Computational Studies of Vitexin isolated from *Vitex negundo* against Rheumatoid Arthritis Targets

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**ABSTRACT**

One chronic inflammatory autoimmune disease that needs attention to be treated is rheumatoid arthritis (RA). Especially, RA encounters more female cases that manifest progressive destruction of bone and cartilage. Expression of more inflammatory cytokines and matrix metalloproteins at the synovial tissue destruct collagen structure and worsen the condition. Generally, anti-inflammatory drugs and immune-modulatory agents are used to deduce the progression of the disease. Such chemical entities have proven many side effects and expensive. This study provides a glimpse of structural based drug designing, using bioactive pharmakon, from a folk chloric herb. *Vitex negundo* is a traditionally available plant that has been reported to possess pharmaco-beneficiary bioactive flavonoids, especially vitexin. Virtual screening involves docking of vitexin with three different targets responsible for triggering the collagenase enzyme achieved using AutoDock. The binding energy of vitexin and TNF α receptor 1 (TNFR1) was found to be slightly higher when compared to docking results of TNF α converting enzyme (TACE) and human inhibitory kinase β (hIKβ) proteins. The molecular interactions were visualized using BIOVIA discovery studio visualizer. This study may become evident that vitexin has enhanced pharmacological benefits that need to be ensured through *in vitro* and *in vivo* assays.

**INTRODUCTION**

Rheumatoid arthritis (RA) is a progressive chronic inflammatory autoimmune disease that leads to the destruction of cartilage and bone. More than 15% of the world’s populations are affected by encountering higher female cases. Since the exact cause is not known, disease-modifying anti-inflammatory drugs (DMARDs) and non-steroidal anti-inflammatory drugs (NSAIDs) are administered even in the advanced stages of RA.[1] DMARDs and NSAIDs are better immuno-modulatory agents reported adverse side effects after prolonged use.[2] Natural molecular entities may replace chemical leads in healing diseases. Biological compounds are cost-effective, ease of production, safety, and efficacy levels also high.[3] *V. negundo* is an annual herb available throughout India, commonly called as “nirgundi” by Ayurvedic pharmacopoeia of India reported excellent anti-inflammatory property, which may occur due to the presence of bioactive pharmakon, vitexin.[4] As an alternate, a biological pharmakon isolated from *V. negundo* may satisfy the above need. Vitexin is a flavone glycoside considered an important flavonoid of interest that possesses ample pharmacological benefits.[5] It is reported that vitexin serves as an excellent anti-inflammatory pharmakon, which reduces the level of cytokine expression at the site of inflammation.[6] TNF α is an important cytokine that plays a crucial role in the immune system’s signaling...
pathways and inflammation.\(^7\) TACE is responsible for the activation of TNF α to bind its receptor TNFR.\(^8\) A human inhibitory kinase (Ikβ) upon activation facilitates the upregulation of translocated NFκβ at the nuclear level and enhances matrix-metallo protein expression.\(^9\) Matrix metallo proteins (MMPs) abundant in synovial tissue, once activated may significantly destruct extracellular matrix, and erode bone and cartilage.\(^10\) Attenuation of TACE, TNFR1, and human Ikβ may reduce the sequestering of more inflammatory cytokines and MMPs.

Structure-based drug discovery promotes a better understanding of three-dimensional protein targets that actively performs in a disease. Computer-aided drug discovery may highlight and scientifically validate the traditional folkloric applications of certain herbs and their biologically active compounds.\(^11\) The efficacy of vitexin to repress the above targets may examine by computational molecular docking analysis. The pharmacokinetic profile of the vitexin has been evaluated to check the pharmacological potency. Virtual screening may help to assure drug-likeness of vitexin and may pay the way for further in vitro and in vivo studies in the treatment of rheumatoid arthritis.

