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ORIGINAL RESEARCH

Investigation of phytoconstituents of *Enicostemma littorale* as potential glucokinase activators through molecular docking for the treatment of type 2 diabetes mellitus

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Abstract

Glucokinase (GK) is an enzyme involved in synthesising glucose into glucose-6 phosphate and serves a crucial function in glucose sensing. Therefore, agents that induce GK activation could be used to treat T2DM. The present work has been carried out to investigate the GK activation potential of phytoconstituents of *Enicostemma littorale* through molecular docking. All the phytoconstituents have been screened through the Lipinski rule of 5, Veber's rule, and ADMET properties. From these initial screening, only Apigenin, Ferulic acid, Genkwanin, p-coumaric acid, Protocatechuic acid, Syringic acid, and Vanillic acid have been selected to perform molecular docking studies. The binding free energy and binding mode of the native ligand in the allosteric site of the enzyme have been considered the reference for the other molecules' validation. The native ligand has exhibited − 7.2 kcal/mol binding free energy, whereas; it has formed four hydrogen bonds with THR-228, LYS-169, ASP-78, and GLY-81. Based on these findings, the interactions of phytoconstituents have been justified. Apigenin, genkwanin, and swertiamarin exhibited − 8.7, − 7.5, and − 8.3 kcal/mol binding free energy, respectively, which indicates better enzyme activation than the native ligand. Swertiamarin has formed 08 hydrogen bonds with allosteric amino acid residues, which confirms the excellent enzyme activation by these phytoconstituents. We concluded that if we can isolate and consume the exact active phytoconstituents (GK activators) from this plant, we can use them effectively to treat T2DM. More GK activators can be developed by considering them as a natural lead moiety.

Keywords *Enicostemma littorale* · Glucokinase activators · Apigenin · Swertiamarin · Verticilliside · Betulin

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Abbreviations

E. littorale	*Enicostemma littorale*
WHO	World Health Organization
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
MD	Molecular docking
UFF	Universal Force Field
PDB	Protein Data Bank
RMSD/UB	Root mean square deviation/upper bound
RMSD/LB	Root mean square deviation/lower bound
Mol. Wt.	Molecular weight
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor

Introduction

The *Enicostemma littorale* Blume (*E. littorale*) plays a critical role in human wellbeing. Parts of the plant *E. littoral* were used historically in therapeutic applications against malaria, skin disorders, leprosy, diabetes, etc. This plant's constituents were beneficial therapeutic compounds because they had low toxicity, environmental friendliness, a long shelf life, and no side effects (Murali et al. 2002; Upadhyay and Goyal, 2004; Vasu et al. 2005). It is a noble source of iron, potassium, sodium, calcium, magnesium, silica, chloride, sulphate, phosphate, and vitamins B and C (Maroo et al. 2003, 2002; Sonawane et al. 2010; Thirumalai et al. 2011).

Numerous phytoconstituents have been isolated from the plant, *E. littorale*. The aerial sections of the plant yielded 34% of the dry alcoholic extract and 15.7% of the ash (Patel et al. 2009; Sadique et al. 1987; Sanmugarajah 2013). It has been stated in the literature that this plant produces five alkaloids, two sterols, and volatile oils(Selvaraj et al. 2014; Vishwakarma et al. 2010). Another sapogenin, betulin, was also isolated from this plant (Indumathi et al. 2014). Monoterpene alkaloids such as enicoflavin, gentiocrucine and seven diverse flavonoids have been extracted from the alcoholic extract and the structures have been categorised as apigenin, genkwanin, isovitexin, wertisin, saponarin, 5-o glucosylwertisin and 5-o glucosylisowertisin have also been isolated by Goshal et al. (1974). For the first time in this species, the occurrence of catechins, saponins, steroids, sapogenin, triterpenoids, flavonoids, and xanthones and a new flavonous C-glucoside called verticilliside was isolated(Jahan et al. 2009). The compound swertiamarin was isolated from *E. littoral* by the alcoholic extract(Alam et al. 2011; Leong et al. 2016; Patel et al. 2013; Sonawane et al. 2010; Vaidya et al. 2009a; Vishwakarma et al. 2004). There have also been six phenolic acids identified: vanillic acid, syringic acid, p-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid, and ferulic acid (Abirami and Gomathinayagam 2011; Rathod and Dhale, 2013; Srinivasan et al. 2005). The methanol extract contained numerous amino acids such as L-glutamic acid, tryptophan, alanine, serine, aspartic acid, L-proline, L-tyrosine, threonine, phenylalanine, L-histidine mono-hydrochloride, methionine (Nagarathnamma et al. 2010; Sawant et al. 2011). Diabetic patients are advised to consume 2g of fresh *E. littorale* leaves on daily basis (Upadhyay and Goyal 2004). Therefore, *E. littorale* has been selected to investigate the antidiabetic potential.

Diabetes mellitus is a metabolic condition that increases the body's blood glucose, also known as diabetes (Pal 2009; Zelent et al. 2005). The hormone insulin converts blood sugar into energy-saving cells. In diabetic conditions, either the body cannot produce enough insulin or the insulin it produces cannot be used efficiently (Grewal et al. 2014; Singh et al. 2016). Two significant forms of diabetes are present; type 1 diabetes mellitus (T1DM) is an autoimmune disorder in the pancreas, where insulin is produced, the immune system targets and kills cells. Type 2 diabetes mellitus (T2DM) happens as the body becomes insulin resistant and the blood accumulates sugar (Grewal et al. 2018; Fyfe and Procter 2009).

