Molecular docking study on the anti-diabetic and anti-inflammatory potential evaluation of phytochemicals in *Ruellia tuberosa* ethanolic extract

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Abstract

Natural products derived from medicinal plants have garnered significant interest as potential therapeutic agents. Ruellia tuberosa, commonly known as Minieroot, is rich in bioactive phytochemicals such as flavones, nonadecatrine and phytol, which exhibit diverse pharmacological properties including antidiabetic and anti-inflammatory effects. This study explores the molecular interactions between Ruellia tuberosa phytochemicals and key proteins associated with diabetes and inflammation using molecular docking simulations. The antidiabetic potential of these compounds was assessed through docking studies against human aldose reductase (PDB: 1C88) and glycogen synthase kinase-3β (PDB: 4QBX), both critical in diabetes-related pathways.

Similarly, their anti-inflammatory properties were evaluated by docking against cyclooxygenase-2 (PDB: 2OYE) and cyclooxygenase-1 (PDB: 6COX), enzymes involved in inflammation and prostaglandin synthesis. The findings provide valuable insights into the molecular interactions of Ruellia tuberosa phytochemicals, supporting their potential as natural drug candidates for managing diabetes and inflammation.

Keywords: *Ruellia tuberosa*, molecular docking, phytochemicals, flavones, nonadecatrine, phytol, antidiabetic activity, anti-inflammatory activity, aldose reductase (1C88), glycogen synthase kinase-3β (4QBX), cyclooxygenase-2 (2OYE), cyclooxygenase-1 (6COX).

Introduction

In recent years, natural products derived from medicinal plants have gained significant attention as potential sources for the development of novel therapeutics for various diseases.[6] Among these plants, *Ruellia tuberosa*, commonly known as Minieroot, stands out for its extensive pharmacological properties.[32] Various studies have reported its effectiveness in managing conditions such as diabetes and inflammation, among others.[31]

In this regard, molecular docking, a computational technique, has emerged as a valuable tool for understanding the interactions between bioactive compounds and their target proteins, aiding in the rational design of new drugs.[21]

Ruellia tuberosa is rich in phytochemicals including flavonoids, alkaloids and terpenoids which contribute to its medicinal properties.[25] Flavones, nonadectrines and phytols are extracted in the ethanol extract. Flavone, a subclass of flavonoids, has been widely studied for its diverse biological activities, including antioxidant, anti-inflammatory and antidiabetic effects.[37] Nonadecatrine, an alkaloid found in *Reel tuberosa*, has shown promising pharmacological properties, including antidiabetic potential.[40] Phytol, a diterpene alcohol, is another bioactive compound found in the plant, with reported anti-inflammatory and antioxidant activities.[10] The growing interest in natural products as potential antidiabetic and anti-inflammatory agents has prompted researchers to explore the molecular mechanisms underlying the therapeutic effects of Ruellia tuberosa phytochemicals.[24]

Molecular docking studies provide valuable insights into the binding interactions between these bioactive compounds and their target proteins, elucidating their potential as drug candidates.[38] For studying the antidiabetic activity of *Ruellia tuberosa* phytochemicals, molecular docking simulations were performed against proteins associated with diabetes-related pathways. Specifically, the phytochemicals were docked against Protein Data Bank (PDB) structures 1C88 and 4QBX, known for their relevance to diabetes research.[30] Protein 1C88 represents the crystal structure of human aldose reductase, an enzyme involved in the polyol pathway while 4QBX corresponds to the active site of human glycogen synthase kinase-3β (GSK-3β), a key regulator of glycogen metabolism and insulin signaling.[5]

In addition to their antidiabetic potential, *Ruellia tuberosa* phytochemicals also exhibit anti-inflammatory properties, making them promising candidates for the management of inflammatory conditions.[51]

To investigate their interactions with proteins relevant to inflammation, molecular docking studies were conducted against PDB structures 2OYE and 6COX. Protein 2OYE represents the crystal structure of human cyclooxygenase-2 (COX-2), an enzyme implicated in the inflammatory response while 6COX corresponds to the active site of human cyclooxygenase-1 (COX-1), another enzyme involved in prostaglandin synthesis and inflammation.[33]

