RESEARCH ARTICLE

Alnustone and Curcumin from *Curcuma xanthorrhiza* Roxb. as Potential Multi-Target Anti-Inflammatory Adjuvants in Pulpitis: An *in silico* Molecular Docking Study

Ferry Sandra[1,*], Melanie Sadono Djamil[1], Priska Natassya[2], Jessica Endriyana[3], Visi Endah Pratitis[4]

[1]Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia
[2]Department of Pharmacology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia
[3]Department of Oral Pathology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia
[4]The Prodia Education and Research Institute, Jl. Kramat Raya No. 150, Jakarta, 10430, Indonesia

*Corresponding author. Email: ferry@trisakti.ac.id

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Abstract

BACKGROUND: Pulpitis is an inflammatory disorder of the dental pulp driven by activation of the toll-like receptor 4 (TLR4)/myeloid differentiation primary response 88 (MyD88)/interleukin-1 receptor-associated kinase 1 (IRAK-1)/tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6)/nuclear factor kappa B (NF-κB) signaling cascade and excessive production of pro-inflammatory cytokines. Bioactive compounds from *Curcuma xanthorrhiza* have demonstrated anti-inflammatory potential, but their molecular interactions with key proteins involved in pulpal inflammation remain unclear. This study aimed to evaluate the binding affinity and interaction profiles of major *C. xanthorrhiza* derived compounds with inflammation-related protein targets using an *in silico* molecular docking approach.

METHODS: The active molecule (arcurcumae, germacrone, curcumin, xanthorrhizol, alnustone, and α-cedrene) were obtained from PubChem, while target proteins (TLR4, MyD88, IRAK-1, TRAF6, inhibitor of kappa B kinase (IKK), NF-κB, interleukin (IL)-1β, TNF-α, and IL-6) were retrieved from the protein data bank. Toxicity and physicochemical properties were predicted using ProTox-3.0 and SwissADME. Active molecule and protein preparation was performed using PyRx and BIOVIA Discovery Studio, respectively. The molecular docking was conducted with CB-Dock 2.0 employing AutoDock Vina.

RESULTS: Alnustone and curcumin exhibited the strongest multi-target binding affinities toward several key proteins involved in the TLR4/MyD88/IRAK-1/TRAF6/IKK/NF-κB inflammatory signaling pathway. Alnustone demonstrated particularly strong interactions with TLR4 (-9.0 kcal/mol), IRAK-1 (-8.7 kcal/mol), NF-κB (-7.3 kcal/mol), TNF-α (-8.3 kcal/mol), and IL-6 (-6.1 kcal/mol), while curcumin showed high binding affinity to TRAF6 (-8.3 kcal/mol) and IL-1β (-8.6 kcal/mol). Interaction analyses indicated stable ligand–protein complexes supported by hydrogen bonding, hydrophobic interactions, and π-alkyl contacts within the active binding sites.

CONCLUSION: Alnustone and curcumin displayed relatively strong multi-target binding to key proteins in the TLR4/MyD88/NF-κB pathway, supporting their possible potential as adjunctive anti-inflammatory agents for pulpitis and warranting further experimental validation.

KEYWORDS: *Curcuma xanthorrhiza*, molecular docking, pulpitis, NF-κB, anti-inflammatory

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Introduction

Pulpitis is a common inflammatory condition of the dental pulp caused by bacterial invasion and the subsequent host immune response, leading to increased expression of inflammatory cytokines and tissue injury.(1-4) Progression of pulpitis involves activation of innate immune mediators and downstream signaling pathways, with nuclear factor kappa B (NF-κB) plays a pivotal role in regulating pro-inflammatory gene expression. Therefore, controlling NF-κB signaling and other inflammatory mediators is essential to prevent pulp damage and alleviating the pain.(5,6)

Recent evidence indicates that pulpitis is closely associated with activation of the toll-like receptor 4 (TLR4)/myeloid differentiation primary response 88 (MyD88)-dependent inflammatory signaling pathway. Bacterial lipopolysaccharides activate TLR4 in dental pulp cells, which subsequently recruits the adaptor protein MyD88 and activates signaling mediators including interleukin-1 receptor-associated kinase 1 (IRAK-1) and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), leading to phosphorylation of the inhibitor of kappa B kinase (IKK) complex and activation of the transcription factor NF-κB.(7,8) Activated NF-κB promotes the transcription of several pro-inflammatory cytokines, particularly interleukin (IL)-1β, TNF-α, and IL-6, which contribute to immune cell recruitment and amplification of inflammatory responses in pulpitis.(9,10) These findings highlight the TLR4/MyD88/IRAK-1/TRAF6/IKK/NF-κB signaling and cytokines as important molecular targets for controlling pulpal inflammation. However, current clinical approaches for managing inflammation often rely on systemic drugs that may cause adverse effects and show limited local efficacy within the dental pulp.