Glucokinase is an enzyme involved in synthesising glucose into glucose-6 phosphate and serves a crucial function in glucose sensing (Charaya et al. 2018; Grewal et al. 2019). Therefore, agents induce glucokinase activation to be used to treat T2DM. The several various groups of compounds that have been discovered to cause glucokinase activation, such as benzamides (Charaya et al. 2018; Grewal et al. 2019; Li et al., 2011; Park et al. 2015), acetamides (Agrawal et al. 2013; Grewal et al. 2014), carboxamides (Grewal et al. 2014), acrylamides (Sidduri et al. 2010), benzimidazoles (Ishikawa et al. 2009), quinazolines, thiazoles (Agrawal et al. 2013), pyrimidines (Pfefferkorn et al. 2011), and urea derivatives (Castelhano et al. 2005; Grewal et al. 2020; Houze et al. 2013; Kohn et al. 2016; Murray et al. 2005; Polisetti et al. 2004; Sarabu et al. 2008).

After knowing the essential value of the activators of glucokinase in the control of T2DM (Filipski et al. 2012; Grewal et al. 2017, 2019; Grimsby et al. 2003; Matschinsky 2004; Zhang et al. 2016), we investigated the effectiveness of phytoconstituents of *E. littorale* as glucokinase activators, as per the literature which reports the hypoglycemic activity of this plant(Babu and Prince 2004; Maroo et al. 2002; Murali et al. 2002; Patel et al. 2009, 2012; Sonawane et al. 2010; Thirumalai et al. 2011; Upadhyay and Goyal 2004; Vaidya et al. 2009b; Vasu et al. 2005; Vijayvargia et al. 2000; Vishwakarma et al. 2010). We tried to identify the potential natural lead compounds from *E. littorale* as glucokinase activators through their binding mode in the

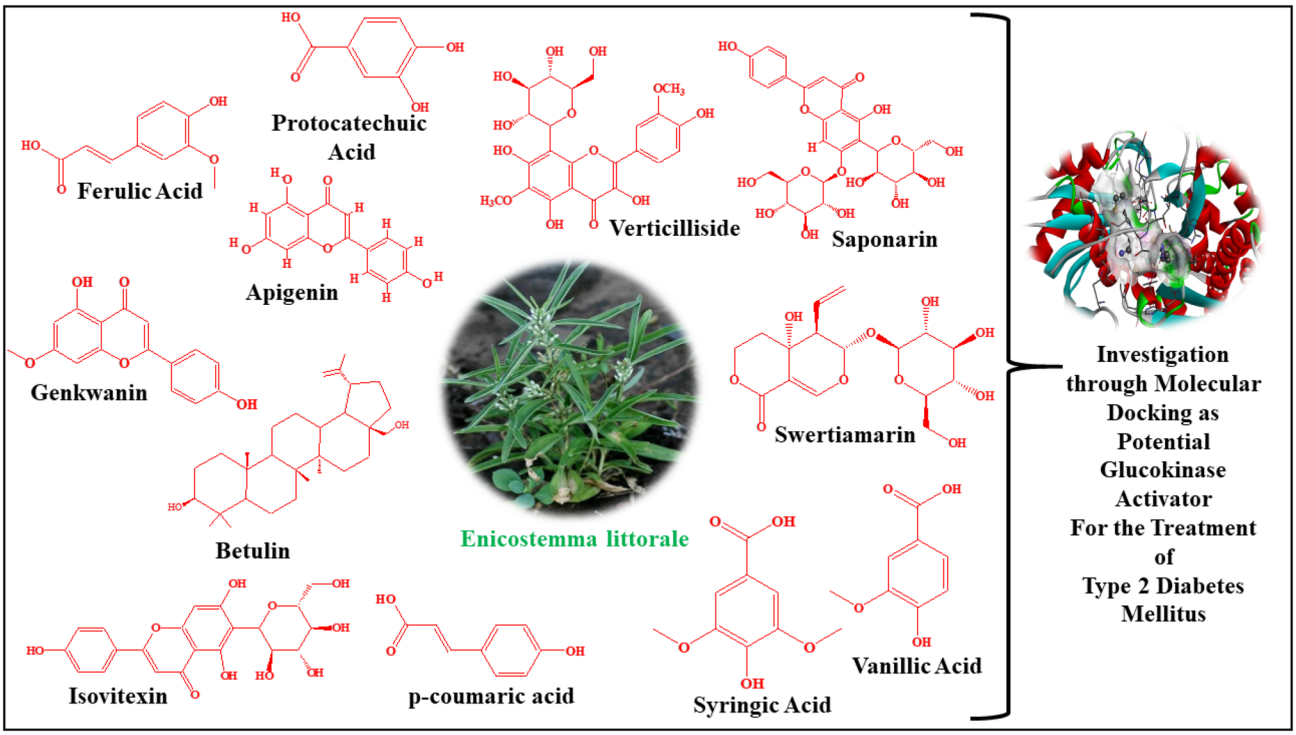

Fig. 1 The structures of all the significant phytoconstituents of *E. littorale*

allosteric site of the enzyme. The structures of all the significant phytoconstituents of *E. littorale* are represented in Fig. 1.

Material and methods

Calculation of Lipinski's rule of five

In order to further optimize the molecules, all the phytoconstituents were tested for violating the Lipinski's rule of five, Veber's rule and the pharmacokinetic (ADMET) characteristics. The properties of all the phytoconstituents were calculated from SwissADME online tool (http://www.swissadme.ch/index.php).

Molecular docking

We conducted molecular docking (MD) on Lenovo ThinkPad T440p using PyRx-Virtual Screening Tool (Dallakyan and Olson, 2015). The structures of all the phytoconstituents and native ligand (.sdf File format) were downloaded from the National Center for Biotechnology Information PubChem (https://pubchem.ncbi.nlm.nih.gov/). The energy minimization (optimization) was performed by Universal Force Field (UFF) (Rappé et al. 1992).

