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LETTERS

Molecular docking-guided screening of phytoconstituents from *Artemisia princeps* as Allosteric Glucokinase Activators

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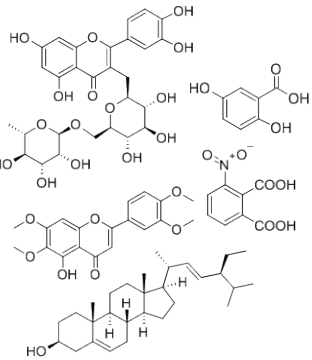
Article

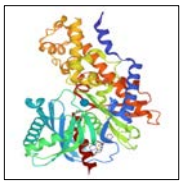
ABSTRACT

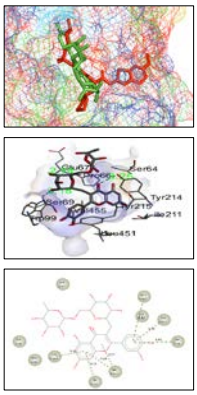


Database of phytoconstituents

Drug-likeness SwissADME web tool and Lipinski's rule of 5

Docking with glucokinase (PDB ID: 3IMX)





Analysis of Docking Results Binding interactions of the ligands with glucokinase

Prediction of Toxicity using pkCSM

Glucokinase (GK) occurs in pancreatic β-cells and liver cells. GK plays a crucial role in whole-body glucose homeostasis. GK is often referred to as a glucose sensor in the β-cells. Small molecule GK activators not only reduce fasting and basal blood glucose levels but also improve glucose tolerance. The present investigation was proposed to screen some phytoconstituents (from *Artemisia princeps*) as allosteric activators of the human GK enzyme using *in silico* molecular docking. A library of phytoconstituents reported in *Artemisia princeps* was evaluated for the prediction of drug-like properties by *in silico* approach. Molecular docking studies of the phytoconstituents with GK were performed using AutoDock vina in order to explore binding interactions between the phytoconstituents and GK enzyme followed by *in silico* prediction of toxicity of these phytoconstituents. The selected phytoconstituents showed good pharmacokinetic parameters for oral bioavailability and drug-likeness as contrived by Lipinski's rule of five. Four compounds (rutin, 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, daucosterol and methyl commate D) showed appreciable binding interactions with the allosteric site residues of the GK enzyme as per docking results. These screened phytoconstituents may serve as promising leads for further development of clinically useful and safe allosteric activators of the human GK enzyme.

Keywords: Allosteric, Artemisia princeps, Docking, Glucokinase, GK activators, Type 2 diabetes.

INTRODUCTION

Diabetes mellitus is probably one of the oldest diseases known to man. It was first reported in an Egyptian manuscript about 3000 years ago.[1] Diabetes mellitus (DM) is often simply regarded as diabetes, a syndrome of disordered metabolism (food) with abnormally high blood glucose levels resulting from a defect in either insulin secretion or insulin action or both. Based on aetiology the terms type 1 DM (T1DM) and type 2 DM (T2DM) were widely used to describe insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus, respectively.[2-3] T2DM is far more common than T1DM; accounting for about 90 % of all cases of DM. Currently, North America, Europe and Japan have the highest prevalence of diabetes and India is supposed to surpass them with 79 million cases of diabetes by 2030. Current anti-diabetic drugs include oral hypoglycaemics, bromocriptine, insulin and insulin analogues.[4-5] Despite the availability of several options to treat T2DM currently, no single oral antidiabetic drug is capable of achieving acceptable, long-

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lasting blood glucose control in the majority of patients thus making it necessary to combine 2-3 drugs to achieve adequate glycemic control. So, the scientific community is currently focusing on developing new, safe and clinically different anti-diabetic agents that can be used as mono-drug therapy with improved efficacy.[6]

Glucokinase (GK) is a type IV isoenzyme belonging to the hexokinase family. GK plays a role in the glucose metabolism of the liver and is a glucose sensor in pancreatic β-cells involved in glucose-dependent insulin release.[7] GK catalyses the conversion of glucose to glucose-6-phosphate (G-6-P), the first step of glucose metabolism. GK is selectively expressed in pancreatic β-cells and liver parenchymal cells (hepatocytes). Indeed, studies in transgenic animals have confirmed that GK plays a crucial role in whole-body glucose homeostasis. GK is often referred to as a 'glucose sensor' in β-cells. Hepatic GK activity and the intracellular location of GK are controlled by a protein produced in hepatocytes called GK regulatory protein (GKRP). Small molecules may activate GK either directly or by destabilizing the GK-GKRP complex.[8] GK activators not only reduce fasting and basal blood glucose levels but also improve glucose tolerance. Although various types of GK activators have been reported in the literature, they suffer from serious side effects, especially hypoglycaemia.[6,7,9] Various natural/plant-derived drugs with potential GK action have recently been reported in the literature.[5,10]

