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An Explicit Computational Approach to the Glucokinase Activating Potentials of Naturally Occurring Phytochemicals for the Treatment of Diabetes Mellitus

Divya Jyothi Palati[1], Prudvi Raj Pulla[2], Banoth Karan Kumar[3], Deepika N.P[1], Muhasina K.M[1], Divya Bhargavi P[1], Duraiswamy Basavan[1*]

1. Department of Pharmacognosy, JSS College of Pharmacy, JSS Academy of Higher Education & Research,Ooty-643001, The Nilagiris, Tamil Nadu, India.
2. Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty-643001, The Nilagiris, Tamil Nadu, India.
3. Medicinal Chemistry Research Laboratory, Department of Pharmacy, Birla Institute of Technology and Science- Pilani, Pilani, Rajasthan, India.

***Corresponding Author:** Dr. B. Duraiswamy, Professor & Head, Department of Pharmacognosy, Jss College of Pharmacy, Ooty, The Nilgiris, Tamilnadu.
E-mail id: duraiswamybasavan@gmail.com

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Abstract

Glucokinase (GK) regulates glucose output and uptake in the liver, as well as insulin release in the pancreas, to maintain glucose homeostasis. In numerous preclinical and clinical studies, allosteric activators of human Glucokinase activators possess activities related to lowering of blood glucose levels. This analysis was aimed to ascertain persuasive Glucokinase activators amongst the 15 selected phytochemicals using molecular docking and molecular dynamic simulation studies. The docking studies with Glucokinase disclosed that among 15 phytochemicals, Rutin and Rosmarinic acid had the best docking scores of -13.92 and -10.59 kcal/mol, and prime MM-GBSA analysis showed free binding energies of -85.016 and -69.865 kcal/mol, respectively. Additionally, top-scoring phytochemicals were subjected to molecular dynamics. These studies exhibited that the molecules stayed moderately stable at the allosteric site of the Glucokinase enzyme. These findings suggest that the identified phytochemicals could be potential lead compounds for developing novel Glucokinase activators. Furthermore, studies are needed to validate their efficacy and safety as therapeutic agents for diabetes.

Key words: Glucokinase, Phytochemicals, Docking studies, Diabetes, Glucose hemostasis.

Introduction

Diabetes is the world's ninth most common cause of mortality. Globally, 537 million individuals were identified with diabetes in 2021, and that figure is anticipated to surge to 783 million by 2045, as reported by the International Diabetes Federation. The predominance of diabetes is rising due to a number of causes, such as food, urbanization, and obesity. The majority of adults worldwide are affected by type 2 diabetes mellitus (T2DM), which is the utmost prevalent variant of the disease (1). T2DM is characterized by insulin resistance, excessive hepatic glucose production (HGP), low glucose-stimulated insulin secretion (GSIS), and high fasting plasma glucose (FPG) (2). Chronic hyperglycemia increases the chance of developing vascular problems like coronary ischemic heart conditions, hemorrhagic stroke, renal disorders, retinal vascular disorder, and diabetic nerve pain and distal polyneuropathy (3). Most of the oral medications used to treat type 2 diabetes work by either decreasing HGP (e.g., biguanides) or enhancing insulin exertion (e.g., thiazolidinediones) or vitalize insulin release (e.g., sulfonylurea drugs), preventing the intestinal assimilation of glucose (e.g.,α -glucosidase inhibitors), or promoting autogenous levels of glucagon-like-peptide (GLP-1) and glucose-dependent insulintropic peptide (GIP) (e.g. sitagliptin and saxagliptin). In the end, insulin. In addition, utmost of these therapeutic medications possess adversarial outcomes such as hypoglycemia, gastrointestinal side effects, obesity and genitourinary infection (4). As a result, there is still an abundant requirement for innovative, safe, and effective therapeutic treatments to alter postprandial and fasting glycemic status.

Glucokinase (GK) (Hexokinase IV, EC 2.7.1.1) is a cytoplasmic bio-catalysts that illustrates a vital part in glucose homeostasis throughout the body (5). Glucokinase is primarily articulated in duct gland β-cells and liver parenchymal cells. GK is the liver's primary glucose-phosphorylating enzyme; its abundance is controlled at several levels, including transcription by insulin and glucagon and post-translationally as a result of the Glucokinase receptor protein (GKRP). As a result, unlike other hexokinase enzymes, GK initiates glycolysis (6).

GK operates as a glucose detector in pancreatic β-cells, leading glucose-stimulated insulin secretion (GSIS) by identifying changes in blood glucose intensities besides inducing the production of insulin. Insulin secretion can be triggered through an increase in glucose concentration due to the activation of adenosine triphosphate (ATP)-sensitive potassium (K_{ATP}) channel closure, β-cell depolarization, besides calcium (Ca2+) inflow through voltage-gated Ca2+ channels.

Glucokinase (GK) holds a role in the liver's increased absorption of glucose and its translation to glycogen for energy stowage. GK is controlled by Glucokinase regulatory protein (GKRP) in the liver. GKRP attaches towards the inactive conformation form of GK in hypoglycemic conditions and stores it in the nucleus. Hyperglycemia triggers GKRP and releases Glucokinase into the cytoplasm, where it can bind to glucose and activate (7). Mutations in genes define new binding sites for the Glucokinase enzyme, which follows in altered enzyme activity. Decreased insulin emission and amplified hepatic glucose production eventually result in critical medical circumstances specifically as maturity-onset diabetes of the young (MODY), persistent

hyperinsulinemia hypoglycemia of infancy (PHHI-GK), and permanent neonatal diabetes mellitus (PNDM) (8).

Biologically active compounds that bind to the allosteric region of the enzyme are known as glucose kinase activators (GKAs), enhancing glucose homeostasis and glycemic control by stimulating secretion of insulin and glycogen synthesis. Furthermore, GKAs govern Glucokinase enzyme super-open and sealed configuration changes, which enhances its ability to metabolize excess glucose levels. (9) GKAs have recently been recognized as novel antidotes towards the managing of type 2 diabetes. In lieu, during clinical trials, it was discovered that the great majority of putative activators had adverse effects. These adverse reactions include hypoglycemia, dyslipidemia, hypertriglyceridemia, hepatic steatosis, induction of fatty liver and other microvascular complications (10). Thus, it is necessary to investigate natural compounds that may well function as an effective Glucokinase activators with minimal adverse effects in the treatment of diabetes.

