RESEARCH PAPER

Computational exploration revealed Nicotiflorin as a potent DPP4 inhibitor for diabetes management: Insights from network pharmacology, molecular docking, and molecular dynamics simulations studies

Priyanka Patil[1*], Laxmi Pattanashetti[2*], Damita L. Cota[3], Nikita N. Kanbarkar[4]

[1]Department of Pharmacology, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Deemed-to-be-University, Belagavi, Karnataka, India

[2]Department of Pharmacology, KLE College of Pharmacy, Hubli, KLE Academy of Higher Education and Research, Deemed-to-be-University, Belagavi, Karnataka, India

[3]Department of Pharmacology, Goa College of Pharmacy, Panaji, Goa, India

[4]Department of Pharmacology, Rani Chennamma College of Pharmacy, Belagavi, Karnataka, India

***Corresponding authors:**

Priyanka Patil, Department of Pharmacology, KLE College of Pharmacy, Airport Road, Basavan Kudchi Belagavi, 591124, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India.

Email: priya.pp556@gmail.com | Orcid: 0000-0002-3402-9114

Laxmi Pattanashetti, Department of Pharmacology, KLE College of Pharmacy, Hubli, KLE Academy of Higher Education and Research, Deemed-to-be-University, Belagavi, Karnataka, India.

Email: lapattanashetti@klecoph.edu.in; pattanashetti.laxmi67@gmail.com | Orcid: 0000-0002-0572-269

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ABSTRACT

Diabetes mellitus is a multifactorial metabolic disorder characterized by chronic hyperglycemia and insulin function impairment. Current treatments often have limitations in terms of efficacy and side effects, highlighting the need for novel therapeutics. This study evaluated the potential of nicotiflorin, a flavonoid glycoside with known antidiabetic properties, using an integrated computational approach. Network pharmacology identified 47 diabetes-associated targets, highlighting Dipeptidyl peptidase 4 (DPP4), TNF, and NOX4 as key modulators of glucose metabolism, inflammation, and oxidative stress. Molecular docking showed Nicotiflorin binds to DPP4 with a higher affinity (−9.3 kcal/mol) than sitagliptin (−8.3 kcal/mol), supported by extensive hydrogen bonding and stable interactions. Molecular dynamics simulations over 200 ns confirmed the stability of the Nicotiflorin–DPP4 complex, with consistent RMSD, hydrogen bonds, and reduced conformational fluctuations. Principal component and free energy landscape analyses indicated lower structural flexibility and stronger binding stability for the binding of Nicotiflorin. These findings suggest that Nicotiflorin acts through multiple targets and mechanisms, offering a promising natural scaffold for future antidiabetic drug development. Further experimental validation is required to confirm its therapeutic efficacy and pharmacokinetic profile.

Keywords: Dipeptidyl peptidase-4, Nicotiflorin, Tumor Necrosis factor, NADPH oxidase 4, Molecular simulation, Diabetes Mellitus.

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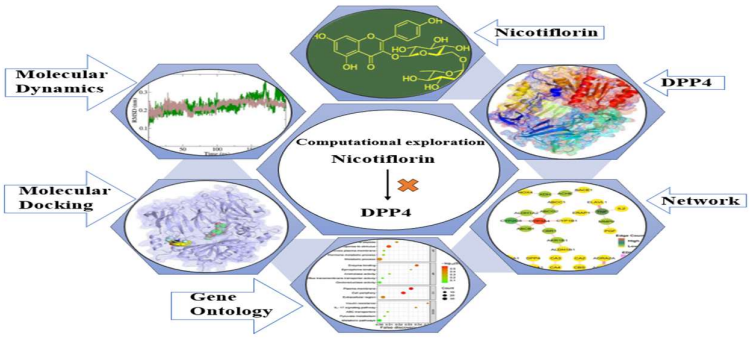


Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from

defects in insulin secretion, insulin action, or both [1-3]. The pathogenesis of diabetes involves a complex interplay of genetic, environmental, and lifestyle factors, leading to impaired glucose homeostasis, insulin resistance, and β-cell dysfunction [4]. Current treatment strategies primarily include oral hypoglycemic agents such as metformin, sulfonylureas, and Dipeptidyl peptidase 4 (DPP4) inhibitors, as well as injectable therapies like insulin and GLP-1 receptor agonists [5]. While these therapies have significantly improved diabetes management, they are often associated with limitations, such as inadequate efficacy, side effects, and the inability to halt disease progression [6]. These limitations underscore the urgent need for the identification and development of novel anti-diabetic agents that can target the underlying molecular mechanisms, offer improved safety profiles, and provide sustained glycemic control to address the growing global burden of diabetes.

Nicotiflorin (kaempferol 3-O-rutinoside), isolated from *Coccinia grandis* leaves, exhibits α-glucosidase inhibitory activity with an IC50 of 235.8 µM, making it 8.6 times more potent than acarbose. This inhibition slows carbohydrate digestion, reducing postprandial blood sugar spikes, supporting its antidiabetic potential [7]. Nicotiflorin (kaempferol-3-O-rutinoside) was identified in the butanolic extract of *Crataegus monogyna* at a concentration of 1.482 ± 0.016 mg/g. This study highlighted the antidiabetic potential of the extract through α-amylase inhibition, with an IC50 value of 91.19 ± 0.10 µg/mL, demonstrating significant inhibitory activity. This suggests that *Crataegus monogyna*, rich in phenolic compounds including nicotiflorin, may serve as a promising natural therapeutic agent for diabetes management [8]. Nicotiflorin, isolated from *Prunus persica* (L.) Batsch flowers, demonstrated glucose-stimulated insulin secretion (GSIS) enhancing activity in INS-1 pancreatic β-cells. At a concentration of 10 µM, nicotiflorin increased insulin secretion, with a glucose stimulation index (GSI) of 3.59 ± 0.07**,** suggesting its potential role in improving pancreatic β-cell function and insulin release. This finding supports its possible antidiabetic activity**,** making it a promising natural compound for diabetes management [9]. The tea infusion extract of *Annona cherimola* Miller leaves, containing nicotiflorin as one of the ingredients, was evaluated for its antihyperglycemic activity in streptozotocin-induced diabetic mice, where it significantly reduced blood glucose levels and improved liver and kidney architecture, mitigating diabetes-associated damage and supporting its potential as a natural antidiabetic agent.[10]. Furthermore, it was identified as a potent α-glucosidase inhibitor with a binding affinity (docking score −29.16 kJ/mol and IC50 = 0.148 mg/mL). Importantly, in vivo, it significantly reduced blood glucose in starch-induced hyperglycemic rats ($p < 0.05$), supporting its potential as a natural antidiabetic agent [11].