Hikino et al., investigated the mechanism of action of ganoderan B (a glycan derived from an aqueous extract of *Ganoderma lucidum* fruit). Ganoderan B boosted hepatic GK, phosphofructokinase, and glucose-6-phosphate dehydrogenase activity while decreasing glycogen synthetase activity.[11] Qian-Cutrone et al., reported GK activators glucolipsin A and B extracted from alcoholic extracts of *Streptomyces purpurogeniscleroticus* and *Nocardia vaccinia*, respectively.[12] Singh et al., investigated the mode of action of coagulanolide (extracted from *Withania coagulans* fruits) on hepatic glucose-regulating enzymes in type 2 diabetic mice. After administration of coagulanolide, hepatic GK activity has risen greatly (approximately 37%) compared to the untreated group.[13] Tatanans A, B, and C, three unique sesquilignans, were extracted from *Acorus tatarinowii* Schot rhizomes and tested for GK-activating potential. With $EC_{1.5}$ values ranging from 0.16 to 1.85 μM, these compounds showed different structural features and a substantial GK-activating effect.[14-15] Kang et al., examined the effect of "eupatilin" obtained from "*Artemisia princeps*" on blood glucose homeostasis and pancreatic β-cell activity. Eupatilin administration improved hepatic GK activity, which resulted in lower fasting sugar levels and elevated hepatic glycogen metabolism.[16] A comparison of the mechanisms of GK activation of five mulberry (*Morus alba*) bioactive components suggested that they might be used to improve postprandial glucose disposal by acting as GK activators. At 12.5 μm, both 1-deoxynojirmycin and resveratrol were observed to increase GK dislocation.[17] Mahmoodi et al., studied the impact of an extract from a hydro-alcoholic content of Persian shallot (*Allium hirtifolium* Boiss) on the glycaemic content of blood, insulin, GK activity and GK expression.[18] In a molecular docking study, quercetin from Persian shallot showed two H-bonds with Arg63 residue (bond lengths of 3.08 Å and 4.49 Å) in the allosteric site of GK.[19] Angadi et al., docked "guggultetrol" isolated from the foliage of the waterlily (*Nymphaea pubescens*) in GK's allosteric site.[20] Mangiferin, a C-glycosylxanthone polyphenol derived from the mango tree (*Mangifera indica*), exhibits anti-glycaemic effects. Mangiferin was reported as a GK activator with an EC_{50} value of 156 μM.[21] Camptothecin showed considerable binding interactions with PPARγ, GK and insulin receptors in docking studies.[22] Ethyl alcohol extract of *Sapium ellipticum* leaves activated GK in induced diabetic rats.[23] Kaempferol (obtained from *Syzygium cumini*) demonstrated two H-bonds (4.9 Å and 4.7 Å) with Arg63 GK protein.[24]

In pursuit of the fact that GK plays an important role in the treatment of T2DM and the role of natural products in GK activation and decreasing blood sugar levels, the present study was planned to screen phytoconstituents from the plant *Artemisia princeps* as allosteric GK activators using molecular docking studies.

EXPERIMENTAL

Design of database

A database of the phytoconstituents present in *Artemisia princeps* including organo-sulphur compounds, alcohols, alkaloids, flavonoids, amino acids, carboxylic acids, esters, fatty acids, fatty acid esters, terpenes, phenols, saponins steroids, and phenolic compounds was prepared for further *in silico* studies (Table S1).

Prediction of drug-like properties

In silico methods for the determination of absorption, distribution, metabolism, and excretion (ADME) parameters depend on the theoretically derived statistical models. The compounds selected for this study were evaluated for their ADME parameters by employing the Swiss online ADME web tool and accessed using Lipinski's rule of five for drug-likeness.[25-26]

Molecular docking

In silico docking studies of the selected compounds were carried out using AutoDock vina and AutoDock tools.[27] The crystal structure of GK (PDB ID: 3IMX) was retrieved from the protein data bank (www.rcsb.org). The crystal structure was prepared individually by removing existing ligands and water molecules while missing hydrogens were added using AutoDock tools. Thereafter, non-polar hydrogens were merged while polar hydrogens were added to the protein file and then saved into docking-ready PDBQT format. 2-D chemical structures of all the ligands (standard as well as test compounds) were retrieved from PubChem and converted into 3D conformation (mol2 format) using the Frog2 server.[28] The ligand molecules were further saved into the docking-ready PDBQT format using AutoDock tools.[27] Docking of the ligands with GK and determination of binding affinities was carried out using AutoDock vina.[29] The grid center for docking was detected as X = 0.380, Y = 2.721, Z = -16.334 with the dimension of the grid box 30 × 30 × 30. The reference ligand was docked with GK and compared with that of the reference activator for determining the accuracy of the docking

protocol. The 3-D optimize d ligands were docked with the refined protein and scored using the scoring function. The binding free energy (ΔG, kcal/mol) for each compound was reported in the log file and the binding interactions of the ligands in the allosteric site of GK protein were analysed using (PyMOL (Schrödinger, Inc.) and Discovery Studio (Dassault Systemes BIOVIA).[30] Coordinates of the complex with the top compound (rutin) having the lowest binding free energy obtained from the docking results were selected to perform molecular dynamics simulations. The molecular dynamics simulation was carried out for 1 ps. The solvation was carried out before doing the dynamics by adding thirty-four Na++ counter ions to neutral the system. The final protein-ligand complex was placed into the periodic boundary conditions in all 3D space. Initially, the energy minimization step

Table 1. ADME properties of the selected phenolic compounds predicted using the SwissADME web server.