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Lamiales
Family:	Acanthaceae
Genus:	*Ruellia*
Species	*tuberosa*

Overall, molecular docking studies on *Ruellia tuberosa* phytochemicals provide valuable insights into their potential mechanisms of action as antidiabetic and anti-inflammatory agents. By elucidating the binding interactions between these bioactive compounds and their target proteins, these computational simulations contribute to the rational design and development of novel therapeutic agents from natural sources.[52]

Material and Methods

***In silico* Molecular docking:** Molecular interaction and binding affinity involved in the activity identified by Molecular docking done using Autodock vina and visualised using Pymol.[44] Process includes 7 different steps:

1. Preparation of Protein and Ligand Structures:

- Retrieve the crystal structures of the target proteins (e.g. 1C88, 4QBX for antidiabetic activity; 2OYE, 6COX for anti-inflammatory activity) from the Protein Data Bank (PDB).
- Remove water molecules and heteroatoms from the protein structures using molecular visualization software (e.g. PyMOL).
- Add hydrogen atoms and assign charges using appropriate force field parameters.
- Save the prepared protein structures in PDB or PDBQT format.
- Obtain the three-dimensional structures of the phytochemicals (flavone, nonadecatrine, phytol) from chemical databases or generate them using molecular modeling software (e.g. Avogadro, ChemDraw).
- Optimize the ligand structures by minimizing energy and checking for stereochemical correctness.
- Save the ligand structures in PDB or PDBQT format.

2. Grid Generation:

- Define the docking search space by generating grid maps around the active sites of the target proteins.
- Specify the dimensions and center coordinates of the grid box to encompass the binding pockets of interest.
- Adjust the grid spacing to ensure adequate coverage of the binding site while minimizing computational resources.

3. Docking Setup:

- Execute AutoDock Vina software through the command-line interface or graphical user interface.
- Specify the input files including the prepared protein and ligand structures, as well as the grid parameters.
- Set the desired exhaustiveness level to control the thoroughness of the docking search (higher values result in more exhaustive sampling but longer computational time).
- Define the output file format to save the docking results including the predicted binding poses and corresponding binding affinities.

4. Docking Simulations:

- Initiate the docking simulations using AutoDock Vina, which employs a stochastic optimization algorithm to explore the conformational space of ligand binding.
- Perform multiple docking runs for each ligand to enhance sampling and improve the reliability of the results.
- Monitor the progress of the docking simulations and ensure convergence of the scoring function across multiple runs.
- Record the docking scores, which represent the predicted binding affinities of the ligands with the target proteins.

5. Analysis and Visualization:

- Analyze the docking results to identify the top-scoring binding poses for each ligand. Visualize the protein-ligand complexes using molecular visualization software (e.g. PyMOL) to inspect the binding interactions.
- Highlight key interactions such as hydrogen bonds, hydrophobic contacts and π-π stacking interactions between the ligands and protein residues.
- Compare the docking poses and interaction patterns across different ligands and target proteins to gain insights into their binding modes and affinities.

6. Validation and Interpretation:

- Validate the docking results by comparing them with experimental data or conducting control docking experiments with known ligands.
- Interpret the observed binding interactions in the context of the reported pharmacological activities of the phytochemicals.
- Correlate the docking scores and binding affinities with the biological effects of the ligands to elucidate their potential as drug candidates.

7. Statistical Analysis:

- Perform statistical analysis to quantify the binding affinities of the ligands with the target proteins and assess the significance of the results.
- Calculate descriptive statistics such as mean, standard deviation and confidence intervals to summarize the docking scores and identify outliers.
- Conduct comparative analysis of the docking results for different ligands and protein targets to evaluate their relative binding affinities and selectivity profiles.

Results and Discussion

Molecular docking results of phytochemicals with the protein tyrosine phosphatase (PDB ID: 1C88): Insulin-dependent (T1DM) and non-insulin-dependent (T2DM) diabetes are the two primary forms of diabetes mellitus (DM). The inability of human pancreatic b-cells to make insulin, a hormone that controls blood sugar, is linked to type 1 diabetes. Children are primarily affected by this kind of diabetes.[42] Insulin resistance and elevated insulin and glucose levels during the initial phases of the disease are associated with type 2 diabetes.