A systematic computational approach is essential for understanding the therapeutic mechanisms of bioactive compounds and their interactions with disease-related targets. Network pharmacology serves as a powerful tool for identifying key molecular targets and pathways associated with diabetes [12]. By integrating multiple biological databases, it constructs a comprehensive network linking the compound to its protein targets, biological functions, and metabolic pathways [13-18]. Following network pharmacology analysis, molecular docking was used to predict the binding affinity and interaction patterns of Nicotiflorin with diabetes-related proteins. It ranks potential targets based on binding energy, facilitating the selection of the most promising protein-ligand complexes [19-21]. However, molecular docking offers only a static view of interactions, which may not fully capture the molecular dynamic recognition in a biological system. To address this, molecular dynamics simulation examines the stability and behavior of Nicotiflorin within the binding pocket under physiological conditions [22]. The integration of network pharmacology, molecular docking, and molecular dynamics simulations provides a comprehensive understanding of the molecular mechanisms of the anti-diabetic effects of Nicotiflorin. This computational workflow aids in target identification, drug repurposing, and rational experimental design, ultimately contributing to novel therapeutic strategies for diabetes treatment.

Materials and Methods

Identification of Nicotiflorin targets for Diabetes

The potential molecular targets of the Nicotiflorin were investigated using various platforms, including Swiss Target Prediction (https://www.swisstargetprediction.ch/), Binding DB (https://www.bindingdb.org/), and the SEA search tool (https://sea.bkslab.org/) [23-25]. To identify diabetes-related targets, a search for "diabetes mellitus" was conducted on GeneCards (https://www.genecards.org/). The identified genes were then compared with nicotiflorin-associated targets using the Venny 2.1 tool (https://bioinfogp.cnb.csic.es/tools/venny/), leading to a refined selection of potential therapeutic targets for diabetes, facilitating further analysis.

Gene set enrichment and Network Analysis of Nicotiflorin in diabetes mellitus related Pathways

The diabetes mellitus-associated targets of nicotiflorin were examined using the STRING database version 12.0 (https://string-db.org/), with "Homo sapiens" as the designated organism [26]. The analysis explored their functional roles across three key Gene Ontology (GO) categories: cellular components, molecular functions, and biological

processes, as well as their involvement in the KEGG signaling pathways. This investigation provided valuable insights into their contributions to diabetes mellitus pathology, as the interaction networks and STRING annotations revealed GO terms closely associated with established disease mechanisms. The identified proteins were found to play active roles in various pathways that influence disease progression. To further illustrate these interactions, a protein-protein interaction network was constructed and visualized using Cytoscape (https://cytoscape.org/), emphasizing multifunctional proteins engaged in multiple pathways as potential hub nodes [27].

Target selection for molecular docking

Among the 47 overlapping targets, dipeptidyl peptidase-4 (DPP4) was selected to evaluate the binding affinity of nicotiflorin [28]. This target was prioritized to determine whether the nicotiflorin exhibits significant binding affinity with one of the well-established targets for diabetes intervention. The 3D structure of the nicotiflorin (PubChem CID: 5318767) was first retrieved from the PubChem database and converted into a .pdb file using Discovery Studio Visualizer (version 2019, https://discover.3ds.com/discover-studio-visualizer-download). The structure was then subjected to energy minimization using the MMFF94 force field and saved in. pdbqt format. Next, DPP4 (PDB: 1J2E) was prepared as a macromolecule by removing the heteroatoms from the protein. Nicotiflorin was subsequently docked to DPP4 using AutoDock Vina [29], which generated nine distinct ligand poses. These poses were assessed based on their binding affinities and interaction counts, as described in previous studies [16]. The pose with the lowest binding energy was selected for ligand-protein interaction visualization and molecular dynamics simulations. Sitagliptin, a well-known DPP4 inhibitor, was used as a control.

Assessment of molecular dynamics simulation for docked complexes

We investigated the stability of the intermolecular interactions by examining the most favorable docked conformations through molecular dynamics simulations using GROMACS (https://www.gromacs.org/) version 2024.1 [30]. To accurately model the molecular behavior, these simulations were conducted for up to 200 ns under explicit solvent conditions using the Amber ff99SB-ildn force field. The preparation steps involved calculating the partial charges for small molecules using the 'bcc' charge model via quantum computations and generating topological parameters for ligands and complexes using the xleap module in Amber Tools. The systems were solvated in a rectangular simulation box with a 10.0 Å buffer around the protein using a three-site water model and neutralized with counter ions to replicate physiological conditions. The preparation phase also included energy minimization, followed by a two-stage equilibration process involving canonical (NVT) and isobaric (NPT) ensembles. Each equilibration phase lasted 1 ns, with the NVT phase maintaining a temperature of 311 K using a modified Berendsen thermostat and the NPT phase stabilizing the pressure at 1 bar using the Parrinello-Rahman barostat. Long-range electrostatic interactions, van der Waals forces, and Coulombic interactions were calculated using the Particle Mesh Ewald (PME) method with a 1-nanometer cutoff, whereas bond constraints were enforced using the LINCS algorithm. After these preparatory steps, a 200-ns production simulation was performed, with system coordinates recorded every 2 femtoseconds for detailed analysis. Post-simulation analysis involved the assessment of key metrics to understand molecular stability and interactions. Tools from GROMACS and other specialized software were used to evaluate metrics such as root mean square deviation (RMSD), root mean square fluctuations (RMSF), hydrophilic interactions, solvent-accessible surface area (SASA), radius of gyration (Rg), and molecular motion modes. Principal component analysis (PCA) was conducted following previous studies [14, 16]. For PCA, the molecules were aligned to a reference structure using least-squares fitting, removing translational and rotational degrees of freedom. A covariance matrix of the Cartesian coordinates was constructed and diagonalized to derive the eigenvectors representing the motion directions and eigenvalues quantifying the energy contributions. Time-dependent projections onto these eigenvectors identified specific vibrational modes, whereas the average projections revealed the atomic contributions to the collective motions. The "gmx covar" and "gmx anaeig" tools in GROMACS facilitated the computation and visualization of the eigenvectors, enhancing the understanding of molecular dynamics. Additionally, the relative free binding energy and residue-specific contributions were estimated using the Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) method with the 'gmx_mmpbsa' tool. [31] This approach includes the decomposition analysis of stable trajectory segments, providing insights into the energetic contributions of individual residues to binding stability. These comprehensive analyses provide a detailed understanding of the molecular interactions and dynamics of each target under study.

