Computational Evaluation of Scopoletin Against Diabetes-Related Targets: Insights from Molecular Docking and Molecular Dynamics Studies

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia and associated complications. The search for safer and more effective antidiabetic agents has led to increasing interest in plant-derived bioactive compounds. The present study investigated the antidiabetic potential of scopoletin through molecular docking and molecular dynamics (MD) simulation approaches. Molecular docking was performed against five key antidiabetic targets, namely α-glucosidase (5NN3), α-amylase (1HNY), dipeptidyl peptidase-4 (DPP-4; 2ONC), PPARγ (6MS7), and pepsin (1PSO). Scopoletin exhibited favorable binding affinities with all target proteins, ranging from −5.9 to −7.2 kcal/mol. Among the investigated targets, DPP-4 (2ONC) demonstrated the strongest binding affinity (−7.2 kcal/mol), supported by hydrogen bonding and hydrophobic interactions within the active site. Consequently, the 2ONC–scopoletin complex was subjected to a 100 ns molecular dynamics simulation using the GROMACS platform. Stability of the complex was evaluated through RMSD, RMSF, radius of gyration (Rg), solvent-accessible surface area (SASA), and hydrogen bond analyses. The complex maintained stable RMSD values (~0.2 nm), low residue fluctuations, consistent Rg values (~2.7 nm), stable SASA profiles (~330 nm²), and a sustained hydrogen-bonding network throughout the simulation period. These findings indicate that scopoletin forms a stable and energetically favorable complex with DPP-4 without causing significant structural perturbations to the protein. Overall, the study highlights scopoletin as a promising natural antidiabetic candidate and supports its potential role as a multi-target inhibitor for diabetes management. Further in vitro and in vivo investigations are warranted to validate its therapeutic efficacy.

Keywords: Scopoletin; Diabetes mellitus; Molecular docking; Molecular dynamics; DPP-4; Antidiabetic agents.

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INTRODUCTION

Among the most common metabolic diseases, diabetes mellitus (DM) is high on the list of priorities due to the severe impact it may have on quality of life and the likelihood of premature death.(Helal et al. 2026). There are two primary types of diabetes: type 1, where the body ceases to produce insulin, and type 2, characterized by a deficiency in insulin secretion and action (Sahu, Jain, and Jain 2025). Diabetes Mellitus involves chronic elevation of glucose levels in the blood (hyperglycemia). As a result, the disease is characterized by disorders in carbohydrate, fat and protein metabolism.(Alyar et al. 2025) Type 2 diabetes also known as insulin-independent diabetes mellitus is the most common and prevalent form of diabetes, which accounts for 90–95 % of diagnosed cases wherein enough insulin is produced by the pancreas but the body unable to effectively use insulin during glucose metabolism. (Patel et al. 2012)**The latest International Diabetes Federation (IDF) Diabetes Atlas (2025) reports that 11.1% – or 1 in 9 – of the adult population (20-79 years) is living with diabetes, with over 4 in 10 unaware that they have the condition.** By 2050, our projections show that 1 in 8 adults, approximately 853 million, will be living

with diabetes, an increase of 46%. This increased prevalence can place a significant burden on healthcare systems and societies because it can lead to diabetes, heart disease, kidney failure, vision problems and other serious complications. Therefore, prevention, early diagnosis and effective management of diabetes are of great importance. Current antidiabetic drugs, such as insulin analogs, metformin, sulfonylureas, and SGLT-2 inhibitors, help regulate blood glucose levels but often come with undesirable side effects such as hypoglycemia, gastrointestinal distress, and long-term metabolic imbalances(Kieffer and Robertson 2020).

The limited efficacy of conventional treatments in preventing diabetes-related complications has driven the search for alternative therapeutic approaches. Despite efforts to curb the prevalence of diabetes mellitus, the disease is still on the rise. A promising approach involves targeting key enzymes such as α-amylase and α-glucosidase, which play a crucial role in carbohydrate metabolism and glucose absorption. Inhibition of these enzymes can significantly reduce postprandial glucose levels, thus helping in the management of diabetes. The α-amylase enzyme facilitates the breakdown of complex carbohydrates into simpler sugars, while α-glucosidase catalyzes the final stages of carbohydrate hydrolysis, leading to the absorption of monosaccharides (More et al. 2025). Dipeptidyl peptidase 4, a serine peptidase is known to act on two important incretin hormones Glucose-dependent insulinotropic polypeptide (GIP) and Glucagon like peptide-1 (GLP-1) that play a crucial role in stimulating the biosynthesis and secretion of insulin and differentiation of β-cells. GIP and GLP-1 promote β-cell proliferation and inhibit apoptosis thereby enhancing β-cell population in pancreas. (Seino, Fukushima, and Yabe 2010)