Despite the decreased insulin sensitivity of the target cells in type 2 diabetic individuals, pancreatic b-cells operate normally.[14] Type 2 diabetes has become more common in young people even though it affects over 90% of adults.[48] The enzymes protein tyrosine phosphatase 1B (PTP1B) and aldose reductase (ALR2) have been discovered as two novel molecular targets implicated in distinct pathways linked to the initiation and progression of type 2 diabetes mellitus (T2DM) and associated comorbidities.

According to Artasensi et al[1], ALR2 is a crucial enzyme of the polyol pathway that may cause an excessive build-up of intracellular reactive oxygen species (ROS) in many tissues which could lead to the development of chronic diabetes problems. According to Eleftheriou et al[11,12], PTP1B is an enzyme that is crucial for the negative regulation of insulin and the emergence of insulin resistance. Numerous reviews have documented various therapeutic compounds that show promise in the treatment of diabetes mellitus when used against the ALR2 and PTP1B enzymes.

The Gibbs free energy of binding (ΔG) between a ligand and a receptor is computed by docking engines and is essential to comprehend intricate systems in molecular biology and biochemistry. Estimates of the total energy of intermolecular forces of attraction, such as hydrogen bonds, electrostatic interactions and van der Waals interactions, serve as the foundation for the computation of ΔG.

According to Jacob et al[18], ligands are ordered according to the computed binding energy value (ΔG). Ligand binding that is more advantageous is indicated by lower binding energy values, while less favorable ligand binding is indicated by higher binding energy values.

The higher negative docking score demonstrated the greater effectiveness of bioactive chemicals by indicating a strong binding affinity between the ligand and receptor molecules. Based on their strong contact with the active site residues and docking energy, the docked ligand molecules were chosen. Since the study mainly focuses on the minimal energy required for forming molecular bonds and interactions, the confirmation with the higher negative energy value is considered as best interaction.

The active phytochemicals of *Ruellia tuberossa* ethanolic extracts are flavone, nonadecatreine and the phytole, while interacting with the protein tyrosine phosphatase, molecular interactions are possible with negative binding energies. Details of binding energy value (ΔG) for each confirmation were recorded and detailed in table 1. Flavone has the best binding energy value of -7.0kcal/mol. The other confirmations also ranged very close and between -6.6kcal/mol to -6.0kcal/mol. As per the literature, the docking value appeared lower than -7.0kcal/mol, considered as best interaction value and the minimum for forming bonds.

The phytochemical nonadecatreine formed a molecular interaction with tyrosine kinase protein, with a minimal binding energy of -4.6kcal/mol. Naonadecatreine has formed eight other confirmations also with the protein, with a very close energy requirement ranging from -4.5kcal/mol to -4.4kcal/mol. This close binding energy in all the conformations exhibits the molecular interaction stability in all possible ways with a very close energy balancing.

Phytols formed molecular interaction and bonds with a minimal binding energy requirement of -3.9kcal/mol. The other confirmations possible between the protein ligand complex are with a binding energy between -3.6kcal/mol to -3.4kcal/mol. Even though both the nonadecatreine and

phytol have not shown much low binding energy value (ΔG), like flavone, all the three ligands have showed good binding affinity with protein tyrosine kinase.

Molecular docking results of phytochemicals with the Human Aldose Reductase (PDB ID: 4QBX): Molecular interaction of ethnic extract derived phytochemicals flavones, nonadecatriene and phytols is analysed in detail with Autodock Vina for studying their molecular interaction pattern and the top nine conformations were recorded in table 2. The major phytochemical flavone has a very superior affinity with the human aldose reductase protein and the molecular binding confirmation formed a very low binding energy of -7.0kcal/mol.