Results

Potential of Nicotiflorin in Diabetes Target proteins Interaction

The identification of the potential targets of nicotiflorin for DM revealed its therapeutic relevance through structural analysis, target overlap, and protein network interactions (Fig. 1). The chemical structure of nicotiflorin consists of multiple hydroxyl groups, contributing to its molecular properties, including an exact mass of 558.16 and a molecular weight of 594.52. A comparison of nicotiflorin-associated targets with the DM-related targets showed a significant overlap, with 47 shared targets, suggesting its potential influence on DM-related pathways, while five targets remained unique to nicotiflorin. The protein-protein interaction network highlights the key targets involved in metabolic and inflammatory processes. CYP3A4 emerged as a central hub with a high interaction strength, signifying its importance in the biological activity of nicotiflorin. Other notable targets include CYP1B1, ABC transporters (ABCB1 and ABCG2), and inflammatory regulators, such as TNF and IL2, which may contribute to its pharmacological effects. The interaction strength and experimentally determined interaction (EDI) values provide insights into the significance of these connections, supporting the potential role of nicotiflorin in DM treatment.

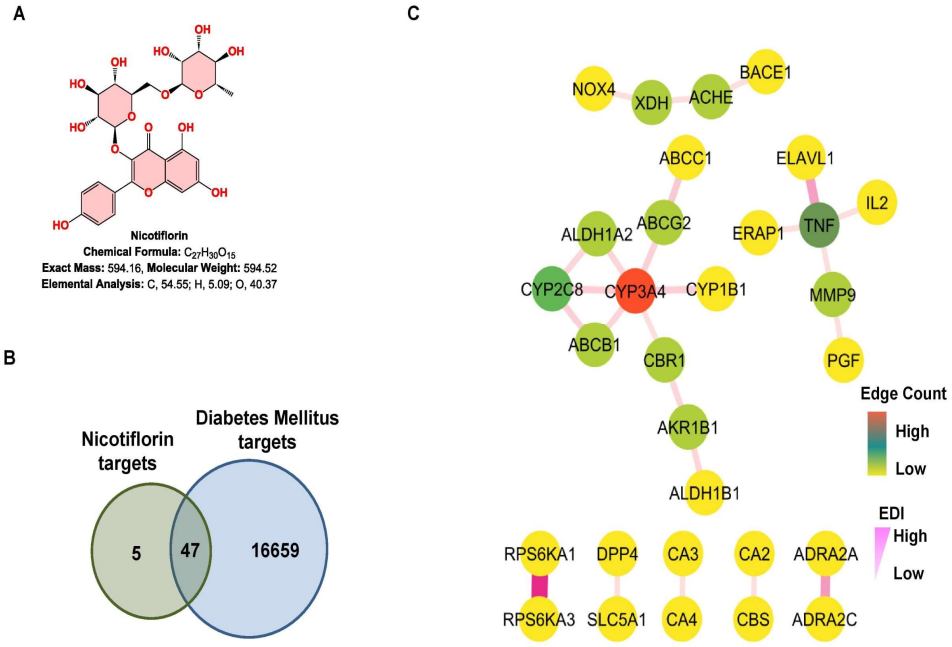
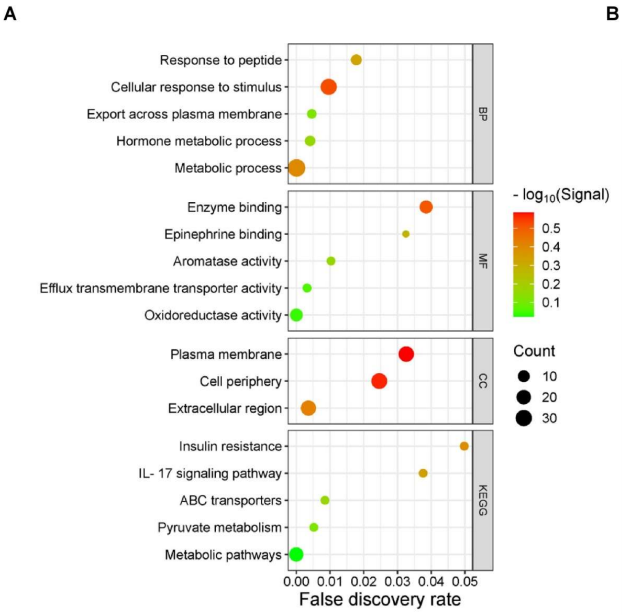


Figure 1: Identification of Nicotiflorin's DM targets and their interactions. (A) Chemical structure and physicochemical properties of Nicotiflorin. **(B)** Venn diagram depicting the overlap between Nicotiflorin targets and Diabetes Mellitus-related targets. **(C)** Protein-protein interaction (PPI) network of Nicotiflorin-related targets exhibiting at least one interaction in the protein-protein interaction network. Nodes represent proteins, and edges indicate interactions. Color coding represents edge count (interaction strength), while experimentally determined interaction (EDI) is depicted using a gradient from low to high.

Nicotiflorin as a Multi Target Approach for Diabetes Treatment

Targeting identified proteins with nicotiflorin presents a promising strategy for diabetes treatment by modulating key biological pathways involved in glucose metabolism, insulin resistance, and inflammation (Fig. 2). Functional enrichment analysis highlighted its influence on metabolic processes, hormone metabolism, and insulin resistance, which are crucial for maintaining glucose homeostasis (Fig. 2A; Table 1). Notably, Nicotiflorin targets proteins such as DPP4, TNF, and RPS6KA3(Fig. 2B), which play significant roles in the pathology of diabetes. DPP4 is a well-established therapeutic target because its inhibition enhances incretin hormone activity, thereby improving insulin secretion and glucose tolerance. The interaction of nicotiflorin with DPP4 suggests its potential to enhance insulin sensitivity and glucose regulation. Similarly, TNF, a pro-inflammatory cytokine that disrupts insulin signaling, contributes to insulin resistance. By targeting TNF (Fig. 2A; Table 1), nicotiflorin may help mitigate chronic inflammation, a key factor in type 2 diabetes progressions. Additionally, its involvement with oxidoreductase activity-related proteins, such as NOX4, ALDH1A2, and XDH (Table 1), suggests a role in reducing oxidative stress, which is a major contributor to pancreatic beta-cell dysfunction and insulin resistance. By minimizing oxidative damage, nicotiflorin can enhance pancreatic function and improve insulin secretion. The enrichment of ABC transporters (ABCC1, ABCB1, and ABCG2) and cell membrane proteins (SLC5A1, ADRA2A, CA4, and BACE1)

