Phytochemicals of Momordica charantia L. as Potential GSK-3 Inhibitors: A Comprehensive In-Silico Study on Antidiabetic Activity and Pharmacokinetic Properties

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Abstract

Diabetes mellitus (DM) is a chronic, non-infectious metabolic disorder that disrupts the normal regulation of carbohydrate, lipid, and protein metabolism. This condition is characterized by sustained hyperglycemia due to insulin dysregulation. DM is primarily classified into two types: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). T1DM is an autoimmune disorder wherein the immune system attacks and destroys insulin-producing β-cells in the pancreas. T2DM, the most prevalent form, arises due to a combination of insulin resistance, inadequate insulin secretion and lifestyle disorders. Globally, T2DM accounts for nearly 90–95% of all diabetes cases, posing a major burden on public health systems. The present study aimed to investigate the inhibitory potential of plant-derived metabolites against Glycogen Synthase Kinase-3 (GSK-3), a key regulatory enzyme implicated in the pathogenesis of type 2 diabetes mellitus (T2DM). GSK-3 plays a critical role in glucose metabolism by phosphorylating and inactivating glycogen synthase (GS), the enzyme responsible for converting glucose into glycogen. In its active state, GSK-3 prevents glycogen synthesis, leading to elevated blood glucose levels. Targeting GSK-3 represents a promising therapeutic strategy for T2DM management. Hence, the identification of novel GSK-3 inhibitors from plant-based bioactive compounds holds significant potential for the development of safer and more effective antidiabetic agents. In recent years, computer-aided drug discovery and design (CADD) tools have played a pivotal role in accelerating new drug discovery process. The current study employed an *in-silico* docking approach to explore the antidiabetic potential of bioactive compounds derived from *Momordica charantiaL.*(bitter melon), a medicinal plant well known for its diverse pharmacological properties, including hypoglycemic, anticancer, antiviral, anti-inflammatory, and cholesterol-lowering effects. Among the ten phytoconstituents evaluated against the diabetes-related target protein GSK-3, five compounds demonstrated superior binding affinities compared to the reference GSK-3 inhibitor drug Tideglusib (binding affinity: –8.2 kcal/mol). These compounds were cucurbitin (–9.4 kcal/mol), cucurbitacins (–8.7 kcal/mol), momordicinin (–8.6 kcal/mol), momordenol (–8.3 kcal/mol), and cycloartenol (–8.3 kcal/mol). Further simulative analysis revealed that these lead compounds exhibit favorable pharmacokinetic and toxicity

profiles, indicating good oral bioavailability and minimal predicted toxicity. These findings suggest that bioactive metabolites of *Momordica charantiaL.*may serve as potent GSK-3 inhibitors with therapeutic potential in the management of type 2 diabetes mellitus.

Keywords - Diabetes mellitus type II, Momordica charantia, GSK-3, Molecular docking, Pharmacokinetics

I. INTRODUCTION

Diabetes mellitus (DM) is a chronic, non-communicable metabolic disorder characterized by dysregulation of carbohydrate, lipid, and protein metabolism[1]**.** It is a prevalent endocrine disease globally, primarily manifested by chronic hyperglycemia resulting from either insufficient insulin production or, more commonly in recent times, reduced insulin sensitivity known as insulin resistance[2]. DM is broadly categorized into two major types: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM)[3]. T1DM, often referred to as autoimmune diabetes, arises from immune-mediated destruction of pancreatic β-cells, leading to absolute insulin deficiency[4]. In contrast, T2DM is primarily associated with insulin resistance and inadequate insulin secretion, compounded by environmental and lifestyle factors such as obesity, physical inactivity, excessive alcohol consumption, smoking, and chronic stress[5]. Currently, T2DM accounts for approximately 90% of all diabetes cases worldwide, making it the most common form of the disease[6].The long-term complications of diabetes are often severe and multi-systemic. Diabetic retinopathy can lead to vision impairment and blindness, while the risk of developing glaucoma and cataracts is significantly elevated[7]. Additionally, diabetes is a leading cause of nephropathy, often progressing to renal failure[8], and neuropathy, which can result in diabetic foot ulcers, and Charcot joint deformities[9]. Recent studies have identified Glycogen Synthase Kinase-3 (GSK-3) as a promising molecular target for the treatment of T2DM[10]. GSK-3 is a serine/threonine protein kinase that plays a key regulatory role in glucose metabolism. It negatively regulates glycogen synthesis by phosphorylating and thereby inactivating glycogen synthase (GS), the enzyme responsible for converting glucose into glycogen. Active GSK-3 inhibits glycogen synthesis, contributing to elevated blood glucose levels [11]**.** Therefore, pharmacological inhibition of GSK-3 can restore GS activity, promote glycogen formation, and reduce hyperglycemia. Thus, GSK-3 represents a compelling target for therapeutic intervention in T2DM[10]. Identifying plant-derived bioactive compounds as GSK-3 inhibitors offers a promising avenue for developing safer and more effective antidiabetic agents.**(Figure 1)** illustrates the process of insulin action and signal transduction pathway. The insulin signaling pathway plays a crucial role in maintaining glucose homeostasis by facilitating glucose uptake into cells and promoting glycogen synthesis[12].This process begins when insulin binds to its specific receptor on the cell membrane, a receptor tyrosine kinase (RTK) composed of α and β subunits[13]**(Step 1)**. The α subunit is responsible for insulin binding **(Step 2)**, which

triggers autophosphorylation of the β subunit. This autophosphorylation event initiates a cascade of intracellular signaling events. Subsequently, insulin receptor substrates (IRS) are phosphorylated, leading to the activation of phosphoinositide 3-kinase (PI3K)**(Step 3)**. PI3K then catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate (PIP2) into the secondary messenger phosphatidylinositol 3,4,5-trisphosphate (PIP3) [14]**(Step 4)**. PIP3 facilitates the recruitment of phosphoinositide-dependent kinase 1 and 2 (PDK1/2) **(Step 5)**, which phosphorylate and activate protein kinase B (AKT/PKB) **(Step 6)**, a central mediator of insulin's metabolic effects[15].Activated AKT promotes the translocation of GLUT-4 (glucose transporter type 4) vesicles to the cell membrane **(Step 7)**, enabling glucose entry into the cell from the bloodstream **(Step 8)**, thereby lowering blood glucose levels [16]. Additionally, AKT phosphorylates and inhibits AS160 (Akt substrate of 160 kDa) **(Step 9)**, which further enhances GLUT-4 mobilization to the plasma membrane [17].AKT also plays a critical role in glycogen metabolism by inactivating glycogen synthase kinase-3 (GSK-3) through phosphorylation [18]**(Step 10)**. Under normal conditions, GSK-3 suppresses the activity of glycogen synthase (GS), the key enzyme responsible for converting glucose to glycogen. Inactivation of GSK-3 **(Step 11)** leads to the activation of GS, thereby promoting glycogen synthesis and glucose storage [19]**(Step 12)**. Collectively, these signaling events ensure efficient glucose uptake and storage, contributing to the maintenance of normal blood glucose levels. Any disruption in this pathway such as impaired insulin signaling, receptor dysfunction, or insulin resistance can lead to metabolic disorders, most notably Type 2 Diabetes Mellitus.

Several synthetic GSK-3 inhibitors, including tideglusib, have been investigated for T2DM management[20].Tideglusib is a synthetic small-molecule drug developed as an irreversible inhibitor of GSK-3β. As with many synthetic drugs, it is associated with certain adverse effects. Synthetic compounds often cause side effects due to off-target interactions and systemic exposure. These side effects, coupled with the high cost of drug development, highlight the urgent need for alternative therapeutic strategies. Notably, approximately 80% of the global population in developing countries relies on plant-based medicines [21]. Furthermore, nearly 30% of all modern pharmaceutical drugs are derived from plants, many of which are used to treat a wide range of ailments [22]. India, with its 5,000-year-old tradition of Ayurvedic medicine, offers a rich repository of medicinal plants[23]. The Himalayan region, particularly Uttarakhand,

is home to a diverse range of medicinal flora. Due to their lower toxicity, affordability, and cultural acceptance[24],Himalayan medicinal plants are increasingly favored for managing chronic diseases, including diabetes. Drug discovery is inherently costly, time-consuming, and prone to high failure rates [25]. In this context, computational approaches such as Computer-Aided Drug Design (CADD) have revolutionized early-stage drug development [26]. Molecular docking and *in-silico* ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) prediction are widely used to screen virtual compound libraries efficiently [27].These methods enable rapid identification of potential lead compounds by assessing their binding affinity to target proteins, predicting pharmacokinetic profiles, and evaluating toxicity all at significantly reduced cost and time. Molecular docking allows researchers to model the interaction between a ligand and its target protein, identifying the most stable binding conformation based on minimum binding energy. This helps predict the likelihood of a compound acting as an inhibitor or activator of a given enzyme or receptor[28]. Additionally, ADMET prediction, which accounts for approximately 40-60% of drug failures in clinical trials, can be effectively performed using in-silico tools, reducing reliance on costly and labor-intensive *in-vitro* experiments[29].

