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Integration of Molecular Docking in the Identification of Natural Antioxidants: Interaction Study of Jackfruit Leaf Flavonoids with NADPH:FMN Oxidoreductase

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Abstract: Oxidative stress plays a critical role in the progression of various degenerative diseases through increased production of reactive oxygen species (ROS), driven in spart by the activity of redox related enzymes such as NADPH:FMN oxidoreductase. Bioactive compounds from jackfruit leaves are known to possess antioxidant potential, yet their molecular mechanisms against specific enzymatic targets remain insufficiently elucidated. This study aimed to evaluate the potential interaction of jackfruit leaf flavonoids morin, oxyresveratrol, and artocarpin with NADPH:FMN oxidoreductase using molecular docking analysis. The 1BKJ protein structure was prepared following standard protocols, and all ligands were optimized prior to performing redocking for method validation. AutoDock Vina 1.2.7 was employed with a 20×20×20 Å grid box area. Redocking produced an RMSD of 0.1469 Å, confirming the reliability of the docking parameters. Docking results revealed that morin (–7.848 kcal/mol) and oxyresveratrol (–7.577 kcal/mol) exhibited stronger binding affinities compared with vitamin C (–5.713 kcal/mol) and artocarpin (–5.577 kcal/mol). The dominant interactions involved Arg15, Arg169, Tyr128, Tyr199, and Tyr200, residues that contribute to the stabilization of ligand protein complexes in silico and may be located near functionally relevant regions associated with redox activity. These findings suggest that jackfruit leaf flavonoids may serve as promising candidates for further investigation as potential modulators of redox related enzymes based on predictive in silico evidence.

Keywords: antioxidant, flavonoid, jackfruit leaf, molecular docking, NADPH:FMN oxidoreductase

1. INTRODUCTION

Oxidative stress is a biological condition in which the production of reactive oxygen species (ROS) exceeds the capacity of endogenous antioxidant systems. This imbalance triggers oxidative damage to membrane lipids, structural and enzymatic proteins, and genetic material, ultimately contributing to the progression of chronic diseases such as cancer, atherosclerosis, neurodegenerative disorders, metabolic syndrome, and premature aging. ROS are generated not only through normal physiological processes such as mitochondrial respiration but also through the activity of redox related enzymes including NADPH oxidase, xanthine oxidase, and NADPH:FMN oxidoreductase. The latter functions as a catalyst for electron transfer from NADPH to the FMN cofactor, a process that under certain conditions may increase electron leakage and promote the formation of superoxide radicals. Due to its strategic role within the cellular redox network, modulation of NADPH:FMN

oxidoreductase represents a promising approach for modulating redox-related processes at the molecular level [1], [2].

In recent years, interest in natural antioxidants has increased significantly due to concerns regarding the long term side effects of synthetic antioxidants. Jackfruit leaves (Artocarpus heterophyllus) have emerged as a valuable source of secondary metabolites, particularly flavonoids, stilbenoids, and phenolics, which have been reported to exhibit a wide range of biological activities including antioxidant, anti inflammatory, and anticancer effects. Among these bioactive compounds, morin, oxyresveratrol, and artocarpin are flavonoids with strong electron donating and radical stabilizing capabilities. Beyond their chemical radical scavenging activity, flavonoids are also known to interact with redox enzymes, influence cellular signaling pathways, and modulate redox homeostasis through either inhibition or activation of specific enzymatic targets. Several studies have reported the antioxidant activity of jackfruit leaf–derived compounds, particularly flavonoids, mainly through radical scavenging mechanisms. This general evidence provides a contextual basis for exploring possible molecular-level interactions of selected flavonoids with redox-related enzymes using in silico approaches. Nevertheless, the specific molecular interactions between jackfruit leaf flavonoids and NADPH:FMN oxidoreductase remain insufficiently understood [3], [4], [5].