further supports the role of nicotiflorin in glucose transport, metabolic regulation, and membrane receptor interactions. ABC transporters are essential for nutrient absorption and drug metabolism, indicating their potential impact on glucose uptake and distribution. Additionally, modulation of IL-17 signaling pathway proteins (MMP9, ELAVL1, and TNF) suggests an immunoregulatory function, potentially reducing diabetes-associated inflammation and improving insulin sensitivity. The involvement of pyruvate metabolism and metabolic pathway regulators (PKM, ALDH1B1, GLO1, and CYP3A4) underscores their influence on cellular energy production, which is essential for glucose metabolism and insulin function (Figure 2A, Table 1). Furthermore, disease-relevant scores and compound-target pairing scores derived from the SEA search server, Binding DB Similarity Index, and SwissTarget Prediction Probability reinforce the therapeutic potential of nicotiflorin (Fig. 2B). The identified targets, including ABCC1, ABCB1, ABCG2, DPP4, NOX4, MMP9, TNF, and CYP enzymes, are implicated in metabolic regulation, oxidative stress response, inflammation, and insulin signaling pathways. High pairing scores for DPP4, NOX4, and TNF suggest that nicotiflorin has the potential to enhance insulin sensitivity and mitigate inflammatory responses in diabetes (Fig. 2B).



B

Targets	DRS	SEASS	BDBSI	STPP
ABCB1	9.39	0.45	-	-
ABCC1	5.38	0.39	-	-
ABCG2	9.00	0.39	-	-
ACHE	13.67	-	0.98	1.00
ADRA2A	49.00	-	0.85	1.00
ADRA2C	5.45	-	-	1.00
AKR1B1	39.72	0.71	0.85	0.53
ALDH1A2	1.27	0.40	-	-
ALDH1B1	2.92	0.40	-	-
BACE1	3.28	-	0.87	-
CA2	9.42	-	0.86	-
CA3	1.53	0.39	-	-
CA4	5.33	0.74	0.85	-
CBR1	2.67	0.32	-	-
CBS	22.00	0.56	-	-
CYP1B1	6.47	0.43	-	-
CYP2C8	4.88	0.39	-	-
CYP3A4	17.06	-	0.80	-
DPP4	38.43	-	0.86	-
ELAVL1	5.84	0.39	-	-
ERAP1	4.32	0.35	-	-
IL2	20.98	0.42	0.80	-
MMP9	24.88	-	0.94	-
NOX4	12.42	0.64	0.85	-
PGF	17.13	0.29	-	-
RPS6KA1	2.66	-	0.83	-
RPS6KA3	1.76	0.76	0.83	-
SLC5A1	20.45	0.36	-	-
TNF	63.23	-	0.80	-
XDH	19.99	0.65	-	-

Figure 2: Functional Enrichment analysis and Nicotiflorin-target(s) pairing score. **(A)** The dot plot represents the results of Gene Ontology (GO) and KEGG pathway enrichment analysis. The false discovery rate (FDR) indicates the significance of enrichment highlighting key biological processes (BP), molecular functions (MF), cellular components (CC), and KEGG pathways. The color gradient represents the -$\log_{10}$(Signal) values, where red indicates higher significance and green indicates lower significance. The dot size corresponds to the count of genes associated with each category. **(B)** The heatmap presents the disease-relevant score (DRS) and compound-target(s) pairing scores identified using SEA search server (SEAA), BindingDB Similarity Index (BDBSI), and SwissTargetPrediction Probability (STPP).

Table 1 Functional enrichment analysis of Nicotiflorin targets in diabetes

Category	Term description	Count	Signal	False discovery rate	Matching proteins in network
GO Process	Metabolic process	38	0.4	7.73E-05	ST6GAL1, IL2, ALDH1A2, NOX4, TYR, ACP1, ADRA2A, CA2, CA3, AKR1B1, CBR1, ALPI, ERAP1, CA4, ACHE, BACE1, PKM, P4HB, CA7, PDE5A, DPP4, CYP2C8, MMP9, GLO1, ALDH1B1, XDH, RPS6KA3, CBS, ABCC1, KDM1A, DAPK1, TNF, RPS6KA1, PTPRS, CYP1B1, AMY2A, ABCG2, CYP3A4