Due to the widespread acceptance in their therapeutic properties and safety, phytochemicals are customarily accustomed for the management of various ailments. Stems, planks, cortex, fruit, peapods, foliage, rootstock, floret, ragweed, and kernels all contain greater quantities of phytoconstituents such alkaloids, cinnamic acids, lignans, coumarins, monoterpenes, diterpenes, flavonoids, phenyl propanoids, triterpenes and tannins. These phytochemicals have been found to have preventive benefits against diseases mediated by oxidative stress, such as diabetes (11). Recent studies have suggested that particular phytochemicals (Table 1) may have regulatory effects on glucose hemostasis (Anethole (12), Berbamine (13), Berberine (14), beta-Caryophyllene (15), Curcumin (16), Daidzein (17), D-Pinitol (18), Eugenol (19), Eugenol (20), Fraxetin (21),

S.no	Compounds	**Dosage**	**Glucose minimizing commotion**	***In-vitro*/ *In-vivo* studies**	**Ref**
1.	Anethole	80mg/kg for 45 days	Regulates glucose homeostasis	Diabetic rat	(12)
2.	Berbamine	100mg/kg for 4 weeks	Regulates glucose homeostasis	Diabetic rat	(13)
3.	Berberine	25µM	Regulates glucose homeostasis	Hepatocytes of Sprague-Dawley rat	(14)
4.	beta-Caryophyllene	200mg/kg for 45 days	Regulates glucose homeostasis	Diabetic rat	(15)
5.	Curcumin	20µM	Regulates glucose homeostasis	Hepatic stellate cells **(**HSCs**)**	(16)
6.	Daidzein	0.02% w/w	Regulates glucose homeostasis	C57BL/KsJlepr**(**db**) (**db/db**)**	(17)
7.	D-Pinitol	50 mg/kg for 30 days	Regulates glucose homeostasis	Diabetic induced rat	(18)

8.	Esculetin	40mg/kg for 45 days	Regulates glucose homeostasis	Diabetic rat	(19)
9.	Eugenol	10mg/kg for 30 days	Regulates glucose homeostasis	Diabetic rat	(20)
10.	Fraxetin	80mg/kg for 30 days	Regulates glucose homeostasis	Diabetic rat	(21)
11.	Genistein	0.02% w/w	Regulates glucose homeostasis	C57BL/KsJlepr**(**db**) (**db/db**)**	(17)
12.	Myricetin	3mg/kg for 3 days	Regulates glucose homeostasis	Diabetic rat	(22)
13.	Rutin	100mg/kg for 45 days	Controls glucose homeostasis	Diabetic induced rat	(23)
14.	Rosmarinic acid	High fat diet food with 100mg/kg for 30 days	Regulates glucose homeostasis	Diabetic rat	(24)
15.	Sinapinic acid	25mg/kg for 30 days	Regulates glucose homeostasis	Diabetic rat	(25)

Genistein (17), Myricetin (22), Rutin (23), Rosmarinic acid (24), and Sinapic acid (25)).

Table 1. Catalogue of selected phytochemicals for *in-silico* molecular docking reviews with human Glucokinase enzyme.

The discovery of molecules with diverse biological activity has been aided by the use of computational tools for drug development, such as molecular docking and molecular dynamics modelling, which have proven to be both cost-effective and efficient. By using simulations of molecular dynamics and molecular docking, it is possible to anticipate the binding stability and affinities of ligands with binding pocket of selected receptors.

Therefore, the motive of this work was to utilize computational techniques to identify putative Glucokinase activators from naturally occurring phytochemicals.

Material and Methods

Selected phytoconstituents

The phytochemicals were selected based on their anti-diabetic activity and regulation of glucose hemostasis. The following phytochemicals were used in this study. Anethole, Berbamine, Berberine, Beta-Caryophyllene, Curcumin, Daidzein, D-Pinitol, Esculetin, Eugenol, Fraxetin, Genistein, Myricetin, Rutin, Rosmarinic acid, Sinapic acid structured shown in Figure.1.

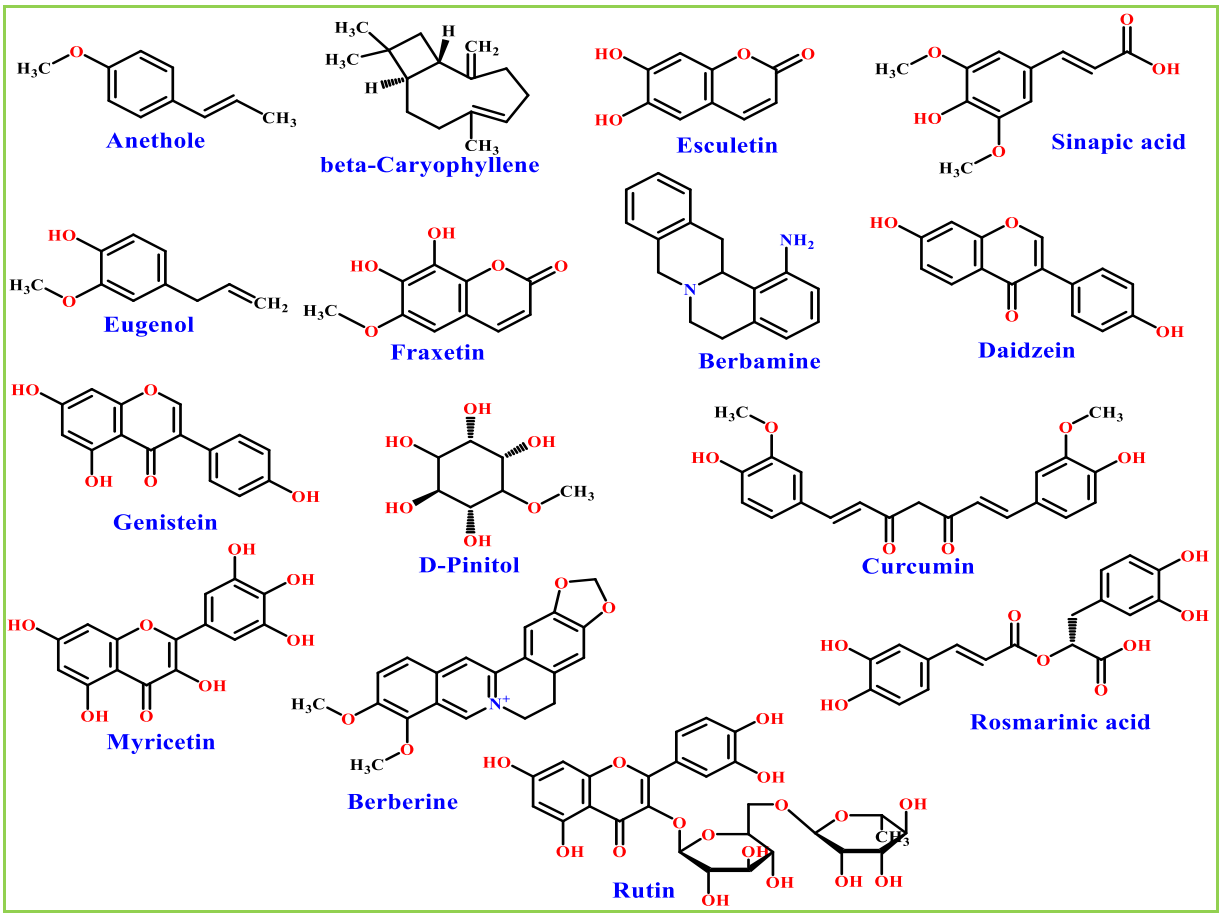

Figure 1. Phytochemicals (15) selected for this study with their structures.