Natural compounds derived from medicinal plants have attention as potential adjunctive anti-inflammatory agents due to their multi-target biological activities and favorable safety profiles.(11) Some studies reported several natural compounds such as *Myrmecodia pendans* extract, chitosan, and aloe vera to improve the healing process of pulpitis due to its anti-inflammatory properties.(12,13) Other than those, *Curcuma xanthorrhiza* Roxb. is traditionally used in Southeast Asia for various inflammatory and digestive disorders contains several bioactive constituents including arcurcumae, germacrome, curcumin, alnustone, alpha cedrene, and xanthorrhizol.(14) Studies have shown that xanthorrhizol and *C. xanthorrhiza* extracts suppress lipopolysaccharide-induced inflammatory factors, including NF-κB, IL-1β, and mitogen-activated protein kinases (MAPK)/activator protein-1 (AP-1) signaling in oral cell models. Curcumin, another major active component, has been demonstrated to inhibit NF-κB p65 phosphorylation and attenuate NLR family pyrin domain containing 3 (NLRP3) inflammasome activation in dental pulp stem cells, suggesting a mechanism that could be relevant for modulating pulpitis inflammation.(15) Previous study have shown that molecular docking analyses demonstrate the anti-inflammatory potential of curcumin through its interactions with pro-inflammatory protein targets.(16)

Despite the reported anti-inflammatory activities of *C. xanthorrhiza*, a comprehensive understanding of how its major phytochemicals interact with inflammation-related molecular targets relevant to pulpitis remains limited. Previous research has largely focused on isolated inflammatory models or individual compounds, without a systematic evaluation of multiple major constituents such as arcurcumae, germacrome, curcumin, alnustone, xanthorrizol, and α-cedrene on molecular targets implicated in pulp inflammation.(17,18) This gap is important because pulpitis is closely associated with activation of the TLR4/MyD88/IRAK-1/TRAF6/IKK/NF-κB signaling pathway and the subsequent production of pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6. This approach provides a broader mechanistic framework for understanding the anti-inflammatory potential of *C. xanthorrhiza* in the context of pulpitis and supports its rational development for dental therapeutic applications.

Therefore, this systematic *in silico* molecular docking analysis examined principal *C. xanthorrhiza* compounds against selected inflammation-associated protein targets, to elucidate their potential binding interactions and anti-inflammatory mechanisms in the context of pulpitis. The *in silico* predictions provide a preliminary basis for subsequent validation through biological activity assays. By identifying active molecule-protein target affinities and interaction profiles, this research will contribute to a deeper mechanistic understanding of *C. xanthorrhiza'*s anti-inflammatory potential and support the future development of herbal-based adjunctive therapeutics for dental inflammation.

Methods

Collection and Preparation of Ligand and Protein Structures

All active molecule data including arcurcumae (Pubchem CID: 3083834), germacrome (Pubchem CID: 6436348),

curcumin (Pubchem CID: 969516), alnustone (Pubchem CID: 5317598), alpha cedrene (Pubchem CID: 6431015), and xanthorrizol (Pubchem CID: 93135) were retrieved from the PubChem database along with their Canonical Simplified Molecular Input Line Entry System (SMILES) representations and downloaded in SDF format. Active molecules selections were based on structural relevance to the intended target compounds for subsequent analysis. Target protein data of TLR4 (PDB ID: 3FXI), MyD88 (PDB ID: 4EO7), IRAK-1 (PDB ID: 2NRU), TRAF-6 (PDB ID: 4K8U), IKK (PDB ID: 3RZF), NFKB (PDB ID: 4Q3J), IL-1β (PDB ID: 4DEP), TNFα (PDB ID: 2AZ5), and IL-6 (PDB ID: 1ALU) were obtained from the Protein Data Bank and downloaded in PDB format. Protein selection criteria included high structural quality, defined by a resolution range of 2.0–2.5 Å, absence of mutations, ≥90% of residues in favored regions, and no residues in disallowed regions.

ProTox-3.0 and SwissADME-Based Toxicity and Pharmacokinetic Evaluation

Toxicity profiles of the active compounds were predicted using ProTox-3.0 (Environmental Protection Agency, Washington. DC, USA, https://tox.charite.de/protox3/). The canonical SMILES of each compound were submitted to evaluate toxicity class, hepatotoxicity, immunotoxicity, mutagenicity, and cytotoxicity. Pharmacokinetic properties were subsequently assessed using SwissADME (Molecular Modelling Group, Lausanne, Switzerland, http://www.swissadme.ch). Canonical SMILES served as input to analyze molecular weight, molar refractivity, lipophilicity, aqueous solubility, number of heavy atoms, rotatable bonds, hydrogen bond acceptors, and hydrogen bond donors.

Preparation and Energy Minimization of Ligands and Proteins

Each active molecule was prepared through energy minimization using PyRx–Virtual Screening Tool (FastSpring, Amsterdam, Netherlands). Molecular structures were imported into the workspace and optimized using the Universal Force Field (UFF). Energy minimization was performed employing the conjugate gradient algorithm with a maximum of 200 iterations. Convergence was achieved when the energy gradient reached <0.01 kcal/mol·Å, ensuring a stable three-dimensional conformation. The optimized structures were subsequently exported in .pdb format for further analysis. Protein preparation was carried out in Biovia Discovery Studio 2016 (Dassault Systèmes, Vélizy-Villacoublay, France) by eliminating water molecules, co-crystallized ligands, and unnecessary heteroatoms to ensure accurate docking simulations.

Molecular Docking and Binding Interaction Analysis

Molecular docking was carried out using CB-Dock 2.0 (https://cadd.labshare.cn/cb-dock2/php/index.php). Prepared ligands and target proteins were uploaded to predict binding interactions. The platform automatically detected up to five potential binding cavities and conducted docking simulations using AutoDock Vina as the docking engine. For each cavity, multiple binding poses were generated, and the optimal pose was selected based on the lowest binding affinity (Vina score, kcal/mol) and cavity suitability. Ligand–protein interactions were subsequently visualized in both three-dimensional (3D) and two-dimensional (2D) formats using BIOVIA Discovery Studio 2016.