Table 1
Molecular docking results of phytochemicals with the Protein tyrosine phosphatase (PDB ID: 1C88)

Ligand	**Flavone**	**Nonadecatriene**	**Phytol**
Molecular formulae	$C_{15}H_{10}O_2$	$C_{19}H_{34}O_2$	$C_{20}H_{40}O$
Molecular weight	222.24	294.5	296.5
Confirmations	Affinity (kcl/mol)		
1	-7	-4.6	-3.9
2	-6.6	-4.5	-3.6
3	-6.6	-4.5	-3.6
4	-6.6	-4.5	-3.6
5	-6.2	-4.5	-3.6
6	-6.1	-4.4	-3.6
7	-6.1	-4.4	-3.5
8	-6.1	-4.4	-3.5
9	-6	-4.4	-3.4

Table 2
Molecular docking results of phytochemicals with the Human Aldose Reductase (PDB ID: 4QBX)

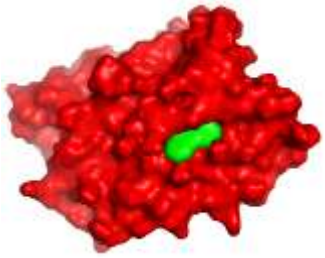
	Flavone	**Nonadecatriene**	**Phytol**
Molecular formulae	$C_{15}H_{10}O_2$	$C_{19}H_{34}O_2$	$C_{20}H_{40}O$
Molecular weight	222.24	294.5	296.5
Confirmations	Affinity (kcl/mol)		
1	-7	-4	-4
2	-6.6	-4	-3.7
3	-6.6	-3.8	-3.6
4	-6.6	-3.8	-3.4
5	-6.2	-3.8	-3.4
6	-6.1	-3.8	-3.4
7	-6.1	-3.7	-3.4
8	-6	-3.7	-3.3
9	-5.9	-3.7	-3.3

Table 3
Molecular docking results of phytochemicals with the Protein Cyclooxygenase-1 (PDB ID: 2OYE)

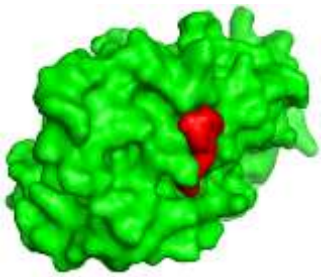
Ligand	**Flavone**	**Nonadecatriene**	**Phytol**
Molecular formulae	$C_{15}H_{10}O_2$	$C_{19}H_{34}O_2$	$C_{20}H_{40}O$
Molecular weight	222.24	294.5	296.5
Confirmations	Affinity (kcl/mol)		
1	-8.9	-5.5	-6
2	-8.2	-5.5	-5.9
3	-7.5	-5.4	-5.9
4	-7.2	-5.3	-5.6
5	-7.2	-5.3	-5.5
6	-7.2	-5.3	-5.5
7	-7	-5.3	-5.5
8	-7	-5.2	-5.3
9	-7	-5.1	-5.3

Table 4
Molecular docking results of phytochemicals with the Protein Cyclooxygenase-2 (PDB ID: 6COX)

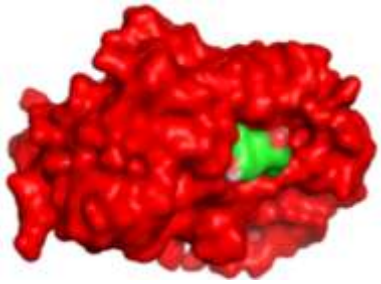
Ligand	**Flavone**	**Nonadecatriene**	**Phytol**
Molecular formulae	$C_{15}H_{10}O_2$	$C_{19}H_{34}O_2$	$C_{20}H_{40}O$
Molecular weight	222.24	294.5	296.5
Confirmations	Affinity (kcl/mol)		
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0	0	0
7	0	0	0
8	0	0	0
9	0	0	0



Tyrosine phosphatase - Flavone Complex

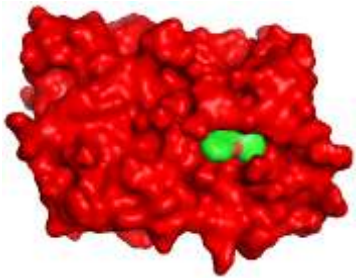


Tyrosine phosphatase - Nonadecatreine Complex

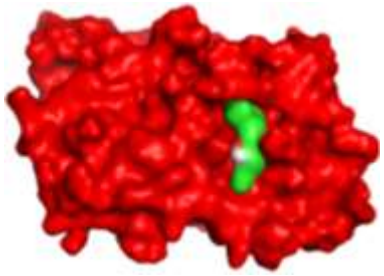


Tyrosine phosphatase – Phytol Complex

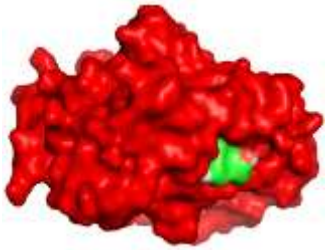
Fig. 1: Molecular docking results of phytochemicals with the protein tyrosine phosphatase (PDB ID: 1C88)