A crystalline human glucokinase structure was obtained as input 1V4S from the Protein Data Bank (PDB) of RCSB (https://www.rcsb.org/structure/1V4S). 1V4S also contained the native ligand 5-(1-methyl-1H-imidazol-2-ylthio)-2-amino-4-fluoro-N-(thiazol-2-yl)benzamide that was used as a reference molecule for MD. In PyRx 0.8, Autodock vina 1.1.2 was used to conduct MD analyses of both the phytoconstituents and native ligands against the crystal structure of glucokinase (Dallakyan and Olson, 2015). With the aid of Discovery Studio Visualizer 2019, the composition of the enzyme was refined, purified, and prepared for MD (San Diego: Accelrys Software Inc. 2012). The specifications of the crystal structure and input compositions of human glucokinase used (PDB ID-1V4S) are provided in Table 1 of the PDB X-ray Structure Validation Report released on 10 August 2020. There were only 5 specific molecules in this entry, and there was one chain (Chain A). The entry comprises 3690 atoms, including 0 hydrogens and 0 deuteriums, which illustrates the need to incorporate hydrogen atoms in protein preparation processes for MD.

The MD was executed by using Vina Wizard Tool in PyRx 0.8. Molecules (PDBQT Files), both ligands and target (human glucokinase), were selected for MD. For the purpose of MD simulation, the three-dimensional grid box (size_x = 43.35 A^0; Size_y = 59.36 A^0; Size_z = 43.92 A^0) was built using Autodock tool 1.5.6 with exhaustiveness

Table 1 The information of the crystal structure and input compositions of human glucokinase used (PDB ID-1V4S)

The details of crystal structure (1V4S):						
Title:	Crystal structure of human glucokinase					
DOI:	10.2210/pdb1V4S/pdb					
Authors:	Kamata, K., Mitsuya, M., Nishimura, T., Eiki, J., Nagata, Y					
Deposited on:	30-03-2004					
Resolution:	2.30 Å(reported)					
Classification:	Transferase					
Organism(s):	Homo sapiens					
Expression System:	*Escherichia coli*					
Method:	X-Ray diffraction					
Residues	Atoms					
The entry composition of 1V4S:						
	Total	C	N	O	S	Na/F
Molecule 1 was a protein called glucokinase isoform 2						
448	3505	2178	609	686	32	0
Molecule 2 was alpha-D-glucopyranose (three-letter code: GLC) (formula: $C_6H_{12}O_6$)						
1	12	6	0	6	0	0
Molecule 3 was SODIUM ION (three-letter code: NA) (formula: Na)						
1	1	0	0	0	0	1 (Na)
Molecule 4 was native ligand 5-(1-methyl-1H-imidazol-2-ylthio)-2-amino-4-fluoro-N-(thiazol-2-yl) benzamide (three-letter code: MRK) (formula: $C_{14}H_{12}FN_5OS_2$)						
1	23	14	5	1	2	1 (F)
Molecule 5 was water						
149	149	0	0	149	0	0

Where, *C* carbon; *N* nitrogen; *O* oxygen; *S* sulphur; *Na* sodium; *F* fluorine

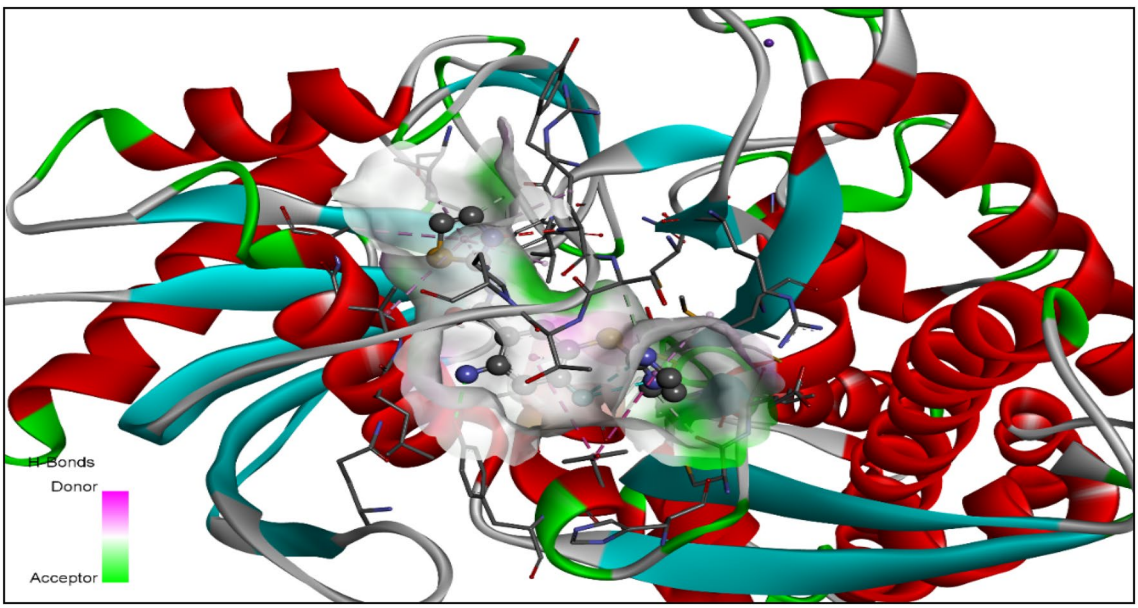

Fig. 2 The cavity of the enzyme is depicted with the co-crystallize ligand molecule (PDB ID: 1V4S)

value of 8 (Dallakyan and Olson, 2015). The active amino acids in the protein were analyzed and illuminated using Visualizer in BIOVIA Discovery Studio (version-19.1.0.18287) (San Diego: Accelrys Software Inc. 2012). The full MD process, the identification of cavity and active amino acid residues, were performed as defined by S. L. Khan et al.

(Chaudhari et al. 2020; Khan and Siddiui 2020; Khan et al. 2020a, b, 2021; Siddiqui et al. 2021). The enzyme cavity is depicted in Fig. 2 with the co-crystallized ligand molecule.