Computational approaches such as molecular docking offer an efficient platform for identifying potential ligand protein interactions at atomic resolution [6]. Docking not only predicts the free binding energy but also reveals key residue interactions such as hydrogen bonding, electrostatic interactions, and π–π stacking that determine complex stability [7], [8]. With adequate redocking validation, this analysis provides an initial prediction of a compound's ability to modulate enzymatic targets prior to more advanced biological assays [9]. Targeting NADPH:FMN oxidoreductase in an in silico framework also offers new insights into developing antioxidants that act via redox modulating mechanisms rather than simple radical scavenging [10], [11].

This study aims to comprehensively explore the potential of jackfruit leaf flavonoids as inhibitors of NADPH:FMN oxidoreductase through a standardized molecular docking approach. By evaluating differences in binding affinity, residue interaction patterns, and ligand orientation within the active site, this research seeks to elucidate the molecular mechanisms underlying the antioxidant activity of these compounds. The findings are expected to provide preliminary in silico insights into the interaction of natural flavonoids with redox-related enzymes, serving as a basis for further computational and experimental investigations.

2. MATERIALS AND METHODS

2.1. Tools and Materials

This study utilized the crystal structure of NADPH:FMN oxidoreductase (PDB ID: 1BKJ), obtained in .pdb format from the Protein Data Bank, as the docking target. The tested compounds included morin, oxyresveratrol, and artocarpin, representing major flavonoids found in jackfruit leaves, while vitamin C served as the positive control. Ligand structures were retrieved from chemical databases in .sdf format, then converted and optimized into .pdbqt format. Protein and ligand preparation was performed using AutoDockTools, while ligand structure conversion and energy minimization were carried out with OpenBabel. Molecular docking simulations were conducted using AutoDock Vina 1.2.7 [12], [13]. Visualization of ligand–protein interactions in both two dimensional and three dimensional forms was conducted using Discovery Studio Visualizer and

PyMOL. Supporting data, including protein and ligand structures, were sourced from online databases such as PubChem and the RCSB PDB [14], [15].

2.2. Preparation of Protein and Ligands

The crystal structure of NADPH:FMN oxidoreductase (PDB ID: 1BKJ) was downloaded from the protein database in .pdb format. All water molecules, ions, and nonessential ligands were removed. The structure was refined by adding polar hydrogens, adjusting residue protonation states, and verifying covalent linkages using standard modeling tools [16], [17]. The protein was then converted to .pdbqt format. The investigated compounds morin, oxyresveratrol, and artocarpin were obtained in three dimensional .sdf format and subjected to geometric optimization and energy minimization. Each ligand was converted to .pdbqt format. Vitamin C was included as a reference antioxidant compound for comparative docking analysis, acknowledging that it primarily functions as a solution-phase radical scavenger rather than a specific enzyme-binding inhibitor [18].

2.3. Method Validation via Redocking

The native ligand present in the 1BKJ structure was isolated and redocked into the active site of the protein. The resulting pose was compared with the original ligand position using RMSD calculations. An RMSD value of 0.1469 Å was obtained, indicating the high accuracy of the applied docking parameters and overall methodology. This validation step served as the foundation for subsequent docking of the test ligands. Docking simulations were performed using AutoDock Vina 1.2.7. The grid box was defined at center_x = –5.783, center_y = –1.835, center_z = 11.177 with dimensions of 20 × 20 × 20 Å to fully encompass the active site. The exhaustiveness was set to 8, and the search algorithm combined iterated local search with BFGS local optimization. Protein and ligand files were executed in AutoDock Vina to determine optimal binding poses based on free binding energy. Vina generated several poses, and the pose with the lowest binding energy was selected for further analysis [6], [12], [19].

2.4. Ligand–Protein Interaction Analysis and Data Interpretation

The best binding pose of each ligand was analyzed using molecular visualization software to identify hydrogen bonds, electrostatic interactions, hydrophobic contacts, and aromatic interactions such as π–π stacking. Key interacting residues were recorded to evaluate their contribution to complex stability. Comparative analysis was conducted between jackfruit leaf flavonoids and the vitamin C control. Binding affinity values, interaction patterns, and ligand orientations within the active site were examined to predict the ability of each compound to modulate NADPH:FMN oxidoreductase activity. These findings were used to construct a preliminary in silico interpretation of ligand–protein interactions related to redox-associated regions, without confirming direct inhibition of electron transfer or ROS formation [10], [11].