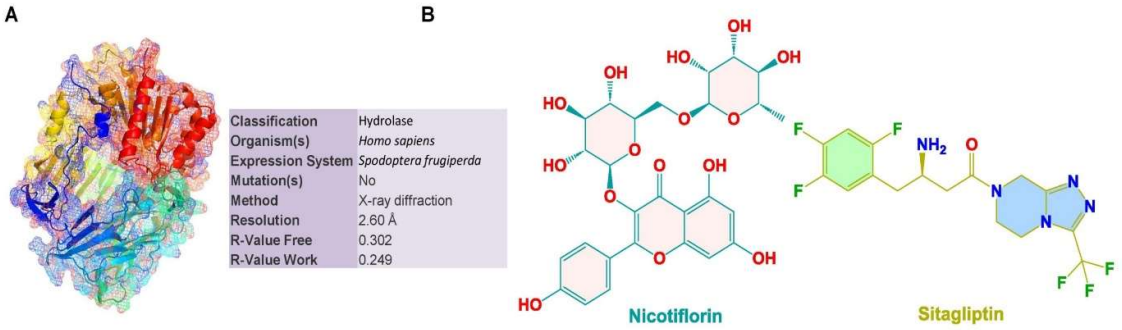
	Hormone metabolic process	6	0.69	0.0041	ALDH1A2, AKR1B1, DPP4, CYP2C8, CYP1B1, CYP3A4
	Export across plasma membrane	4	0.77	0.0046	KCND3, ABCC1, ABCB1, ABCG2
	Cellular response to stimulus	29	0.3	0.0096	IL2, ALDH1A2, NOX4, ADRA2A, CA2, AKR1B1, CBR1, ERAP1, ACHE, BACE1, PKM, P4HB, PDE5A, DPP4, CYP2C8, MMP9, RPS6KA3, CHRM5, CBS, ABCC1, KDM1A, ADRA2C, DAPK1, TNF, RPS6KA1, PGF, CYP1B1, ABCG2, CYP3A4
	Response to peptide	7	0.47	0.0178	CA2, BACE1, PKM, MMP9, ABCC1, TNF, CYP1B1
GO Function	Oxidoreductase activity	13	0.91	2.95E-05	ALDH1A2, NOX4, TYR, AKR1B1, CBR1, P4HB, CYP2C8, ALDH1B1, XDH, CBS, KDM1A, CYP1B1, CYP3A4
	Efflux transmembrane transporter activity	3	0.88	0.0032	ABCC1, ABCB1, ABCG2
	Aromatase activity	3	0.69	0.0103	CYP2C8, CYP1B1, CYP3A4
	Epinephrine binding	2	0.53	0.0325	ADRA2A, ADRA2C
	Enzyme binding	14	0.31	0.0385	CD22, NOX4, ADRA2A, ALPI, BACE1, P4HB, DPP4, RPS6KA3, CBS, KDM1A, ELAVL1, TNF, ABCB1, CYP3A4
GO Component	Extracellular region	24	0.38	0.0036	CD22, ST6GAL1, IL2, SLC5A1, ACP1, CA2, AKR1B1, CBR1, ALPI, ERAP1, CA4, ACHE, PKM, P4HB, DPP4, MMP9, GLO1, XDH, ABCC1, TNF, PGF, PTPRS, ABCB1, AMY2A
	Cell periphery	27	0.27	0.0246	CD22, NOX4, SLC5A1, ACP1, ADRA2A, CA2, ALPI, ERAP1, CA4, ACHE, BACE1, KCND3, PKM, P4HB, DPP4, CYP2C8, MMP9, GLO1, SLC28A3, CHRM5, ABCC1, ADRA2C, DAPK1, TNF, PTPRS, ABCB1, ABCG2
	Plasma membrane	25	0.26	0.0326	CD22, NOX4, SLC5A1, ACP1, ADRA2A, CA2, ALPI, ERAP1, CA4, ACHE, BACE1, KCND3, P4HB, DPP4, CYP2C8, GLO1, SLC28A3, CHRM5, ABCC1, ADRA2C, DAPK1, TNF, PTPRS, ABCB1, ABCG2
KEGG	Metabolic pathways	19	0.95	8.47E-08	ST6GAL1, ALDH1A2, TYR, CA2, CA3, AKR1B1, CBR1, ALPI, CA4, PKM, CA7, PDE5A, CYP2C8, GLO1, ALDH1B1, XDH, CBS, AMY2A, CYP3A4
	Pyruvate metabolism	3	0.76	0.0052	PKM, GLO1, ALDH1B1
	ABC transporters	3	0.69	0.0085	ABCC1, ABCB1, ABCG2
	IL-17 signaling pathway	3	0.46	0.0376	MMP9, ELAVL1, TNF
	Insulin resistance	3	0.41	0.0498	RPS6KA3, TNF, RPS6KA1

Docking Analysis of Nicotiflorin as a DPP4 Inhibitor

Molecular docking analysis of the binding affinity and interaction profile of the flavonoid glycoside, nicotiflorin, with DPP4 (PDB: 1J2E) was conducted and compared with sitagliptin, a known DPP4 inhibitor. The docking results indicated that the binding energy of nicotiflorin was-9.3 kcal/mol, which was more favorable than that of sitagliptin (-8.3 kcal/mol), suggesting a stronger interaction with the active site of DPP4. Interaction analysis revealed that Nicotiflorin forms a total of 9 hydrophilic

interactions with key residues, including Arg125 (2.53 Å), Glu206 (2.17 Å), Tyr547 (2.49 Å), Trp629 (2.94 Å), Ser630 (2.07 Å), Tyr662 (2.47, 2.85 Å), and His740 (2.43, 2.51 Å). Additionally, 3 hydrophobic interactions between the nicotiflorin and Phe357 (3.82, 4.22 Å) and Trp629 (3.61 Å) contributed to its stable binding within the active site. In contrast, sitagliptin exhibited only two hydrophilic interactions, specifically with Glu452 (2.07 Å) and Pro510 (2.21 Å), but formed a higher number (10) of hydrophobic interactions with residues such as Arg452 (3.60 Å), Arg453 (3.99, 4.90 Å), Pro475 (3.17, 3.21, 5.09 Å), Lys512 (4.13 Å), Ile529 (5.05 Å), Phe559 (5.14 Å), and Arg560 (3.18 Å). Notably, some hydrophobic interactions in sitagliptin binding involve halogen participation (Fig. 3). The enhanced binding affinity and extensive hydrophilic interactions of nicotiflorin suggest its potential as a promising DPP4 inhibitor. These findings highlight the importance of hydrogen bonding and hydrophobic contacts in ligand binding and pave the way for further investigations into the therapeutic potential of nicotiflorin against DPP4-mediated metabolic disorders.

Figure 3: Molecular docking of Nicotiflorin with DPP4 (PDB: 1J2E). (A) Structure of DPP4 (PDB: 1J2E). **(B)** Structure of Nicotiflorin (test) and sitagliptin (control, DPP4 inhibitor). **(C)** Binding affinity and docking score of Nicotiflorin (-9.3 kcal/mol) and sitagliptin (-8.3 kcal/mol). Nicotiflorin exhibited 9 hydrophilic interactions (Arg125 (2.53), Glu206 (2.17), Tyr547 (2.49), Trp629 (2.94), Ser630 (2.07), Tyr662 (2.47, 2.85), His740 (2.43, 2.51)) and 3 hydrophobic (Phe357 (3.82, 4.22), Trp629 (3.61)). Similarly, Sitagliptin exhibited 2 hydrophilic interactions (Glu452 (2.07), Pro510 (2.21)) and 10 hydrophobic (Arg452 (3.60)*, Arg453 (3.99, 4.90), Pro475



C

	Ligand type	Binding energy	Number of hydrophilic interactions	Hydrophilic interaction residues	Number of hydrophobic interactions	Hydrophobic interaction residues
Nicotiflorin	Test	-9.3	9	Arg125 (2.53), Glu206 (2.17), Tyr547(2.49), Trp629 (2.94), Ser630 (2.07), Tyr662 (2.47, 2.85), His740 (2.43, 2.51)	3	Phe357(3.82, 4.22), Trp629 (3.61)
Sitagliptin	Control	-8.3	2	Glu452 (2.07), Pro510 (2.21)	10	Arg452 (3.60)*, Arg453 (3.99, 4.90), Pro475 (3.17, 3.21*, 5.09), Lys512 (4.13), Ile529 (5.05), Phe559 (5.14), Arg560 (3.18)*

(3.17, 3.21*, 5.09), Lys512 (4.13), Ile529 (5.05), Phe559 (5.14), Arg560 (3.18)*), *halogen involved with these interactions.