Preparation of Ligands

ChemDraw was used to tidy up the assembly for bond alignment and to draw all ligands that were used as contribution for the docking exploration. The ligands were loaded into the workstation, and the OPLS3e (Optimized Potentials for Liquid Simulations) force field in Ligprep was used to minimize the energy (Version 2019-1, Schrodinger). This trivialization aids with docking studies by facilitating bonding order consignment, hydrogen bond accumulation to ligands and the transformation of a 2D structure into a 3D structure. The final outcome (Best ligand conformations) has been introduced into a docking experiment (26).

Protein Preparation

Schrodinger's protein preparation wizard (2019-1 version) was the most useful tool for minimizing protein structure. Hydrogen atoms and charges have been supplemented to the target protein. Using Epik, we were able to generate Het states between pH 7.0 and 2.0. Protein pre-processing, enhancement, and modification using workspace water molecule analysis. All other molecules were destroyed except for the heteroatoms in water, which remained unchanged. After that, the OPLS3 force field was employed to trivialize the protein. The RCSB protein data bank provided information regarding X-ray crystal structure of the GK protein bound to an allosteric activator (27). After a comprehensive examination of multiple entries, the optimal entry (PDB code: 3IMX)

was handpicked based upon improved resolution and essential binding interfaces amongst Glucokinase (GK) and small molecule GK activators (28).

***In-silico* physicochemical parameters**

The phytochemicals must satisfy the drug likeness test to be employed in molecular docking. The Schrodinger drug discovery software's Qikprop tool of was utilized to evaluate drug similarity tests for all compounds. Schrodinger software's Qikprop is a swift, precise, and simple to execute absorption, distribution, metabolism, and excretion forecast application (28).

Molecular docking studies

For the docking studies, we used an Intel XenonW3565 processor running Ubuntu enterprises 14.04 with the Glide module of Schrodinger software. To determine the target protein's structure, researchers employed the RCSB protein data library. ChemDraw 16.0 PerkinElmer software was used to draw targeted ligands.

Molecular Docking

Molecular docking was executed utilizing the previously mentioned ligand (small molecule) and protein. The results of the molecular docking study (Version 2019-1, Schrodinger) were evaluated using XP Visualizer. Schrodinger's Glide module was utilized for the Docking investigations on the selected phytochemicals. All docking investigates were performed out in XP mode (Extra Precision). Atoms in the protein were given partial atomic charges of less than 0.15 and scaling coefficients of 0.8. The Glide docking values were utilized to find the output with the best docked substantiate. The interfaces between these docked substantiates were explored remoter via XP visualizer.

MM-GBSA analysis

The MM-GBSA method was adopted to calculate the free binding energies of complexes of protein-ligand. Utilizing the prime module of the Schrodinger software, the conglomerate with the lowly docking value had their free binding energy computed. For this study, we used the VSGB 2.0 model, which we modified based on physics to account for p-p interactions, hydrophobic interactions, and hydrogen bonding self-contact interfaces (26).

Molecular dynamics (MD) simulations

The MD simulations experiment was carried out by the System Builder Panel of the Schrodinger software's Desmond module (Schrodinger Release, 2019) with a least remoteness of 10 among the protein superficial and the solvent superficial. The orthorhombic simulation box was created through the simple point-charge (SPC) explicit water model. The orthorhombic SPC model was used for solving complexes of docked proteins and ligands (29). A 0.15 M physiological salt concentration was maintained, and counter ions were accustomed to nullify the solvated arrangement. The OPLS3 force field was accustomed to model the receptor-ligand conglomerate arrangement. The simulation was carried out aimed at 100 ns at 300 K and 1.013 bars of atmospheric pressure using the NPT ensemble (Isothermal-Isobaric ensemble, constant warmth, constant pressurize and continuous digit of particles) ensemble with the default relaxation parameters (30). A simulation time of 100 ns was used for the MD simulation, which was carried out using the MD simulation tool. The _out.cms file was also incorporated in order to interpretation

of the trajectory data and create a movie. A higher resolution (1280_1024) and higher quality video was exported from the video. The MD simulation's 2002 frames were used to build the trajectory. To acknowledge the steadiness of the complex during MD conglomerate, the protein backbone frames were positioned in relation to the spinal column of the starting frame. After incorporating the _out.cms file and choosing Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) in the analysis type, the simulated interface diagram was finally examined.

Outcomes and discussion

Glucokinase activators (GKA) reduce glycemia levels by increasing glucose absorption within hepatocytes and stimulating insulin emanation from pancreatic beta cells, making them an attractive molecular target for diabetes treatment. The docking studies were executed by exploiting the allosteric active site of Glucokinase (PDB entry: 3IMX) and validated by docking of 3IMX and its co-crystal molecule in the allosteric position. Between the crystallographic alignment and the finest-docked posture, RMSD values were calculated. RMSD standards amongst the crystallographic alignment and the best-docked posture were calculated. Root mean square deviation (RMSD) was examined in XP mode subsequently the final docking process with the co-crystal molecule to authorize the protein, as well as the RMSD ranges of the selected target was determined to be 1.2 Å. (Figures 2). The reduced RMSD result suggests that the docking methodology might be trustworthy for the concluding docking investigations of the chosen molecules in contradiction of the validated target.

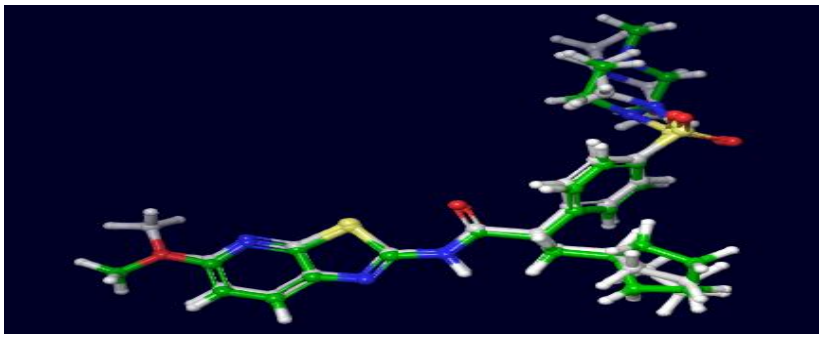

Figure 2. Superimposed perspective of the X-ray earliest pose of the molecule/ligand and its docked pose in the allosteric site of the objective PDB-3IMX (RMSD- 1.2 Å)

Colour clarification – Green- Docked pose and White –X-ray earliest *pose of the ligand.*

The investigations using docking demonstrated the best docking scores and showed excellent binding patterns in the objective protein's active region. The results were unforeseen: the selected target protein co-crystal ligand had lower docking score than the top molecule (Rutin). Table 2 presents the docking evaluations of co-crystal molecule and molecules.