3. RESULTS AND DISCUSSION

Docking parameters, the algorithms applied, and the validation of redocking results are essential components for ensuring the reliability of any in silico study. In this research, the docking of jackfruit leaf compounds against the NADPH:FMN oxidoreductase receptor (PDB ID: 1BKJ) was performed

using AutoDock Vina version 1.2.7. The grid box was configured at center_x = –5.783, center_y = –1.835, and center_z = 11.177 with dimensions of 20 × 20 × 20 Å. This grid size was selected to fully cover the binding pocket, allowing the ligands to explore various orientations and conformations around the active site. An exhaustiveness value of 8 was chosen to maintain a balance between search thoroughness and computational efficiency. This parameter determines how comprehensively the algorithm samples the conformational space, where a higher exhaustiveness increases the likelihood of identifying the optimal pose, albeit with greater computational cost [6], [9].

AutoDock Vina utilizes an Iterated Local Search (ILS) algorithm combined with the Broyden–Fletcher–Goldfarb–Shanno (BFGS) local optimization method. ILS performs a global exploration of the conformational landscape to generate initial ligand orientations within the binding pocket, while BFGS refines these orientations locally to achieve energetically favorable conformations [12], [20]. This combination ensures efficient yet precise pose prediction, increasing the likelihood that the resulting ligand conformations reflect realistic molecular interactions within a biological system [12], [21].

The reliability of the docking results was evaluated through redocking, in which the native ligand from the crystal structure was removed and docked back into the binding pocket. The predicted pose was then compared with the original crystallographic pose using Root Mean Square Deviation (RMSD). The redocking produced an RMSD value of 0.1469 Å, well below the widely accepted validation threshold of 2.0 Å [22]. This exceptionally low RMSD confirms that the docking protocol accurately reproduces the native ligand orientation, validating the overall methodology and supporting the credibility of the docking outcomes for the tested ligands [23]. Consequently, differences in binding affinities and interaction patterns observed among the test compounds are unlikely to be computational artifacts but instead represent genuine molecular interaction tendencies [6], [19].

NADPH:FMN oxidoreductase (1BKJ) was selected as the target receptor due to its essential role in catalyzing electron transfer from NADPH to the FMN cofactor, a crucial step in various metabolic pathways. This enzyme is central to maintaining cellular redox balance, and modulation of its activity may significantly influence electron flow and ROS formation. Ligand binding within the active or cofactor binding regions can disrupt electron transfer or stabilize a less redox active enzyme state, ultimately reducing ROS production [22], [24]. For these reasons, this receptor serves as an appropriate model for evaluating the antioxidant potential of jackfruit leaf bioactive compounds. Vitamin C was included as a positive control due to its well established function as a natural antioxidant, providing a meaningful point of comparison for the tested compounds.

Figure 1. Overlay of the native ligand before and after redocking, showing an RMSD value of 0.1469 Å

The docking results presented in Table 1 show that morin and oxyresveratrol exhibit lower (more favorable) binding affinity values compared with vitamin C and artocarpin. However, these differences should be interpreted cautiously, as molecular docking provides relative and predictive interaction scores rather than absolute binding free energies or direct measures of enzymatic inhibition. Morin (–7.848 kcal/mol) and oxyresveratrol (–7.577 kcal/mol) form stronger and more stable interactions with the receptor, whereas vitamin C (–5.713 kcal/mol) as the positive control and artocarpin (–5.577 kcal/mol) demonstrate comparatively weaker predicted binding affinity, which may be attributed to its small size and high polarity, making it less suitable for stable enzyme binding in docking simulations. The superior binding ability of morin and oxyresveratrol is largely attributed to their aromatic rings and hydroxyl groups, which enable the formation of hydrogen bonds, electrostatic interactions, and π–π stacking with aromatic residues within the active site. Residue analysis reveals the consistent involvement of positively charged Arg residues (Arg A:15 and Arg A:169) and aromatic Tyr residues (Tyr A:128, Tyr A:199, Tyr A:200) in ligand interactions. Arg residues contribute strong electrostatic stabilization to the polar functional groups of the ligands, while Tyr residues facilitate aromatic interactions that enhance binding strength through charge delocalization. The combination of these interactions results in lower predicted binding affinity, thereby increasing the stability of the ligand receptor complexes [25], [26]. Binding affinity values represent single docking scores generated by AutoDock Vina.