Assessing molecular dynamics simulation of ligand-protein complex

Atomic deviation assesses the structural stability of a protein-ligand complex over the simulation trajectory, with Nicotiflorin and Sitagliptin bound to DPP4 exhibiting values of 0.226 ± 0.039 nm and 0.223 ± 0.024 nm, respectively (Fig. 4A). Both complexes maintained stable RMSD values, suggesting minimal conformational deviations throughout the simulation; however, the standard deviation of the RMSD of the sitagliptin complex was slightly higher, indicating marginally greater fluctuations in the backbone structure compared to those of the sitagliptin complex. Similarly, RMSF measures the flexibility of individual residues in the protein-ligand complex, with nicotiflorin showing an RMSF of 0.097 ± 0.067 nm, whereas sitagliptin exhibited slightly greater fluctuations at 0.116 ± 0.102 nm (Fig. 4B**)**. This suggests that sitagliptin binding induces a marginal increase in residue

mobility in certain regions of DPP4, potentially affecting ligand stability and binding efficiency. Hydrogen bonding is crucial for stabilizing ligand-protein interactions. Notably, nicotiflorin forms an average of 3.56 ± 1.63 hydrogen bonds with DPP4, whereas sitagliptin forms only 0.14 ± 0.52 hydrogen bonds (Fig. 4C). This significant difference indicates that the nicotiflorin establishes much stronger and more consistent hydrogen bonding, contributing to a higher binding affinity and stability. Furthermore, SASA measures the extent of a protein-ligand complex exposed to the solvent and provides insights into conformational changes upon ligand binding, with the binding of the nicotiflorin at 334.42 ± 3.75 nm², which is slightly higher than sitagliptin binding 331.15 ± 3.82 nm² (Figure 4D). The small difference between the two suggests that both complexes exhibit comparable solvent exposure, although the more exposed conformation of DPP4 is induced by the presence of nicotiflorin. Likewise, the radius of gyration quantifies protein compactness, which was 2.737 ± 0.008 nm for nicotiflorin and 2.732 ± 0.011 nm for sitagliptin binding, indicating that both complexes remained stable and compact with minimal variations (Fig. 4E). However, Nicotiflorin exhibited slightly lower residual fluctuations (Fig. 4B), suggesting a more rigid and structurally constrained conformation, which could contribute to enhanced binding stability. PCA provides insights into the global motion of the protein-ligand complexes by analyzing essential dynamics through principal components (PC1 and PC2), which represent dominant collective motions during the simulation. The PCA results for the complex of Nicotiflorin and Sitagliptin with DPP4 revealed distinct differences in motion, with nicotiflorin exhibiting a standard deviation of 1.86 along PC1 and 1.45 along PC2, whereas sitagliptin demonstrated considerably higher fluctuations with a standard deviation of 4.19 along PC1 and 1.87 along PC2 (Fig. 4F). The variance observed in PC1 was significantly greater for sitagliptin, suggesting that its binding to DPP4 induces higher conformational flexibility and large-scale motions in the protein-ligand complex. In contrast, the fluctuations of nicotiflorin along both principal components were relatively restrained, indicating a more stable interaction with DPP4. The constrained movement in the sitagliptin complex suggests that it stabilizes the protein structure more effectively, limiting large-scale dynamic shifts, whereas the sitagliptin complex undergoes more pronounced conformational changes, which may suggest a weaker or more transient binding mode. This demonstrates Nicotiflorin for stronger and more stable binding to DPP4 than Sitagliptin, as evidenced by its significantly higher hydrogen bonding interactions and more rigid conformation, which contribute to limited large-scale conformational changes. In contrast, sitagliptin exhibited greater flexibility, as reflected by its higher RMSF values, suggesting weaker structural stabilization upon binding. Both ligands maintained overall structural stability, as indicated by similar RMSD and radius of gyration values, ensuring minimal perturbations in the DPP4 backbone. These findings suggest that the stronger hydrogen bonding and constrained conformation of nicotiflorin enhance its inhibitory effect on DPP4, making it a promising candidate for further drug optimization.