S.No	Selected natural molecules	Glide score (Kcal/mol)	Glide energy (Kcal/mol)
1	**Rutin Hydrate**	**-13.92**	**-67.01**
2	**Rosmarinic acid**	**-10.59**	**-54.51**

3	**Myricetin**	**-9.44**	**-39.91**
4	**Curcumin**	**-9.23**	**-44.95**
5	**Berberine**	**-8.49**	**-32.2**
6	Genistein	-8.35	-35.48
7	Daidzein	-7.38	-34.11
8	D-Pinitol	-6.94	-19.8
9	Fraxetin	-6.84	-26.37
10	beta-Caryophyllene	-6.68	-22.5
11	Esculetin	-6.42	-26.26
12	Sinapic acid	-6.35	-27.93
13	Eugenol	-5.79	-24.26
14	Anethole	-4.72	-18.85
15	Berbamine	-2.92	-33.58
16	**Co-crystal ligand**	**-13.06**	**-73.14**

Table 2. The docking results of the selected phytochemicals against PDB-3IMX

The co-crystal ligand of the PDB Id: 3IMX i.e., ((2R)-3-cyclopentyl-N-(5-methoxy [1,3] thiazolo[5,4-b] pyridin-2-yl)-2-4-[(4-methylpiperazin-1-yl) sulfonyl] phenyl propanamide) relieved ARG-63 amino acid residue showed two hydrogen bond interactions with NH (secondary amine) and Thiazole nitrogen (tertiary), and single Pi-Pi interaction with TYR-214. The co crystal ligand showed significant docking value of -13.06 kcal/mol within the target protein active domain for all those crucial amino-acid residue contacts/interactions (Figure 3).

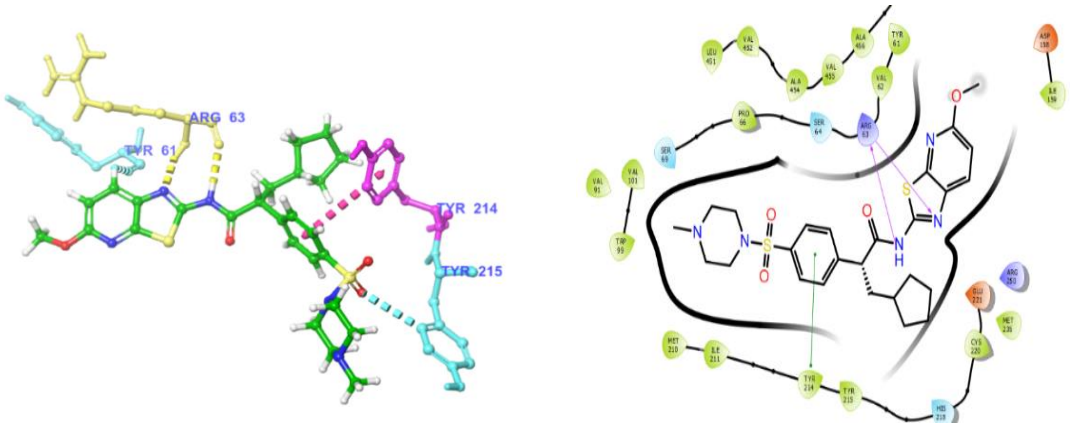

Figure 3. Amino-acid residue contacts demonstrated by the co-crystal ligand in the allosteric site of the objective protein PDB-3IMX. (A) 3D interaction images of the co-crystal molecule displaying the hydrogen bond contacts in black, aromatic bond in blue colour (LEFT). (B) 2D interaction images of the co-crystal molecule displaying the hydrogen bond contacts in magenta colour (RIGHT)

As paralleled to the Co-crystal ligand, Rutin had the maximum dock score of -13.92kcal/mol in the active domain of the receptor 3IMX. Rutin (vitamin P) is a bioflavonoid found in a variety of

plants, including the Fagopyrum esculentum, Sophora japonica and Ruta graveolens. This flavonoid has been associated with a several of pharmacological maneuver, comprising anti-diabetic, nephroprotective, anti-swelling, antioxidant, and hepatoprotective activity (31).

The exertion of Glucose-6-Phosphatase in both hepatocytes and nephron of diabetic rats was reduced with rutin (100 mg/kg). In addition, Rutin shrunk the commotion of fructose-1, 6-bisphosphatase, and a essential gluconeogenic enzyme, in hepatic, renal, muscles and also, boosted the activity of hexokinase (23).

Four hydrogen bonds, four aromatic bonds, and three Pi-Pi interactions were generated at the active domain of the receptor protein by this molecule. ARG-63, PRO-66, and TYR-215 showed hydrogen bond interactions with the active site and TYR-215, TRP-99 showed Pi-Pi interactions

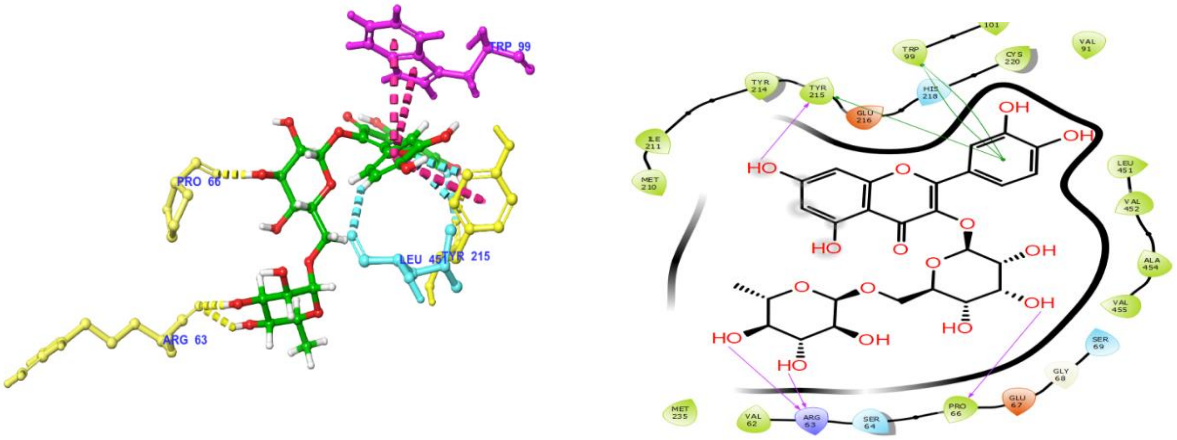

Figure 4. Amino-acid residue contacts demonstrated by the Rutin Hydrate in the allosteric site of the objective protein PDB-3IMX. (A) 3D interaction images of the co-crystal molecule displaying the hydrogen bond contacts in black, aromatic bond in blue colour (LEFT). (B) 2D interaction images of the co-crystal molecule displaying the hydrogen bond contacts in magenta colour (RIGHT)