Sr. No.	Compound	Mol. Wt.	Log P	HBAs	HBDs	TPSA	GI ab.	BBB permeant
1	5-Desmethyl-sinensetin	358.34	3.45	7	1	87.36	High	No
2	Jaceosidin	330.29	2.36	7	3	109.36	High	No
3	Acacetin	284.26	2.56	5	2	79.90	High	No
4	Genkwanin	284.26	2.48	5	2	79.90	High	No
5	Eupatilin	344.32	3.11	7	2	98.36	High	No
6	Eupafolin	316.26	2.13	7	4	120.36	High	No
7	Apigenin	270.24	1.89	5	3	90.90	High	No
8	Hispidulin	300.26	2.27	6	3	100.13	High	No
9	Chrysoplenetin	374.34	3.36	8	2	107.59	High	No
10	3,5,4'-Trihydroxy-6,7,3'-trimethoxy flavone	360.31	2.58	8	3	118.59	High	No
11	5,4'-Dihydroxy-6,7,3',5'-tetramethoxyflavone	374.34	3.39	8	2	107.59	High	No
12	Rutin	608.54	0.21	15	10	260.20	Low	No
13	5,4'-Dihydroxy-6,7,3'-trimethoxyflavone	344.32	3.07	7	2	98.36	High	No
14	6-Acetyl-2,2-dimethylchroman-4-one	218.25	2.28	3	0	43.37	High	Yes
15	Sesamin	354.35	3.46	6	0	55.38	High	Yes
16	Salicylic acid	138.12	1.13	3	2	57.53	High	Yes
17	m-Hydroxy benzoic acid	138.12	0.86	3	2	57.53	High	Yes
18	p-Hydroxy benzoic acid	138.12	0.85	3	2	57.53	High	Yes
19	Vanillic acid	168.15	1.40	4	2	66.76	High	No
20	Gentisic acid	154.12	0.49	4	3	77.76	High	No
21	Protocatechuic acid	154.12	0.66	4	3	77.76	High	No
22	Syringic acid	198.17	1.54	5	2	75.99	High	No
23	Phloroglucinol	126.11	0.66	3	3	60.69	High	Yes
24	6-Methyl-3-isopropylphenol	150.22	2.24	1	1	20.23	High	Yes
25	2,6-Di-tert-butyl-4-methylphenol	220.35	3.33	1	1	20.23	High	Yes
26	Phenol	94.11	1.24	1	1	20.23	High	Yes
27	1,2-Benzenediol (catechol)	110.11	1.13	2	2	40.46	High	Yes
28	1,4-Benzenediol (hydroquinone)	110.11	0.92	2	2	40.46	High	Yes
29	1,3-Benzenediol (resorcinol)	110.11	0.96	2	2	40.46	High	Yes
30	2,6-Dimethoxy phenol	154.16	1.85	3	1	38.69	High	Yes
31	Diethyl phthalate	222.24	2.26	4	0	52.60	High	Yes
32	Dibutyl phthalate	278.34	2.97	4	0	52.60	High	Yes
33	Butyl isobutyl phthalate	292.37	3.86	4	0	52.60	High	Yes
34	Diisooctyl phthalate	390.56	5.42	4	0	52.60	High	No
35	3-Nitrophthalic acid	211.13	0.09	6	2	120.42	High	No
36	Stigmasterol	412.69	5.08	1	1	20.23	Low	No
37	β-Sitosterol	414.71	5.05	1	1	20.23	Low	No
38	Ergosterol peroxide	428.65	4.60	3	1	38.69	High	No
39	Daucosterol	576.85	5.17	6	4	99.38	Low	No
40	γ-Sitosterol	414.71	5.07	1	1	20.23	Low	No
41	Friedelin	426.72	4.49	1	0	17.07	Low	No
42	β-Amyrin	426.72	4.63	1	1	20.23	Low	No
43	β-Amyrin acetate	468.75	4.86	2	0	26.30	Low	No
44	Wrightial	400.64	4.10	2	1	37.30	High	No
45	Wrightial acetate	442.67	4.50	3	0	43.37	Low	No
46	Sajabalal acetate	468.67	4.77	3	0	43.37	Low	No
47	Ursolic acid	456.70	3.95	3	2	57.53	Low	No
48	Methyl commateD	486.73	4.08	4	2	66.76	High	No
49	3-Keto-urs-12-ene	424.70	4.58	1	0	17.07	Low	No
50	α-Amyrin (viminalol)	426.72	4.80	1	1	20.23	Low	No

Topological surface area; GI ab.: Gastro-intestinal absorption; BBB permeant: Blood-brain barrier permeation.

was carried out with 500 steps, followed by heating for 4ps for 300 K temperature and saved the result at every 2ps. To equalize the system equilibration step was carried out for 10ps and the final production was done for 10ps.

Prediction of toxicity

All the compounds were evaluated *in silico* for the prediction of the possible toxicity of these compounds using the "pkCSM" online computer program.[31]

RESULTS AND DISCUSSION

Prediction of drug-likeness

"Drug-like" molecules usually follow these five criteria: molecular weight (Mol. Wt.) up to 500, logP = 5 (milog), up to 10 and 5 hydrogen bond acceptors (HBAs) and donors (HBDs), respectively. The compounds that do not violate these rules can be further analysed to select the best drug candidate. The results obtained from the ADME study revealed that the selected compounds showed good pharmacokinetic parameters for oral bioavailability (Table 1) and drug-likeness as contrived by Lipinski's rule of five.[32] However, rutin failed Lipinski's rule of five for drug-likeness (with 3 violations, i.e., Mol. Wt, HBAs and HBDs). Amongst the compounds tested, only a few compounds (rutin, stigmasterol, β-sitosterol, daucosterol, γ-sitosterol, friedelin, β-amyrin, β-amyrin acetate, wrightial acetate, 27-norcycloart-20(21)-ene-25-al-3β-ol acetate, ursolic acid, 3-keto-urs-12-ene and α-amyrin) were predicted to be having low GI absorption.