Results

Toxicological Profiles and Physiochemical of Active Molecule

The toxicity prediction analysis using Pro-Tox III indicated that all investigated *C. xanthorrhiza*-derived exhibited a generally favorable safety profile. Arcurcumae, germacrome, curcumin, xanthorrizol, alnustone, and α-cedrene were classified as low-toxicity compounds, while germacrone and α-cedrene showed the lowest predicted acute toxicity among the tested active molecules. Notably, all compounds were predicted to be inactive to hepatotoxicity, mutagenicity, and cytotoxicity, suggesting a low risk of systemic and cellular toxicity. Although curcumin showed a predicted immunotoxic activity, the remaining active molecules were classified as immunologically inactive, indicating that most *C. xanthorrhiza* constituents may exert anti-inflammatory effects without triggering adverse immune responses (Table 1).

Physicochemical profiling using the SwissADME platform further supported the drug-likeness and suitability of these compounds for oral applications. All active molecules demonstrated molecular weights and lipophilicity values within ranges commonly associated with favorable membrane permeability, which is advantageous for local delivery in the oral cavity. Germacrone stood out due to its rigid structure with no rotatable bonds. Curcumin, while larger and more flexible than the other compounds, displayed a balanced profile in terms of hydrogen bond donors and acceptors, supporting its known multi-target biological activity (Table 2).

Table 1. Toxicity prediction profiles of active molecule using Pro-Tox III.

Toxicity Test	Active Molecule					
	Arcurcumae	Germacrome	Curcumin	Xanthorrizol	Alnustone	Alpha Cedrene
Toxicity Class (LD_{50})	Class 4 (2000 mg/kg)	Class 5 (2950 mg/kg)	Class 4 (2000 mg/kg)	Class 4 (2000 mg/kg)	Class 4 (2000 mg/kg)	Class 5 (5000 mg/kg)
Hepato-toxicity (probability)	Inactive (prob: 0.77)	Inactive (prob: 0.71)	Inactive (prob: 0.61)	Inactive (prob: 0.68)	Inactive (prob: 0.63)	Inactive (prob: 0.83)
Immuno-toxicity (probability)	Inactive (prob: 0.99)	Inactive (prob: 0.98)	Active (prob: 0.92)	Inactive (prob: 0.97)	Inactive (prob: 0.86)	Inactive (prob: 0.98)
Mutagenicity (probability)	Inactive (prob: 0.92)	Inactive (prob: 0.85)	Inactive (prob: 0.88)	Inactive (prob: 0.88)	Inactive (prob: 0.74)	Inactive (prob: 0.83)
Cytotoxicity (probability)	Inactive (prob: 0.85)	Inactive (prob: 0.90)	Inactive (prob: 0.88)	Inactive (prob: 0.84)	Inactive (prob: 0.95)	Inactive (prob: 0.74)

Interactions of *C. xanthorrhiza* Roxb.-derived Active Molecules with TLR4/MyD88/IRAK-1/TRAF6/IKK/NF-Kb Inflammatory Signaling Pathway

The molecular docking simulations revealed that bioactive molecules derived from *C. xanthorrhiza* Roxb. formed stable complexes with key proteins involved in the TLR4/MyD88/IRAK-1/TRAF6/IKK/NF-κB inflammatory signaling cascade. At the receptor level, strong interactions were observed between several active molecules and TLR4. Among the tested molecules, alnustone exhibited the strongest binding affinity with a Vina score of −9.0 kcal/mol, forming a stable docking conformation within the receptor binding cavity. The interaction profile demonstrated extensive hydrophobic contacts between the aromatic backbone of alnustone and hydrophobic residues lining the TLR4 binding pocket. In addition, π-alkyl interactions were observed between the conjugated ring system of alnustone and nearby aliphatic residues, accompanied by multiple van der Waals interactions that stabilized the active molecule orientation within the binding site of protein. Arcurcumae, which displayed a binding affinity of −8.0 kcal/mol, also formed stable interactions with TLR4. The α-Cedrene interacted with TLR4 with a binding affinity of −7.7 kcal/mol, primarily through alkyl and hydrophobic interactions generated by its terpene framework.

Following receptor interactions, active molecules interactions were also identified with the adaptor protein MyD88, which mediates signal transmission of TLR4. The strongest binding affinity toward MyD88 was observed for xanthorrizol (−7.0 kcal/mol). Docking configuration demonstrated that the xanthorrizol established hydrophobic interaction while forming additional stabilizing van der Waals interactions along the protein surface. Curcumin (−6.8 kcal/mol) and alnustone (−6.6 kcal/mol) also formed stable complexes with MyD88 (Table 3).

At the kinase signaling stage, strong active molecule interactions were observed with IRAK-1, a key kinase responsible for propagating inflammatory signals of MyD88. Alnustone showed the strongest binding affinity (−8.7 kcal/mol), occupying the catalytic pocket of the

Table 2. Physiochemical properties of active molecule using SwissADME web-based platform.

Physiochemical	Active Molecule					
	Arcurcumae	Germacrome	Curcumin	Xanthorrizol	Alnustone	Alpha Cedrene
Molecular weight (g/mol)	202.34	218.33	363.38	218.33	262.35	204.35
Number of heavy atoms	15	16	27	16	20	15
Number of rotatable bonds	4	0	8	4	6	0
Number of HBA	0	1	6	1	1	0
Number of HBD	0	0	2	1	0	0
Molar refractivity	69.55	70.88	102.80	71.57	84.78	66.88
Lipophilicity (Log P)	4.86	3.60	3.03	4.31	4.32	4.36
Water solubility (Log S / ESOL)	-4.52	-3.37	-3.94	-4.65	-4.39	-4.02

Table 3. Binding affinity values (Vina score, kcal/mol) of *C. xanthorrhiza* Roxb. bioactive compounds interacting with key proteins in the TLR4/MyD88/IRAK-1/TRAF6/IKK/NF-κB inflammatory signaling pathway.