In the active site of the 3IMX, Rosmarinic acid had the second-best dock score of -10.59kcal/mol. Various common comestible aromatic plants, similar to those in the Lamiaceae, Boraginaceae, and Anthocerotaceae families, contain this powerful antioxidant. Antiviral, antibacterial, antioxidant, antimutagenic, and anti-inflammatory are just a few of the biological properties of RA (32). The activity of liver specific pathological enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were reduced in diabetic induced rats treated using rosmarinic acid (100 mg/kg) (2014). In addition, the operations of crucial carbohydrate metabolizing enzymes including hexokinase, pyruvate kinase, glucose-6-\phosphatase, fructose 1,6-bisphosphatase, glucose-6-phosphate dehydrogenase, glycogen synthase, and glycogen phosphorylase in the hepatic cellular tissue of diabetic rats were altered ($P < 0.05$) in the liver tissue of diabetes persuaded rats (24).

This molecule has four hydrogen bond contacts exists between amino acids ARG-63, PRO-66, and TYR-61. The identical compound exhibited no aromatic or Pi-Pi interactions (Figure 5).

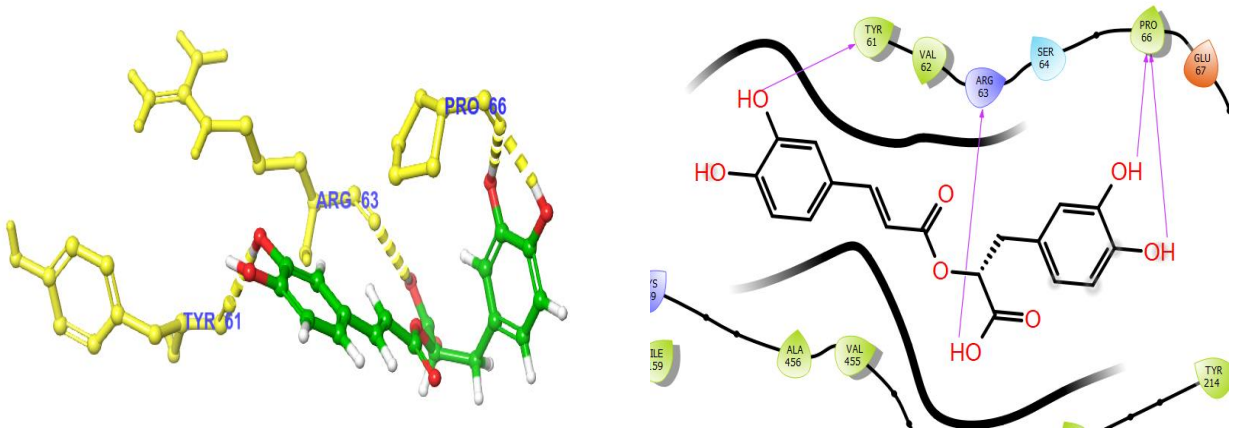

Figure 5. Amino-acid residue contacts demonstrated by the Rosmarinic acid in the allosteric site of the objective protein PDB-3IMX. (A) 3D interaction images of the co-crystal molecule displaying the hydrogen bond contacts in black, aromatic bond in blue colour (LEFT). (B) 2D interaction images of the co-crystal molecule displaying the hydrogen bond contacts in magenta colour (RIGHT)

Myricetin, a top three molecule, has a dock score of -9.44 kcal/mol in the target 3IMX's active region. Tea, berries, fruits, vegetables, and medicinal plants all contain Myricetin (3, 5, 7, 3'4', 5' -hexahydroxyflavone). It has been studied for its antioxidant, anticancer, anti-inflammatory, anti-amyloid genic, antibacterial, antiviral, and antidiabetic properties (33). Subsequently 2 days of therapy with (3 mg/12 h) of Myricetin, hyperglycaemia in diabetic rodents was decreased by 50%, and hypertriglyceridemia was lowered. It is also increased the amount of glycogen and glucose-6-phosphate (G-6-P) in the hepatocytes (22).

With the active domain of the receptor protein, this molecule released four hydrogen bond interactions, one Pi-Pi interaction and one aromatic bond interaction. This molecule has four hydrogen bond connections containing amino acid sequences ARG-63, HIS-218, LEU-451 and 2 Pi-Pi bond contacts with the amino acid residues TYR-215, TRP-99. (Figure 6).

Curcumin is ranked fourth on the list, with a dock score of -9.23 kcal/mol in the target 3IMX's active site. Curcumin (1, 7–bis (hydroxyl-3-methoxyphenyl)-1.6-heptadiene-3, 5-dione is one of the effective polyphenolic component in Curcuma longa (34).

Curcumin is having a therapeutic potential for a wide range of diseases and disorders, such as, Alzheimer's disease, myelodysplastic syndromes, oral cancers, bowel cancer, mastitis, pancreatic cancer, psoriasis, diabetic kidney disease, parodontosis disease, pre-cancerous lesions and recurrent apthous stomatitis (35).

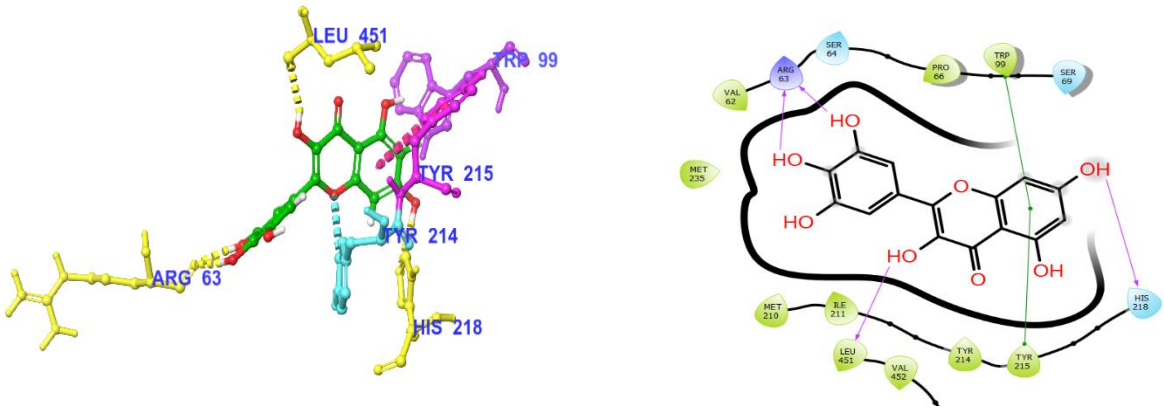

Figure 6. Amino-acid residue contacts demonstrated by the Myricetin in the allosteric site of the objective protein PDB-3IMX. (A) 3D interface images of the co-crystal molecule displaying the hydrogen bond contacts in black, aromatic bond in blue colour (LEFT). (B) 2D interface images of the co-crystal molecule displaying the hydrogen bond contacts in magenta colour (RIGHT)