Molecular docking

In silico virtual screening is an effective technique to find a safe and effective solution to major disorders like T2DM. The main objective of the present research was to identify potential allosteric activators of human GK (an enzyme involved in T2DM). In silico molecular docking studies were performed to explore the affinity and binding interactions of the designed molecules using AutoDock vina in the active site of GK (PDB ID: 3IMX). The docking protocol used in this study was first validated by redocking the co-crystallized ligand of GK. The re-docked GK activator produced a pose similar to that of the co-crystallized GK activator in the allosteric site of GK with ΔG of -11.1 kcal/mol, validating the accuracy of the docking methodology used (Figure S1A). The 3IMX ligand showed strong hydrogen bond interactions with Ser69 (3.20 Å) and Arg63 (3.50 and 3.20 Å) residues in the allosteric site of the GK protein (Figure S1B).

The selected phytoconstituents were docked in the allosteric site of GK, which is surrounded by the b1 strand and a5 helix of the large domain, the C-terminal a13 helix of the small domain, and the GK-specific connecting region I (Ser64-Gly72). The allosteric site of GK comprises Arg63, Ser69, Tyr215, Met210, Tyr214, Val452 and Val455 residues. Binding energy (ΔG) is an important factor for predicting a potential drug candidate against any disease. The lower the binding energy, the more stable will be the complex. Some of the docked compounds showed significant binding interactions in the allosteric site of GK as established by analysing their bonding interactions and ΔG (kcal/mol) of the best-docked poses (Table 2). Out of the phytoconstituents docked with GK in this study, rutin, 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, daucosterol and methyl commate D showed appreciable binding in the allosteric site of GK as determined by analyzing the hydrogen bond and hydrophobic interactions of the best-docked poses. On the basis of their lowest binding free energy (kcal/mol) and docking interactions in the allosteric site, these compounds were further analyzed in detail by PyMOL and Discovery Studio visualiser. An overlay of the docked pose of these compounds (rutin, 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, daucosterol and methyl commate D) with that of the 3IMX ligand showed that these

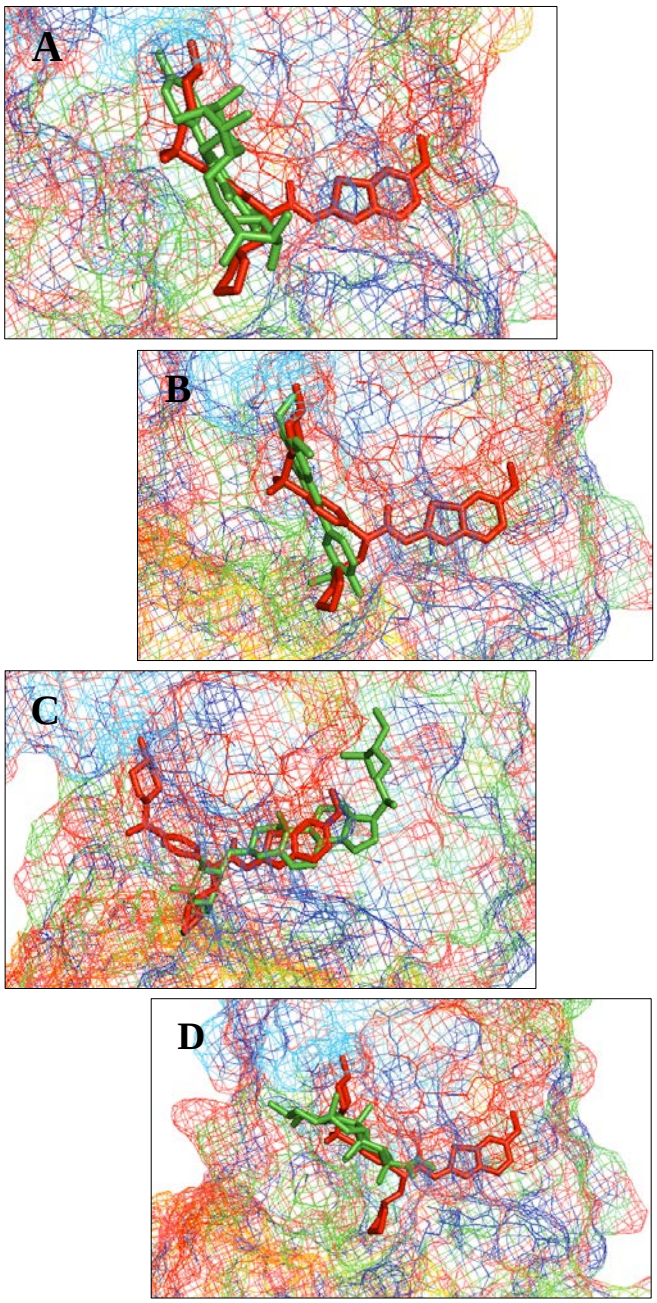

Figure 1. Overlay of the docked poses of rutin (A), 5,4'-dihydroxy-6,7,3'-trimethoxyflavone (B), daucosterol (C) and methyl commate D (D) (green sticks) with that of 3IMX ligand (red sticks) in the allosteric site of GK protein.

compounds had a similar binding pattern in the allosteric site of GK enzyme as that of the co-crystallized ligand (Figure 1). The docking studies of these molecules suggested a complementary fit in the allosteric site of GK.