**MATERIALS AND METHODS**

**Protein Preparation**

The three-dimensional structure file of drug targets, such as, TACE, TNFR1, and hIKβ was retrieved from the Research Collaboratory for Structural Bioinformatics-Protein data Bank (RCSB-PDB) database. All water molecules were removed, and Gasteiger charges were assigned to the protein structure.\(^12\)

**Ligand Preparation**

SMILES structure of vitexin retrieved from PubChem was submitted in SwissADME, an online tool that predicts the selected compound’s efficiency by checking various physicochemical properties.\(^13\) In silico toxicity predictor tool predicts the lethal dose 50 (LD\(_{50}\)) value in mg/kg body weight. Protox II, an online tool, predicts and assesses the toxicity of vitexin (Table 1).\(^14\) 2D structure of vitexin was drawn using ChemSketch from the SMILES data available in the PubChem database. The 3D structure of vitexin was generated, optimized, and saved in a .mol file. Further conversions were made using the open babel molecular converter program and saved in PDB format.\(^12\)

**Protein-Ligand Interaction Prediction**

AutoDock is one of the best suites of automated docking tools. The software is used for modeling flexible small molecules, such as, drug molecule binding to target proteins of known structure. Genetic algorithms are used in this suite for checking the conformational search. AutoDock is a user-friendly tool to perform blind docking, where the location of the binding site is not known. Molecular docking was performed using the AutoDock Tools 4.2 graphical user interface, which generates ten conformations (poses) of the protein-ligand complex customized in the order from lowest to highest binding free energy (ΔG).

**BIOVIA Discovery Studio Visualizer**

BIOVIA visualization tool helps to visualize the docking interactions between vitexin and different protein targets of interest in both 2D and 3D. Emphasized images of different interactions are easily visualized in the Discovery Studio tool.\(^16\)

**RESULT AND DISCUSSION**

**Vitexin Structure and its Properties**

The 2D (Fig. 1) and 3D structure (Fig. 2) of vitexin were retrieved and saved in PDB format. Membrane permeability and bioavailability of vitexin are always assessed with some basic molecular properties, such as, log p (partition coefficient), molecular weight (MW), or counts of hydrogen bond acceptors and donors in a molecule.\(^17\) These molecular properties were used in formulating the “rule of five.”\(^18\) The rule states that most molecules with good membrane permeability have molecular weight ≤ 500, calculated octanol-water partition coefficient, log P ≤ 5, hydrogen bond donors ≤ 5, and acceptors ≤ 10.\(^19\)

**Table 1:** LD\(_{50}\) and toxicity class for vitexin

| Ligand name | LD\(_{50}\) mg/kg | Toxicity class |
|-------------|------------------|---------------|
| Vitexin     | 1,213            | 4             |

\(^{17}\) TACE is responsible for the activation of TNF α to bind its receptor TNFR.\(^8\) A human inhibitory kinase (Ikβ) upon activation facilitates the upregulation of translocated NFκβ at the nuclear level and enhances matrix-metallo protein expression.\(^9\) Matrix metallo proteins (MMPs) abundant in synovial tissue, once activated may significantly destruct extracellular matrix, and erode bone and cartilage.\(^10\) Attenuation of TACE, TNFR1, and human Ikβ may reduce the sequestering of more inflammatory cytokines and MMPs.

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**Fig. 1:** 2D structure of vitexin

**Fig. 2:** 3D structure of vitexin
Therefore, Lipinski's rule of five was used to test the bioavailability characteristics, such as, absorption, distribution, metabolism, elimination (ADME) of the lead compounds, which was predicted by molinspiration[20] and SwissADME.