Curcumin inhibited GLUT4 membrane translocation caused by leptin by interfering with the insulin receptor (IR) substrates/phosphatidyl inositol 3-kinase/AKT signaling cascade. Curcumin also increased glucose transformation into Glucose-6-Phosphate by stimulating Glucokinase activity (16).

This molecule has 1 hydrogen bond contact with the ARG-63 amino acid sequences, along with one Pi-Pi bond interface with the TYR-215 amino acid sequences.

In comparison to the previously described molecule and co-crystal ligand, this molecule showed lower docking scores (Figure 7).

The next compound on the list, Berberine, had a dock score of -8.49 kcal/mol in the target 3IMX's dynamic site. This plant alkaloid, Berberine Isoquinoline, has a wide range of pharmacologic actions, including protection against some stomach ulcers, therapy of inflammatory, cardiovascular, or lipid illnesses, and glucose-related ailments (36). Berberine therapy elevated Gk and inhibited Pck1 and G6pc expression in ZF hepatocytes without insulin (14). Berberine forms four Pi-Pi bond contacts with the TYR-215, TRP-99 amino acid residues (Figure 8).

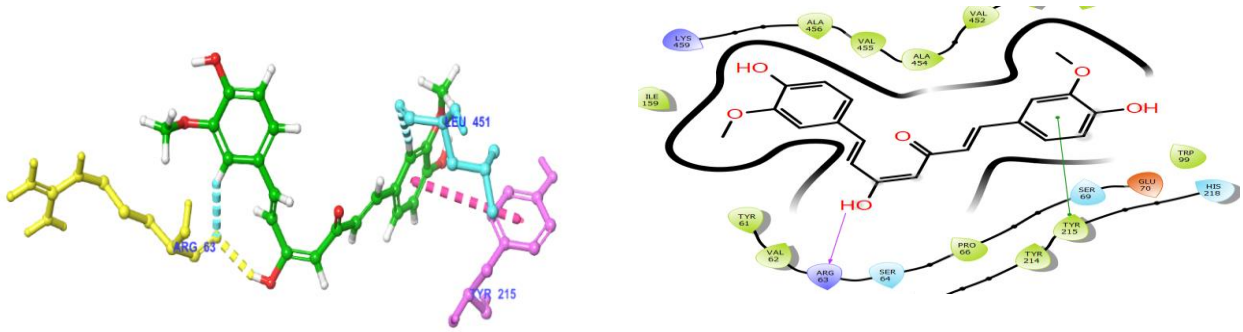

Figure.7 Amino-acid residue contacts demonstrated by the Curcumin in the allosteric site of the objective protein PDB-3IMX. (A) 3D interface images of the co-crystal molecule displaying the hydrogen bond

contacts in black, aromatic bond in blue colour (LEFT). (B) 2D interface images of the co-crystal molecule displaying the hydrogen bond contacts in magenta colour (RIGHT)

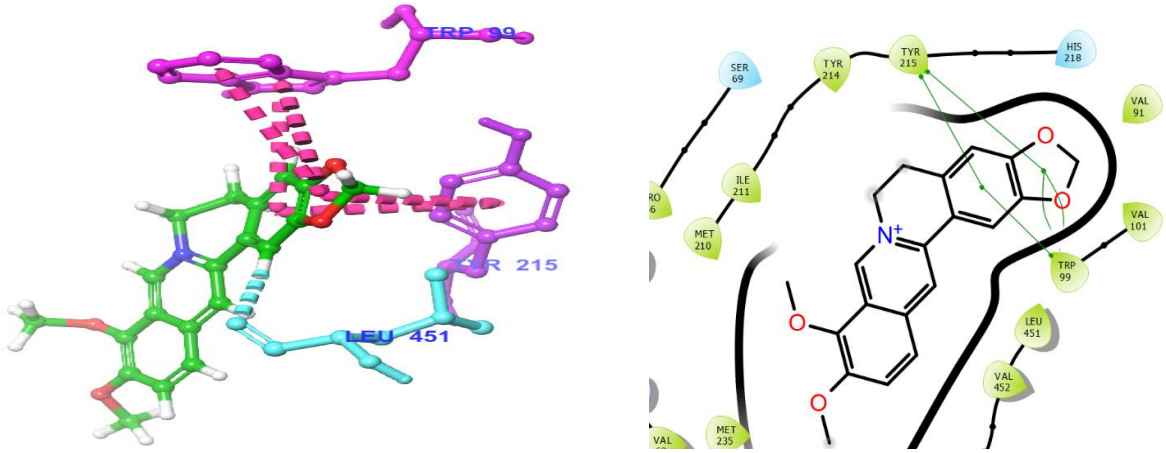

Figure 8. Amino-acid residue contacts demonstrated by the Berberine ligand in the allosteric site of the objective protein PDB-3IMX. (A) 3D interface images of the co-crystal molecule displaying the hydrogen bond contacts in black, aromatic bond in blue colour (LEFT). (B) 2D interface images of the co-crystal molecule displaying the hydrogen bond contacts in magenta colour (RIGHT)

In addition, the MM-GBSA segment examined the binding energy estimation of the best molecules established on their binding similarity to the allosteric domain binding pocket of the target protein. MM-GBSA scores of the highest molecules were -85.0163, -69.865, -65.9935, and -51.5478, respectively. These results, revealed that the free binding energy value of Rutin and Rosmarinic acid was better in comparison to the Myricetin and curcumin compounds (Table 3).

S.NO	Name of the compounds	MMGBSA score (Kcal/mol)
1.	Rutin hydrate	-85.0163
2.	Rosmarinic acid	-69.865
3.	Myricetin	-65.9935
4.	Curcumin	-51.5478

Table 3. The calculated (MM-GBSA) binding free energies of the designated molecules in contrast to Glucokinase enzyme.