The present study was conducted to evaluate the GSK-3 inhibitory potential of bioactive compounds isolated from *Momordica charantiaL.,* (bitter melon), a medicinal plant known for its hypoglycemic, anticancer, anti-inflammatory, and cholesterol-lowering properties[30].Molecular docking was employed to assess the binding affinity of these compounds to GSK-3, and *in-silico* ADMET profiling was performed to predict their pharmacokinetic behavior and drug-likeness. To the best of our knowledge, this study is among the first to report detailed molecular docking and ADMET evaluation of *Momordica charantiaL.,*compounds targeting GSK-3. The results offer promising insights for future research and may contribute valuable data to the global search for novel, plant-based antidiabetic agents.

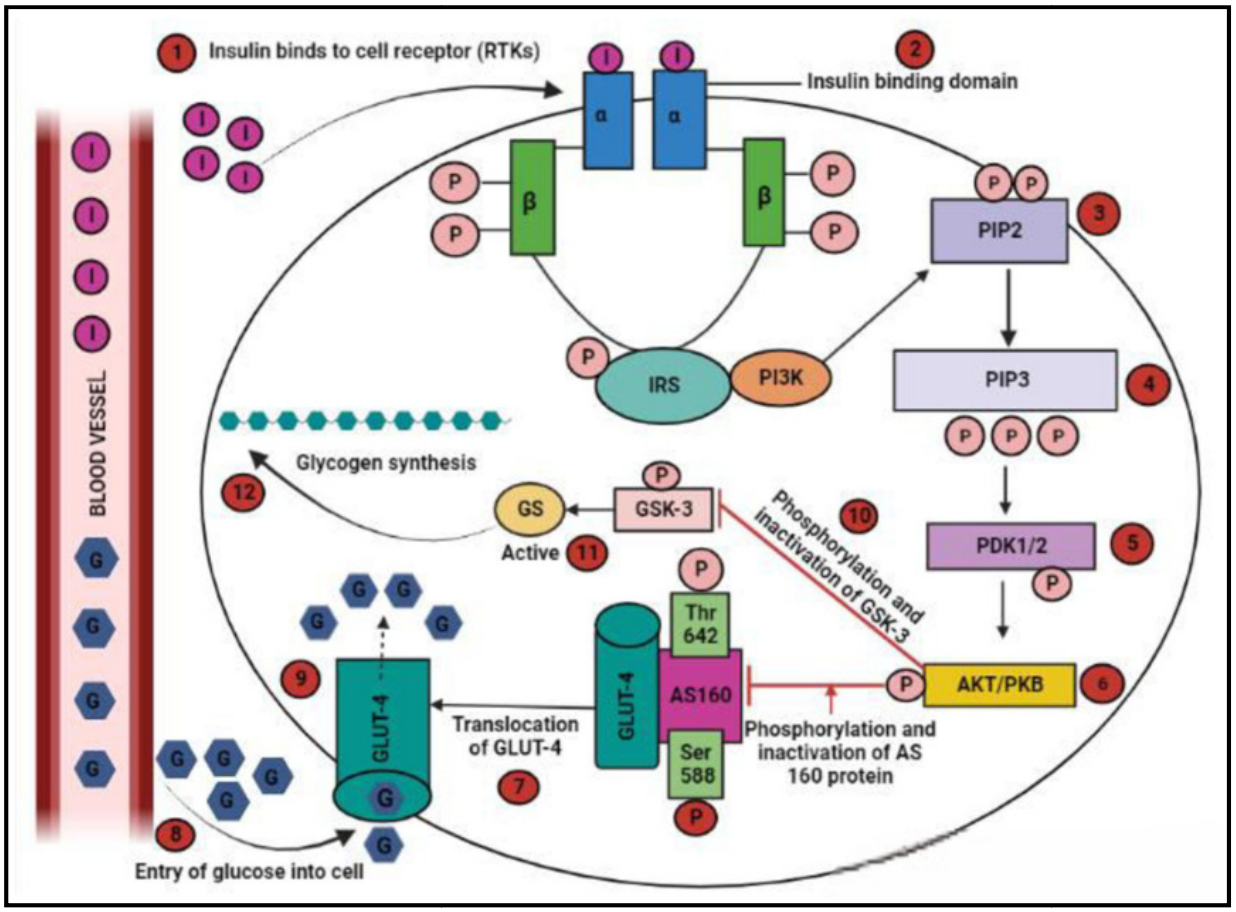


Figure 1.Schematic Representation of the Insulin Signaling Pathway Highlighting GSK-3 as a Therapeutic Target

II. MATERIALS AND METHODS

2.1 Target protein selection and preparation

The crystal structure of the Glycogen synthase kinase-3 (GSK-3) was retrieved from the RCSB (Research Collaboratory for Structural Bioinformatics) in PDB format(**Fig.1**). The protein had a resolution of 2.10 Å, using Pymol[31], the ligand was isolated from the respective PDB structure, followed by the removal of water molecules. This receptor structure was then uploaded into PyRx, where it was subjected to energy minimization and was furtherconverted to PDBQT format for analysis[32].

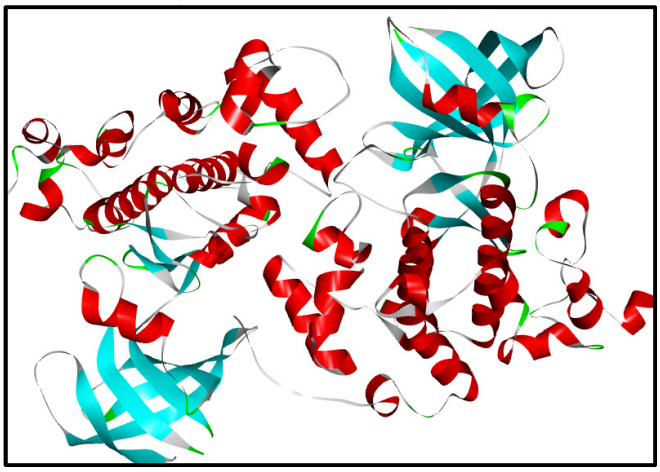


Figure 2. Three-dimensional (3D) structure of GSK-3 (PDB ID: 1Q41)

The PROCHECK Ramachandran plot and ERRAT were used to validate the protein structure[33].Te results indicate that there is two amino acid in the disallowed region, and 89.4% of residues are in the most favored region, the result is still within an acceptable range and indicates that the model possesses a reasonably good stereochemical quality. A good quality model typically has>90% of amino acids in the most favored region [34].**Figure 3** illustrates the Ramachandran plot of 1q41. ERRAT shows a quality factor of 96.478%, indicating that the protein structure is of high quality(**Fig.4**). A quality factor>50% is considered indicative of a good quality model [35].