Physicochemical properties depicting oral bioavailability was predicted using SwissADME (Table 2), in which lipophilicity was calculated in terms of XLOGP3 as 0.21. Insolubility log value depicted as -2.84 indicates solubility of vitexin, and unsaturation as per Csp3 was found to be 0.29 (Fig. 3).[21] Properties, such as, G-protein coupled receptor (GPCR) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor, and enzyme inhibitor, were predicted with Molinspiration, and bioactive scores were tabulated (Table 3).[22]

**Molecular Docking of Vitexin against Drug Targets**

AutoDock 4.2 suite was used to perform molecular docking analysis of vitexin against TACE, TNFR1, and hIKβb. Hydrogen atoms were added, water molecules removed, and Gasteiger charges were added to the ligand and protein. The rotatable bonds of the ligand were altered using the “choose torsions” option. Further flexible bonds of the ligand were checked to dock with rigid protein targets. Blind docking was performed using grid point value (X, Y, and Z) of 126 Å and spacing between the grid points was 0.375 Å. The Lamarckian genetic algorithm was selected for ligand conformational searching,[23] and default docking parameters were used. A total of 10 docking configurations were determined in each docking calculation.

A preferable docking configuration was chosen based on the lowest empirical binding free energy and the most frequent cluster. AutoDock results are ranked according to the highest negative binding free energies and corresponding root mean square deviation (RMSD) values from the experimentally determined binding site. AutoDock shares functional commonalities, including the global optimization of the scoring function, pre-calculation of grid maps, and the pre-calculation of distant dependent pair-wise energetics between each atom type. However, they employ a different scoring function and algorithms to obtain binding free energies and should be considered as different programs.[24] The docked protein-ligand complex (Table 4) visualized using BIOVIA Discovery Studio visualizer, and the distances of interactions are calculated (Table 5).

**Visualization of Protein-Vitexin Complex Interactions**

The best-docked ligand models were selected according to the lowest binding energy. Two and three-dimensional conformational structures of the ligand-protein complexes were visualized using BIOVIA Discovery Studio Visualizer v4.5[25] to investigate the binding modes. The ligand binds at the target protein by non-covalent interactions, such as, H-bonding, alkyl, alkyl-π, π-π, π-σ, and van der Waals interactions. Simplified visualization is illustrated in 2D, which displays the H-bonding, van der Waals forces, carbon-oxygen dipole-dipole interaction, alkyl-pi interactions, T-shaped pi-pi stacking, and pi-pi stacking. Images

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**Table 2**: SWISSADME-predicted Lipinski rule values, including TPSA, bioavailability, and water solubility for vitexin

| Properties       | Predicted value |
|------------------|-----------------|
| Mi Logp          | 0.52            |
| TPSA             | 181.05          |
| Number of atoms  | 31              |
| Mw               | 432.38          |
| H bond acceptor  | 10              |
| H bond donor     | 7               |
| Number of violations | 1            |
| Number of rotational bonds | 3         |
| Volume           | 355.2           |
| Bioavailability  | 0.55            |
| Water solubility | Soluble         |

**Table 3**: Bioactivity score for vitexin

| Bioactivity               | Probability score |
|---------------------------|-------------------|
| GPCR ligand               | 0.13              |
| Ion channel modulator     | 0.14              |
| Kinase inhibitor          | 0.19              |
| Nuclear receptor ligand   | 0.23              |
| Protease inhibitor        | 0.03              |
| Enzyme inhibitor          | 0.46              |

**Fig. 3**: Bioavailability radar (pink zone depicts suitable physicochemical space for oral bioavailability) for vitexin (LIPO indicates lipophilicity in terms of XLOGP3, SIZE indicates in terms of molecular weight, POLAR indicates polarity in terms of topological polar surface area, INSOLU depicts insolubility in water in terms of log S scale, INSATU refers to unsaturation as per fraction of carbons in the sp3 hybridization, and finally FLEX indicates flexibility as per rotatable bonds)
Interactions between TNFR1 and vitexin (Fig. 4) showed the lowest binding energy when compared to TACE-vitexin interactions (Fig. 5) and vitexin-hIKβb kinase protein binding interactions (Fig. 6).