Results

Pharmacokinetic characteristics are an important component of drug development because it enable researchers to assess the biological aspects of medication candidates. In order to establish whether or not the compound optimal for oral bioavailability, Lipinski's rule of five and Veber's rules was utilized (Table 2). All the phytoconstituents were studied for their ADMET characteristics better to grasp their pharmacokinetics profiles and drug-likeness qualities (Table 3). The ligand energies (kcal/mol), binding free energy (kcal/mol), root mean square deviation/upper bound (rmsd/ub), and root mean square deviation/lower bound (rmsd/lb) of the conformers generated of all the docked phytoconstituents are tabulated in Table 4. The active amino residues, reactive atom of ligands, bond length (A^0), and type of interactions of phytoconstituents with glucokinase enzyme are depicted in Table 5. The 2D- and 3D-docking poses of all the docked molecules are represented in Figs. 3, 4, 5, 6.

Where: GI, gastrointestinal; BBB, blood brain barrier; P-gp, p-glycoprotein.

Discussion

We tried to identify the potential natural lead compounds from *E. littorale* as glucokinase activators through the binding mode in the enzyme's allosteric site and binding free energies. In accordance with Lipinski's and Veber's rules (Table 2), many of the phytoconstituents did not demonstrated the drug-likeness characteristics and violated both the rules. Amongst all the molecules, betulin has a log P value of 8.28, which violates the Lipinski rule of 5 and indicates poor lipophilicity. An essential aspect of the compound that influences its function in the human body is lipophilicity. The compound's Log P value shows the permeability of the drugs in the body to enter the target tissue (Krzywinski and Altman 2013; Lipinski et al. 2012). Isovitexin was found to have 7 hydrogen bond donors, which violates the Lipinski rule of 5. Saponarin has a molecular weight of 594.52 Da, 15 hydrogen bond acceptors, and 10 hydrogen bond donors, with 3 violations of the Lipinski rule of 5. It is preferable to look for substances that exceed the Lipinski limit of 500 Da, since this will only boost absorption. Still, there are several reports of relatively more significant compounds that are transported effectively through the cells. The remaining phytoconstituents, including native ligand, had fortunately not violated the Lipinski rule of 5, indicating better absorption and/or lipophilicity of the molecules. Many phytoconstituents violated the Veber's rule with total polar surface area (TPSA, should be less than 140) values and the number of rotatable bonds (which should be less than 10) that do not fall within the acceptable range for oral

Table 2 The molecular formula, Lipinski rule of five and Vebers's rule

Molecule Name	Molecular Formula	Lipinski rule of 5					Veber's rule	
		Mol. Wt.[a]	HBA[a]	HBD[a]	LogP	Violation	Total polar surface area (Å^2)	No. of rotatable bonds
Native Ligand	$C_{14}H_{12}FN_5OS_2$	349	04	02	2.00	0	139.37	5
Apigenin	$C1_5H_{10}O_5$	270.24	05	03	3.02	00	90.90	1
Betulin	$C_{30}H_{50}O_2$	442.72	02	02	8.28	01	40.46	2
Ferulic acid	$C_{10}H_{10}O_4$	194.18	04	02	1.51	00	66.76	3
Genkwanin	$C_{16}H_{12}O_5$	284.26	05	02	3.35	00	79.90	2
Isovitexin	$C_{21}H_{20}O_{10}$	432.38	10	07	0.21	01	181.05	3
p-coumaric acid	$C_9H_8O_3$	164.16	03	02	1.46	00	57.53	2
Protocatechuic acid	$C_7H_6O_4$	154.12	04	03	1.15	00	77.76	1
Saponarin	$C_{27}H_{30}O_{15}$	594.52	15	10	− 1.60	03	260.20	6
Swertiamarin	$C_{16}H_{22}O_{10}$	374.34	10	05	− 2.00	00	155.14	4
Syringic acid	$C_9H_{10}O_5$	198.17	05	02	1.04	00	75.99	3
Vanillic acid	$C_8H_8O_4$	168.15	04	02	1.43	00	66.76	2
Verticilliside	$C_{23}H_{24}O_{13}$	508.43	13	08	0.00	00	219.74	5

[a]*Mol. Wt.* molecular weight; *HBA* hydrogen bond acceptor; *HBD* hydrogen bond donor

Table 3 The pharmacokinetic and drug-likeness properties of selected phytoconstituents

Parameters	Compound names												
	Native Ligand	Apigenin	Betulin	Ferulic acid	Genkwanin	Isovitexin	p-coumaric acid	Protocatechuic acid	Saponarin	Swertiamarin	Syringic acid	Vanillic acid	Verticilliside
Pharmacokinetics													
GI absorption	Low	High	Low	High	High	Low	High	High	Low	Low	High	High	Low
BBB permeation	No	No	No	Yes	No	No	Yes	No	No	No	No	No	No
P-gp substrate	No	No	No	No	No	No	No	No	Yes	No	No	No	No
CYP1A2 inhibitor	Yes	Yes	No	No	Yes	No	No	No	No	No	No	No	No
CYP2C19 inhibitor	Yes	No	No	No	No	No	No	No	No	No	No	No	No
CYP2C9 inhibitor	Yes	No	No	No	Yes	No	No	No	No	No	No	No	No
CYP2D6 inhibitor	Yes	Yes	No	No	Yes	No	No	No	No	No	No	No	No
CYP3A4 inhibitor	Yes	Yes	No	No	Yes	No	No	Yes	No	No	No	No	No
Log K_p (skin permeation, cm/s)	− 6.59	− 5.80	− 3.12	− 6.41	− 5.66	− 8.79	− 6.26	− 6.42	− 11.06	− 10.00	− 6.77	− 6.31	− 9.40
Drug-likeness													
Ghose	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes	No
Egan	No	Yes	No	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No
Muegge	Yes	Yes	No	No	Yes	No	No	No	No	No	No	Yes	No
Bioavailability Score	0.55	0.55	0.55	0.85	0.55	0.55	0.85	0.56	0.17	0.11	0.56	0.85	0.17

Table 4 The ligand energies (kcal/mol), binding free energy (kcal/mol), rmsd/ub, and rmsd/lb of the conformers generated of all the docked phytoconstituents