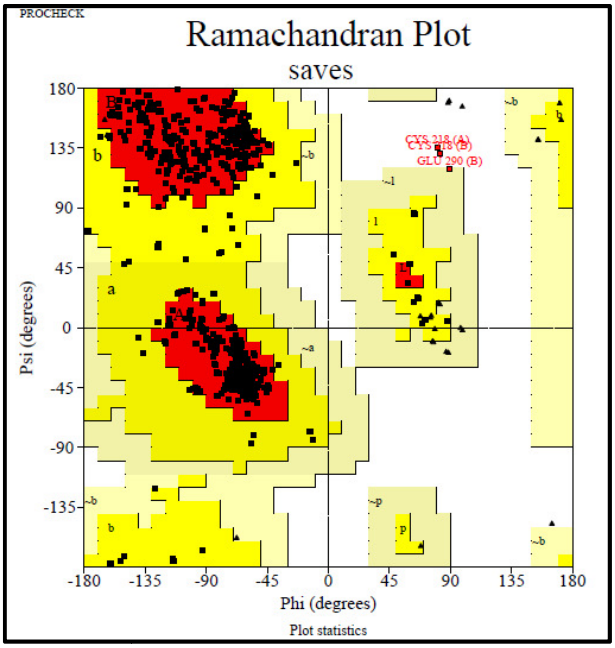


Figure 3. Ramachandran plot of GSK-3 protein

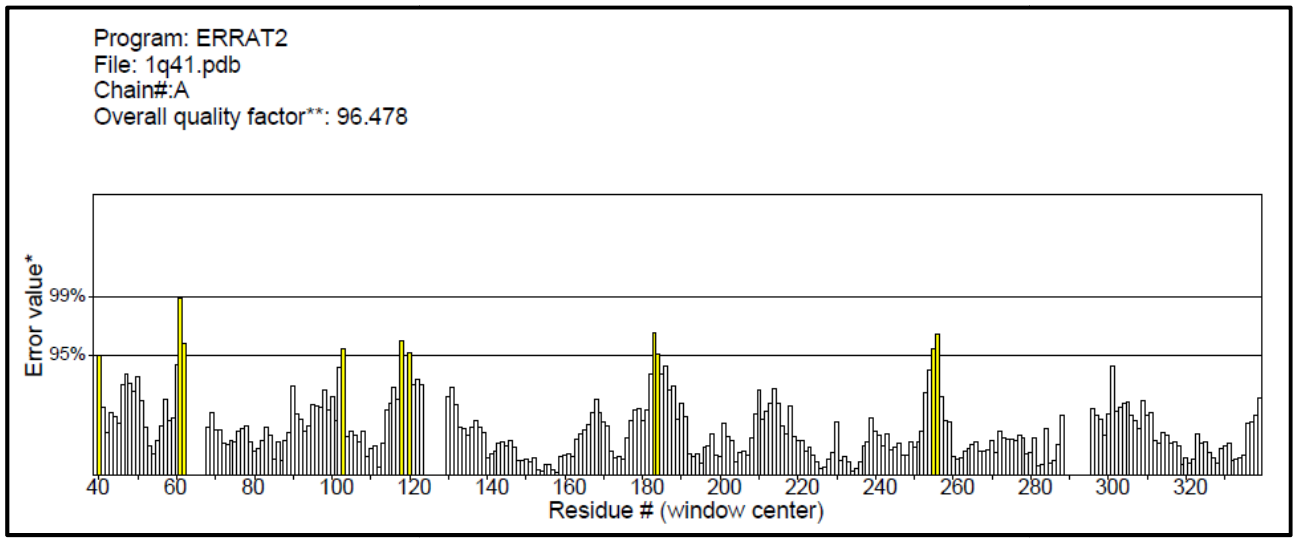


Figure 4. ERRAT-based structural validation of GSK-3 protein

2.2 Ligands preparation -

All the phytochemicals or ligands were obtained from the PubChem database in sdf format (URL: https://pubchem.ncbi.nlm.nih.gov)[36]. Further by utilizing OpenBabel 3.1.1 software, these ligands were converted from their SDF format to the PDB format for further virtual screening[37]. Before testing the potentiality of the*Momordica charantia*L.compounds against GSK-3(PDB ID: 1q41), Anti-diabetic drug i.e., Tideglusib with PubChem CID-11313622wasalso used as a control.**Figure 5** illustrates the chemical structures of selected phytoconstituents from *Momordica charantia* L. evaluated in the present study for their potential inhibitory activity against GSK-3.

2.3 In-Silico Prediction of Biological and Pharmacological Activities of Phytochemicals -

The biological and pharmacological activities of the selected phytocompounds were predicted using the PASS Online (Prediction Activity Spectra for Substances) web server (https://www.way2drug.com/passonline/). This tool estimates the potential bioactivity of compounds based on their structural similarity to known biologically active molecules. The analysis provides two key parameters: the probability of activity (Pa) and the probability of

inactivity (Pi). A Pa value greater than 0.7 indicates a high likelihood of biological activity, suggesting strong therapeutic potential. Compounds with Pa values between 0.3 and 0.7 are considered moderately active and may require further validation. Pa values below 0.3 reflect low predicted activity [38].These predictions serve as a preliminary screening tool to prioritize phytocompounds for further computational and experimental evaluation in the drug discovery process.

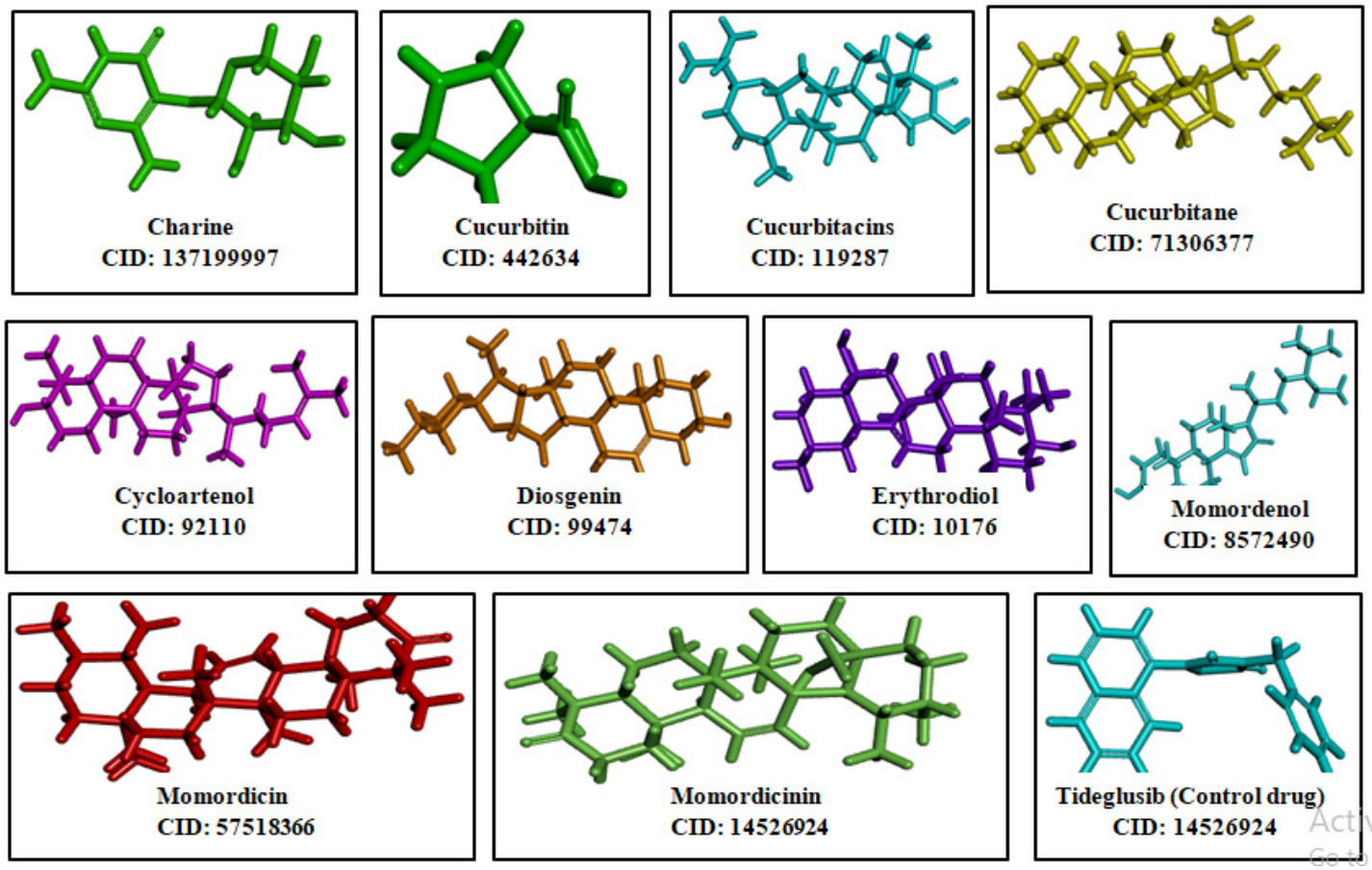


Figure 5. Chemical Profiles of *Momordica charantia* L. Compounds

2.4 Prediction of Allergenicity of Phytochemicals –

Following the prediction of biological activities, the allergenic potential of the selected phytocompounds was assessed using ChAlPred (Chemical Allergen Prediction) (https://webs.iiitd.edu.in/raghava/chalpred/). This tool classifies compounds as either allergenic or non-allergenic based on their chemical structure and known allergenic profiles[39]. Compounds identified as allergenic were excluded from further consideration, while non-

allergenic compounds were retained for potential therapeutic application. The integration of PASS Online for bioactivity prediction with ChAlPred for allergenicity assessment provided a systematic and comprehensive screening approach, ensuring that only phytocompounds with both therapeutic potential and favorable safety profiles progressed to subsequent stages of validation.

2.5 Computational Identification of Active Sites for Accurate Molecular Docking –

Active sites of proteins are critical for facilitating specific ligand binding and molecular interactions, particularly in docking studies. To accurately predict and validate these functional regions in the target protein Glycogen Synthase Kinase-3 (GSK-3)**,** the CASTp server was employed. CASTp analysis enabled the identification of key amino acid residues forming the active binding pockets, while also providing detailed geometric and topological characteristics of the protein surface[40]. This information ensured the selection of precise and accessible bindingsitessuitable for molecular docking simulations. The identified active sites, including their structural configurations and binding cavities, are visually depicted in **Figures 6**, offering a comprehensive view of potential ligand interaction regions essential for structure-based drug design.