Active Molecules	Protein (Vina Score)					
	TLR4	MyD88	IRAK-1	TRAF6	IKK	NF-κB
Arcurcumae	**-8.0**	-5.4	-7.7	-7.1	-7.1	-6.4
Germacrome	-7.2	-6.2	-7.2	**-7.3**	**-8.3**	-6.1
Curcumin	-7.6	**-6.8**	**-8.5**	**-8.3**	**-7.9**	**-7.1**
Alnustone	**-9.0**	**-6.6**	**-8.7**	**-7.8**	**-8.2**	**-7.3**
Alpha cedrene	**-7.7**	-6.5	-7.1	-7.1	-7.7	-6.3
Xanthorrizol	-7.5	**-7.0**	**-7.8**	-7.0	-7.0	-6.0

Values shown in bold indicate the strongest binding affinity (lowest Vina score) of the active molecules toward each target protein.

kinase and forming multiple hydrophobic interactions along the binding cavity, π-alkyl interactions between the active molecule's aromatic structure and surrounding residues further stabilized the docking conformation. Curcumin exhibited a similarly strong interaction with IRAK-1 (−8.5 kcal/mol). Xanthorrizol (−7.8 kcal/mol) also showed stable binding within the IRAK-1 binding pocket (Table 3).

Docking simulations demonstrated active molecule interactions with TRAF6, a key adaptor protein involved in signal amplification. Curcumin exhibited the strongest binding affinity toward TRAF6 (−8.3 kcal/mol) (Table 3). The docking complex showed hydrogen bonding interactions involving the hydroxyl groups of curcumin, together with π-π stacking and hydrophobic contacts between the aromatic rings of curcumin and residues within the TRAF6 binding pocket. Alnustone and germacrome also formed a strong interaction with TRAF6, stabilized through hydrophobic contacts and π-alkyl interactions.

The active molecule of *C. xanthorrhiza* Roxb interactions were observed with IKK, a central kinase responsible for the phosphorylation of IκB prior to NF-κB activation. The docking results showed that germacrome exhibited the strongest interaction with IKK (−8.3 kcal/mol). Alnustone (−8.2 kcal/mol) and curcumin (−7.9 kcal/mol) also formed stable complexes with IKK. Curcumin displayed hydrogen bonding interactions in addition to hydrophobic contacts, while alnustone interacted predominantly through hydrophobic and π-alkyl interactions (Table 3, Figure 1).

At the transcription factor level, Active molecule of *C. xanthorrhiza* Roxb. interactions with NF-κB were also observed. Alnustone demonstrated the strongest binding affinity (−7.3 kcal/mol), forming hydrophobic interactions and π-alkyl contacts within the protein binding interface. Curcumin (−7.1 kcal/mol) also formed a stable docking complex characterized by hydrogen bonding interactions together with aromatic stacking interactions. Other active molecule of *C. xanthorrhiza* Roxb. exhibited moderate binding affinities toward NF-κB (Table 3, Figure 1).

Interactions of *C. xanthorrhiza* Roxb.-derived Active Molecules with Pro-Inflammatory Cytokines After the NF-κB Activation

Docking interaction also showed stable active molecule binding with pro-inflammatory cytokines after NF-κB activation, including IL-1β, TNF-α, and IL-6. Among the analyzed active molecules, curcumin demonstrated the strongest interaction with IL-1β with a Vina score of −8.6 kcal/mol (Table 4). The docking conformation showed hydrogen interactions between the phenolic hydroxyl groups of curcumin and residues within the cytokine binding interface (Figure 2). Alnustone exhibited the second strongest binding affinity toward IL-1β (−7.9 kcal/mol), forming hydrophobic interactions and π-alkyl contacts with residues within the cytokine binding pocket. Germacrome also displayed notable interaction with IL-1β (−7.4 kcal/mol) (Table 4).

For TNF-α, the docking analysis showed that alnustone exhibited the strongest binding affinity (−8.3 kcal/mol). The interaction profile revealed hydrophobic contacts, π-alkyl interactions, and van der Waals forces contributing to stabilization of the active molecule within the TNF-α binding interface. Curcumin also formed a strong complex with TNF-α (−8.2 kcal/mol), where hydrogen bonds and aromatic interactions supported the active molecule binding conformation (Table 4, Figure 2).

Interactions were also observed with IL-6, although the binding affinities were relatively lower compared with

