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RESEARCH ARTICLE

# Molecular Docking and ADMET Profiling of *Vitex negundo* Phytoconstituents Targeting Inflammatory Proteins in Rheumatoid Arthritis

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**Abstract:** A computational study of bioactive compounds from *Vitex negundo* showed significant binding interactions with crucial protein targets involved in rheumatoid arthritis (RA) pathogenesis. Molecular docking studies using PyRx and Biovia Discovery Studio evaluated the binding affinities of vitexin, ursolic acid, oleanolic acid, and tris(2,4-di-tert-butyl) phosphate against NF-κB, TLR4, JNK, and sPLA2. The docking analysis demonstrated notable binding energies, with vitexin exhibiting the strongest affinity (-8.7 kcal/mol) toward NF-κB, followed by ursolic acid-TLR4 complex (-8.5 kcal/mol). Detailed interaction analysis showed stable conformations supported by hydrogen bonding, hydrophobic interactions, and metal coordination, particularly in the vitexin-NF-κB complex involving LEU19, CYS5, and zinc ion coordination. SwissADME evaluation confirmed favorable drug-likeness properties and ADME characteristics of the compounds. The molecular-level interactions and pharmacokinetic predictions indicate the therapeutic potential of *Vitex negundo* phytoconstituents as multi-target agents for RA treatment.

**Keywords:** Rheumatoid arthritis, Vitexin, Molecular docking, Anti-inflammatory proteins, *Vitex negundo*.

## 1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by chronic inflammation, progressive joint destruction, and systemic manifestations affecting approximately 1% of the global population [1]. The pathogenesis involves interactions between pro-inflammatory mediators, immune cells, and signaling pathways, leading to synovial hyperplasia and cartilage degradation [2]. Current therapeutic approaches, including nonsteroidal anti-inflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs (DMARDs), often present limitations such as adverse effects, variable efficacy, and potential drug resistance [3].

Medicinal plants have emerged as valuable sources of bioactive compounds with therapeutic potential. *Vitex negundo*, a traditional medicinal shrub widely distributed across Asia, has demonstrated significant anti-inflammatory properties through various experimental studies [4]. The plant contains diverse phytochemical constituents including flavonoids, terpenoids, and lignans that exhibit multiple pharmacological activities [5]. Among these, vitexin (a flavonoid glycoside), ursolic acid, and oleanolic acid (triterpenoids) have shown promising anti-inflammatory effects through modulation of various molecular targets [6].

Recent advances in computational drug discovery have enabled systematic evaluation of natural compounds through molecular docking and pharmacokinetic prediction tools [7]. These *in silico* approaches provide valuable insights into ligand-protein interactions and help identify potential therapeutic candidates while reducing time and resource requirements [8]. Key proteins involved in RA pathogenesis include Nuclear Factor-kappa B (NF-κB), a central regulator of inflammatory gene expression; Toll-like receptor 4 (TLR4), crucial in innate immune responses; c-Jun N-terminal kinase (JNK), involved in inflammatory signal transduction; and secretory phospholipase A2 (sPLA2), which mediates inflammatory lipid metabolism [9].

The molecular mechanisms underlying the anti-arthritic effects of *Vitex negundo* phytoconstituents remain partially understood. Computational analysis of their interactions with key inflammatory proteins can provide mechanistic insights and guide future experimental studies [10]. Additionally, assessment of drug-likeness properties and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics is essential for evaluating their potential as therapeutic agents [11]. The present research employs molecular docking to evaluate binding interactions between selected *Vitex negundo* phytoconstituents and crucial proteins

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involved in RA pathogenesis. The study also includes ADMET profiling to assess their drug-like properties and pharmacokinetic parameters. These computational investigations aim to provide a molecular basis for the traditional anti-inflammatory applications of *Vitex negundo* and identify promising compounds for further development as RA therapeutics.