Hydrogen bond interactions of the selected phytoconstituents (rutin, 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, daucosterol and methyl commate D) with the allosteric site residues of the GK protein (PDB ID: 3IMX) are presented in Figure 2. Rutin showed three hydrogen bonds with Ser64, Glu67 and Ser69 residues of GK (with bond distances of 2.74, 3.18 and 3.25 Å, respectively). 5,4'-Dihydroxy-6,7,3'-trimethoxyflavone showed two hydrogen bonds with Arg63 and Ser69 residues of GK (with bond distances of 2.86 and 2.97 Å, respectively). Daucosterol showed one hydrogen bond with Tyr214 residue of GK (with bond distance of 2.97 Å). Methyl commate D also showed one hydrogen bond with Arg63 residue of GK (with bond distance of 3.05 Å).

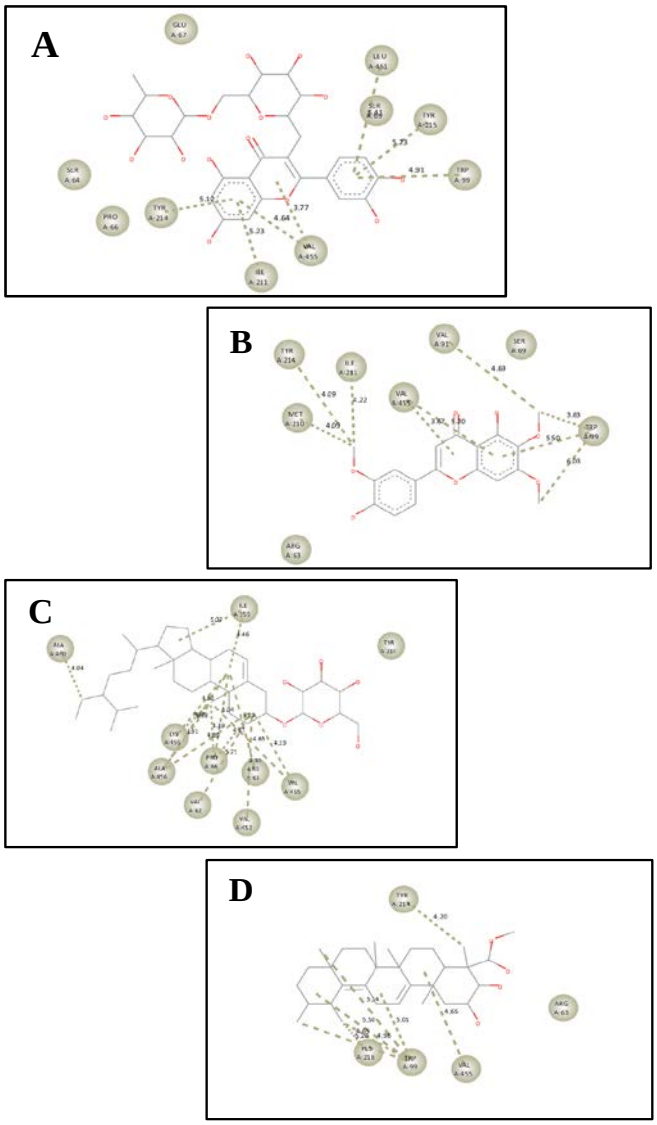

Figure 3. Docked poses showing hydrophobic interactions of rutin (A), 5,4'-dihydroxy-6,7,3'-trimethoxyflavone (C), daucosterol (C) and methyl commate D (D) in the allosteric site of human GK enzyme (PDB ID: 3IMX).

Hydrophobic interactions of the selected phytoconstituents with the allosteric site residues of human GK protein (PDB ID: 3IMX) are presented in Figure 3. These 4 phytoconstituents protruded in the hydrophobic pocket of GK to form strong hydrophobic interactions. Rutin showed hydrophobic interactions with Trp99, Ile211, Tyr214, Tyr215, Leu451 and Val455 residues in the allosteric site of GK. 5,4'-Dihydroxy-6,7,3'-trimethoxyflavone displayed strong hydrophobic interactions with Val91, Trp99, Met210, Ile211, Tyr214 and Val455 residues in the allosteric site of GK. Daucosterol showed hydrophobic interactions with Val62, Arg63, Pro66, Ile159, Val452, Val455, Ala456, Lys459 and Ala460 residues in the allosteric site of GK. Methyl commate D showed hydrophobic interactions with Trp99, Tyr214, His218 and Val455 residues in the allosteric site of GK. These molecular docking studies thus helped us in predicting that these