In-silico predicted physicochemical parameters:

Absorption, Distribution, Metabolism, and Excretion (ADME) is an important concept in medicinal chemistry. The drug-likeness of the molecules will be determined by these criteria. In-silico the Schrodinger's QikProp module was used to forecast the physico-chemical (Lipinski's rule of five and Jorgensen's rule of three) parameters of all chemicals from the docking investigation. (Table 4). Summarizes the parameters. The molecules were found to have a lower molecular weight than expected. The log Po/w values were projected, and they fall between the prescribed ranges of -2.0 to 6. Violations can result in a penalty of up to four points. The maximum violation

for all expected molecules is only three. These findings indicated that the molecule may have drug-like properties.

The compounds had decent solubility, as evidenced by the Log S values, which were all within the prescribed range. With the exception of curcumin, the in-silico predicted Caco-2 cell permeability for the selected molecules turned out to be quite low, indicating that absorption may be difficult. It was discovered that the variety of possible metabolic reactions for the molecules is quite high. Overall, the results are satisfactory, except for the regulations that have less violation

Compound name	Lipniski rule of five					Jorgensen rules of three			
	M.W	Donor HB	Accept HB	LogPo/W	Violations	Logs	PCaco	Metab	Violations
Rutin Hydrate	610.52	9	20.55	-2.467	3	-2.24	0.82	10	2
Rosmarinic acid	360.32	5	7	1.159	0	-3.47	1.507	6	1
Myricetin	318.24	5	6	-0.307	1	-2.64	6.563	6	1
Curcumin	368.39	2	7	3.609	0	-4.59	145.6	5	0
Berberine	608.73	1	8	10.3	2	-4.1	189.8	12	1
Genistein	270.24	2	3.75	1.686	0	-3.05	161.8	3	0
Daidzein	254.24	2	4	1.784	0	-3.01	376.3	2	0
D-Pinitol	194.18	5	10.2	-1.862	0	-1.08	133.4	5	0
Fraxetin	208.17	2	4.75	0.244	0	-1.74	215.6	3	0
beta-Caryophyllene	264.41	0	2	4.302	0	-4.86	5321	0	0
Esculetin	178.14	2	4	0.11	0	-1.34	2E+05	2	0
Sinapic acid	224.21	2	4.25	1.512	0	-2.16	66.54	3	0
Eugenol	164.2	1	1.5	2.67	0	-2.36	3231	3	0
Anethole	148.2	0	0.75	3.166	0	-2.93	9906	2	0
Berbamine	608.73	1	8	5.779	2	-4.1	189.8	12	1

Table 4. *In silico* projected Physico-chemical Properties considerations of all selected compounds

Molecular dynamics

To elucidate the protein-ligand interaction of the bound compounds as well as assess the stability of the ligand binding in the active domain of the target receptor, a molecular dynamic simulation investigation was conducted on the leading two compounds from the Protein Data Bank (3IMX). The protein complex was engrossed in an orthorhombic box of SPC water molecules containing Rutin and Rosmarinic acid compounds. To neutralize the solvated system, one Na^+ counter ion was added. To achieve system equilibrium, solutes were put through to an NPT ensemble. Lastly the entire system was subsequently tested to a 100-ns MD simulation at 300 K and 1 bar. The simulation outcomes were evaluated by utilizing the backbone RMSD values. In the specified target, all conformations revealed significant RMSD values. Rutin and rosmarinic acid have maximum RMSD values of 4.6 and 3.8, respectively, according to the target (Figure 9). Ligand and Protein contacts were observed during the course of the simulation and these interactions are depicted in Figure 10.

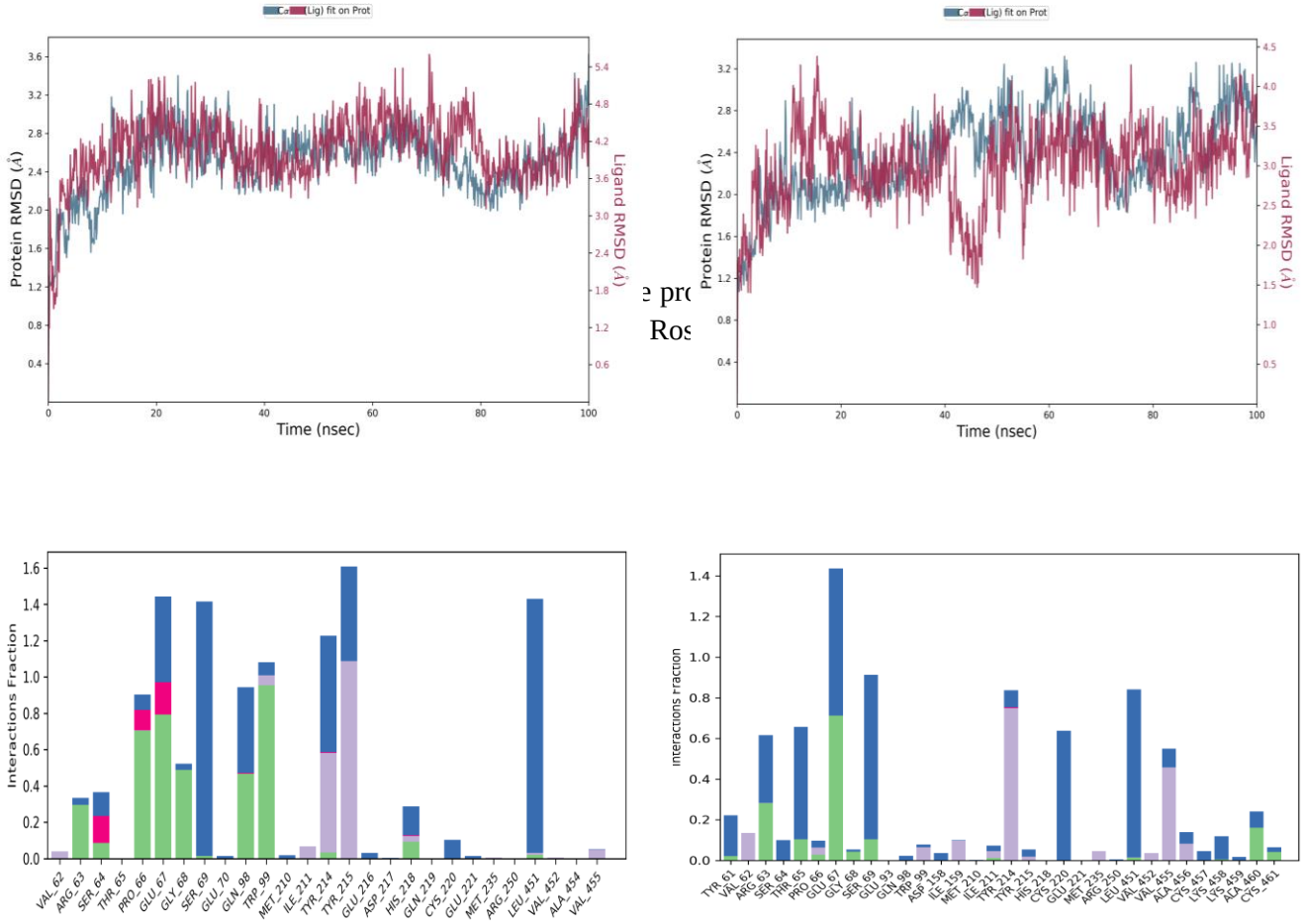

Figure 10. Plot (stacked bar charts) of Protein contacts with the selected molecule directed during the simulation of the molecule Rutin (LEFT), and Rosmarinic acid (RIGHT)