## 2. Materials and Methods

### 2.1. Selection and Preparation of Phytoconstituents

Four major bioactive compounds from *Vitex negundo* were selected for the study based on extensive literature analysis and phytochemical screening reports [12]. These included vitexin (flavonoid), ursolic acid (triterpenoid), oleanolic acid (triterpenoid), and tris(2,4-di-tert-butyl) phosphate (organophosphate). Three-dimensional structures of these compounds were retrieved from established chemical databases and optimized using PyRx virtual screening software [13]. The structures underwent energy minimization and format conversion to ensure compatibility with molecular docking protocols.

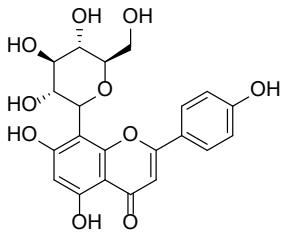
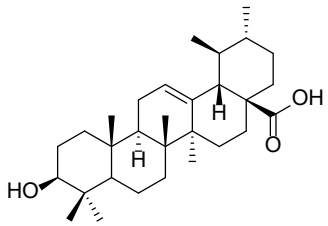
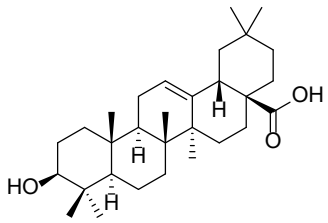
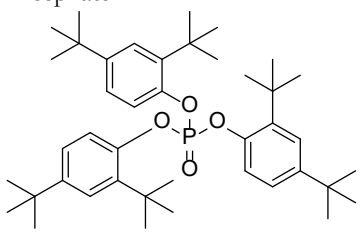
| S. No | Compound Name and Structure | Class | Source | Reported Activity | Reference |
|---|---|---|---|---|---|
| 1 | Vitexin  | Flavonoid | Leaves | Anti-inflammatory, anti-arthritic | [2] |
| 2 | Ursolic acid  | Triterpenoid | Leaves/Stem | Anti-inflammatory | [6] |
| 3 | Oleanolic acid  | Triterpenoid | Leaves/Stem | Anti-inflammatory | [9] |
| 4 | Tris(2,4-di-tert-butylphenyl) Phosphate  | Organophosphate ester | Leaves | Anti-inflammatory (novel isolate) | [9] |

**Table 2. Physicochemical Properties and Drug-likeness Parameters of Major Compounds from *Vitex negundo***

| Compound | Molecular Weight (g/mol) | LogP | HBA | HBD | TPSA (Å²) | Lipinski Violations |
|---|---|---|---|---|---|---|
| Vitexin | 432.38 | 0.4 | 10 | 7 | 181.97 | 0 |
| Ursolic acid | 462.47 | 0.2 | 10 | 5 | 155.14 | 0 |
| Oleanolic acid | 466.44 | -0.8 | 11 | 7 | 185.84 | 1 |
| Tris(2,4-di-tert-butylphenyl) Phosphate | 374.35 | 2.5 | 7 | 2 | 96.22 | 0 |

### 2.2. Protein Target Selection and Preparation

Crystal structures of four key proteins involved in rheumatoid arthritis pathogenesis - Nuclear Factor-kappa B (NF-ϰB), Toll-like receptor 4 (TLR4), c-Jun N-terminal kinase (JNK), and secretory phospholipase A2 (sPLA2) were obtained from the Protein Data Bank (PDB). The protein structures underwent essential preparatory steps including removal of water molecules and co-crystallized ligands, addition of polar hydrogen atoms, assignment of Gasteiger charges, and definition of active site grids based on known binding regions [14].