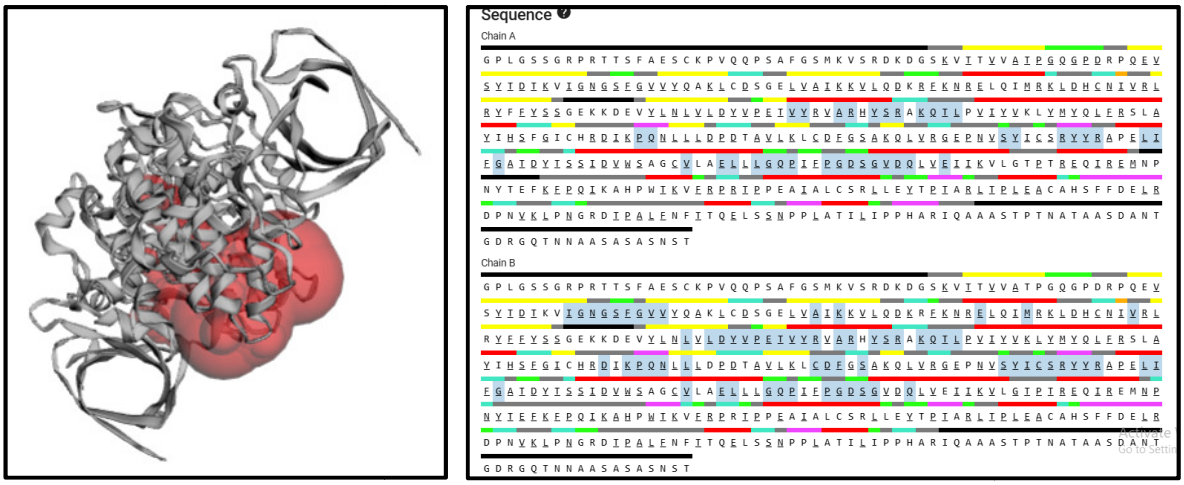


Figure 6. Active site of the GSK-3. (Left view) Red sphre denoting the active sites. (Right view) Amino acid residues in the active site (shaded)

2.5Molecular Docking and Visualization -

Molecular docking studies were conducted using the PyRx virtual screening tool, which incorporates the AutoDock Vina engine for docking simulations. All docking calculations employed the Lamarckian Genetic Algorithm (LGA) to predict optimal ligand–receptor interactions[41]. The exhaustiveness parameter, which determines the thoroughness of the search, was set to 8 to ensure reliable conformational sampling. A grid box was defined to encompass the active site of the target protein, with the following coordinates and dimensions: center (X, Y, Z) = (27.96020, 6.2504, 28.9949) Å; size (X, Y, Z) = (77.6442, 68.2910, 64.7983) Å. These parameters ensured adequate coverage of the active binding pocket for all ligand docking runs. Binding affinity (expressed in kcal/mol) and root mean square deviation (RMSD) values were used as the primary criteria for evaluating docking results. Ligand conformations with RMSD values less than 1.0 Å were considered to exhibit favorable and consistent binding poses[42]. Ligand molecules were automatically docked into the active site of the receptor protein, and the conformers with the highest binding affinities were subjected to post-docking analysis. Visualization and interaction profiling of the selected ligand–protein complexes were performed using Discovery Studio Visualizer version 3.0, enabling detailed assessment of molecular interactions within the active site [43].

2.6Drug-likeness and in silico ADMET prediction -

ADMET analysis encompassing absorption, distribution, metabolism, excretion, and toxicity is a critical step in the early stages of drug development to assess the pharmacokinetic and safety profiles of candidate compounds. One of the key criteria for evaluating drug-likeness is Lipinski's Rule of Five, which predicts the oral bioavailability of a compound based on its physicochemical properties. According to this rule, an orally active drug candidate should not violate more than one of the following parameters: molecular weight $\leq$ 500 Da, partition coefficient (MLogP) $\leq$ 5, hydrogen bond donors $\leq$ 5, and hydrogen bond acceptors $\leq$ 10. To enhance the efficiency and cost-effectiveness of this study, Lipinski's Rule of Five was applied to assess the drug-likeness of the top-ranking compounds identified through molecular docking. These compounds, along with the reference drug tideglusib, were further subjected to comprehensive ADMET analysis to evaluate their pharmacokinetic and toxicity profiles. ADME parameters were predicted using SwissADME, a widely used computational tool that evaluates

drug-likeness, physicochemical properties, and pharmacokinetics[44].Toxicological assessments, including median lethal dose (LD_{50}) predictions and classification of toxicity, were conducted using ProTox-3.0[45], which provides in silico toxicity profiling of chemical compounds. This integrated approach allowed for the selection of potential lead compounds with favorable ADMET characteristics and minimal predicted toxicity, thereby supporting their candidacy for further *in-vitro* and *in-vivo* validation as GSK-3 inhibitors.

III. RESULTS AND DISCUSSION

3.1 In-Silico Prediction of Selected Phytocompounds Based on Biological and Pharmaceutical Activity-

In-silico prediction of the biological and pharmacological activities of phytochemicals was summarized in **Table 1**. A total of ten bioactive compounds were selected from *Momordica charantia* L., a medicinal plant native to the Uttarakhand Himalayan region. These compounds were evaluated using PASS Online, which predicts biological activity based on structural similarity to known bioactive molecules. The analysis revealed that several compounds possess significant therapeutic potential based on their Probability of Activity (Pa) values. Charine exhibited notable activity as a lactase inhibitor (Pa = 0.769) and sweetener (Pa = 0.623). Cucurbitin demonstrated potential as a sugar-phosphatase inhibitor (Pa = 0.729), insulinase inhibitor (Pa = 0.624), and saccharopepsin inhibitor (Pa = 0.591), suggesting possible applications in glycemic regulation. Cucurbitacins showed strong hepatoprotective potential (Pa = 0.879) and efficacy in hepatic disorder treatment (Pa = 0.847). Similarly, cucurbitane was predicted to be hypolipemic (Pa = 0.816), a cholesterol antagonist (Pa = 0.800), and an insulin promoter (Pa = 0.606). Cycloartenol exhibited the highest Pa scores for hypolipemic action (Pa = 0.964), cholesterol antagonism (Pa = 0.947), and hepatoprotection (Pa = 0.852), indicating broad therapeutic value. Diosgenin displayed robust activity as a cholesterol antagonist (Pa = 0.973), hypolipemic agent (Pa = 0.950), and β-glucosidase inhibitor (Pa = 0.602). Erythrodiol also showed insulin-promoting effects (Pa = 0.970), along with hypolipemic (Pa = 0.829) and hepatoprotective (Pa = 0.806) properties. Momordenol was active as a cholesterol antagonist (Pa = 0.898), hypolipemic agent (Pa = 0.873), and sugar-phosphatase inhibitor (Pa = 0.833). Additionally, momordicin exhibited moderate anti-inflammatory activity (Pa = 0.565), while

momordicinin was predicted to be hypolipemic (Pa = 0.707) and anti-inflammatory (Pa = 0.669). Overall, these findings highlight the multifunctional bioactivity of several *M. charantia* compounds, with cycloartenol, diosgenin, and erythrodiol emerging as particularly promising candidates for managing type 2 diabetes and its related metabolic disorders.These findings provide valuable insights into the potential bioactive compounds that could be further explored for drug discovery and development.

Table-1Prediction of Biological and Pharmacological Potential of Selected Phytochemicals against Type-2 Diabetes Mellitus (T2DM)

S. No.	Compound Name	Pa	Pi	Predicted Pharmacological Activity
1	**Charine**	0.769 0.623	0.004 0.003	Lactase inhibitor Sweetener
2	**Cucurbitin**	0.729 0.624 0.591	0.028 0.005 0.078	Sugar-phosphatase inhibitor Insulinase inhibitor Saccharopepsin inhibitor
3	**Cucurbitacins**	0.879 0.847	0.003 0.003	Hepatic disorders treatment Hepatoprotectant
4	**Cucurbitane**	0.816 0.800 0.606	0.005 0.004 0.014	Hypolipemic Cholesterol antagonist Insulin promoter
5	**Cycloartenol**	0.964 0.947 0.852	0.003 0.002 0.003	Hypolipemic Cholesterol antagonist Hepatoprotectant
6	**Diosgenin**	0.973 0.950 0.602	0.001 0.003 0.002	Cholesterol antagonist Hypolipemic Beta-glucosidase inhibitor
7	**Erythrodiol**	0.970 0.829 0.806	0.001 0.006 0.004	Insulin promoter Hypolipemic Hepatic disorders treatment
8	**Momordenol**	0.898 0.873 0.833	0.003 0.005 0.012	Cholesterol antagonist Hypolipemic Sugar-phosphatase inhibitor
9	**Momordicin**	0.565	0.039	Antiinflammatory
10	**Momordicinin**	0.707 0.669	0.013 0.020	Hypolipemic Antiinflammatory

3.2 ChAlPred-Based Prediction of Allergenic and Non-Allergenic Phytocompounds -

The allergenic potential of the selected phytochemicals was assessed using ChAlPred**,** a computational tool designed to predict chemical allergenicity based on structural features. A total of ten phytochemicals from *Momordica charantia* L., along with the control drug tideglusib,

were analyzed. Out of these, nine compounds were predicted to be non-allergenic**,** indicating their potential safety for therapeutic use. The non-allergenic compounds and their respective scores were: Charine (0.25)**,** Cucurbitin (0.12)**,** Cucurbitacins (0.24)**,** Cycloartenol (0.22)**,** Diosgenin (0.17)**,** Erythrodiol (0.26)**,** Momordenol (0.22)**,** Momordicin (0.28)**,** and Momordicinin (0.25)**.** In contrast, only one compound, Cucurbitane**,** was predicted to be allergenic with a score of 0.31**,** suggesting a potential risk of hypersensitivity reactions and thus requiring caution in further evaluation. The control drug Tideglusib was also classified as non-allergenic with a score of 0.28**,** validating the reliability of the prediction model. This allergenicity screening helps to refine the selection of phytocompounds for further in-silico and experimental validation, ensuring that only safe, non-allergenic candidates are prioritized for drug development. A detailed summary of these findings is presented in **Table 2**, supporting the identification of low-risk, plant-derived therapeutic agents for potential clinical use.