Human Aldose Reductase - Flavone Complex

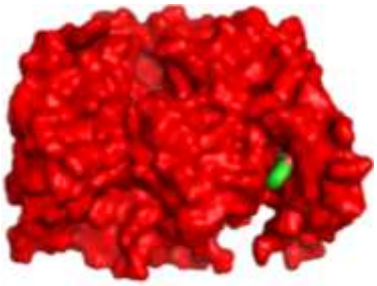


Human Aldose Reductase - Nonadecatreine Complex

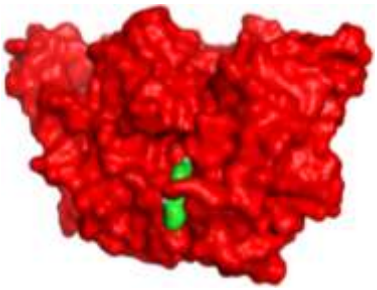


Human Aldose Reductase - Phytol Complex

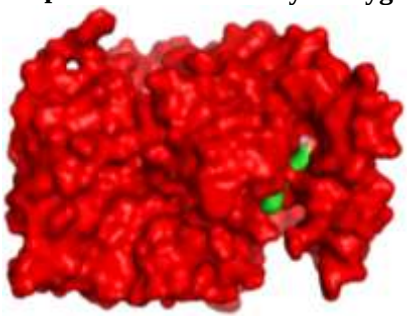
Fig. 2: Molecular docking results of phytochemicals with the Human Aldose Reductase (PDB ID: 4QBX)



Cyclooxygenase-1 - Flavone Complex



Cyclooxygenase-1 - Nonadecatreine Complex



Cyclooxygenase-1 - Phytol Complex

Fig. 3: Molecular docking results of phytochemicals with the Cyclooxygenase-1 (PDB ID: 2OYE)

Like its interaction with tyrosine reductase protein, here also the flavone exhibits an interaction pattern with -7.0kcal/mol, which indicates its high antidiabetic potential. The eight other confirmations also happened with in an energy requirement range of -6.6kcal/mol to -5.9kcal/mol.

Nonadecatreine exhibited its ability to form two different conformations with the same binding energy value (ΔG) of -4.0kcal/mol. Two different confirmations at the same energy level indicate their close nature for molecular bonds and the same is further confirmed with the binding energy requirement of other confirmations. The binding energy required for other close interactions was -3.8kcal/mol and -3.7kcal/mol. Human aldose reductase protein has formed a molecular interaction with ligand phytol with a minimal energy value requirement of -4.0kcal/mol as similar to nonadecatreine. The other possible molecular interactions were reported with an energy requirement range of -3.7kcal/mol to -3.3kcal/mol.

Molecular docking results of phytochemicals with the Cyclooxygenase-1 (PDB ID: 2OYE): Inflammation is a complex process with many different mediators including prostaglandins.[8] Prostaglandins are important mediators of the body's response to pain and inflammation and are formed from essential fatty acids found in cell membranes. This reaction is catalysed by cyclooxygenase (COX). The cyclooxygenase enzyme occurs in two isoforms, COX-1 and COX-2.[15] The constitutively expressed COX-1 is present in cells under physiological conditions and produces protective substances for the stomach and kidney.