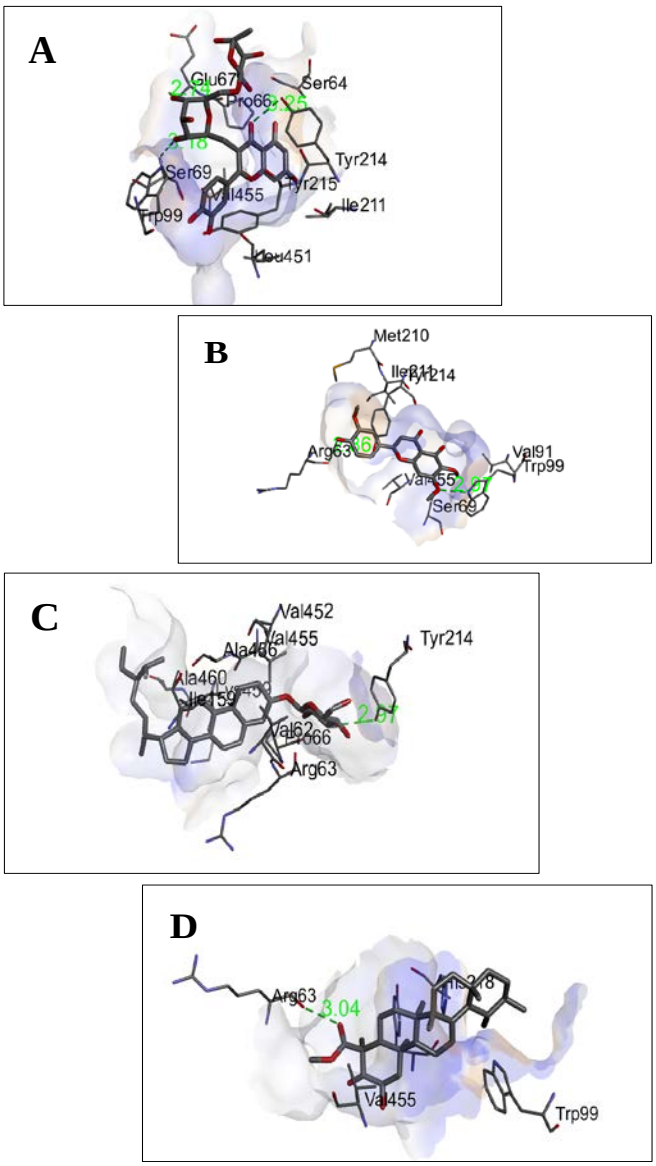

Figure 2. Docked poses showing hydrogen bond interactions of rutin (A), 5,4'-dihydroxy-6,7,3'-trimethoxyflavone (C), daucosterol (C) and methyl commate D (D) in the allosteric site of human GK enzyme (PDB ID: 3IMX).

phytoconstituents could act as potential allosteric activators of the human GK enzyme.

The molecular dynamics simulation of the best-docked pose of rutin (best-docked compound) was carried out to elucidate the stability of the docked complex. The plot between the total energy of the complex with respect to the time(ps) showed the stability of the complex (rutin-GK) sustained during the molecular dynamics simulations (Figure 4). The plot between temperature applied during the simulations and time (ps) confirmed the stability and sustainability of the complex with

Table 2. Hydrogen bond interactions and docking score (ΔG, kcal/mol) of the best docked poses of phytoconstituents with GK.

Sr. No.	Ligand (compound)	ΔG (kcal/mol)	No. of H-bonds	Residue(s) involved in H-bonds	Bond length (Å)
1	5-Desmethyl-sinensetin	-7.4	1	Arg63	3.25
2	Jaceosidin	-7.5	1	Arg63	2.88
3	Acacetin	-7.2	0	-	-
4	Genkwanin	-6.9	0	-	-
5	Eupatilin	-7.6	1	Arg63	2.70
6	Eupafolin	-7.2	1	Arg63	2.80
7	Apigenin	-7.2	1	Arg63	3.55
8	Hispidulin	-7.4	1	Arg63	2.77
9	Chrysoplenetin	-7.0	1	Arg63	3.08
10	3,5,4'-Trihydroxy-6,7,3'-trimethoxy flavone	-7.0	1	Arg63	2.71
11	5,4'-Dihydroxy-6,7,3',5'-tetramethoxyflavone	-7.2	1	Arg63	2.94
12	Rutin	-8.0	3	Ser64, Glu67, Ser69	2.74, 3.18, 3.25
13	5,4'-Dihydroxy-6,7,3'-trimethoxyflavone	-8.4	2	Arg63, Ser69	2.86, 2.97
14	6-Acetyl-2,2-dimethylchroman-4-one	-7.6	1	Arg63	3.47
15	Sesamin	-7.6	1	Arg63	3.83
16	Salicylic acid	-5.1	1	Arg63	3.03
17	m-Hydroxy benzoic acid	-5.2	1	Ser69	3.08
18	p-Hydroxy benzoic acid	-5.0	1	Arg63	4.01
19	Vanillic acid	-5.1	1	Arg63	2.84
20	Gentisic acid	-5.4	1	Arg63	2.78
21	Protocatechuic acid	-5.2	1	Arg63	2.84
22	Syringic acid	-5.2	1	Arg63	3.01
23	Phloroglucinol	-4.6	1	Arg63	3.21
24	6-Methyl-3-isopropylphenol	-5.7	1	Arg63	3.10
25	2,6-Di-tert-butyl-4-methylphenol	-6.8	0	-	-
26	Phenol	-4.5	0	-	-
27	1,2-Benzenediol (catechol)	-4.4	1	Arg63	2.99
28	1,4-Benzenediol (hydroquinone)	-4.3	1	Arg63	3.25
29	1,3-Benzenediol (resorcinol)	-4.4	1	Arg63	3.16
30	2,6-Dimethoxy phenol	-4.6	1	Arg63	3.14
31	Diethyl phthalate	-6.3	0	-	-
32	Dibutyl phthalate	-6.6	0	-	-
33	Butyl isobutyl phthalate	-6.9	0	-	-
34	Diisooctyl phthalate	-6.6	0	-	-
35	3-Nitrophthalic acid	-5.9	0	-	-
36	Stigmasterol	-7.6	0	-	-
37	β-Sitosterol	-6.9	0	-	-
38	Ergosterol peroxide	-7.0	0	-	-
39	Daucosterol	-8.6	1	Tyr214	2.97
40	γ-Sitosterol	-7.5	0	-	-
41	Friedelin	-7.6	0	-	-
42	β-Amyrin	-7.7	0	-	-
43	β-Amyrin acetate	-7.8	0	-	-
44	Wrightial	-6.9	0	-	-
45	Wrightial acetate	-7.0	0	-	-
46	Sajabalal acetate	-6.5	1	Ser69	3.09
47	Ursolic acid	-7.6	0	-	-
48	Methyl commate D	-8.5	1	Arg63	3.04
49	3-Keto-urs-12-ene	-7.5	0	-	-
50	α-Amyrin (viminalol)	-6.8	0	-	-
51	Reference GK activator*	-11.1	3	Arg63, Ser69	3.20 and 3.50, 3.20