Compound name	Ligand energies (kcal/mol)	Binding free energies of conformers (kcal/mol)	rmsd/ub	rmsd/lb
Native Ligand	689.51	– 7.2	0	0
		– 7.1	11.569	9.551
		– 7	3.954	3.154
		– 6.9	14.845	11.338
		– 6.8	10.337	7.01
		– 6.7	1.683	1.445
		– 6.6	7.21	2.806
		– 6.5	17.14	14.594
		– 6.4	6.309	2.65
Apigenin	192.64	– 8.7	0	0
		– 7.4	10.327	4.068
		– 7.3	10.821	4.626
		– 7.2	21.133	19.956
		– 6.9	35.515	34.274
		– 6.9	35.782	34.596
		– 6.8	33.332	31.774
		– 6.8	10.029	4.368
		– 6.7	9.059	6.294
Ferulic Acid	470.67	– 6.8	0	0
		– 6.4	6.204	1.962
		– 5.9	19.764	19.019
		– 5.9	20.297	19.456
		– 5.6	39.562	37.223
		– 5.5	35.688	35.127
		– 5.4	36.304	35.659
		– 5.1	22.572	21.564
		– 5.1	20.025	18.771
Genkwanin	206.69	– 7.5	0	0
		– 7.1	24.283	22.091
		– 7	37.334	34.875
		– 6.5	25.831	23.254
		– 6.5	25.252	22.573
		– 6.5	24.856	21.891
		– 6.5	25.269	23.189
		– 6.4	24.678	23.526
		– 6.2	25.372	23.061
p-Coumaric acid	86.43	– 6.4	0	0
		– 6.2	5.781	1.352
		– 5.8	36.105	35.604
		– 5.6	19.899	19.203
		– 5.5	5.985	4.033
		– 5.3	20.45	19.509
		– 5.1	19.394	18.094
		– 5	46.168	46.046
		– 5	5.274	3.587

Table 4 (continued)

Compound name	Ligand energies (kcal/mol)	Binding free energies of conformers (kcal/mol)	rmsd/ub	rmsd/lb
Protocatechuic acid	69.09	– 5.9	0	0
		– 5.8	3.823	2.267
		– 5.7	20.234	19.688
		– 5.7	37.453	36.16
		– 5.3	37.892	36.5
		– 5.3	20.033	19.486
		– 5.1	34.46	34.289
		– 5	34.43	34.254
		– 5	45.895	45.58
Syringic acid	837.9	– 5.7	0	0
		– 5.4	3.552	0.385
		– 5.3	5.175	2.812
		– 5.2	4.587	2.804
		– 5.2	15.57	13.004
		– 5.1	22.174	19.227
		– 5.1	15.49	13.102
		– 5.1	22.457	19.465
		– 5	3.615	3.153
Vanillic acid	85.01	– 5.5	0	0
		– 5.5	28.978	28.041
		– 5.5	4.174	1.335
		– 5.3	28.558	27.034
		– 5.2	28.09	26.65
		– 5	21.792	20.446
		– 5	29.168	28.414
		– 5	22.059	20.823
		– 4.9	29.605	27.718

availability. Isovitexin, Saponarin, Swertiamarin, and Verticilliside violated the Veber's rule.

For further optimization, all the molecules have been subjected to calculations of pharmacokinetics and drug-likeness properties. All the molecules did not show BBB penetration potential which is not favorable property for the drugs to be targeted for central nervous system. Unfortunately, many molecules did exhibited optimum log *Kp* (skin permeation, cm/s) and bioavailability scores. Many molecules violated the Ghose, Egan, and Muegge filters (Table 3). The molecules which displayed low GI absorption and violations of Lipinski and Veber's rules have been eliminated from further optimization. Also, native ligand displayed low GI absorption. Therefore, only Apigenin, Ferulic acid, Genkwanin, p-coumaric acid, Protocatechuic acid, Syringic acid, and Vanillic acid have been selected to perform molecular docking studies on the GK enzyme.

A total 9 conformers were generated through MD for each molecule (Table 4). The conformer with zero rmsd/ub and rsmd/lb values has been treated as the best fit model for the glucokinase enzyme activation. The binding free energy and binding mode of the native ligand in the allosteric site of the enzyme have been considered a reference for validating the other molecules (Table 5 and Figs. 3, 4, 5, 6). Native ligand has binding free energy of − 7.2 kcal/mol and has formed 4 hydrogen bonds (3 conventional and 1 carbon-hydrogen bond) with ASP78 (2.04258 A^0), LYS169 (2.60065 A^0), and THR228 (2.20855 A^0, 3.75081 A^0). The hydrogen of a free primary amino group from the native ligand has formed a hydrogen bond ASP78, and the fluorine atom has formed a hydrogen bond with LYS169. THR228 has reacted with

Table 5 The active amino residues, reactive atom of ligands, bond length (A^0), and type of interactions of phytoconstituents with glucokinase enzyme (1V4S)