Table-2*In-Silico* Prediction of Non-Allergenic Phytocompounds as Potential Therapeutics for Type-2 Diabetes Mellitus (T2DM)

S. No.	Compound	Score	Prediction
1	Charine	0.25	Non-allergen
2	Cucurbitin	0.12	Non-allergen
3	Cucurbitacins	0.24	Non-allergen
4	Cucurbitane	0.31	Allergen
5	Cycloartenol	0.22	Non-allergen
6	Diosgenin	0.17	Non-allergen
7	Erythrodiol	0.26	Non-allergen
8	Momordenol	0.22	Non-allergen
9	Momordicin	0.28	Non-allergen
10	Momordicinin	0.25	Non-allergen
Control drug	Tideglusib	0.28	Non-allergen

3.3 Molecular Docking and Virtual Screening of Phytocompounds:

A (1). Docking Analysis and Comparative Docking Study of Screened Phytocompounds with Reference Drug for GSK-3 -

Molecular docking was performed using the PyRx virtual screening tool**,** integrating AutoDock Vina and AutoDock 4.2**,** to evaluate the binding affinities of 10 selectedphytocompounds against GSK-3. The docking results revealed that several phytocompounds exhibited strong binding affinities**,** suggesting their potential as GSK-3 inhibitors. Among these, the compounds demonstrated binding affinities ranging from -5.3 kcal/mol to -9.4 kcal/mol**,** indicating their significant interaction with the target receptor. Comparative docking analysis was performed to identify potential hit phytocompounds with lower binding energy than control drugs, including Tideglusib. Among the 10 screened phytocompounds docked against the GSK-3 receptor, five phytocompounds exhibited lower binding energy than Tideglusibas reported in **Table 3**. This comparative computational analysis underscores the therapeutic relevance of phytocompounds in drug discovery.

A (2). Post Docking and Protein-Ligand Interaction Studies

3.3.1 Interaction Analysis of the Screened Phytocompouds with GSK-3 -

Following molecular docking, the identified hit phytocompounds from *Momordica charantia* L. were further evaluated through non-covalent interaction analysis with the target protein Glycogen Synthase Kinase-3 (GSK-3) to assess binding stability and interaction specificity. The docking results, including binding affinities, RMSD values, interacting residues, and hydrogen bond formation, are summarized in **Table 3**.A binding affinity of −7 kcal/mol or lower (i.e., more negative) is generally regarded as indicative of a strong and potentially significant interaction between a ligand and its target protein, making it a promising starting point for further drug development and optimization[46].Among the ten compounds analyzed, Cucurbitin demonstrated the highest binding affinity at –9.4 kcal/mol, with a key hydrogen bond formed with Asp A:260, suggesting a strong and stable interaction. Similarly, Cucurbitacins exhibited a binding energy of –8.7 kcal/mol and formed a hydrogen bond with Arg B:141, indicating favorable binding characteristics.Momordicinin also showed a notable binding affinity of –8.6

kcal/mol, primarily interacting with Leu B:252 through hydrophobic forces. Cycloartenol and Momordenol both recorded binding energies of –8.3 kcal/mol, with cycloartenol interacting with residues Phe B:93, Arg B:96, and Lys A:292, and momordenol interacting with Arg B:96, reflecting moderate but stable binding conformations. Erythrodiol showed a binding affinity of **–** 8.0 kcal/mol, engaging with Arg B:141, His B:145, and Tyr B:157, suggestive of multiple stabilizing interactions.Charine demonstrated a binding energy of –7.8 kcal/mol, with key interactions involving Asp B:200 and hydrogen bonding with Asn B:64, which may contribute to enhanced binding stability. Momordicin and Cucurbitane displayed binding energies of –7.4 kcal/mol and –6.9 kcal/mol, respectively, with interactions involving Leu B:252 and Phe B:67, though without hydrogen bonding, indicating relatively weaker affinities. Interestingly, Diosgenin showed the lowest binding affinity among the selected compounds at –5.3 kcal/mol, but still formed a hydrogen bond with Asn B:95, along with hydrophobic interactions involving Val B:87, Phe B:93, and Pro A:294.In comparison, the control drug Tideglusib exhibited a binding affinity of **–**8.2 kcal/mol, with key interactions involving Lys A:292 and hydrogen bonding with Arg B:96. Notably, several phytocompounds outperformed Tideglusib in terms of binding affinity, suggesting their potential as more effective GSK-3 inhibitors. The docking poses and interaction profiles of top 5 hit compounds, along with comparative analysis against the control, are illustrated in **Figure 7 to 12**, providing further insight into their therapeutic relevance.

Table-3 Binding interaction of selected hits with active site amino acid residues of GSK-3

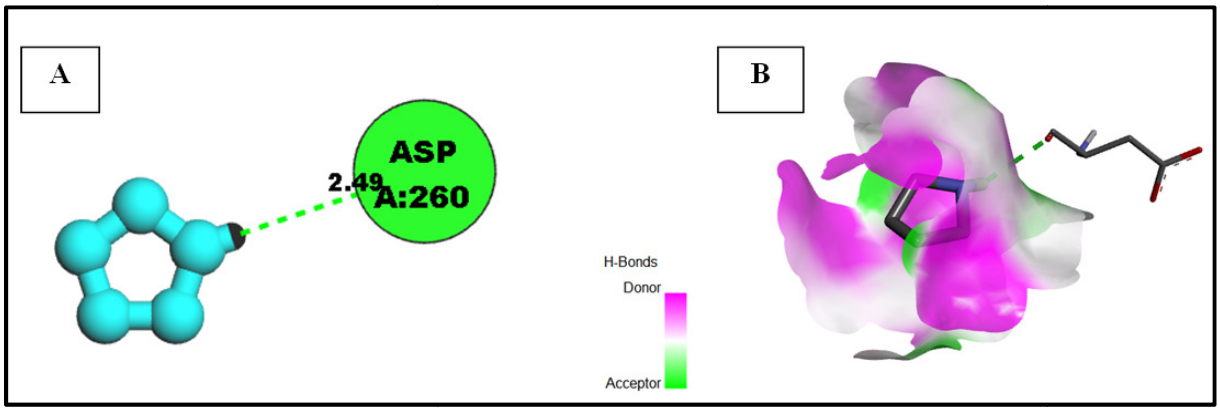
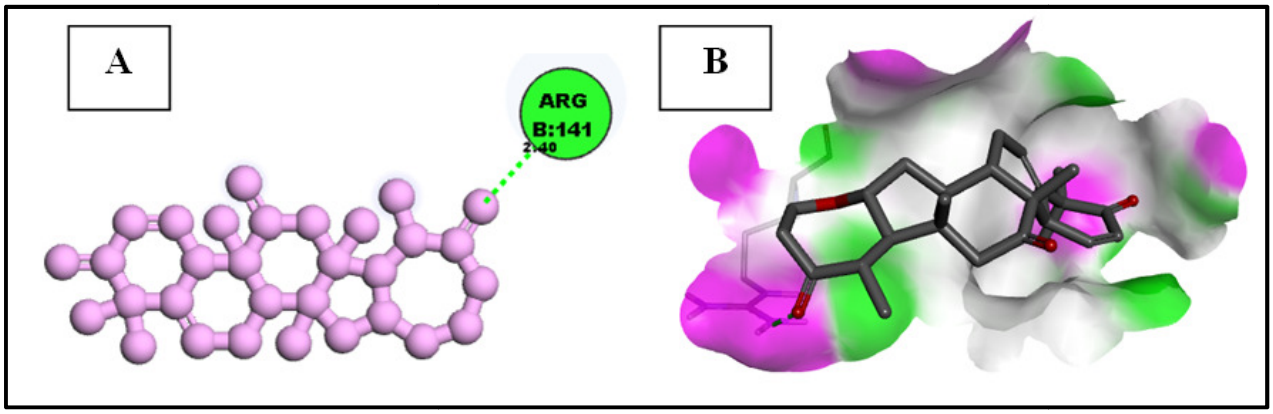