Natural products, particularly those derived from medicinal plants, have gained attention for their potential multi-target effects, which could offer a safer and more effective strategy for diabetes management.(Sri Peni Fitrianingsih 1 2 et al. 2025). Medicinal plants have long been valued for their therapeutic properties, including their antioxidant, anti-inflammatory, and hypoglycemic activities, which make them promising candidates for diabetes treatment(Formagio et al. 2013). Plants, being an exemplary source of bioactive compounds, are a better therapeutic alternative to managing diabetes. Thus, identifying and validating the biological effects of these secondary metabolites would be an advancement in the field of pharmaceuticals. (T.M. Archana a et al. 2024). (Gunny et al. 2024). One such bioactive compound is scopoletin also known as *7-hydroxyl-6-methoxycoumarin*, is reported to have antifungal, hypotensive, vasorelaxant, antioxidative, antithyroid, and antihyperglycemic activities (Jang, Park, and Han 2020). Being a plant-derived coumarin, scopoletin is non-toxic and can regulate multiple target molecules, indicating its promising pharmacological potential (Qnais et al. 2025)

Molecular docking has gained increasing importance as a method in drug discovery (Hasan et al., 2024). It allows for atomic-level predictions of molecular ligand-target interactions and helps identify potential therapeutic targets. This approach is widely used to study the binding of proteins to ligands and assess compounds for discovering new lead candidates. In recent years, the application of molecular docking to explain the relevant mechanism of action has become a trend in the research and development of new drugs. (Pinzi & Rastelli, 2019; Ruiz Puentes et al., 2022). In current studies molecular docking and molecular stimulations studies of scopoletin was done. Five key antidiabetic enzymes like α-glucosidase, α- amalyse, DPP-4, PPARγ were used as target protein. The findings of this study would be employed as a novel approach for forming antidiabetics drug.

MATERIAL AND METHODOLOGY

Molecular Docking studies

Molecular docking has accelerated the drug discovery by providing the structure-based interactions between ligand and receptor protein(Arif et al. 2021). The MD analysis of the scopoletin was carried out to predict the preferred binding orientation when it interacts with key antidiabetic enzymes like α- glucosidase, α- amalyse, DPP-4, PPARγ, pepsin with PDB IDs 5NN3, 1HNY, 2ONC, 6MS7 and 1PSO respectively, helping to estimate the strength and nature of the interaction.(Sri Peni Fitrianingsih 1 2 et al. 2025) It works by exploring the conformational space of both protein and ligand to identify the most energetically favorable binding pose within the active site. Docking algorithms use scoring functions to approximate binding affinity based on intermolecular forces such as hydrogen bonding, hydrophobic interactions, electrostatic interactions, and van der Waals forces. The process typically involved preparing the protein and ligand structures, defining the binding pocket, generating multiple poses and ranking them according to binding energy.

2.1.1 Preparation of Protein

Protien purification of 5NN3, 1HNY, 2ONC, 6MS7 and 1PSO enzymes was performed in BIOVIA Discovery Studio by loading each PDB structure and retaining a single amino acid chain to reduce structural complexity while maintaining stability. All water molecules, ions, ligand groups and unnecessary heteroatoms were

removed. Polar hydrogens were added to ensure accurate charge distribution followed by optimization of polar groups for stable interactions.

2.1.2. Protein-Ligand preparation

Protein–ligand docking was performed in PyRx by first loading the purified protein structure and converting it into a macromolecule in PDBQT format. The Scopoletin ligand file was imported, converted from PDB to PDBQT and energy-minimized using the built-in minimization tool to obtain stable conformations. A grid box was then generated around the active site of the protein to define the search space for docking. Docking was executed using AutoDock Vina integrated within PyRx, and multiple binding poses were obtained for each ligand. The best docking model was selected based on the lowest binding affinity score and an RMSD value of 0, indicating the most reliable and consistent binding pose.