Active amino residue	Atom from ligand	Bond length (A^0)	Bond category	Bond types
Native ligand				
ASP78	H	2.04258	Hydrogen bond	Conventional hydrogen bond
LYS169	F	2.60065	Hydrogen bond; halogen	Conventional hydrogen bond; halogen (Fluorine)
THR228	S	2.20855	Hydrogen bond	Conventional hydrogen bond
THR228	F	3.75081		Carbon hydrogen bond
ARG85	Pi-Orbitals	3.56863	Electrostatic	Pi-Cation
ASP205		3.9455		Pi-Anion
ASP409		3.70544		
Apigenin				
TYR215	O	2.06298	Hydrogen bond	Conventional hydrogen bond
TYR215	O	2.15918		
TYR214	H	3.00628		Pi-Donor hydrogen bond
VAL455	Pi-Orbitals	3.86479	Hydrophobic	Pi-Sigma
TYR214		5.11899		Pi-Pi T-shaped
PRO66		4.99697		Pi-Alkyl
ILE211		5.46378		
VAL455		4.74718		
ILE211		4.92959		
VAL62		4.80355		
PRO66		5.06		
VAL452		5.03778		
ALA456		4.9239		
Ferulic acid				
TYR61	H	2.0266	Hydrogen bond	Conventional hydrogen bond
ILE211	Pi-Orbitals	4.61546	Hydrophobic	Pi-Alkyl
VAL452		4.8904		
VAL455		4.64867		
Genkwanin				
ILE159	C-H	3.78006	Hydrophobic	Pi-Sigma
ILE159	C-H	3.77297		
VAL455	C-H	3.87537		
ALA456	Pi-orbitals	4.63516		Pi-Alkyl
ALA456		4.89221		
LYS459		5.06083		
VAL62		5.08219		
PRO66		5.00443		
ILE159		5.34614		
VAL452		5.4002		
ALA456		4.7483		
p-Coumaric acid				
TYR61	H	1.86828	Hydrogen bond	Conventional hydrogen bond
TYR215	O	2.07668		
ILE211	Pi-Orbitals	4.58608	Hydrophobic	Pi-Alkyl
VAL452		4.8732		
VAL455		4.66716		

Table 5 (continued)

Active amino residue	Atom from ligand	Bond length (A^0)	Bond category	Bond types
Protocatechuic acid				
THR65	O	2.3016	Hydrogen bond	Conventional hydrogen bond
TYR215	O	2.70823		
VAL452	O	3.71031		Carbon hydrogen bond
ILE211	Pi-Orbitals	3.47995	Hydrophobic	Pi-Sigma
TYR214		5.12043		Pi-Pi T-shaped
Syringic acid				
ASP205	H	2.69294	Hydrogen bond	Conventional hydrogen bond
ARG85	O	2.33855		
ARG85	O	2.66692		
LYS169	O	2.52862		
ASP409	Methyl C	3.28918		Carbon Hydrogen Bond
ASN83		3.59997		
ASP78	Pi-Orbitals	3.71563	Electrostatic	Pi-Anion
Vanillic acid				
LEU25	O	2.5621	Hydrogen bond	Conventional hydrogen bond
SER373	O	1.95412		
THR376	Pi-Orbitals	3.61887	Hydrophobic	Pi-Sigma

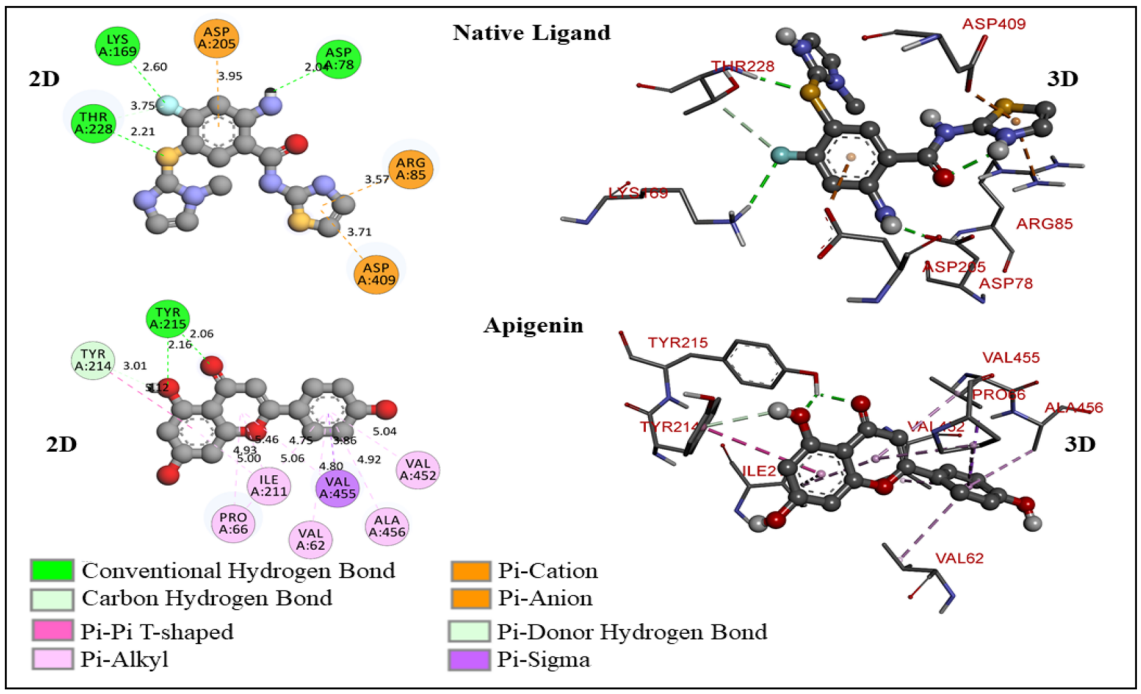

Fig. 3 The 2D- and 3D-molecular interaction poses of native ligand and apigenin with the glucokinase enzyme

sulfur and fluorine simultaneously with forming one conventional hydrogen bond and one carbon-hydrogen bond. Native ligand showed electrostatic interactions with ARG85 (3.56863 A^0), ASP205 (3.9455 A^0), and ASP409 (3.70544 A^0) through Pi-orbitals of the aromatic ring system (Fig. 3).