Table 1. Predicted binding affinity scores obtained from AutoDock Vina docking of jackfruit leaf compounds against NADPH:FMN oxidoreductase

Ligand	Binding Affinity (kcal/mol)
Vitamin C	-5.713
Morin	-7.848
Artocarpin	-5.577
Oxyresveratrol	-7.577

Mechanistically, the involvement of Arg residues particularly Arg A:169, which consistently appears in the interaction profiles indicates its strategic role in positioning the ligand near the electron transfer pathway or the FMN binding region. Such interactions may be spatially relevant to redox-associated regions of the enzyme and could potentially influence enzyme behavior, although direct interference with electron transfer cannot be confirmed without dynamic or experimental studies. In addition, interactions with Tyr residues play an important role in enhancing the ligands' ability to stabilize charge through π–π stacking, which strengthens their radical scavenging capacity. Consequently, morin and oxyresveratrol not only display lower predicted binding affinity values than the positive control vitamin C but also exhibit interaction patterns that may be associated with redox-related regions of the enzyme. Although vitamin C is well known for its antioxidant activity through electron donation mechanisms in solution, its small and flexible structure limits its ability to establish multiple stable interactions with key residues in 1BKJ. Artocarpin, despite forming interactions with several important residues (Arg, Lys, Tyr), demonstrates weaker affinity compared with morin and oxyresveratrol, likely due to less optimal binding orientation that fails to maximize synergistic interactions. Overall, the in silico findings confirm that jackfruit leaf flavonoids, particularly morin and oxyresveratrol, exhibit lower predicted binding affinity values in silico

compared with vitamin C, indicating a higher potential for interaction with the target protein under docking conditions. Their potential mechanism of action, based on in silico predictions, may extend beyond chemical radical scavenging and involve interactions with redox-related enzymes, which require further validation [2].