2.1.3.Protein-Ligand visualization

The top-ranked protein–ligand complex was then imported into BIOVIA Discovery Studio, where detailed 2D and 3D interaction analyses were performed to visualize hydrogen bonds, hydrophobic contacts, and overall binding orientation.

Molecular Dynamics (MD) Simulation

Molecular Dynamics Simulations (MDS) serve as an essential tool in computational biology for assessing the temporal stability and interaction dynamics of biomolecular protein. For this investigation, the GROMACS simulation software was utilized to study the dynamics of the 2ONC(DPP4) protein and ligand (scopoletin) in complex with the protein receptor (2ONC i. e DPP-4). This analysis was predicated on prior docking results, which highlighted strong interactions in the protein.

The simulation commenced with the generation of a precise topological model for ligand using the Swiss Param tool. Ensuring accurate molecular representation is critical for reliable simulation outcomes. The widely adopted Charmm27 force field was selected to simulate the molecular forces and dynamics, encapsulating the complex and protien in a cubic simulation box with dimensions of 90 Å on each side to simulate a naturalistic molecular environment. Initial system preparation involved an energy minimization step using the steepest descent method to minimize potential energy, setting a solid foundation for dynamic simulations. This was followed by the introduction of the Single Point Charge Extended (SPCE) water model for system solvation, chosen for its precision in simulating water molecule behaviour. To replicate physiological ionic conditions and maintain a balanced pH, appropriate concentrations of sodium and chloride ions were added.

The equilibration phase was divided into two segments. Initially, a 10-nanosecond temperature equilibration was conducted under the NVT ensemble using the v-rescale temperature coupling method, stabilizing the system at 300 K. This phase included positional restraints to ensure proper orientation of solvent molecules around the complex and protein. It was succeeded by a 10-nanosecond pressure equilibration phase under the NPT ensemble, where positional restraints were progressively lifted to achieve pressure stabilization. The final phase entailed a comprehensive 100-nanosecond production simulation, which is crucial for observing the naturalistic molecular motions and interactions under stable conditions of temperature (300 K) and pressure (1 bar). Throughout this period, important dynamic parameters such as Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Radius of Gyration (Rg), Solvent Accessible Surface Area (SASA), and hydrogen bonding activity were meticulously recorded to elucidate the stability and interaction dynamics of the ligand with Protein.

RESULT AND DISCUSSION

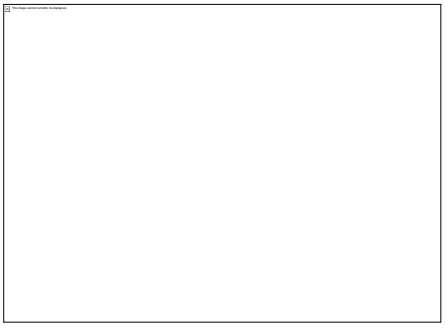
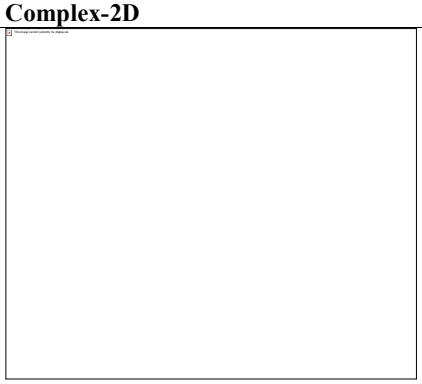
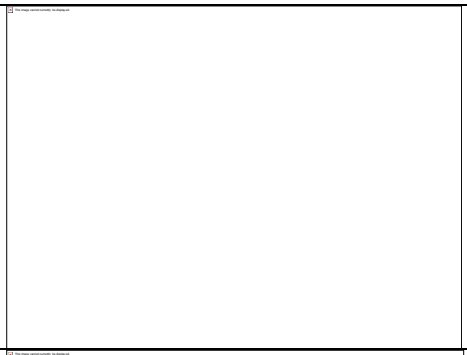
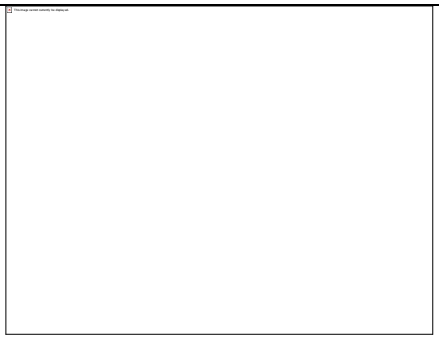
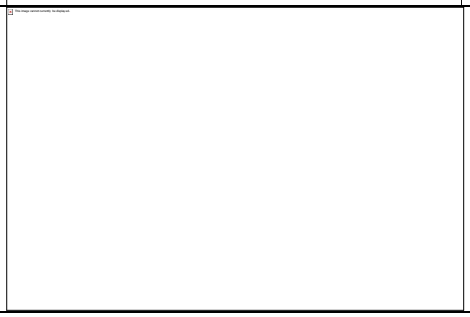
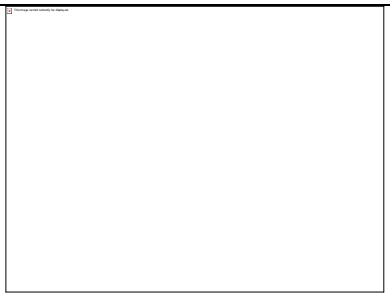
Molecular Docking