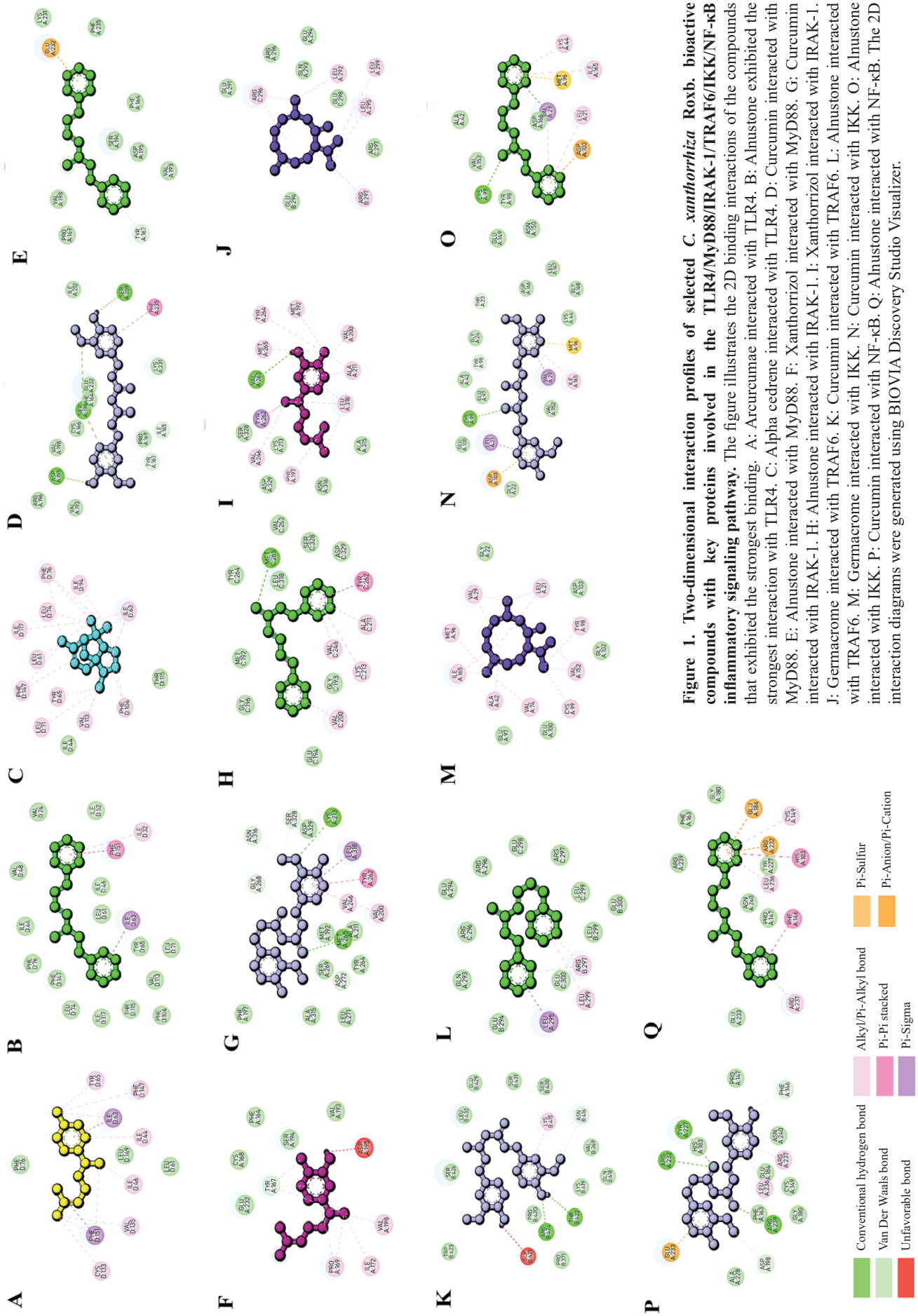

Figure 1. Two-dimensional interaction profiles of selected *C. xanthorrhiza* Roxb. bioactive compounds with key proteins involved in the TLR4/MyD88/IRAK-1/TRAF6/IKK/NF-κB inflammatory signaling pathway. The figure illustrates the 2D binding interactions of the compounds that exhibited the strongest binding. A: Arcurcumae interacted with TLR4. B: Alnustone exhibited the strongest interaction with TLR4. C: Alpha cedrene interacted with TLR4. D: Curcumin interacted with MyD88. E: Alnustone interacted with MyD88. F: Xanthorrizol interacted with MyD88. G: Curcumin interacted with IRAK-1. H: Alnustone interacted with IRAK-1. I: Xanthorrizol interacted with IRAK-1. J: Germacrome interacted with TRAF6. K: Curcumin interacted with TRAF6. L: Alnustone interacted with TRAF6. M: Germacrome interacted with IKK. N: Curcumin interacted with IKK. O: Alnustone interacted with IKK. P: Curcumin interacted with NF-κB. Q: Alnustone interacted with NF-κB. The 2D interaction diagrams were generated using BIOVIA Discovery Studio Visualizer.

Table 4. Binding affinity values (Vina score, kcal/mol) of *C. xanthorrhiza* Roxb. bioactive compounds interacting with pro-inflammatory cytokines after NF-κB activation.

Active Molecules	Protein (Vina Score)		
	IL-1β	TNFα	IL-6
Arcurcumae	-6.9	-7.0	-5.8
Germacrome	**-7.4**	-7.4	-5.9
Curcumin	**-8.6**	**-8.2**	-5.9
Alnustone	**-7.9**	**-8.3**	**-6.1**
Alpha cedrene	-6.9	-7.5	-5.8
Xanthorrizol	-7.0	-6.8	**-6.1**

Values shown in bold indicate the strongest binding affinity (lowest Vina score) of the active molecules toward each target protein.

IL-1β and TNF-α. The strongest interactions were observed for alnustone and xanthorrizol, each displaying a Vina score of −6.1 kcal/mol. These complexes were stabilized by hydrophobic interactions and van der Waals contacts within the IL-6 binding region.