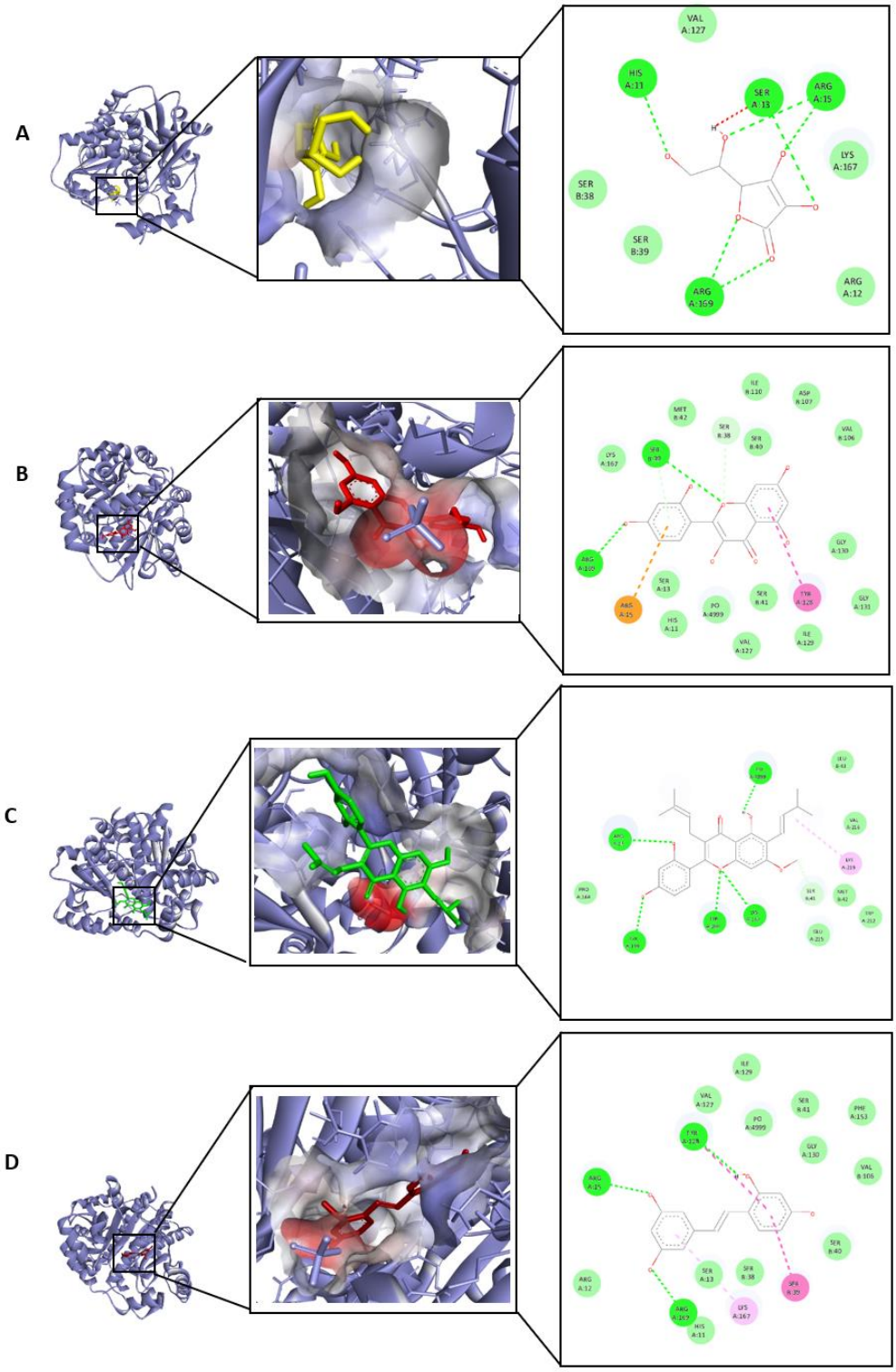

Figure 2. Visualization of the molecular docking simulation in 3D and 2D using Discovery Studio (A: Vitamin C interaction; B: Morin interaction; C: Artocarpin interaction; D: Oxyresveratrol interaction)

These results support the development of jackfruit leaf compounds as promising natural antioxidant candidates. To strengthen these findings, further studies such as molecular dynamics simulations to evaluate complex stability, more accurate predicted binding affinity calculations, and in vitro assays to validate enzymatic inhibition and radical scavenging activity are required. This study is limited by the use of static molecular docking without molecular dynamics simulations or predicted binding affinity refinement. Therefore, the stability and persistence of the predicted ligand–protein interactions over time cannot be assessed and should be addressed in future studies.

4. CONCLUSION

This study demonstrates that flavonoid compounds from jackfruit leaves, particularly morin and oxyresveratrol, exhibit stronger binding affinity toward the NADPH:FMN oxidoreductase receptor compared with vitamin C as the positive control and artocarpin. Morin (–7.848 kcal/mol) and oxyresveratrol (–7.577 kcal/mol) form stable interactions with key Arg and Tyr residues, which are located near regions associated with electron transfer and redox-related activity of the enzyme. The very low redocking RMSD value (0.1469 Å) confirms the validity of the docking protocol employed. Mechanistically, these interactions suggest a potential association with redox-related regions of the enzyme based on in silico predictions, which warrants further validation through molecular dynamics simulations and experimental assays. Therefore, morin and oxyresveratrol represent promising flavonoid candidates for further investigation based on preliminary in silico evidence.

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