The binding of scopoletin to α glycosidase, α- amylase, DPP-4, PPAR-ϒ and pepsin was analyzed using molecular docking and the results are shown in table No 1 and fig 1**.** The molecular docking analysis of ligand with 5NN3 receptor reveled a binding affinity of -6.2 kcal/mol. The ligand formed hydrogen bond with 2 amino acid residues (ARG A:594 and HIS A: 584), carbon hydrogen bond with ARG A:268, along with pi-pi T shaped bond with HIS A:717 as shown in fig 1 (A). These interaction suggest moderately stable complex. (Wang, Wei, and Huang 2026). Additionally the docking analysis of ligand with 1HNY suggests binding affinity of -6.1 kcal/mol slightly less than 5NN3. Fig 1 (B) reveled multiple stabilizing interactions within the active site. A conventional hydrogen bond was formed with GLN63, while carbon-hydrogen bonds were observed with GLU233, ASP300, and HIS101. Additionally, aromatic π–π stacking interaction with TYR62 and π–alkyl interaction with LEU165 contributed to the stabilization of the protein–ligand complex. These interactions indicate favorable accommodation of the ligand within the binding pocket of the target protein. Moreover the highest binding affinity was shown by the protein 2ONC that is – 7.2. As shown in fig 1 (C) ligand forms conventional hydrogen bond with TRP (A: 201) and Carbon hydrogen bond with TRP A: 124 and THR A: 129. Ligand shows binding affinity of -6.5 with 6MS7 forming conventional hydrogen bond with GLN A:255 and ARG A:280. Additionally it has formed alkyl and pi-

alkyl bong with LEU A:255, CYS A: 285, ILE A:281, ILE A:341, AND MET A: 348. One Pi cation bong is also formed with ARG A:288. (Najmi et al. 2023). The lowest binding affinity of -5.9 was seen between the ligand and 1 PSO. According to the fig 1 (E) ligand formed carbon hydrogen bond with GLY E: 34 and ASP E: 215. Similarly it form pi alkyl bond with ILE E:120 and Pi sigma bond with TYR E: 75. Overall molecule docking result suggest that ligand forms moderately stable bond with all the proteins associated with anti diabetic mechanism. Amongst all 2ONC showed highest affinity and hence selected for molecular stimulation studies.

Table No 1: Molecular docking scores

Complex	Binding affinity (kcal/mol)	RMSD
5NN3-Scopoletin	-6.2	0
1HNY- Scopoletin	-6.1	0
2ONC- Scopoletin	-7.2	0
6MS7- Scopoletin	-6.5	0
1PSO- Scopoletin	-5.9	0