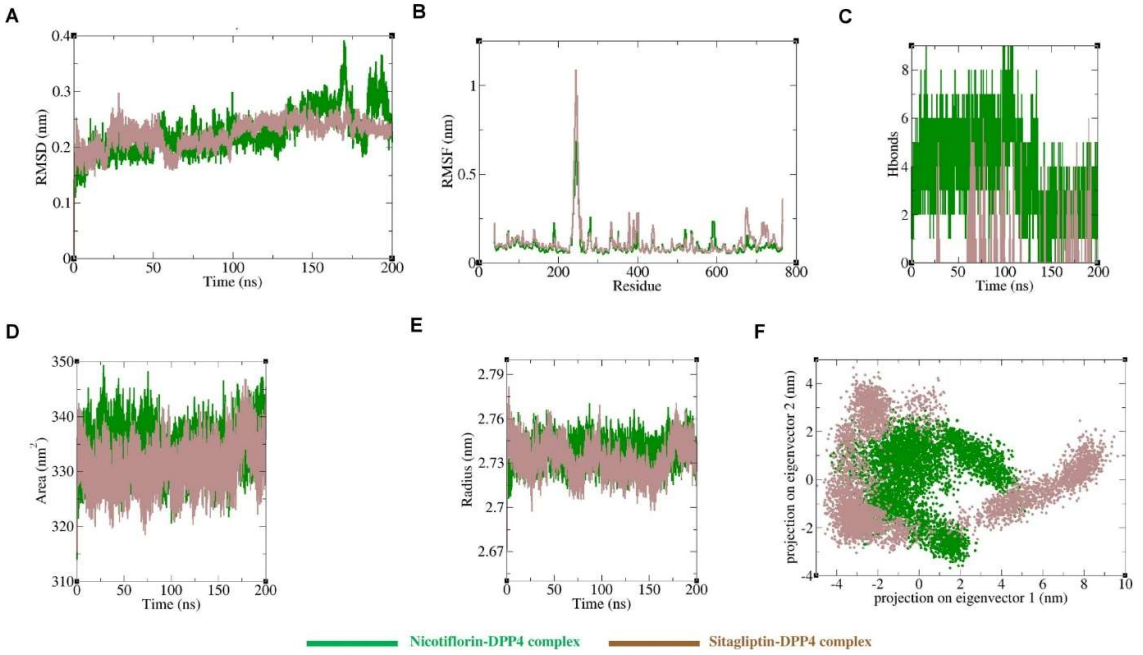


Figure 4: Molecular dynamics simulation analysis of Nicotiflorin-DPP4 and Sitagliptin-DPP4 Complexes. **(A)** Root Mean Square Deviation (RMSD) represents structural stability of protein-ligand complexes over time indicating the deviation of the protein backbone from its initial structure. **(B)** Root Mean Square Fluctuation (RMSF) measures the flexibility of individual residues throughout simulation in which peaks correspond to highly flexible regions while lower values indicate more stable structures. **(C)** Hydrogen Bond Analysis represents number of hydrogen bonds formed between the ligand and the protein over time in which a higher number of stable hydrogen bonds suggest strong ligand binding, contributing to complex stability. **(D)** Solvent Accessible Surface Area (SASA) determines the degree of exposure of the protein to the solvent. Changes in SASA over time indicate conformational rearrangements that may affect ligand binding and protein stability. **(E)** Radius of Gyration assesses the compactness of the protein-ligand complex in which a relatively stable value suggests a well-folded structure, whereas significant variations may indicate unfolding or conformational changes. **(F)** Principal Component Analysis plot visualizes the motion of protein along first two principal components (eigenvectors) where clustering of points suggests limited conformational transitions, while a dispersed pattern indicates significant structural rearrangements during simulation

Energy Landscape of Nicotiflorin and Sitagliptin Binding to DPP4

Molecular dynamics simulation analysis provided a comparative evaluation of the binding behavior of sitagliptin and nicotiflorin with DPP4. The representation of the simulation system demonstrated the solvated protein-ligand complex, ensuring a biologically relevant environment for interaction assessment (Fig. 5A). The potential energy values calculated after the simulation indicated the overall system stability of both the Nicotiflorin-DPP4 and Sitagliptin-DPP4 complexes (Fig. 5B). A lower potential energy suggests that the system has settled into a stable state, which is crucial for understanding ligand binding efficiency and protein dynamics. Energy landscape analysis offers further insights into the conformational dynamics of these complexes. The relationship between the RMSD of the average structure and the radius of gyration provides an understanding of the structural deviations and compactness of the system throughout the simulation (Fig. 5C and 5D). The Gibbs free energy contour plots illustrated the distribution of low-energy states, indicating the most favorable conformations adopted during the simulation. The surface variations in the energy landscape reflect how each complex explores different conformational states over time, with some regions showing greater structural stability, whereas others show increased flexibility. Together, these analyses provide a comprehensive understanding of how Nicotiflorin and Sitagliptin interact with DPP4 under dynamic conditions. The differences in energy landscapes, RMSD variations, and radius of gyration suggest that each ligand stabilizes the protein in a distinct manner, influencing the overall conformational space explored during the simulation. This comparative assessment contributes to the understanding of the molecular mechanisms governing ligand binding and stability, which can be valuable for future drug design and optimization efforts.

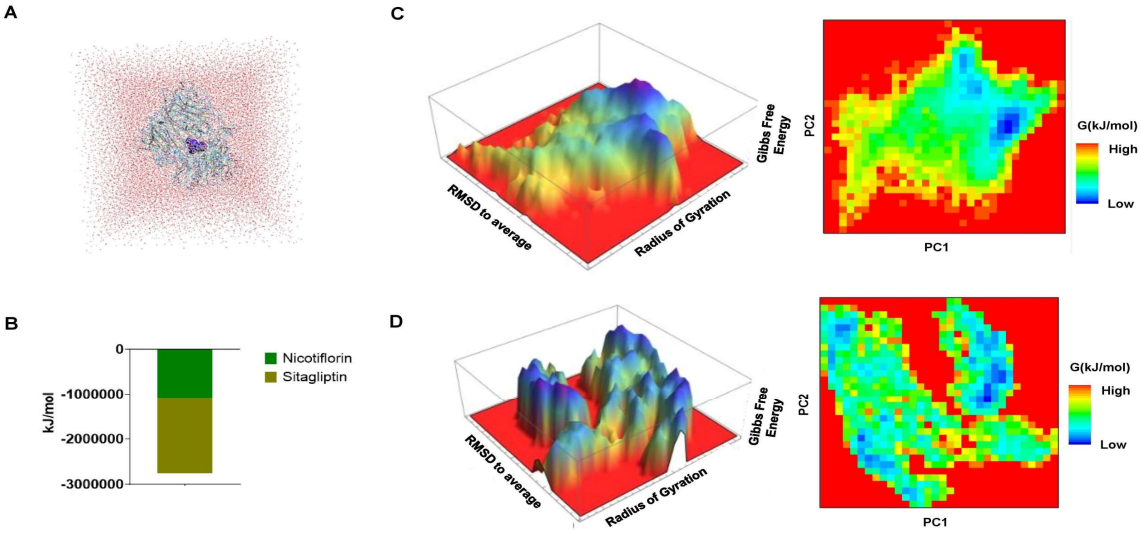


Figure 5: Comparative Molecular Dynamics Simulation Analysis of Nicotiflorin and Sitagliptin Binding to DPP4. **(A)** Representation of molecular dynamics simulation system showing solvated protein-ligand complex. **(B)** The potential energy of system after simulation for Nicotiflorin- and Sitagliptin-DPP4 complexes indicating

system stability. **(C)** Energy landscape of the Nicotiflorin-DPP4 complex, depicting the relationship between RMSD to the average structure and the radius of gyration, along with the Gibbs free energy contour plot. **(D)** Energy landscape of the Sitagliptin-DPP4 complex, visualizing the RMSD-radius of gyration relationship and the corresponding Gibbs free energy distribution.