Computational analysis is less laborious, easy to perform, and yield quick results than conventional drug designing techniques. Cost-effective computational approaches may also seem to screen docking hits to produce small novel herbal-based drugs. AutoDock Vina docking enrolls better-performing speed simultaneously, elucidate better result accuracy. The resulting docking complexes are expected to produce an excellent pharmacological effect in the treatment of RA. This investigation may serve as a tool for the evaluation of drug-like properties of pharmakon from folkloric herb

Table 4: Docking score predictions of protein-vitexin complex

| S. No. | Conformation parameters | TACE     | TNFR1    | hIKβb    |
|--------|-------------------------|----------|----------|----------|
| 1      | Binding energy          | -5.71    | -6.1     | -4.7     |
| 2      | Inhibitory constant (µm) | 65.22    | 33.94    | 360.35   |
| 3      | van der Waal interaction | -8.39    | -8.64    | -7.32    |
| 4      | Electrostatic energy    | -0.3     | -0.44    | -0.36    |
| 5      | Torsional energy        | 2.98     | 2.98     | 2.98     |
| 6      | Unbound energy          | -7       | -6.66    | -7.54    |
| 7      | Amino acids             | SER287, LEU285, TYR238, GLU463, PRO218 | CYS158, CYS127, GLN131, ASN130 | GLN548, ARG549, ARG452, GLN548, GLU149, CYS99 |

Table 5: Predicted distance and interacted amino acid residues of protein targets with vitexin

| Vitexin (ligand) | AA-residue | Distance (Å) | Docking energy (kcal/mol) |
|------------------|------------|--------------|---------------------------|
| TACE (protein)   | SER287     | 3.36         | -5.71                     |
|                  | LEU285     | 2.94         |                           |
|                  | TYR238     | 2.186        |                           |
|                  | GLU463     | 2.086        |                           |
|                  | PRO218     | 2.149        |                           |
|                  | PRO218     | 1.983        |                           |
| TNFR1 (protein)  | CYS158     | 2.07         | -6.1                      |
|                  | CYS127     | 2.017        |                           |
|                  | CYS127     | 2.48         |                           |
|                  | CYS127     | 1.82         |                           |
|                  | GLN131     | 1.822        |                           |
|                  | ASN130     | 3.3          |                           |
| hIKβb (protein)  | GLU149     | 2.71         | -4.7                      |
|                  | GLU97      | 2.96         |                           |
|                  | CYS99      | 2.93         |                           |
|                  | GLN548     | 1.956        |                           |
|                  | ARG549     | 2.042        |                           |
|                  | ARG452     | 1.817        |                           |
|                  | GLN548     | 2.199        |                           |

were generated using Discovery Studio Visualizer 4.5.[26] Interactions between TNFR1 and vitexin (Fig. 4) showed the lowest binding energy when compared to TACE-vitexin interactions (Fig. 5) and vitexin-hIKβb kinase protein binding interactions (Fig. 6).

Fig. 4: 3D and 2D representations of vitexin-TACE protein interactions visualized by BIOVIA Discovery Studio visualizer; a) 3D interactions: TACE (protein)-line model; vitexin (ligand)-ball and stick model; green dotted lines-hydrogen bond interactions; b) 2D diagram: Ligand line model and protein interactions are colored depending on their type: conventional hydrogen bonds are colored in green, van der Waals interactions are colored in light green, and π-alkyl interactions are colored in light pink, respectively; the blue halo surrounding the interacting residues represents solvent-accessible surface that is proportional to its diameter.
V. negundo. It can be further utilized for the beneficiary of mankind after in vivo and clinical trial studies.

**CONCLUSION**

The present computational studies, concludes that vitexin might serve as better potent herbal pharmakon in repressing the activated inflammatory protein targets of RA such as, TACE, TNFR1, and hIKβb. Vitexin from V. negundo might serve as an efficient pharmaco-beneficiary lead in replacement of commercial drugs due to its lesser side effects and cost effectiveness, thereby the folk-chloric bioactive compound vitexin might act as a promising lead in therapeutics of Rheumatoid arthritis.

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