	Protein	Complex-3D	Complex-2D
A	5NN3		
B	1HNY		
C	2ONC		

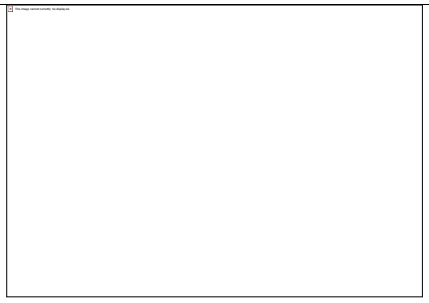
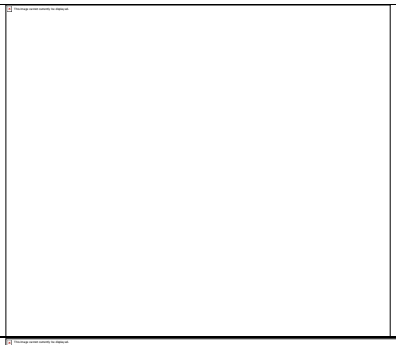
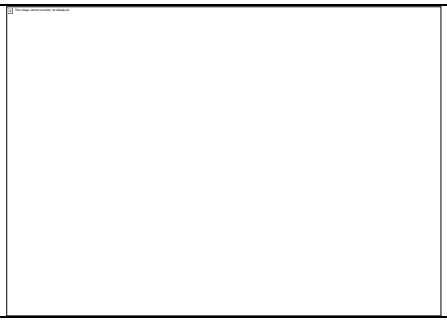
D	**6MS7**		
E	**1PSO**		

Fig 1: Molecular Docking studies 3D and 2D interactions

Molecular Stimulation Studies:

Molecular dynamics (MD) simulations provide information on the atomic-level behavior of biomolecules, facilitating the investigation of dynamic interactions between putative therapeutic candidates and proteins. According to, MD simulations offer a more profound comprehension of binding affinities and the molecular intricacies of drug action.(Hamim et al. 2025). In this work MD simulation was performed for 100 Nanoseconds (ns), to understand the stability of mentioned proteins and protein ligand complex and the average value of RMSD, RMSF, Rg, H-bonds and SASA has been tabulated in Table 2. Overall study suggest that scopoletin does not make any significant alterations in the protein structure and forms stable complex.

Table 2: Molecular Dynamics Analysis of complex and protein.

S.No	Complex	RMSD (nm)	RMSF (nm)	RG (nm)	SASA (nm2)	H bonds (number)
1	2ONC-Scopoletin	0.2 +/- 0.01	0.2+/- 0.05	2.7+/- 0.02	330	500
2	2ONC	0.2+/- 0.01	0.18+/- 0.05	2.73/- 0.02	325+/- 2.20	540

The information provided describes the structural properties of the complex, which are identified by their unique protein. The "Average RMSD" (root mean square deviation) and "Average RMSF" (root mean square fluctuation) values are measures of the overall conformational stability and flexibility of the protein. A lower RMSD value indicates that the protein has a more stable structure, while a higher RMSF value indicates that the protein is more flexible. The "Radius of gyration" value measures the protein's size, with a larger radius indicating a larger protein. The "Average SASA" (solvent accessible surface area) value is a measure of the amount of surface area of the protein that is exposed to the solvent (such as water). A larger SASA value indicates that more of the protein is exposed to the solvent.

3.2.1 Root mean square deviation

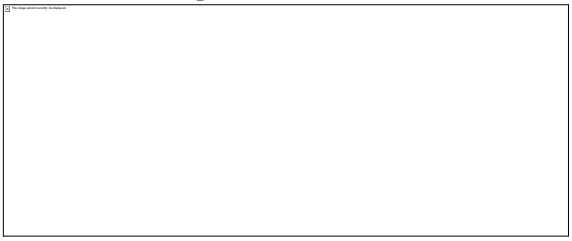


Fig.2. RMSD images of the all the complex

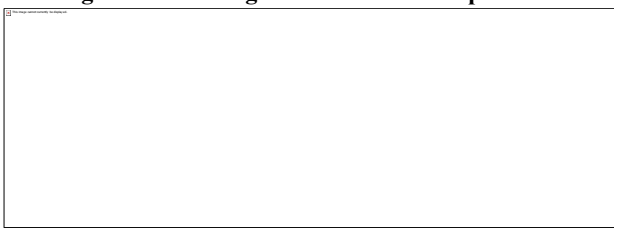


Fig 3. Root mean square deviation analysis of (apo)protein.

Root Mean Square Deviation (RMSD) is an important parameter used to assess the structural stability of proteins and protein–ligand complexes during molecular dynamics simulations. The RMSD values of the apo protein (2ONC) and the 2ONC–Scopoletin complex were calculated over a 100 ns simulation period and are presented in Table 2. The protein backbone RMSD profile of the 2ONC–Scopoletin complex exhibited an initial increase during the early stage of the simulation, followed by gradual stabilization throughout the remaining trajectory. The RMSD values fluctuated within a narrow range of approximately 0.15–0.30 nm, indicating only minor conformational adjustments required for ligand accommodation.

