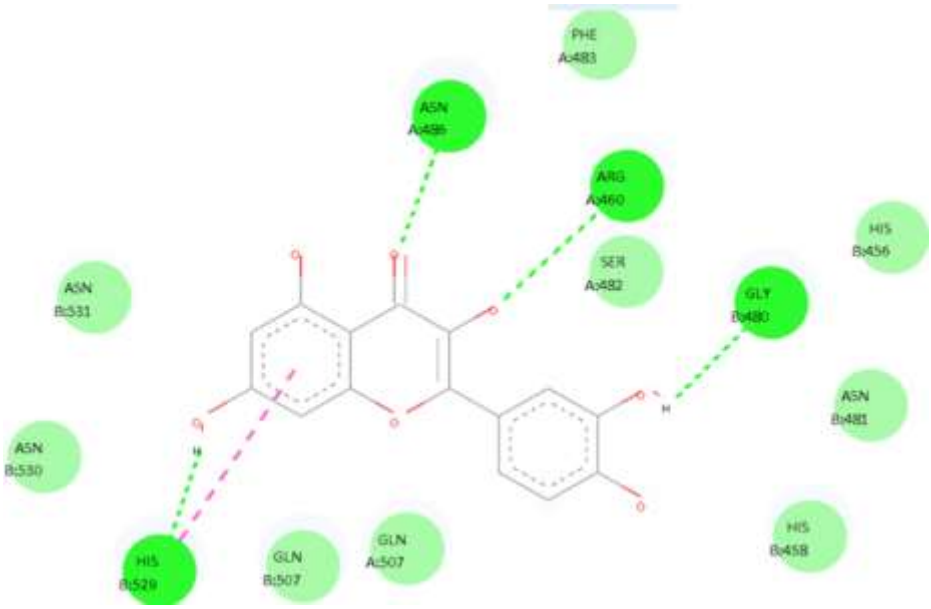

Figure 2. Interaction of Hydrogen Bonds and Active Residues of TLR4 with Quercetin Compounds Based on BIOVIA 2D Analysis

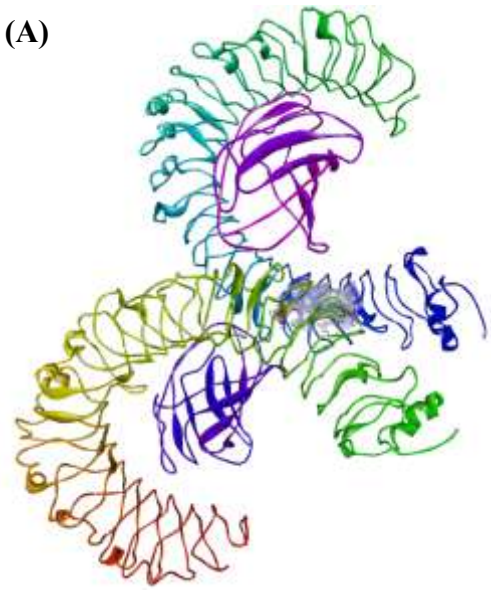

Figure 3. (A) The three-dimensional structure of the TLR4 protein-ligand complex after interacting with the bioactive compounds resulting from *molecular docking*.

Comparison of TLR4 Flavonoid and Phenolic Inhibitor Studies

Various *in vitro* and *in vivo studies* have shown that activation of Toll-like Receptor 4 (TLR4) plays a central role as a trigger of inflammation in both infectious and non-infectious conditions, while inhibition of TLR4 has been shown to be able to reduce the production of pro-inflammatory cytokines and the formation of *reactive oxygen species* (ROS) in various models of degenerative and metabolic diseases. Polyphenols, particularly flavonoids such as quercetin and catechin, have been known to have antioxidant and anti-inflammatory activity through ROS scavenging, NF-κB inhibition, and modulation of the Nrf2 pathway (Cheol *et al.,* 2021*).* The combination of polyphenol ligands and metabolic precursors (e.g. citric acid, malate, succinate)

acts as complementary. Polyphenols induce the expression of antioxidant genes, while organic acids support the redox capacity of cells through the provision of NADPH and TCA intermediates. The approach in this study also affirms the concept of synergy at the TLR4 level where flavonoids act as high-affinity TLR4 inhibitors, while organic acids provide antioxidant metabolic support.

As a docking-based *in silico* study, these findings are still predictive. Docking only models static ligand–receptor interactions and has not considered protein dynamics, solubility effects, metabolism, and systemic toxicity. Therefore, clinical claims cannot be made at this stage. Experimental validation through *in vitro* assays (e.g. measurements of TLR4 phosphorylation, NF-κB activation

This *in silico* study successfully demonstrates that bioactive compounds from ramie (*Boehmeria nivea*) leaf kombucha possess the potential to target the TLR4 signaling pathway, offering a molecular rationale for their reported anti-inflammatory and antioxidant effects. Through *network pharmacology*, TLR4 was identified as a central *hub protein* in the relevant pathological network. Molecular docking revealed that flavonoids, particularly catechin (-7.8 kcal/mol) and quercetin (-7.4 kcal/mol), exhibit strong and stable binding affinities to the TLR4 active site, characterized by a combination of hydrogen bonds and hydrophobic interactions with key amino acid residues. This predicted binding suggests a mechanism by which these compounds could act as direct antagonists, inhibiting downstream pro-inflammatory and oxidative stress cascades. Organic acids, while showing moderate binding, may play a complementary role by supporting cellular metabolism and redox balance. Collectively, these findings provide a novel molecular blueprint, positioning ramie kombucha as a promising multi-component *nutraceutical* candidate that targets the critical nexus of inflammation and oxidative stress in degenerative diseases.

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