Discussion

The present study highlights the potential of nicotiflorin as a promising antidiabetic agent by integrating network pharmacology, molecular docking, and molecular dynamics simulations. Using network pharmacology, key molecular targets related to diabetes mellitus were identified, with a focus on DPP4, owing to its established role in glucose metabolism [28].

Molecular docking analysis demonstrated that nicotiflorin exhibits a strong binding affinity with DPP4 (-9.3 kcal/mol), which is more favorable than sitagliptin (-8.3 kcal/mol). This binding was stabilized by multiple hydrogen bonds and hydrophobic interactions. Additionally, Nicotiflorin formed multiple hydrogen bonds and hydrophobic interactions with key residues, contributing to its stable binding, whereas sitagliptin's interaction relied more on hydrophobic contacts. Molecular dynamics simulations further validated the stability and interaction behavior of nicotiflorin in the binding pocket of DPP4, showing minimal structural deviations, consistent hydrogen bonding, and a stable dynamic profile, supporting its potential as a natural DPP4 inhibitor for diabetes management [32].

Molecular dynamics simulations further confirmed that nicotiflorin maintained RMSD and root mean square fluctuation RMSF values, suggesting a more stable and constrained conformation than sitagliptin. The increased hydrogen bonding and reduced conformational flexibility indicates that it may exert a prolonged inhibitory effect on DPP4. Moreover, unlike sitagliptin, which primarily targets DPP4, Nicotiflorin exhibits multi-target activity as shown in Fig. 1, interacting with additional proteins involved in glucose metabolism, oxidative stress, and inflammation, which may provide additional therapeutic benefits in diabetes management [33]. This broader spectrum of activity suggests that nicotiflorin could serve as a more comprehensive therapeutic agent with potential advantages over existing DPP4 inhibitors.

Furthermore, nicotiflorin exerts antidiabetic effects through multiple mechanisms by modulating glucose metabolism, enhancing insulin secretion, and mitigating oxidative stress and inflammation [9]. As a DPP4 inhibitor, nicotiflorin prolongs incretin hormone activity, enhancing insulin secretion from pancreatic β-cells and improving glucose homeostasis. Additionally, its interaction with key metabolic regulators suggests a broader role in maintaining insulin sensitivity and reducing hyperglycemia. Beyond DPP4 inhibition, Nicotiflorin has been shown to interact with inflammatory mediators such as TNF (Fig. 1C), which is implicated in insulin resistance [34]. By downregulating pro-inflammatory pathways (Fig. 2A), nicotiflorin may help preserve β-cell function and improve insulin signaling. Furthermore, its interactions with oxidative stress-related proteins, including NOX4 and ALDH1A2 (Fig. 1C), suggest a potential role in reducing cellular damage caused by hyperglycemia-induced oxidative stress. By minimizing oxidative damage and inflammation in diabetes [35], this active compound may protect pancreatic β-cells from dysfunction and apoptosis, thereby contributing to sustained insulin production. These multifaceted effects highlight the potential of nicotiflorin as a natural compound capable of addressing key pathological mechanisms in diabetes, making it a promising candidate for further drug development.

Likewise, molecular dynamics simulations demonstrated that the structural stability of the compound is maintained under physiological conditions, with minimal deviations in RMSD and radius of gyration values throughout the simulation. The consistent hydrogen bonding interactions observed during the trajectory indicate that nicotiflorin within the DPP4 binding site was stable, suggesting strong retention and potential long-term inhibitory activity. However, while *in silico* studies support its stability, its pharmacokinetic properties require further investigation to determine its bioavailability and metabolic stability. As a flavonoid glycoside, nicotiflorin may undergo extensive metabolism, potentially affecting its systemic availability and therapeutic efficacy. Structural modifications or formulation strategies can be explored to enhance stability and improve oral bioavailability. Future studies should focus on *in vivo* pharmacokinetic profiling and preclinical evaluations to validate the therapeutic potential of this compound. Additionally, clinical studies are essential to assess its safety, efficacy, and potential integration into diabetes treatment regimens, either as a monotherapy or in combination with existing anti-diabetic drugs.

The multi-target approach in diabetes management is increasingly recognized as an effective strategy for addressing the complex pathophysiology of the disease [36, 37]. Nicotiflorin, with its ability to inhibit DPP4 while also modulating inflammatory and oxidative stress pathways, presents a promising candidate for diabetes treatment beyond conventional single-target therapies. Its interaction with key regulators of glucose metabolism, insulin

secretion, and β-cell function (Fig. 1C) suggests that it could provide a more comprehensive therapeutic effect compared to existing DPP4 inhibitors. Given its favorable binding affinity, stability, and potential pharmacological benefits, Nicotiflorin could serve as a lead compound for further drug development, with opportunities for structural optimization to enhance its potency and bioavailability. Additionally, its integration with current treatment regimen [38], such as in combination with metformin or GLP-1 receptor agonists, could offer synergistic effects, improve glycemic control while potentially reduce adverse effects associated with existing drugs. Further experimental validation and clinical studies will be necessary to establish its efficacy and safety, paving the way for its potential translation into a novel therapeutic agent for diabetes management.

Conclusion

The computational analyses provide valuable insights into Nicotiflorin's potential as an anti-diabetic agent; they have inherent limitations in accurately predicting its in vivo efficacy. Molecular docking and dynamics simulations offer a detailed understanding of binding affinity, stability, and interaction patterns, but they cannot fully account for factors such as metabolism, bioavailability, and off-target effects in a biological system. Therefore, experimental validation through in vitro enzymatic assays, cell-based studies, and animal models is essential to confirm its DPP4 inhibitory activity, effects on insulin secretion, and overall therapeutic potential. Additionally, structural modifications may be necessary to improve Nicotiflorin's drug-like properties, including enhanced absorption, stability, and metabolic resistance. Further research can explore analog development or nano formulation strategies to optimize its pharmacokinetic profile. The integration of advanced computational tools, such as free energy calculations, machine learning-based screening, and quantum mechanics simulations, could further refine drug discovery approaches and facilitate the rational design of more potent and selective Nicotiflorin derivatives. Future studies should focus on bridging the gap between computational predictions and experimental validation to accelerate the development of Nicotiflorin-based therapeutics for diabetes management.

Declarations

Conflict of Interest: The authors declared no conflict of interest

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