A comparison of the RMSD values of the free protein and the protein–ligand complex revealed similar average RMSD values, suggesting that Scopoletin binding did not induce significant structural perturbations in the protein. The absence of abrupt fluctuations or large deviations throughout the simulation indicates that both the apo protein and the ligand-bound complex maintained stable conformations. Furthermore, the relatively low RMSD values observed for the complex demonstrate the formation of a stable protein–ligand interaction and confirm that Scopoletin binding does not destabilize the protein backbone.

3.2.2Root mean square fluctuations

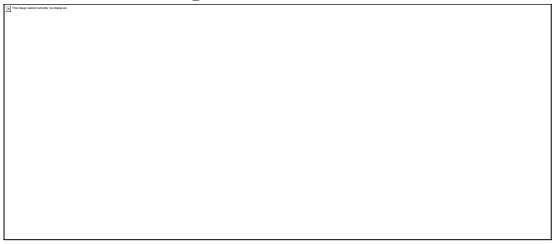


Fig.4. RMSD images of the all the complex

Fig 5. Root mean square fluctuation analysis of apo protien

Root Mean Square Fluctuation (RMSF) analysis was performed to evaluate the flexibility and dynamic behavior of individual amino acid residues in both the apo protein (2ONC) and the 2ONC–Scopoletin complex during the 100 ns molecular dynamics simulation. The average RMSF values for the protein and protein–ligand complex are presented in Table 1, and the corresponding RMSF profiles are shown in Fig. 4 and Fig 5..

Comparison of the RMSF profiles revealed that the 2ONC–Scopoletin complex exhibited lower residue fluctuations than the apo protein across most regions of the protein structure. In both systems, a few peaks were observed at specific residues, which are likely associated with loop regions and terminal segments that are naturally more flexible. However, the majority of residues displayed low fluctuations (<0.2 nm), indicating that the overall protein framework remained stable throughout the simulation.

Notably, residues located within or near the ligand-binding site showed reduced fluctuations in the presence of Scopoletin compared to the apo protein, suggesting that ligand binding restricts residue mobility and enhances the stability of the binding pocket. Although minor fluctuations were observed in certain flexible regions, no substantial destabilization of the protein structure occurred during the simulation. Overall, the RMSF analysis demonstrates that the binding of Scopoletin contributes to the stabilization of the 2ONC protein by reducing residue-wise flexibility and maintaining structural integrity throughout the 100 ns simulation period.

3.2.3 Radius of gyration:

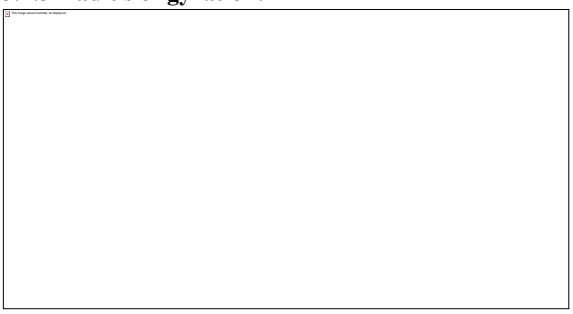


Fig.6. RG images of the all the complex

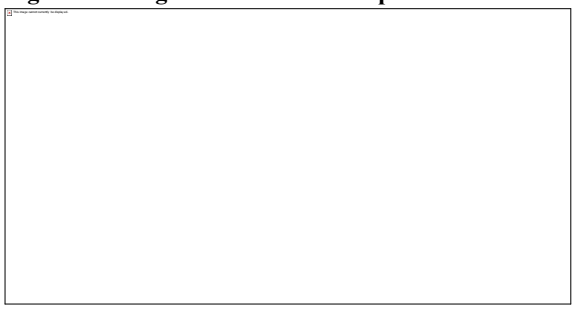


Fig 7. Radius of gyration analysis of apo protein

Radius of gyration is defined as the mass-weighted root mean square distance of atoms from their common center of mass. The Rg values of the apo protein (2ONC) and the 2ONC–Scopoletin complex were calculated over a 100 ns simulation period, the average values of Rg (nm) and the corresponding profiles are presented in Table 2 and Fig.6 and 7 respectively.