Figure 7 A. Docked Conformation of Cucurbitin with GSK-3: (A) 2D Interaction Map, (B) 3D Binding Pose



S. No.	Compound Name	Binding Energy Kcal/mol	rmsd/ub	rmsd/lb	Interactive residues	Hydrogen Bonds
1	Cucurbitin	-9.4	0	0	-	ASP A:260
2	Cucurbitacins	-8.7	0	0	-	ARG B:141
3	Momordicinin	-8.6	0	0	LEU B:252	-
4	Cycloartenol	-8.3	0	0	PHE B:93, ARG B:96, LYS A:292	-
5	Momordenol	-8.3	0	0	ARG B:96	-
6	Erythrodiol	-8	0	0	ARG B:141, HIS B:145, TYR B:157	-
7	Charine	-7.8	0	0	ASP B:200	ASN B:64
8	Momordicin	-7.4	0	0	LEU B:252	-
9	Cucurbitane	-6.9	0	0	PHE B:67	-
10	Diosgenin	-5.3	0	0	VAL B:87, PHE B:93, PRO A:294	ASN B:95
Control	Tideglusib	-8.2	0	0	LYS A:292	ARG B:96

Figure 8 A. Docked Conformation of Cucurbitacins with GSK-3: (A) 2D Interaction Map, (B) 3D Binding Pose

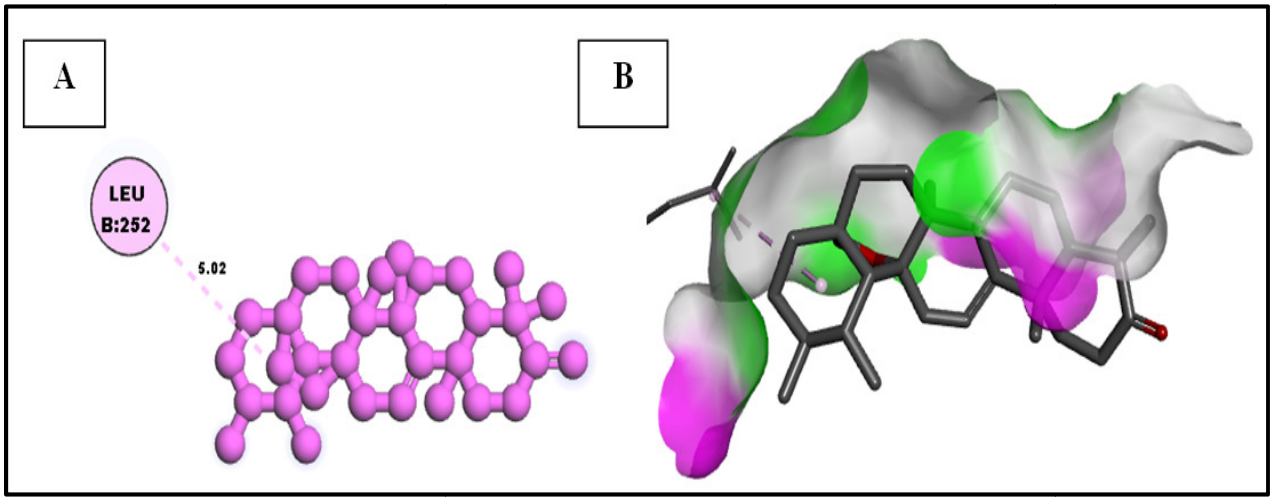


Figure 9 A. Docked Conformation of Momordicinin with GSK-3: (A) 2D Interaction Map, (B) 3D Binding Pose

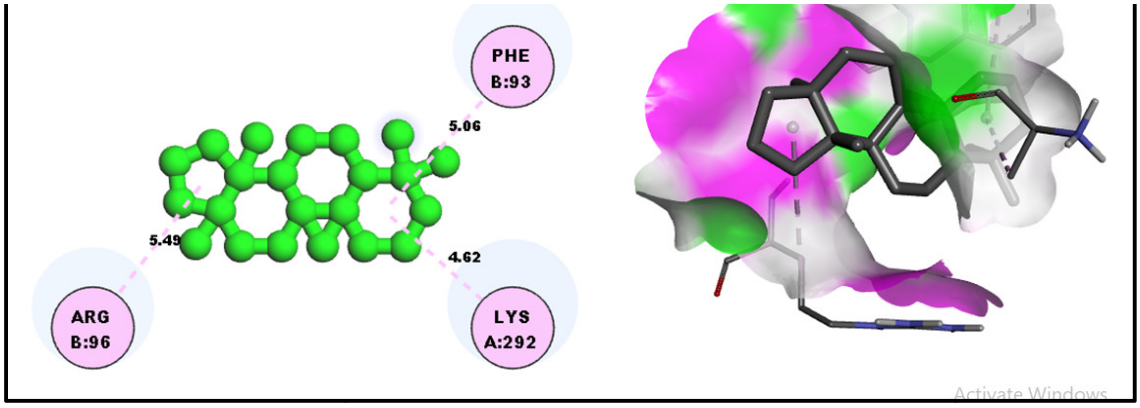


Figure 10 A. Docked Conformation of Cycloartenol with GSK-3: (A) 2D Interaction Map, (B) 3D Binding Pose

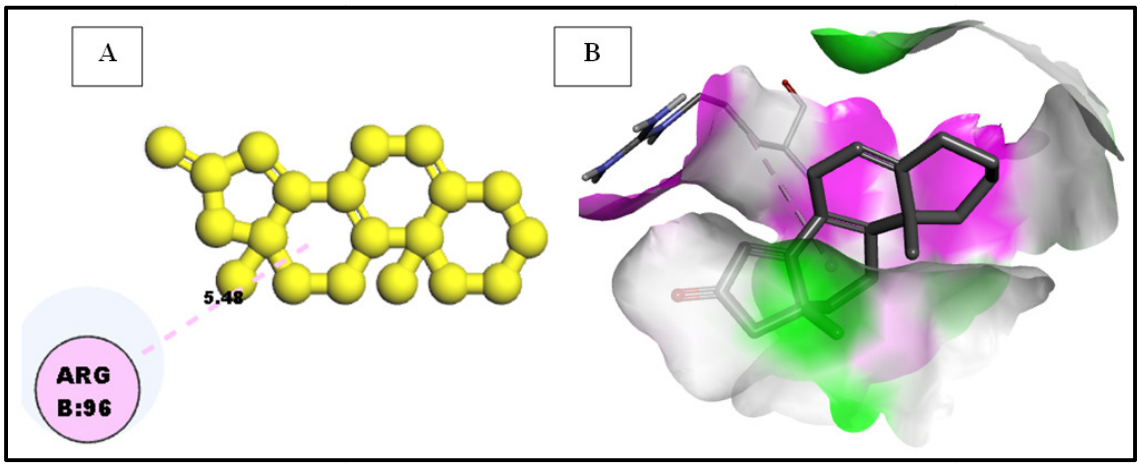


Figure 11 A. Docked Conformation of Momordenol with GSK-3: (A) 2D Interaction Map, (B) 3D Binding Pose

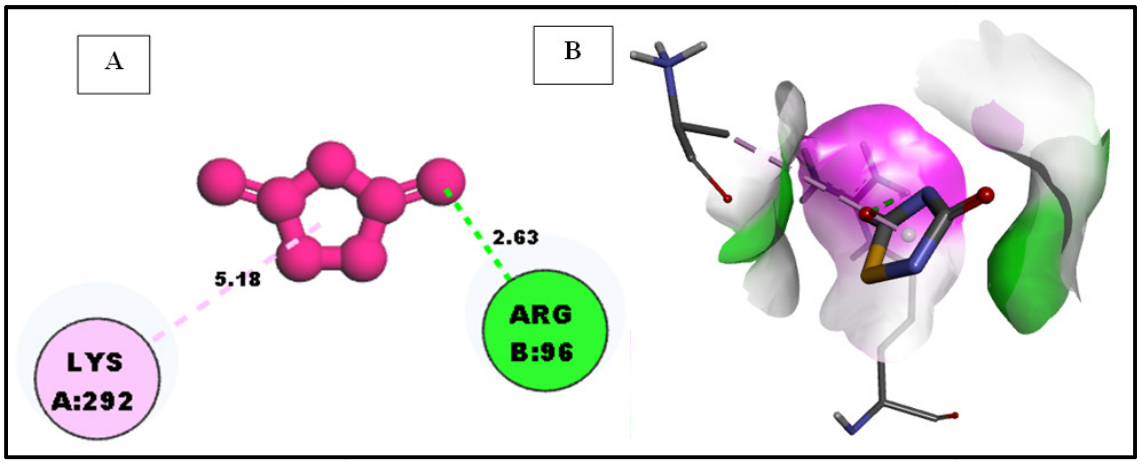


Figure 12 A. Docked Conformation of Tideglusib (Control drug) with GSK-3: (A) 2D Interaction Map, (B) 3D Binding Pose