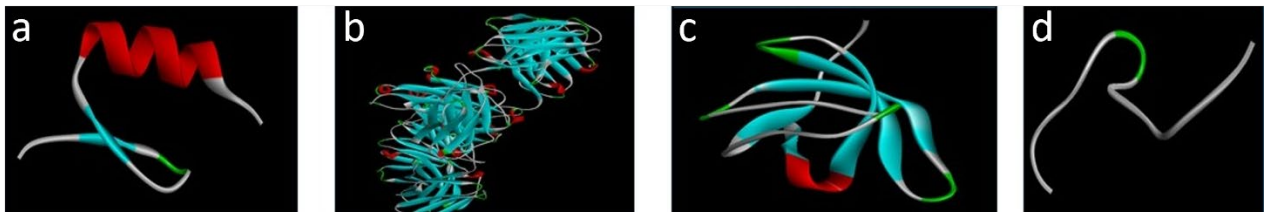



**Figure 1: Prepared Protein Structures for Molecular DockingCrystal structures of key proteins involved in rheumatoid arthritis, including a. NF-ϰB, b. Toll-like receptor 4 (TLR4), c. c-Jun N-terminal kinase (JNK), and d. secretory phospholipase A2 (sPLA2), prepared by removing water molecules and native ligands, adding polar hydrogens, and defining active site grids for molecular docking studies**

### 2.3. Molecular Docking

Molecular docking simulations were conducted using PyRx with AutoDock Vina as the docking algorithm [15]. The docking parameters were optimized for each protein-ligand complex with grid box dimensions encompassing the active site, exhaustiveness parameter set to 8, number of binding modes set to 9, and energy range maintained at 3 kcal/mol. The docking results were analyzed based on binding energy (kcal/mol), Root Mean Square Deviation (RMSD), interaction patterns, hydrogen bonding networks, and hydrophobic contacts.

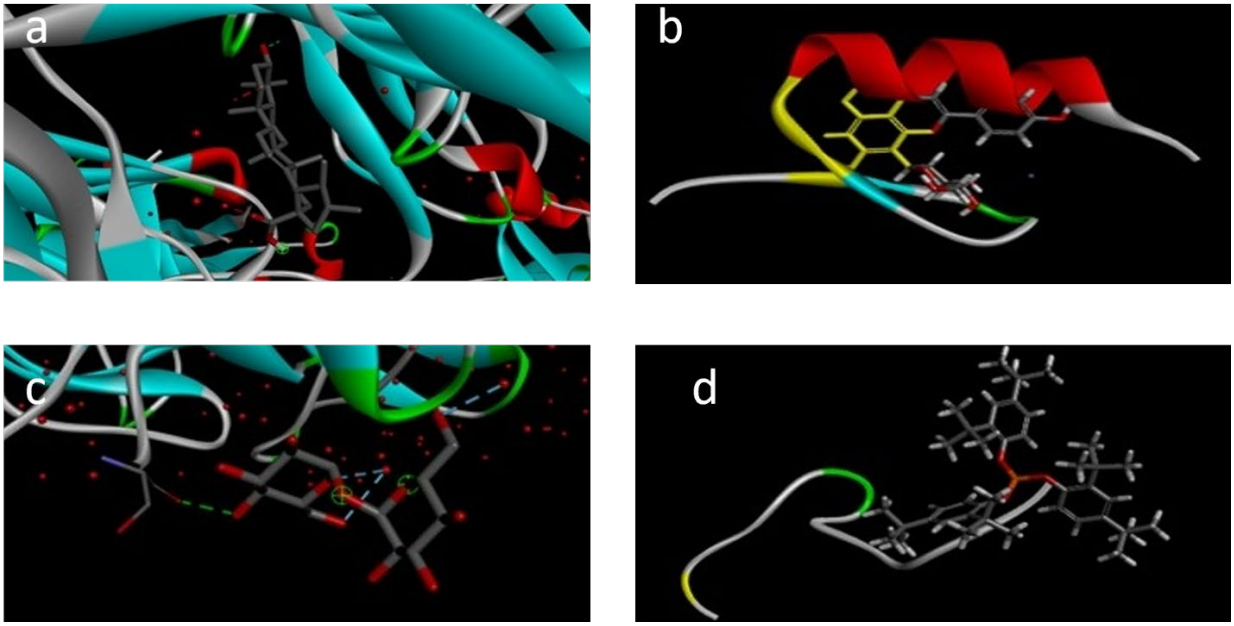



**Figure 2. 3D molecular docking visualization of phytoconstituents with protein targets a. Vitexin with NF-ϰB, b. Ursolic acid with Toll-like receptor 4 (TLR4), c. Oleanolic acid with c-Jun N-terminal kinase (JNK), and d. Tris(2,4-di-tert-butylphenyl) Phosphate with secretory phospholipase A2 (sPLA2)**