A comparative analysis of the Rg profiles revealed that both the apo protein and the 2ONC–Scopoletin complex exhibited a similar pattern of fluctuations throughout the simulation, indicating the maintenance of their overall structural architecture. The 2ONC–Scopoletin complex displayed minor fluctuations around an average Rg value of approximately 2.7 nm, suggesting a stable and compact protein structure during the entire simulation period. No significant increase in Rg was observed, indicating the absence of protein unfolding or structural collapse.

Compared with the apo protein, the ligand-bound complex exhibited slightly lower and more stable Rg values during the middle phase of the simulation, suggesting transient compaction induced by Scopoletin binding. This reduction in Rg reflects a more compact protein conformation and enhanced structural stability. Subsequently, the Rg values stabilized and remained nearly constant until the end of the simulation, demonstrating the maintenance of a well-folded and structurally stable state.

Overall, the comparative Rg analysis indicates that both the free protein and the protein–ligand complex retained their compactness throughout the 100 ns simulation. However, the 2ONC–Scopoletin complex exhibited slightly greater structural compactness and stability, suggesting that Scopoletin binding contributes to preserving the structural integrity and folded conformation of the protein.

3.2.4 Solvent Accessible Surface

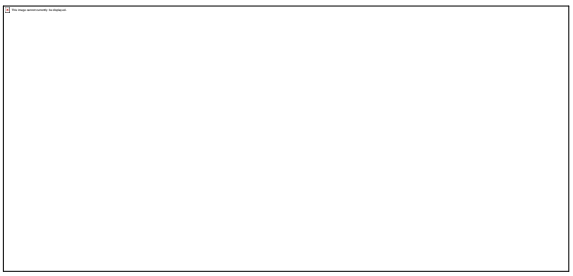


Fig.8 SASA images of the all the complex

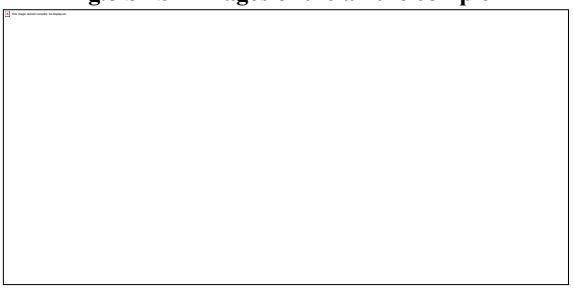


Fig 9. Solvent accessible surface area analysis of apo protein

Solvent Accessible Surface Area (SASA) analysis was performed to evaluate changes in the protein surface exposed to the solvent and to assess the compactness of the hydrophobic core during the molecular dynamics simulation. The average SASA values of the apo protein (2ONC) and the 2ONC–Scopoletin complex over the 100 ns simulation period are presented in Table 2, while the corresponding SASA profiles are shown in Fig.8 and Fig 9.

Comparative analysis of the SASA profiles revealed that both the apo protein and the 2ONC–Scopoletin complex maintained relatively stable solvent-accessible surface areas throughout the simulation, indicating the absence of major structural alterations. The SASA values of the 2ONC–Scopoletin complex fluctuated moderately within the range of approximately 315–335 nm², demonstrating a stable equilibrium between solvent exposure and structural compactness.

An initial decrease in SASA was observed for the ligand-bound complex compared with the apo protein, suggesting partial burial of surface residues and tighter packing of the protein structure following Scopoletin binding. This reduction in solvent-accessible area indicates enhanced compactness of the protein and stabilization of the hydrophobic core. Subsequently, the SASA values remained relatively constant, reflecting the attainment of an equilibrium conformation and maintenance of a stable protein structure throughout the remainder of the simulation.

The apo protein also exhibited a stable SASA profile; however, the protein–ligand complex showed slightly lower SASA values, indicating reduced solvent exposure due to ligand-induced conformational stabilization. No

abrupt increases or decreases in SASA were detected in either system, suggesting the absence of significant unfolding, collapse, or abnormal exposure of buried residues during the simulation.

Overall, the comparative SASA analysis demonstrates that both the free protein and the 2ONC–Scopoletin complex remained structurally stable during the 100 ns simulation. The slightly lower and more stable SASA values observed in the ligand-bound complex indicate that Scopoletin binding promotes tighter packing of the protein structure, preserves the integrity of the hydrophobic core, and enhances the overall structural stability of the protein.