*Reference GK activator: 3IMX Ligand, 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.

Table 3. ADME properties of the selected phenolic compounds predicted using the SwissADME web server.

Sr. No.	AMES toxicity	Max. tolerated dose	hERG I inhibition	hERG II inhibition	Oral rat acute toxicity	Oral rat chronic toxicity	Hepato-toxicity	Skin toxicity
1	No	0.679	No	Yes	2.744	1.576	No	No
2	No	0.989	No	No	2.310	2.476	No	No
3	No	0.573	No	Yes	2.350	1.448	No	No
4	No	0.006	No	No	1.923	1.843	No	No
5	Yes	0.993	No	Yes	2.473	2.364	No	No
6	No	0.868	No	No	2.059	2.288	No	No
7	No	0.555	No	No	2.225	1.653	No	No
8	No	0.555	No	No	2.225	1.653	No	No
9	No	0.733	No	Yes	2.142	1.532	No	No
10	No	0.538	No	No	2.020	2.725	No	No
11	No	0.419	No	No	1.960	1.516	No	No
12	No	0.49	No	Yes	2.482	5.126	No	No
13	No	0.547	No	Yes	2.572	1.513	No	No
14	No	0.801	No	No	1.652	2.096	No	No
15	Yes	0.299	No	No	3.170	1.614	No	No
16	No	0.605	No	No	2.048	2.213	No	No
17	No	0.993	No	No	1.863	2.978	No	No
18	No	1.002	No	No	1.862	2.971	No	No
19	No	1.408	No	No	2.203	1.982	No	No
20	No	0.027	No	No	2.016	2.366	No	No
21	No	1.379	No	No	2.180	1.950	No	No
22	No	1.652	No	No	2.086	2.470	No	No
23	No	0.456	No	No	1.891	2.241	No	No
24	No	0.522	No	No	2.334	2.229	Yes	Yes
25	No	0.201	No	No	2.207	2.255	No	Yes
26	No	1.113	No	No	1.910	1.981	No	Yes
27	No	0.610	No	No	2.144	2.172	No	Yes
28	No	0.610	No	No	2.144	2.172	No	Yes
29	No	1.020	No	No	1.983	2.769	No	Yes
30	No	1.245	No	No	1.924	2.249	No	No
31	No	1.136	No	No	1.950	2.680	No	No
32	No	1.289	No	No	1.855	2.291	No	No
33	No	1.265	No	No	1.655	2.249	No	No
34	No	1.112	No	Yes	1.249	2.695	No	No
35	No	0.635	No	No	2.039	2.513	No	No
36	No	0.318	No	Yes	2.454	1.125	No	No
37	No	0.272	No	Yes	2.474	1.108	No	No
38	No	0.013	No	No	2.041	1.868	Yes	No
39	No	0.546	No	No	2.598	3.023	No	No
40	No	0.272	No	Yes	2.474	1.108	No	No
41	No	0.260	No	Yes	2.454	1.063	No	No
42	No	0.018	No	Yes	2.373	1.274	No	No
43	No	0.375	No	Yes	2.322	2.149	No	No
44	No	0.735	No	No	2.702	2.222	No	No
45	No	0.275	No	Yes	2.441	2.036	No	No
46	No	0.172	No	Yes	2.293	2.178	No	No
47	No	0.650	No	No	4.086	2.043	Yes	No
48	No	0.017	No	No	2.382	1.763	No	No
49	No	0.140	No	Yes	2.071	1.038	No	No
50	No	0.186	No	Yes	2.383	1.280	No	No

AMES toxicity: Mutagenic (or carcinogenic) potential; Max. tolerated dose: Maximum tolerated human dose (log mg/kg/day); hERG I & hERG II inhibition (cardio-toxicity); Oral rat acute toxicity: (LD_{50}) (mol/kg); Oral rat chronic toxicity: log mg/kg_bw/day; Skin toxicity: Skin sensitization.

respect to the temperature (Figure 5). These molecular dynamics simulations confirmed the stability of the ligand-protein complex (i.e., rutin-GK protein).