Apigenin (4′,5-trihydroxyflavone), a flavonoid, falls under the flavone class that is the aglycone of many naturally-occurring glycosides [(Ali et al. 2017; Baumann 2008; Salehi et al. 2019; Shukla and Gupta 2010)]. It has shown − 8.7 kcal/mol of binding free energy and formed

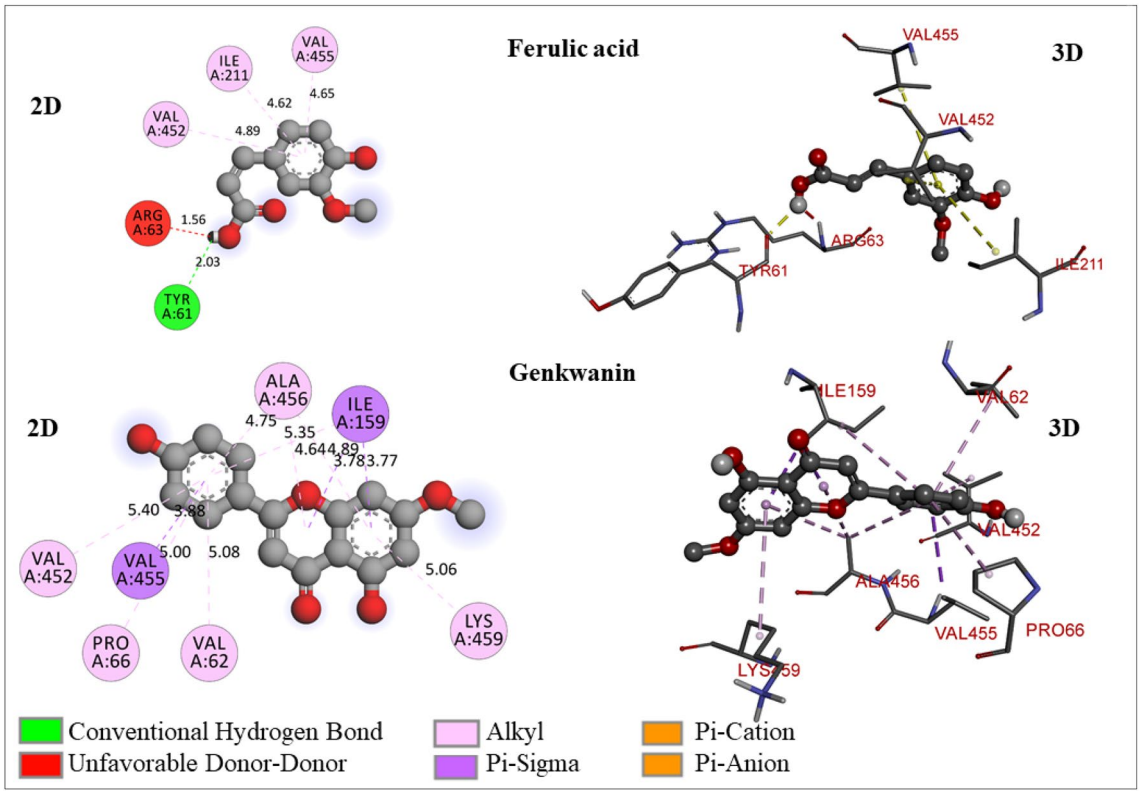

Fig. 4 The 2D- and 3D-molecular interaction poses of ferulic acid and genkwanin with the glucokinase enzyme

3 hydrogen bonds (2 conventional and 1 Pi-donor hydrogen bond) with TYR215 (2.06298 A^0, 2.15918 A^0), and TYR214 (3.00628 A^0) (Fig. 3). It has formed two hydrogen bonds with TYR215 through hydroxyl and carbonyl oxygen atoms. One free hydroxyl group in apigenin has formed one Pi-donor hydrogen bond with TYR214 through hydrogen atom. It has shown many hydrophobic interactions due to Pi-orbitals of aromatic ring systems with VAL455 (3.86479 A^0), TYR214 (5.11899 A^0), PRO66 (4.99697 A^0), ILE211 (5.46378 A^0), VAL455 (4.74718 A^0), ILE211 (4.92959 A^0), VAL62 (4.80355 A^0), PRO66 (5.06 A^0), VAL452 (5.03778 A^0), and ALA456 (4.9239 A^0).

Ferulic acid is an organic compound; chemically, it is 3-methoxy-4-hydroxycinnamic acid. In plant cell walls a rich phenolic phytochemical is present covalently attached to arabinoxyls as side chains (Mathew and Abraham 2006; Wu et al. 2018). It exhibited -6.8 kcal/mol of binding free energy, which is less than native ligand, and therefore, this molecule does not possess potential to activate glucokinase enzyme. It has also formed an unfavorable donor-donor bond with ARG63 (1.56 A^0) through hydroxyl hydrogen atom (Fig. 4).

Genkwanin is a monomethoxyflavone, which is a derivative of apigenin. It has been biosynthesized by apigenin in plants by methylation of the hydroxyl group at 7th position (Lee et al. 2015; Nasr Bouzaiene et al. 2016). Genkwanin has shown − 7.5 kcal/mol of binding free energy with glucokinase enzyme and possesses stable ligand energy of 206.69 kcal/mol. It exhibited hydrophobic interactions (Pi-sigma and Pi-alkyl) with ILE159 (3.78006 A^0, 3.77297 A^0, 5.34614 A^0), VAL455 (3.87537 A^0), ALA456 (4.63516 A^0, 4.89221 A^0, 4.7483 A^0), LYS459 (5.06083 A^0), VAL62 (5.08219 A^0), PRO66 (5.00443 A^0), and VAL452 (5.4002 A^0) (Fig. 4). As it has not formed any hydrogen bond, which may result in poor activation of the enzyme.

p-Coumaric acid is a hydroxyl derivative of cinnamic acid and widely distributed in many plant species(Pei et al. 2016). It has shown − 6.4 kcal/mol of binding free energy and formed 2 conventional hydrogen bonds with TYR61 (1.86828 A^0), TYR215 (2.07668 A^0), whereas hydrophobic interactions (Pi-alkyl) with ILE211(4.58608 A^0), VAL452 (4.8732 A^0), VAL455 (4.66716 A^0) (Fig. 5).