3.4 Pharmacokinetic and Drug-Likeness Evaluation:

3.4.1 ADMET and Drug-Likeness Predictions for Screened Phytocompounds -

The drug-likeness of the top five phytocompounds, selected based on their superior binding affinities compared to the control drug Tideglusib**,** was evaluated using SwissADME**.** These compounds were assessed using Lipinski'sRule of Five (Ro5)**,** which serves as a key predictor of oral bioavailability and drug-likeness. The parameters included molecular weight (<500 Da), lipophilicity (MLogP<5), hydrogen bond donors (<5), hydrogen bond acceptors (<10), and no more than one rule violation. All five phytochemicals Cucurbitin**,** Cucurbitacins**,** Cycloartenol**,** Momordenol**,** and Momordicinin showed acceptable profiles, with no more than one Ro5

violation**.** CucurbitinandCucurbitacinsexhibited optimal drug-likeness with no violations**,** while Cycloartenol**,** Momordenol**,** and Momordicinin each had **a** single violation due to high MLogP values**,** which may influence solubility but remain within acceptable limits for drug-like compounds. Notably, allfive compounds were classified as Ro5-compliant**,** suggesting favorable oral bioavailability. In comparison, Tideglusibalso followed Ro5 without any violations. The drug-likeness assessment results are summarized in **Table 4**, confirming that these compounds are potential candidates for further development in antidiabetic therapy targeting GSK-3. Predictions for human gastrointestinal absorption (HIA), passive blood-brain barrier (BBB) permeation, and P-glycoprotein (P-gp) substrate properties are illustrated using the BOILED-Egg diagram **(Fig. 13)**. In this plot, the white region represents a high probability of gastrointestinal absorption**,** while the yellow region (yolk) indicates potential BBB penetration. Compounds are marked with blue circles (PGP+) if they are predicted to be actively effluxed by P-gp**,** meaning they are likely to be transported out of cells**,** reducing their intracellular retention and therapeutic effectiveness. In contrast, red circles (PGP−) indicate compounds that are non-substrates of P-gp, suggesting better cellular retention and potentially enhanced bioavailability.

In this study, Tideglusib was predicted to be well absorbed (positioned within the white region) and capable of crossing the blood-brain barrier (located within the yellow yolk), with no active efflux by P-glycoprotein **(**PGP−**)**, suggesting good bioavailability and potential central nervous system (CNS) activity[44]. Cucurbitinwas located in the white region, indicating good gastrointestinal absorption, but outside the BBB-permeant zone, and it was also predicted to be PGP−, meaning it is not actively effluxed from cells, which may enhance its retention and activity. Cucurbitacins, on the other hand, were situated within the white region (indicating highabsorption potential) but outside the BBB zone, and were predicted to be PGP+, suggesting that they may be actively expelled from cells, potentially reducing their intracellular efficacy. Both MomordicininandMomordenol were found outside the white and yellow regions, indicating poor absorptionand no BBB penetration, and were also PGP−, meaning they are not substrates for active efflux, but their bioavailability may still be limited due to poor permeability. Notably, Cycloartenolwas found outside the defined range of the BOILED-Egg plot, reinforcing its unsuitability for oral delivery or CNS targeting based on these in silico pharmacokinetic predictions.

3.5 Prediction of Toxicity Analysis & Median Lethal Dose (LD50):

3.5.1 Prediction of Computational Toxicity for Screened Phytocompounds -

Toxicity evaluation is a critical step in the early stages of drug development to ensure the safety of potential therapeutic candidates. In this study, the ProTox-3.0 server was employed to assess the toxicological profiles of the top five hit phytocompounds Cucurbitin, Cucurbitacins, Cycloartenol, Momordenol, and Momordicinin, in comparison with the control drug Tideglusib**.** The analysis focused on key toxicity parameters including hepatotoxicity, neurotoxicity,carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity**,** which are essential for predicting adverse effects in vivo. The results revealed that Cucurbitinwas predicted to be non-toxic across all evaluated endpoints**,** indicating a highly favorable safety profile. Cucurbitacinswere found to be active for both carcinogenicity and immunotoxicity**,** suggesting a potential risk that warrants further investigation. Cycloartenolwas non-toxic in most parameters but exhibited immunotoxicity**,** which may influence its suitability for long-term use. Momordenol showed neurotoxicity and immunotoxicity**,** while Momordicinin displayed only immunotoxicity, making them relatively safer but requiring cautious evaluation. In comparison, the control drug Tideglusibexhibited both neurotoxicity and carcinogenicity, highlighting the potential advantage of plant-derived compounds in reducing side effects. These findings, summarized in **Table 5**, suggest that several *M. charantia*-derived phytoconstituents, particularly Cucurbitin**,** demonstrate excellent toxicity profiles and could be promising candidates for further development as safer GSK-3 inhibitors.

Table-4Drug-Likeness Assessment of Top Five Hit Phytocompounds Based on Lipinski's Rule of Five

Lipinski's Rule of five (Ro5)							
S. No.	**Compound**	**Molecular Weight (g/mol)**	**Lipophilicity (MLogP)**	**H-bond donors**	**H-bond acceptors**	**No. of rule violations**	**Drug-Likeness Lipinski's**
		Less than 500 Da	Less than 5	Less than 5	Less than 10	Less than 2 violations	Rule Follows
1.	**Cucurbitin**	130.15	-3.44	3	4	0	Yes
2.	**Cucurbitacins**	498.65	2.24	6	1	0	Yes
3.	**Cycloartenol**	426.72	6.92	1	1	1	Yes
4.	**Momordenol**	426.67	5.60	1	2	1	Yes
5.	**Momordicinin**	438.69	5.89	0	2	1	Yes
Control drug	**Tideglusib**	334.39	2.88	0	2	0	Yes

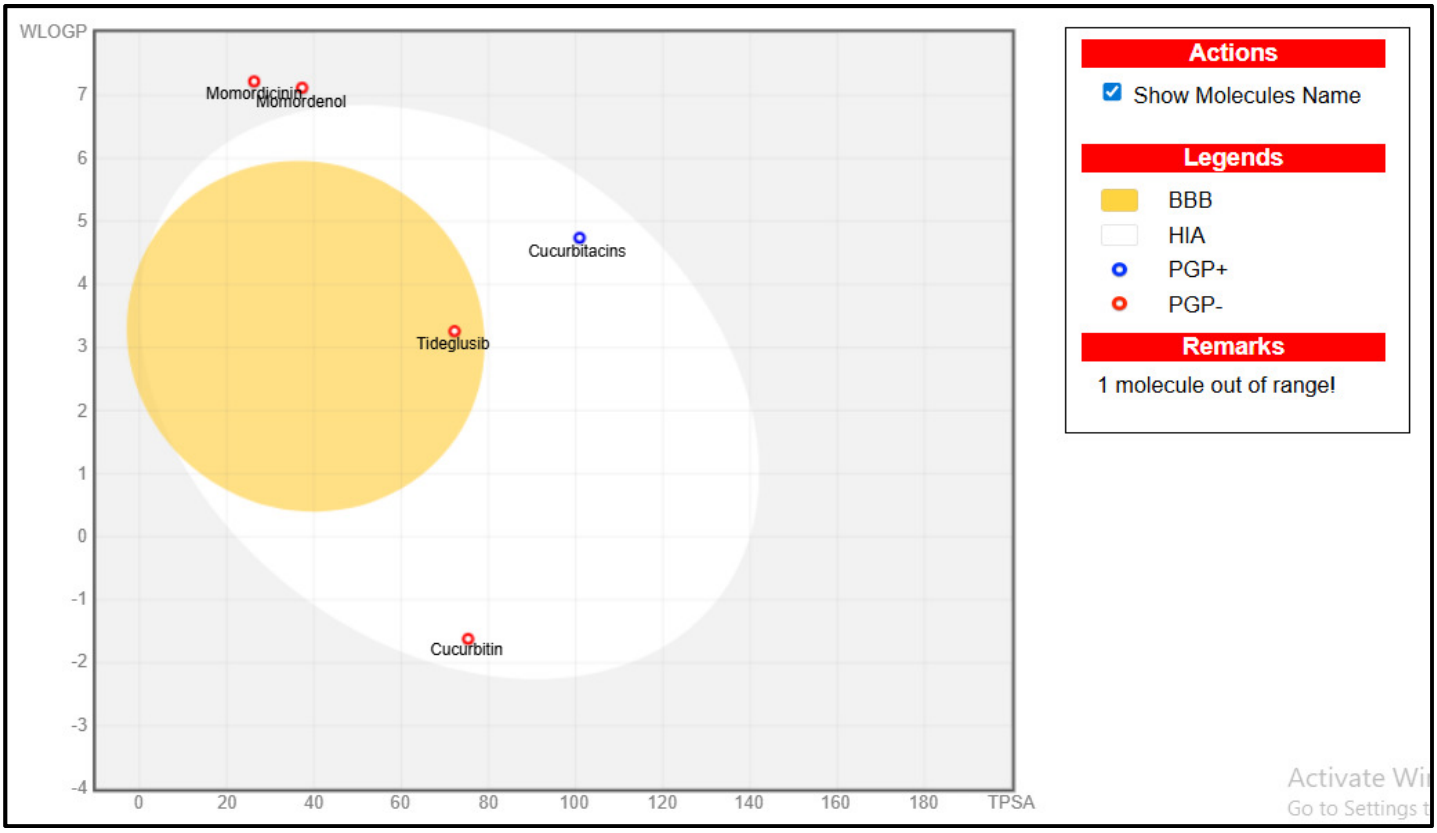


Figure 13. BOILED-Egg Model Representing Gastrointestinal (GI) Absorption, Blood-Brain Barrier (BBB) Penetration, and P-Glycoprotein (PGP) Activity of Target Compounds and Control drug (Tideglusib)