Discussion

The predicted toxicity and physicochemical profiles further support the potential of these compounds as anti-inflammatory candidates. ProTox-III analysis classified all active molecules within low toxicity categories and predicted them to be inactive for hepatotoxicity, mutagenicity, and cytotoxicity. Although curcumin was predicted as active in immunotoxicity, this does not necessarily indicate adverse effects but rather reflects its immunomodulatory bioactivity (Table 1); therefore, its application should be approached with careful consideration of appropriate concentration and dosage. Furthermore, SwissADME evaluation indicated drug-like physicochemical properties, including appropriate molecular weight, lipophilicity, and hydrogen bonding capacity, which are important for molecular stability and membrane permeability (Table 2). Such characteristics have been associated with favorable pharmacokinetic behavior and therapeutic potential of natural anti-inflammatory compounds.(18,19)

Inflammation of the dental pulp is primarily initiated by microbial invasion that activates innate immune signaling pathways. One of the most important molecular mechanisms involved in pulpitis is the TLR4/MyD88/IRAK-1/TRAF6/NF-κB signaling pathway, which regulates the transcription of inflammatory mediators in pulpal tissues. Lipopolysaccharide derived from Gram-negative bacteria stimulates TLR4 activation, leading to recruitment of the adaptor protein MyD88 and activation signaling kinases such as IRAK-1 and TRAF6, resulting in nuclear translocation of NF-κB and transcription of pro-inflammatory genes.(20,21) The molecular docking results in the present study showed that several bioactive compounds derived from *C. xanthorrhiza* interact with multiple proteins involved in this signaling cascade (Table 3, Figure 1). Notably, alnustone and curcumin consistently exhibited the strongest interaction profiles across several key protein targets, indicating their potential importance as the principal anti-inflammatory constituents of *C. xanthorrhiza*. This observation is consistent with previous pharmacological studies reporting that plant-derived phytochemicals frequently exert anti-inflammatory activity through multi-target modulation of signaling pathways such as TLR4 and NF-κB rather than through a single molecular target.(22)

At the signaling pathway, the docking analysis indicated strong interactions between several *C. xanthorrhiza* compounds and TLR4 and MyD88, suggesting potential modulation of early inflammatory signaling. Among the tested molecules, alnustone showed particularly strong interactions with the TLR4 receptor, while curcumin also demonstrated stable binding interactions with several proteins within the receptor and adaptor complex (Figure 1). The TLR4/MyD88 pathway is recognized as a central mechanism driving inflammatory responses in pulpitis, and inhibition of this pathway has been shown to significantly reduce activation of downstream inflammatory signaling cascades.(23)

IRAK-1 and TRAF6 function as essential mediators of signal amplification, linking receptor activation to kinase signaling and NF-κB activation. In the present study, curcumin and alnustone displayed strong interactions with these signaling proteins, highlighting their potential role in modulating intracellular inflammatory signaling (Table 3). Previous studies have demonstrated that curcumin can suppress activation of multiple inflammatory pathways by interfering with upstream kinase signaling and blocking NF-κB activation.(24,25)

Activation of the IKK complex represents a crucial regulatory step that leads to phosphorylation and degradation of IκB, allowing NF-κB to translocate into the nucleus and initiate transcription of inflammatory mediators. The docking results revealed stable binding interactions between *C. xanthorrhiza* phytochemicals and the IKK protein, with alnustone and curcumin again demonstrating prominent