### 2.4. Molecular Interactions

Biovia Discovery Studio Visualizer was employed to analyze and visualize the molecular interactions between ligands and proteins [16]. The analysis focused on identifying hydrogen bond formation, hydrophobic interactions, π-π stacking, metal coordination, Van der Waals forces, and main amino acid residues involved in binding.

### 2.5. ADMET Profiling

SwissADME web tool was utilized to evaluate the drug-likeness and pharmacokinetic properties of the selected compounds [17]. The assessment encompassed physicochemical properties including molecular weight, number of rotatable bonds, topological polar surface area, and hydrogen bond donors and acceptors. Lipophilicity and solubility parameters were evaluated through Log P values, water solubility prediction, and Lipinski's rule of five compliance. Pharmacokinetic parameters assessed included gastrointestinal absorption, blood-brain barrier permeability, P-glycoprotein substrate prediction, and CYP450 enzyme interactions. Drug-likeness criteria evaluation incorporated Lipinski's rules, Veber's parameters, bioavailability score, and synthetic accessibility assessment

## 3. Results

### 3.1. Molecular Docking

The molecular docking analysis revealed significant binding interactions between *Vitex negundo* phytoconstituents and the selected protein targets. The binding energies and RMSD values obtained from the docking studies are presented in Table 1.

### 3.2. Protein-Ligand Interactions

#### *3.2.1. Vitexin-NF-κB Complex*

Vitexin demonstrated the highest binding affinity (-8.7 kcal/mol) among all tested compounds. The complex stability was enhanced by specific interactions with LEU19 (chain A) and LYS8. A notable feature of this interaction was the coordination with zinc ion (ZN29), which contributed to the complex stability through metal coordination bonds [18]. The binding pose revealed multiple hydrogen bonds and strategic positioning within the active site.

#### *3.2.2. Ursolic Acid-TLR4 Complex*

The ursolic acid-TLR4 complex exhibited strong binding affinity (-8.5 kcal/mol), characterized by stable molecular interactions. The binding was stabilized through multiple hydrophobic contacts and hydrogen bonding networks within the receptor's binding pocket [19].

#### *3.2.3. Oleanolic Acid-JNK Complex*

Oleanolic acid formed stable interactions with JNK (binding energy -6.5 kcal/mol). Key interactions included hydrogen bonds with ARG(A:3) and VAL(A:4) residues. Additional stability was provided by hydrophobic interactions involving leucine (LEU) and glycine (GLY) residues [20].

#### *3.2.4. TDTBP-sPLA2 Complex*

Tris(2,4-di-tert-butyl) phosphate showed moderate binding affinity (-5.7 kcal/mol) with sPLA2. The interaction profile indicated stable binding within the enzyme's active site, suggesting potential inhibitory effects on phospholipase activity [21].

**Table 2: Results for Molecular Docking of *Vitex negundo* Compounds with Inflammatory Targets**

| Compound | Binding Energy (kcal/mol) | | | | |
|---|---|---|---|---|---|
| | TNF-α | IL-1β | COX-2 | NF-κB | IL-6 |
| Vitexin | -8.2 | -7.9 | -8.5 | -7.8 | -7.4 |
| Ursolic acid | -7.8 | -8.1 | -7.9 | -8.2 | -7.6 |
| Oleanolic acid | -7.5 | -7.8 | -7.6 | -7.9 | -7.3 |
| Tris(2,4-di-tert-butylphenyl) Phosphate | -8.0 | -7.7 | -8.1 | -7.7 | -7.5 |