Table-5*In-Silico* Organ Toxicity Profiles of Top Five Phytocompounds and Control Drug (Tideglusib)

	Organ Toxicity					
Compound	**Hepatotoxicity**	**Neurotoxicity**	**Carcinogenicity**	**Immunotoxicity**	**Mutagenicity**	**Cytotoxicity**
Cucurbitin	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Cucurbitacins	Inactive	Inactive	Active	Active	Inactive	Inactive
Cycloartenol	Inactive	Inactive	Inactive	Active	Inactive	Inactive
Momordenol	Inactive	Active	Inactive	Active	Inactive	Inactive
Momordicinin	Inactive	Inactive	Inactive	Active	Inactive	Inactive
Tideglusib (Control drug)	Inactive	Active	Active	Inactive	Inactive	Inactive

3.5.2 Prediction of LD50 and Safety Profile Assessment for Screened Phytocompounds for Further Validation -

To evaluate the **acute oral toxicity** potential of the selected phytoconstituents from *Momordica charantia* L., *in-silico* LD_{50} predictions were carried out using the ProTox 3.0 web server (https://tox.charite.de/), a machine-learning-based tool that predicts toxicological parameters based on chemical structure and known toxicity datasets. The compounds were classified according to the Globally Harmonized System (GHS) into six toxicity classes ranging from Class I (extremely toxic) to Class VI (non-toxic)[45]. Among the phytochemicals analyzed, Momordicinin was predicted to have an LD_{50} value of 6000 mg/kg body weight**,** categorizing it as non-toxic(Class VI). Similarly, CycloartenolandCucurbitin demonstrated LD_{50} values of 3450 mg/kgand 2700 mg/kg**,** respectively, and were classified under Class V (practically non-toxic)**.** Momordenol exhibited a moderate toxicity profile with an LD_{50} of 1000 mg/kg, placing it in Class IV (slightly toxic). Notably, Cucurbitacinsshowed the highest toxicity among the tested compounds, with an LD_{50} of 100 mg/kg, falling under Class III (moderately toxic). In comparison,thecontrol drug Tideglusibwas also predicted to be slightly toxic (Class IV) with anLD_{50} of 1000 mg/kg, aligning with the profile of Momordenol. Theaverage structural similarityof the compounds to known toxic substances ranged from 40.52% for Tideglusib to82.71% for Cycloartenol, and prediction accuracy varied between 54.26% and 70.97%**,** indicating moderate to high confidence in the toxicity estimates. These toxicity predictions suggest that Momordicinin, Cycloartenol, and Cucurbitin exhibit low toxicity potential, thereby

supporting their candidacy as safe plant-derived GSK-3 inhibitors for the management of type 2 diabetes mellitus. Moreover, these findings contribute to the preliminary safety evaluation of phytocompounds, which is essential for advancing them to further pharmacological and clinical studies. **Table 6** presents the predicted LD_{50} values, toxicity classification, and associated prediction accuracy for the selected phytocompounds derived from *Momordica charantia* L., alongside the control drug Tideglusib. The data highlight significant variation in toxicity potential among the compounds, with most falling into low or non-toxic categories, thus supporting their therapeutic safety profile for further drug development targeting GSK-3 inhibition.

Table-6 LD50 Prediction and Safety Assessment of Screened Phytocompoundsand Control Drug (Tideglusib)

Compound	Oral Toxicity Prediction Results			
	Predicted LD50 (mg/Kgbw)	Predicted Toxicity Class	Average Similarity (%)	Prediction Accuracy (%)
Cucurbitin	2700	V	58	67.38
Cucurbitacins	100	III	64.88	68.07
Cycloartenol	3450	V	82.71	70.97
Momordenol	1000	IV	78.57	69.26
Momordicinin	6000	VI	75.2	69.26
Tideglusib (Control drug)	1000	IV	40.52	54.26

***Class I:** Fatal if swallowed (LD50≤5 mg/kg)
Class II: Fatal if swallowed (5<LD50≤50 mg/kg)
Class III: Toxic if swallowed (50<LD50≤300 mg/kg)
Class IV: Harmful if swallowed (300<LD50≤2000 mg/kg)
Class V: May be harmful if swallowed (2000<LD50≤5000 mg/kg)
Class VI: Non-toxic (LD50>5000 mg/kg)

IV. CONCLUSION

In this research, Glycogen Synthase Kinase-3 (GSK-3) was chosen as the target protein due to its key involvement in the pathogenesis of type 2 diabetes mellitus (T2DM). To inhibit GSK-3, we selected *Momordica charantiaL.* (Bitter melon), a medicinal plant known for its antidiabetic, anticancer, antiviral, antioxidant, and anti-inflammatory properties, as well as its low or negligible side effects. In contrast, the currently used GSK-3 inhibitor, Tideglusib, though effective, is associated with side effects such as neurotoxicity and carcinogenicity, motivating the

search for safer plant-derived alternatives using *in-silico*drug design methods, which offer low cost, rapid screening, and time efficiency. In current study, ten bioactive compounds are screened from *Momordica charantiaL.* namely-Charine, Cucurbitin, Cucurbitacins, Cucurbitane, Cycloartenol, Diosgenin, Erythrodiol, Momordenol, Momordicin, and Momordicinin. Initial biological activity prediction using PASS online revealed diverse predicted pharmacological activities, including hypolipidemic, hepatoprotective, and anti-inflammatory effects. Allergenicity prediction identified nine compounds as non-allergenic, with only Cucurbitanepredicted as an allergen, while Tideglusib was also non-allergenic. Molecular docking analysis showed that five compoundsnamely -Cucurbitin (−9.4 kcal/mol), Cucurbitacins (−8.7 kcal/mol), Momordicinin (−8.6 kcal/mol), Cycloartenol (−8.3 kcal/mol), and Momordenol (−8.3 kcal/mol)exhibited higher binding affinity to GSK-3 relative to the control drug Tideglusib (−8.2 kcal/mol).SwissADME analysis revealed that all five top compounds followed Lipinski's Rule of Five, with zero or one violation, indicating good oral drug-likeness. In terms of toxicity, ProTox-II analysis showed that Cucurbitin was non-toxic across all parameters and had an LD_{50} of 2700 mg/kg (Class V), while Momordicinin had the highest LD_{50} of 6000 mg/kg (Class VI), also showing no organ toxicity. Cucurbitacins, although potent, showed carcinogenicity and immunotoxicity with a lower LD_{50} of 100 mg/kg (Class III). The control Tideglusib was found to be neurotoxic and carcinogenic, with an LD_{50} of 1000 mg/kg (Class IV). Overall, the findings suggest that compounds from *Momordica charantia L.*, especially Cucurbitin, demonstrate strong GSK-3 inhibition potential, along with excellent safety, non-allergenicity, and drug-like properties, making them promising candidates for the development of safe and effective therapies for type 2 diabetes mellitus. Further *in-vitro* and *in-vivo* studies are recommended to validate these computational results and explore their clinical potential.

ABBREVIATIONS

ΔG: Binding Energy

ADME: Absorption, Distribution, Metabolism, Excretion

CADD: Computer Aided Drug Design

DM: Diabetes Mellitus

GSK-3: Glycogen Synthase Kinase-3

LD50: Median Lethal Dose 50

PDB: Protein Data Bank

PubChem: Public Chemical Database

RCSB: Research Collaboratory for Structural Bioinformatics

RMSD: Root Mean Square Deviation

SDF: Structure Data File

T2DM: Type 2 Diabetes Mellitus

Author Contributions

Suraj Joshi conducted the computational simulations and was primarily responsible for manuscript drafting. **Vineeta Sharma** and **Suman Bhandari** carried out the literature review and contributed to manuscript writing. **Dr. Mukesh Samant** assisted in simulation execution and provided support in manuscript development. **Dr. Shankar Mondal** conceptualized the study, offered critical technical guidance, and supervised the overall preparation and finalization of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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