Artemisia genus has been reported as a rich source of flavonoids, including eupatilin, eupatorine and rutin. Several lines of evidence suggest that flavonoids that originated from vegetables and medicinal plants have beneficial effects on diabetes by improving glycaemic control, lipid profile, and antioxidant status. Eupatilin showed strong hydrogen bond interaction with Arg63 residue of GK (binding energy of -7.6

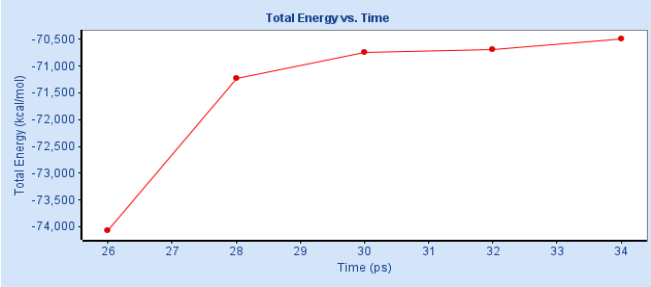

Figure 4. Plot between the total energy of the complex with respect to the time(ps)

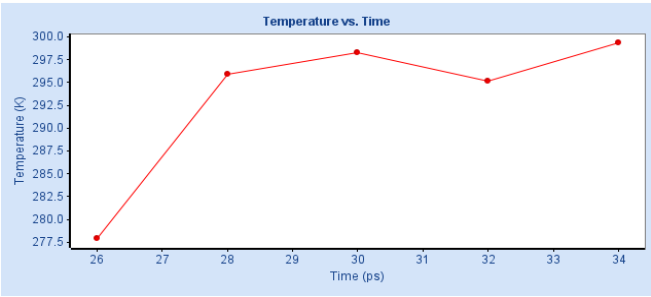

Figure 5. Plot between temperature applied during the simulations and time (ps).

kcal/mol) *in silico* supporting the *in vitro* GK activation and *in vivo* glucose lowering activity reported earlier.[13] Rutin is a flavonoid found in many plants and shows a wide range of biological activities including anti-inflammatory, antioxidant, neuroprotective, nephroprotective and hepatoprotective effects.[33-36] Cirsilineol (5,4'-dihydroxy-6,7,3'-trimethoxyflavone) administration at 7.5 and 15 mg/kg for 28 days reduced the blood glucose level to a significant level in streptozotocin-induced diabetic animals.[37-38] Daucosterol, a natural phytosterol-like compound isolated from *Costus pictus* (after enteric coating) significantly reduced blood glucose levels and increased insulin release in rats at a very low dose.[39-40]

Prediction of toxicity

The possible toxicity (mutagenic, carcinogenic, cardiotoxicity, immunotoxicity, skin irritation and reproductive toxicity) for the selected compounds was accessed using the pkCSM online tool (Tables 3). pkCSM is a freely accessible web server which relies on graph-based signatures and provides an integrated platform to rapidly evaluate the pharmacokinetic and toxicity properties of small molecules.[31] Mutagenicity (AMES toxicity) was predicted for eupatilin and sesamin. Cardiac toxicity (hERG II inhibition) was predicted for 17 compounds (5-desmethyl-sinensetin, acacetin, eupatilin, chrysoplenetin, rutin, 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, diiso-octyl phthalate, stigmasterol, β-sitosterol, γ-sitosterol, friedelin, β-amyrin, β-amyrin acetate, wrightial acetate, 27-norcycloart-20(21)-ene-25-al-3β-ol acetate, 3-keto-urs-12-ene and α-amyrin. Hepatotoxicity was predicted for 6-methyl-3-isopropylphenol, ergosterol peroxide and 27-norcycloart-20(21)-ene-25-al-3β-ol acetate). Skin sensitivity was predicted for 6-methyl-3-isopropylphenol, 2,6-di-tert-butyl-4-methylphenol, phenol, 1,2-benzenediol, 1,4-benzenediol and 1,3-benzenediol. In this context, the preliminary assessment conducted *in silico* can complement future studies on the safety of these compounds.

CONCLUSION

Molecular docking investigations using AutoDock vina were performed to explore the binding mechanism of the phytoconstituents of *Artemisia princeps* with the human GK (an enzyme involved in T2DM). In current *in silico* docking studies, results clearly demonstrated that amongst the phytoconstituents tested in silico, some compounds (rutin, 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, daucosterol and methyl commate D) showed appreciable binding in the allosteric site of GK enzyme. *In silico* study is actually an added advantage for screening allosteric GK activators and natural compounds may serve as useful leads for the development of clinically useful and safe GK activators. However, structural modifications and further studies on these phytoconstituents are required to develop safe and effective activators of human GK.

SUPPLEMENTARY INFORMATION

The supplementary information file contains the list of phytoconstituents selected for the *in silico* docking studies against the GK enzyme the database of phytoconstituents (Table S1), validation of the docking protocol adopted in this study (Figure S1) and the results of molecular dynamics simulation (Figures S2 and S3).

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