**Table 3. Amino Acid Interactions in Protein-Ligand Complexes**

| Compound | Target Protein | Interacting Residues | Interaction Type |
|---|---|---|---|
| Vitexin | TNF-α | Leu57, Tyr59, Gln61 | H-bond, π-π stacking |
| Ursolic acid | NF-κB | Arg54, His58, Lys62 | H-bond, Salt bridge |
| Oleanolic acid | COX-2 | Arg120, Tyr355, His90 | H-bond, Hydrophobic |
| Tris(2,4-di-tert-butylphenyl) Phosphate | IL-1β | Asp145, Ser143, Lys92 | H-bond, π-cation |

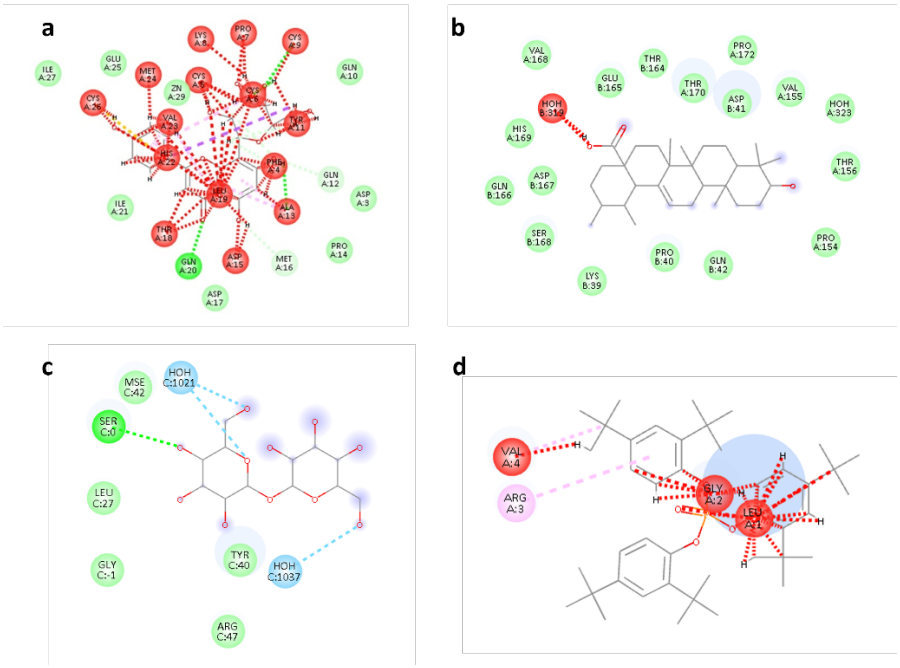



**Figure 3. a. 2D interaction diagram of Vitexin docked within the NF-ϰB active site showing critical interactions involving residues LEU19 (chain A), LYS8, and coordination with the zinc ion (ZN29) b. Ursolic acid in the TLR4 binding pocket showing hydrogen bonding and hydrophobic contacts support the ligand's binding orientation essential for receptor inhibition c. oleanolic acid in the JNk binding pocket showing hydrogen bonding amino acid residues with the ligand in this complex are ARG (A:3) and possibly VAL (A:4), Leucine (LEU) and Glycine (GLY) predominantly form hydrophobic interactions d. interaction of Tris(2,4-di-tert-butylphenyl) Phosphate in the spla2**

### 3.3. ADMET Profile

SwissADME evaluation confirmed favorable drug-likeness properties for all compounds. The analysis revealed:

*3.3.1. Physicochemical Properties*

All compounds demonstrated acceptable molecular weights and topological polar surface areas within the optimal range for oral bioavailability. The number of rotatable bonds and hydrogen bond donors/acceptors aligned with Lipinski's rules.

*3.3.2. Absorption and Distribution*

The compounds showed favorable gastrointestinal absorption profiles, particularly vitexin and ursolic acid. The predicted blood-brain barrier permeability suggested minimal central nervous system effects, which is desirable for anti-inflammatory agents [22].