3.2.5 Hydrogen Bond Analysis

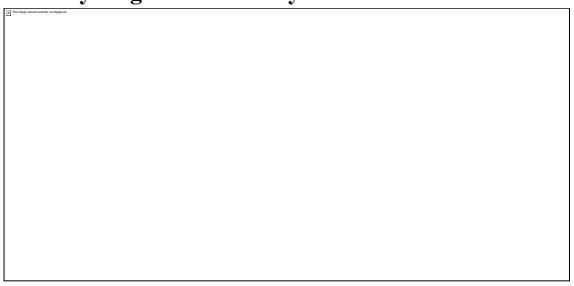


Fig.10. Hydrogen bond images of the all the complex

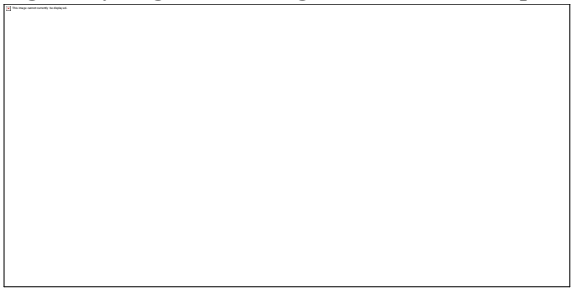


Fig 11. Hydrogen bonds analysis of apo protein

Hydrogen bond analysis was performed to evaluate the stability of the protein structure and the influence of ligand binding on the internal hydrogen-bonding network during the 100 ns molecular dynamics simulation. The average number of hydrogen bonds observed during the simulation is presented in Table 2 .

A comparative assessment of the apo protein (2ONC) and the 2ONC–Scopoletin complex revealed that both systems maintained a relatively stable hydrogen-bonding network throughout the simulation period. The protein–ligand complex exhibited approximately 480–500 hydrogen bonds, with only minor fluctuations around the mean value. This consistent hydrogen-bonding pattern indicates the preservation of the protein's structural framework and suggests that the overall conformation remained stable during the simulation.

Compared with the apo protein, the 2ONC–Scopoletin complex showed a similar or slightly higher number of hydrogen bonds, indicating that ligand binding did not disrupt the intrinsic hydrogen-bonding network of the protein. Instead, the presence of Scopoletin appeared to support the maintenance of favorable intermolecular and intramolecular interactions, thereby contributing to enhanced structural stability. The absence of significant decreases in hydrogen bond numbers throughout the simulation further confirms that the protein retained its native folding and structural integrity in the ligand-bound state.

Overall, the hydrogen bond analysis demonstrates that both the free protein and the 2ONC–Scopoletin complex remained structurally stable during the 100 ns simulation. The sustained hydrogen-bonding network observed in the ligand-bound complex suggests that Scopoletin binding stabilizes the protein structure without perturbing its internal interactions, thereby supporting the formation of a stable and energetically favorable protein–ligand complex.

CONCLUSION

The present study demonstrates the potential of scopoletin as a promising natural antidiabetic compound through comprehensive molecular docking and molecular dynamics simulation analyses. Molecular docking revealed favorable interactions of scopoletin with multiple diabetes-related targets, including α-glucosidase, α-amylase, DPP-4, PPARγ, and pepsin, with the strongest binding affinity observed against DPP-4 (2ONC) at −7.2 kcal/mol. Detailed interaction analysis confirmed the involvement of hydrogen bonds and hydrophobic interactions in stabilizing the protein–ligand complexes.

To further evaluate the stability of the most favorable complex, a 100 ns molecular dynamics simulation of the 2ONC–scopoletin complex was performed. The RMSD, RMSF, radius of gyration, SASA, and hydrogen bond analyses collectively demonstrated that scopoletin binding did not induce significant conformational changes in the protein structure. Instead, ligand binding contributed to enhanced structural stability, reduced residue flexibility, maintained protein compactness, and preserved the internal hydrogen-bonding network throughout the simulation period.

Overall, the findings suggest that scopoletin possesses significant potential as a natural DPP-4 inhibitor and may serve as a promising lead molecule for the development of novel antidiabetic therapeutics. However, further experimental validation through in vitro, in vivo, and clinical studies is necessary to confirm its efficacy, safety, and mechanism of action in diabetes management.

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