Hydrogen bonds, Hydrophobic contacts, Ionic interactions, and Water bridges are the four types of protein-ligand interactions. The type of amino acid sequences existing in the allosteric domain

of the targeted receptor is critical meant for the stability of the protein-ligand composite/complex. Results of the MD simulation investigation demonstrate that the compounds Rutin and Rosmarinic acid were slightly steady in the protein-ligand composite. Docking tests demonstrated that the amino acid residues TRP-99 and GLY-68 made hydrogen bond contact with the molecule Rutin along the trajectory, with 94 and 37% interaction, respectively. The amino acid residues ARG-63 (28%), PRO-66 (67%), GLU-67 (54%, 20%), and GLN-98 (33%) were found to be involved in the creation of hydrogen bonds with Rutin. The second hit molecule Rosmarinic acid demonstrated substantial hydrogen bond interaction with amino acid sequences ARG-63 (13%) & GLU-67(32%, 233%) during simulation (Figures 11).

All of the amino acid contacts/interactions that were identified throughout the docking investigations of the target molecules were detected in the course of the subsequent dynamic simulation studies. The results indicate that the permanence of the protein molecule interaction was maintained throughout the simulation period with small modifications to the backbone. Established on the MD simulations, it was observed that the hit molecules exhibited a higher frequency of water-mediated couplings and amino-acid-mediated water bridges. Figures 12 depicts timeline links featuring amino acid residues present in the targets. The greater the number of amino acid linkages shows darker the colour.

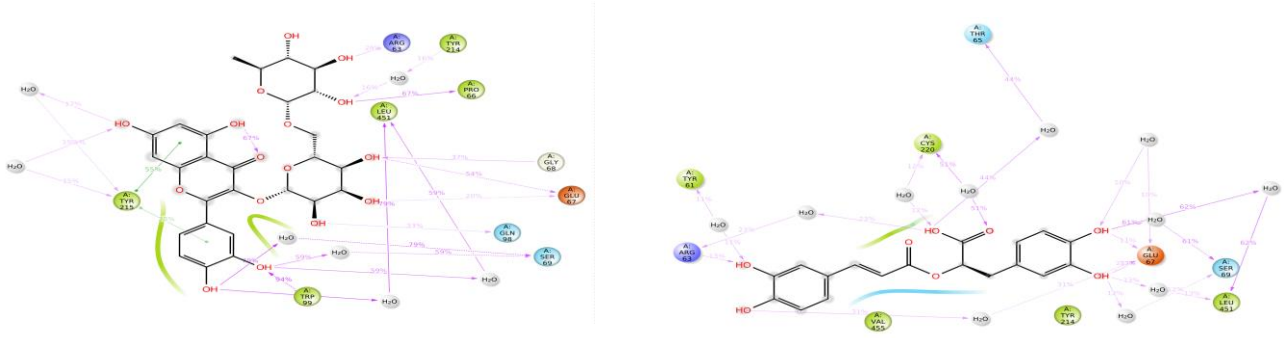

Figure 11. Illustrates the atomic interactions of the ligand Rutin (LEFT), and Rosmarinic acid (RIGHT) with the targeted residues in PDB-3IMX

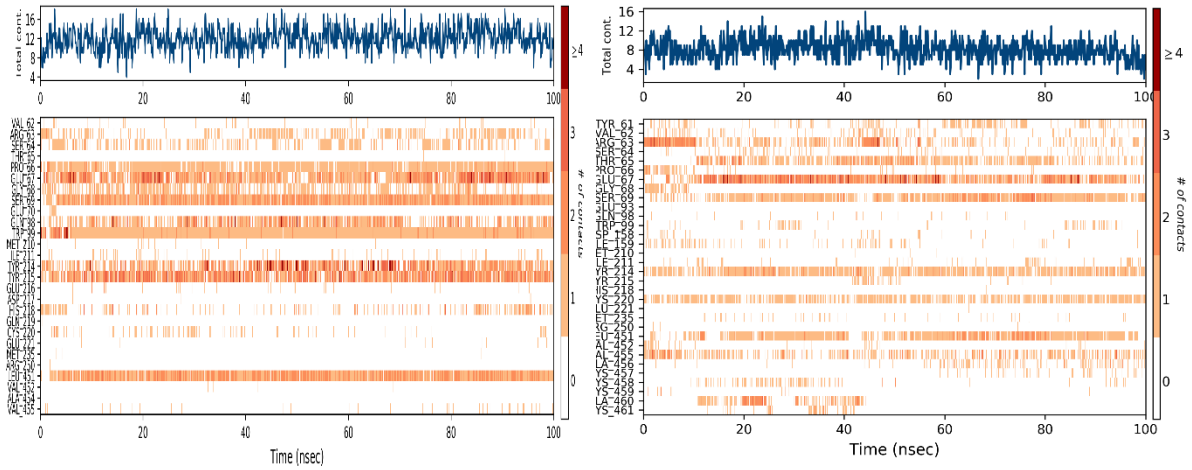

Figure 12. Throughout the trajectory, the protein makes specific interactions with the ligand. (Darker colour indicates more precise ligand interaction) Rutin (LEFT), and Rosmarinic acid (RIGHT) (PDB-3IMX).

Conclusion

In this study, computational approaches were employed to elucidate the roles of phytochemicals as Glucokinase activators. Rutin and Rosmarinic acid were predicted to have the most promise as Glucokinase activators based on results from molecular docking and MM-GBSA analyses. Additional evidence supporting these findings comes from molecular dynamic simulations, which elucidated the possible binding mechanisms of Rutin and Rosmarinic acid in the Glucokinase active site. According to the results of the studies, Rutin and Rosmarinic acid are two potential pharmaceutical agents for triggering the Glucokinase enzyme and regulating blood glucose levels. This investigation results lent credence to the use of these phytochemicals in diabetes management and hinted that activating Glucokinase could be one of their potential mechanisms of action. Additional exploration is obligatory to endorse the results of the Insilco investigations to confirm the safety and efficacy of Rutin and Rosmarinic acid as potent Glucokinase activators.

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