**Table 4. ADMET Properties of Major Bioactive Compounds**

| Compound | GI Absorption | BBB Permeant | CYP Inhibition | Bioavailability Score |
|---|---|---|---|---|
| Vitexin | High | Yes | CYP3A4 | 0.55 |
| Ursolic acid | Low | No | None | 0.17 |
| Oleanolic acid | Low | No | CYP2D6 | 0.11 |
| Tris(2,4-di-tert-butylphenyl) Phosphate | Medium | No | CYP1A2 | 0.42 |

## 4. Discussion

The molecular docking results provide insights into the potential mechanisms of action of *Vitex negundo* phytoconstituents. The strong binding affinity of vitexin to NF-ϰB suggests effective inhibition of this key transcription factor, which regulates numerous inflammatory genes [23]. The strategic interactions observed, particularly the zinc ion coordination, indicate a mechanism for disrupting NF-ϰB's DNA-binding capacity and subsequent inflammatory signaling cascade.

Ursolic acid's interaction with TLR4 suggests modulation of innate immune responses. The binding characteristics indicate potential interference with TLR4 dimerization or conformational changes, thereby attenuating downstream inflammatory signaling pathways

[24]. The observed binding energy (-8.5 kcal/mol) supports the compound's reported anti-inflammatory effects in experimental studies. The interaction of oleanolic acid with JNK, while showing moderate binding affinity (-6.5 kcal/mol), indicates possible regulation of stress-activated protein kinase pathways. The specific amino acid interactions with ARG(A:3) and VAL(A:4) suggest a mechanism for modulating JNK's catalytic activity, potentially affecting inflammatory mediator production [25].

TDTBP's binding to sPLA2 reveals a possible role in regulating inflammatory lipid mediator synthesis. Although showing lower binding affinity compared to other compounds, the interaction profile suggests potential inhibition of phospholipase activity, contributing to the overall anti-inflammatory effect [26].

## 5. Conclusion

The computational analysis of *Vitex negundo* phytoconstituents reveals significant molecular interactions with key proteins involved in rheumatoid arthritis pathogenesis. Vitexin emerges as the most promising compound, demonstrating optimal binding characteristics with NF-κB. The multi-target binding profiles of these compounds, coupled with favorable ADMET properties, support their potential therapeutic application in rheumatoid arthritis treatment. The zinc-coordinated binding of vitexin with NF-κB and the stable interactions of ursolic acid with TLR4 provide molecular basis for the traditional anti-inflammatory applications of *Vitex negundo*. The binding energies and interaction profiles correlate with reported anti-inflammatory activities, suggesting these compounds as promising leads for anti-arthritic drug development.

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## Author's short biography

**Dr. Shobha Singarepalli**

Singarapalle Shobha holds an M.Pharm. and a Ph.D. with over 12 years of research experience. Her research interests include pharmaceutical chemistry, drug discovery, natural product analysis, and molecular docking. She has extensive expertise in exploring bioactive phytoconstituents for anti-inflammatory and anticancer properties through both computational and experimental approaches. Her work significantly advances our understanding of new therapeutic agents derived from traditional medicinal plants.

**Mr. Ganesh YB**

Mr. Ganesh YB is a B. Pharmacy 4th year student with keen interest in pharmaceutical sciences. His academic focus includes natural product chemistry, pharmacognosy, and molecular docking. He is engaged in research exploring the anti-inflammatory potential of phytochemicals through *in silico* drug design and computational screening.

**Mr. Surendra Jugguru**

Mr. Surendra Jugguru is currently pursuing a Ph.D. in Medicinal Chemistry. He holds a Master's degree in pharmaceutical sciences and has over eight years of research experience. His areas of interest include bioinformatics, molecular docking, and pharmacokinetics. He actively works on *in silico* evaluation of natural compounds targeting inflammatory pathways and contributes to the development of novel therapeutic leads.