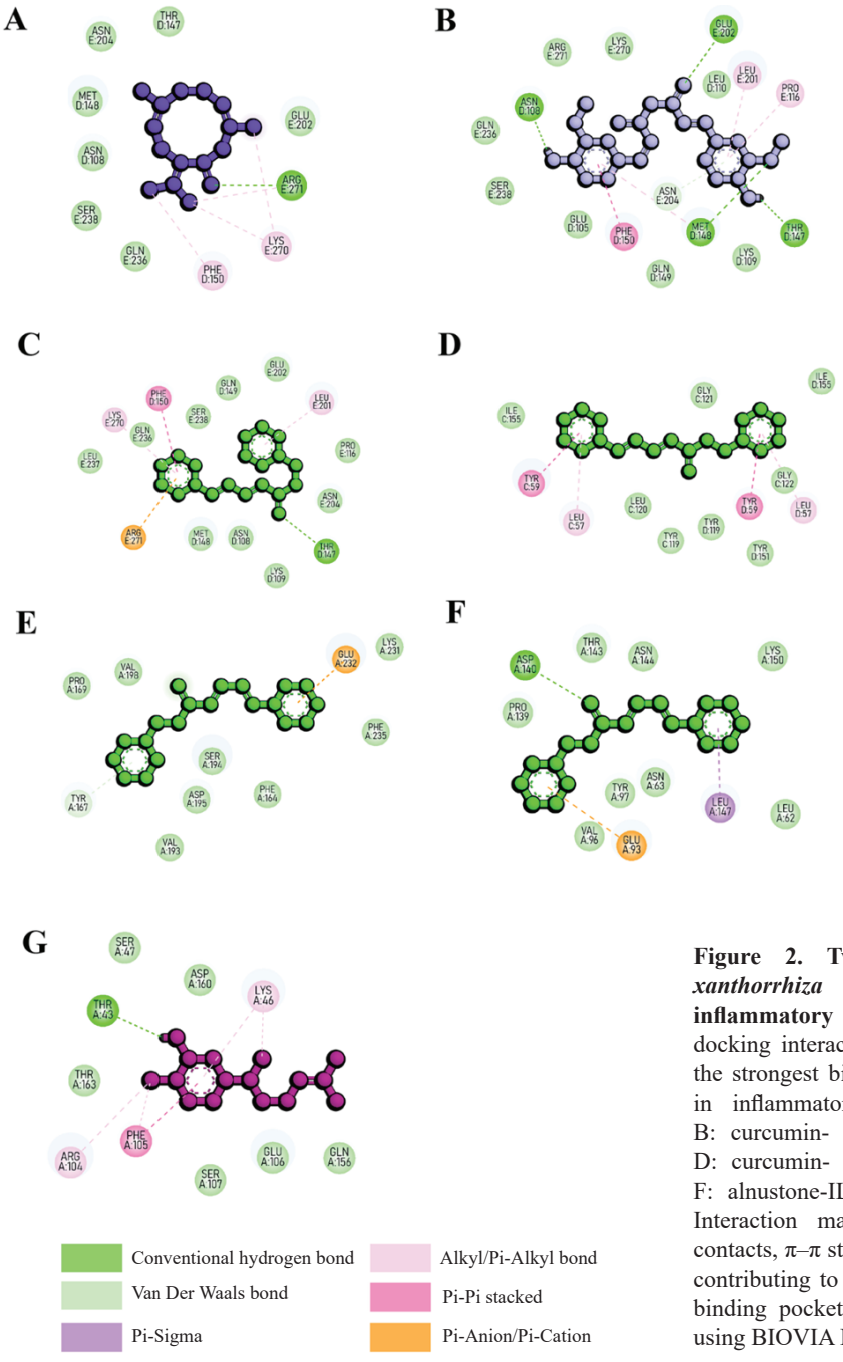

Figure 2. Two-dimensional interaction profiles of *C. xanthorrhiza* Roxb. bioactive compounds with pro-inflammatory cytokine after NF-κB activation. 2D molecular docking interaction diagrams of selected compounds exhibiting the strongest binding affinities toward cytokine targets involved in inflammatory signaling. A: germacrone–IL-1β complex; B: curcumin- IL-1β complex; C: alnustone- IL-1β complex; D: curcumin- TNFα complex; E: alnustone- TNFα complex; F: alnustone-IL-6 complex, and G: alnustone--IL-6 complex. Interaction maps illustrate hydrogen bonding, hydrophobic contacts, π–π stacking, π-sigma, and π-anion/π-cation interactions contributing to active molecule stabilization within the cytokine binding pockets. The 2D interaction diagrams were generated using BIOVIA Discovery Studio Visualizer.

interaction profiles (Figure 1). NF-κB is widely recognized as a master regulator of inflammatory gene expression and plays a major role in numerous inflammatory diseases, including pulpitis.(26)

The NF-κB activation involve increased expression of pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6, which amplify inflammatory responses within pulpal tissues. The docking results described in the cytokine interaction subsection showed that curcumin displayed strong interactions with IL-1β, while alnustone demonstrated strong interactions with TNF-α and IL-6 (Table 4). These cytokines are widely recognized as key mediators of pulpal inflammation because they promote leukocyte recruitment and amplify inflammatory signaling in dental tissues.(3,9)

Previous studies have also demonstrated that curcumin suppresses production of inflammatory cytokines by inhibiting NF-κB activation and reducing the transcription of cytokines including IL-1β, TNF-α, and IL-6.(27) Taken together, the present molecular docking findings suggest that alnustone and curcumin represent the most active

compounds among *C. xanthorrhiza* phytochemicals, exhibiting strong interactions across multiple proteins involved in the TLR4/MyD88/IRAK-1/TRAF6/IKK/NF-κB signaling cascade and its cytokine pro-inflammatory network. The ability of these compounds to interact with proteins at several stages of the inflammatory pathway supports the concept that phytochemicals may exert therapeutic effects through multi-target modulation of complex inflammatory signaling networks. This multi-level interaction profile highlights the potential of *C. xanthorrhiza* bioactive compounds as promising candidates for adjunctive anti-inflammatory strategies in pulpitis therapy. Further experimental validation through *in vitro* and *in vivo* studies will be necessary to confirm the biological significance of these predicted molecular interactions.

Conclusion

This molecular docking study demonstrated that bioactive compounds derived from C. xanthorrhiza Roxb. interact with multiple proteins involved in the TLR4/MyD88/IRAK-1/TRAF6/IKK/NF-κB signaling pathway and pro-inflammatory cytokines associated with pulpitis. Alnustone and curcumin exhibited the strongest and most consistent interactions across several key protein targets, including TLR4, IRAK-1, TRAF6, IKK, NF-κB, and downstream cytokines such as IL-1β and TNF-α. These findings indicate that C. xanthorrhiza phytochemicals, particularly alnustone and curcumin, may exert anti-inflammatory effects through multi-target modulation of inflammatory signaling pathways, supporting their potential as adjunctive therapeutic candidates for managing pulpitis.

Authors Contribution

FS conceptualized and planned the study, while MSD, PN, and JE contributed additional insights. FS and VEP conducted data collection and analysis, prepared the manuscript draft, and created the figures. All authors participated in critically revising the manuscript.

Ethical Statement

Ethical approval and informed consent were not required for this work.

Conflict of Interest

All authors declare that they have no competing interests.