COX-2 is effectively absent in healthy tissue and is induced in migratory and other cells by proinflammatory agents, such as cytokines, mitogens and endotoxins under pathological conditions such as inflammation. The main target of anti-inflammatory drugs is the enzyme cyclooxygenase. Inhibition of COX-1 and 2 can provide relief from the symptoms of inflammation and pain[45]. The most common non-steroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of inflammation including diclofenac, indomethacin and ketoprofen.[49]

Docking engines calculate the Gibbs free energy of binding (ΔG) between a ligand and a receptor, which is fundamental to the understanding of complex systems in biochemistry and molecular biology. The calculation of ΔG is based on estimates of the total energy of intermolecular forces of attraction including van der Waals interactions, hydrogen bonding and electrostatic interactions. Ligands are ranked by the calculated binding energy value (ΔG); lower binding energy values correspond to more favorable ligand binding where higher binding energy values are less favorable ligand binding.[19]

After all, the molecular docking studies the minimal energy required for forming molecular interactions and forming bonds between molecules. So the lowest binding energy value (ΔG) indicates the easiest way to form molecular interactions. The docked ligand molecules were selected based on docking energy and good interaction with the active site residues and the results are shown in table 1. The plant phytochemicals flavones, nonadecatrienes and phytols exhibited very low binding energy. Flavones' interaction with cyclohexagease-1 happened with a lowest binding energy of -8.9kcal/mol. Along with this lowest possible interaction, there are 8 other confirmations also noted with very low binding energy ranging between -7kcal/mol to -8.2kcal/mol. Another important phytochemical constituent, Nonadecatriene, also expressed low binding energy -5.5kcal/mol with cyclooxygenase-1. It was interesting to note that two different interaction patterns can be possible

with a same binding energy of -5.5kcal/mol between the protein and ligand. Thus, there are two different confirmation possibilities with the same binding energy value (ΔG). All the other binding energies were recorded in close range from 5.4kcal/mol to 5.5kcal/mol. The phytochemical phytol, also showed similar interaction potential with Cyclooxygenase-1. The lowest binding energy required for forming a molecular interaction is -6kcal/mol. Other confirmations are also possible with binding energy ranging from -5.3kcal/mol to -5.9kcal/mol.

Molecular docking results of phytochemicals with the Protein Cyclooxygenase-2 (PDB ID: 6COX): Flavone, nonadecatriene and the phytol, the three major phytocemical constituents of *Ruellia tuberossa* ethanolic extract, have not shown any major or notable bonding energy even at the blind dock performed with Auto dock Vina. The binding energy has shown zero with all the nine confirmations where the distances from rmsd and best rmsd modes ranged from 0 to 20.73 and 0 to 23.574 respectively for flavones. For nonadecateriene and phytols, the binding energy is zero in all the trials and the distance from rmsd remained 0 to 23 and 0 to 26 respectively. The binding interactions of all compounds have shown hydrogen bonding and hydrophobic interactions with the target protein.

The docking studies confirmed the anti-inflammatory activity of flavone, nonadecatreine and phytole and thereby inhibition of target protein as cyclooxygenase-2 (PDB ID: 6COX) through the binding interactions. Even though the compounds have not shown interactive potential against the 6COX, they showed good interaction with very low binding energy against 2OYE, which indicates their anti-inflammatory potential.

Conclusion

The study has been conducted to analyse the antidiabetic and anti-inflammatory potential of major phytochemicals derived from the ethanol extract of *Ruellia tuberossa* using molecular docking. Since molecular docking majorly concentrates on the molecular interaction possibilities and binding energy requirement, the activities were analyzed based on the possibility and minimal energy requirement for forming the interaction. The major phytochemicals flavone, nonadecatreine and phytols were considered as ligands in the study and two major proteins for andi diabetic and anti-inflammatory activity were considered as protein units.

From this study, it can be concluded that the phytochemical flavone has formed good molecular interaction with both turbine Kinase and Human Aldose Reductase with a very low binding energy requirement and showed good anti diabetic potential. The nonadecatreine and phytol even exhibited slightly higher energy requirements, but they also contributed well to the anti diabetic potential of the extract. So it can be concluded that the ethanol extract of *Ruellia tuberossa* has an anti diabetic potential and all three phytochemicals contribute towards the same.

While considering the anti-inflammatory potential, the phytochemicals were docked against cyclooxygenase-1 and cyclooxygenase-2. The flavone has expressed very low binding energy with cyclooxygenase-1. Nona decatriene and phytol also exhibited good molecular interaction with cyclooxygenase-1, but all three phytochemicals have not exhibited any negative binding energy value against the cyclooxygenase-2. The results can be summarized as the phytochemical extract expressing anti-inflammatory potential, but only against cyclooxygenase-1.

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