References

1. Mente J, Geletneky B, Ohle M, Koch MJ, Friedrich Ding PG, Wolff D, Dreyhaupt J, Martin N, Staehle HJ, Pfefferle T. Mineral trioxide aggregate or calcium hydroxide direct pulp capping: an analysis of the clinical treatment outcome. J Endod. 2010; 36(5): 806-13.
2. Sandra F, Ranggaini D, Hendamin LA, Dewi NM, Djamil MS. Caffeic acid inhibits mass formation in MG-63 cells-induced mice. Indones Biomed J. 2022; 14(4): 416–20.
3. Khorasani MMY, Hassanshahi G, Brodzikowska A, Khorramdelazad H. Role(s) of cytokines in pulpitis: Latest evidence and therapeutic approaches. Cytokine. 2020; 126: 154896. doi: 10.1016/j.cyto.2019.154896.
4. Sandra F, Sutanto A, Wulandari W, Lambertus R, Celinna M, Dewi NM, *et al.* Crucial triad in pulp-dentin complex regeneration: Dental stem cells, scaffolds, and signaling molecules. Indones Biomed J. 2023; 15(1): 25–46.
5. Pohl S, Akamp T, Smeda M, Uderhardt S, Besold D, Krastl G, *et al.* Understanding dental pulp inflammation: From signaling to structure. Fronti Immunol. 2024; 15: 1474466. doi: 10.3389/fimmu.2024.1474466.
6. Walsh D, Quigley R, Ekperuoh A, Duncan HF. Objectively diagnosing pulpitis: Opportunities and methodological challenges in the development of point-of-care assays. Int J Mol Sci. 2025; 27(1): 355. doi: 10.3390/ijms27010355.
7. Azam S, Jakaria M, Kim IS, Kim J, Haque ME, Choi DK. Regulation of toll-like receptor (TLR) signaling pathway by polyphenols in the treatment of age-linked neurodegenerative diseases: Focus on TLR4 signaling. Front Immunol. 2019; 10: 1000. doi: 10.3389/fimmu.2019.01000.
8. Deguine J, Barton GM. MyD88: A central player in innate immune signaling. F1000Prime Rep. 2014; 6: 97. doi: 10.12703/P6-97.
9. Khaiboullina S. Special Issue: The role of cytokines in disease. Int J Mol Sci. 2026; 27(2): 876. doi: 10.3390/ijms27020876.
10. Kokkas A, Goulas A, Stavrianos C, Anogianakis G. The role of cytokines in pulp inflammation. J Biol Regul Homeost Agents. 2011; 25(3): 303-11.
11. Sovia E, Anggraeny D. Sugar palm fruits (Arenga pinnata) as potential analgesics and anti-inflammatory agent. Mol Cell Biomed Sci. 2019; 3(2): 107-14.
12. Tifani AS, Rachmawati R, Nugraeni Y, Fauzi A, Rahayu RF. Chitosan–Aloe vera combination enhances STRO-1, DSPP, and reparative dentin formation in a rat model of reversible pulpitis. Indones Biomed J. 2025; 17(6): 585-93.
13. Sudiono J, Hardina M. The effect of myrmecodia pendans ethanol extract on inflamed pulp: Study on sprague dawley rats. Mol Cell Biomed Sci. 2019; 3(2): 115-21.
14. Rahmat E, Lee J, Kang Y. Javanese turmeric (Curcuma xanthorrhiza Roxb.): Ethnobotany, phytochemistry, biotechnology, and pharmacological activities. Evid Based Complement Alternat Med. 2021; 2021: 9960813. doi: 10.1155/2021/9960813.
15. Zhang DD, Jin Y, Wu JZ, Qin P, Liu C, Lu Y, *et al*. Stigmasterol: a comprehensive review on its pharmacological, pharmacokinetic,

and molecular mechanisms. Front Pharmacol. 2022; 13: 903479. doi: 10.3389/fphar.2022.903479.
16. Sohilait MR, Pranowo HD, Haryadi W. Molecular docking analysis of curcumin analogues with COX-2. Bioinformation. 2017; 13(11): 356-9. doi: 10.6026/97320630013356.
17. Rahaman MM, Rakib A, Mitra S, Tareq AM, Emran TB, Shahid-Ud-Daula AFM, *et al.* The genus Curcuma and inflammation: Overview of the pharmacological perspectives. Plants. 2020; 10(1): 63. doi: 10.3390/plants10010063.
18. Rahmat E, Lee J, Kang Y. Javanese turmeric (Curcuma xanthorrhiza Roxb.): Ethnopharmacology, phytochemistry, and biological activities. Plants. 2021; 10(5): 1001. doi: 10.1155/2021/9960813.
19. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017; 7: 42717. doi: 10.1038/srep42717.
20. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010; 11(5): 373-84.
21. Hamerman JA, Pottle J, Ni M, He Y, Zhang ZY, Buckner JH. Negative regulation of TLR signaling in myeloid cells--implications for autoimmune diseases. Immunol Rev. 2016; 269(1): 212-27.
22. Zhao Y, Wu J, Liu X, Chen X, Wang J. Decoding nature: Multi-target anti-inflammatory mechanisms of natural products in the TLR4/NF-κB pathway. Front Pharmacol. 2025; 15: 1467193. doi: 10.3389/fphar.2024.1467193.
23. Kang Y, Su G, Sun J, Zhang Y. Activation of the TLR4/MyD88 signaling pathway contributes to the development of human hepatocellular carcinoma via upregulation of IL-23 and IL-17A. Oncol Lett. 2018; 15(6): 9647-54. doi: 10.3892/ol.2018.8586.
24. Peng Y, Ao M, Dong B, Jiang Y, Yu L, Chen Z, Hu C, Xu R. Anti-inflammatory effects of curcumin in the inflammatory diseases: Status, limitations and countermeasures. Drug Des Devel Ther. 2021; 15: 4503-25.
25. He Y, Yue Y, Zheng X, Zhang K, Chen S, Du Z. Curcumin, inflammation, and chronic diseases: how are they linked? Molecules. 2015; 20(5): 9183-213.
26. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017; 2: 17023. doi: 10.1038/sigtrans.2017.23.
27. Liu M, Wang J, Song Z, Pei Y. Regulation mechanism of curcumin mediated inflammatory pathway and its clinical application: A review. Front Pharmacol. 2025; 16: 1642248. doi: 10.3389/fphar.2025.1642248.