Protocatechuic acid is a type of phenolic acid that is naturally present and over 500 plants have it or its derivatives (active constituents), and these substances have different therapeutic potential. It has structural similarities with gallic acid, caffeic acid, vanillic acid, and syringic acid, which are well-known antioxidants found in foods and other items (Kakkar and Bais 2014). Protocatechuic acid has shown − 5.9 kcal/mol of binding free energy and formed

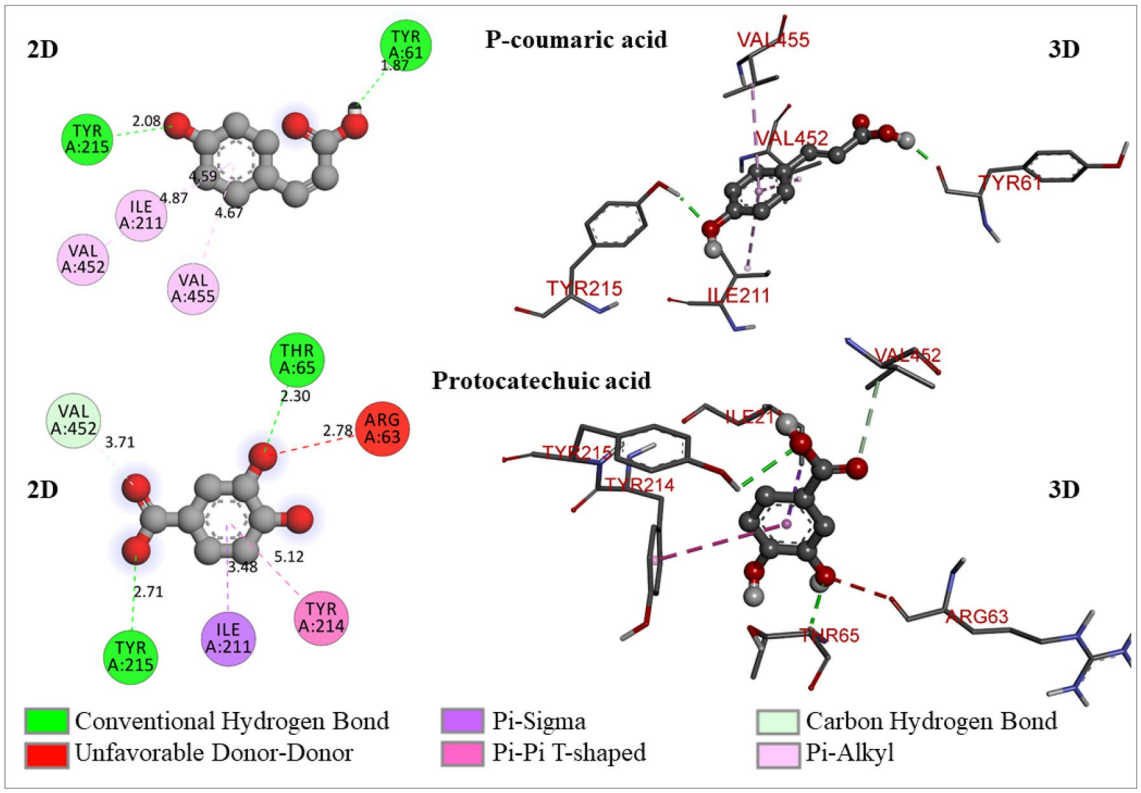

Fig. 5 The 2D- and 3D-molecular interaction poses of p-coumaric acid and protocatechuic acid with the glucokinase enzyme

3 hydrogen bonds (2 conventional and 1 carbon-hydrogen bond) with THR65 (2.3016 A^0), TYR215 (2.70823 A^0), and VAL452 (3.71031 A^0). It has demonstrated 2 hydrophobic bonds (Pi-sigma and Pi-Pi T-shaped) with ILE211 (3.47995 A^0) and TYR214 (5.12043 A^0) (Fig. 5). From these results, it can be concluded that protocatechuic acid does not have much potential to activate the glucokinase enzyme.

Syringic acid is a phenolic substance that is mostly present in fruits and vegetables. This compound is made by the shikimic acid process and is found in plants. It shows a wide variety of clinical applications in preventing diabetes, coronary disorders, cancer, ischemic stroke, etc. It can shield brain tissue from free radical injury, delay the development of diabetes, and is hepatoprotective medicine (Srinivasulu et al. 2018). It has shown -5.7 kcal/mol of binding free energy and formed 6 hydrogen bonds (4 conventional and 2 carbon-hydrogen bonds) with ASP205 (2.69294 A^0), ARG85 (2.66692 A^0, 2.33855 A^0), LYS169 (2.52862 A^0), ASP409 (3.28918 A^0), ASN83 (3.59997 A^0). It has formed 1 electrostatic (Pi-anion) bond with ASP78 (3.71563 A^0) (Fig. 6). It demonstrated less binding free energy but exhibited a good number of hydrogen bonds, which may effectively activate the glucokinase enzyme. Vanillic acid has exhibited − 5.5 kcal/mol of binding free energy and formed 2 conventional hydrogen bonds with LEU25 (2.5621 A^0), and SER373 (1.95412 A^0) (Fig. 6).

Conclusion

Glucokinase is an enzyme involved in synthesising glucose into glucose-6 phosphate and serves a crucial function in glucose sensing. Therefore, agents that induce glucokinase activation could be used to treat T2DM. The *Enicostemma littorale* Blume (*E. littorale*) plays a critical role in human wellbeing. Parts of the plant *E. littoral*, were used historically in therapeutic applications against malaria, skin disorders, leprosy, and mostly antidiabetic activity of this plant have been reported in many literatures as well as it has been recommended in diabetic patients in Ayurveda system of medicine. The present work has been carried out to investigate the glucokinase activation potential of phytoconstituents of *E. littorale* through MD. All the phytoconstituents have been screened through the Lipinski rule of 5, Veber's rule, and ADMET properties. From this initial screening, only Apigenin, Ferulic acid, Genkwanin, p-coumaric acid, Protocatechuic acid, Syringic acid, and Vanillic acid have been selected to perform molecular docking studies on the GK enzyme.

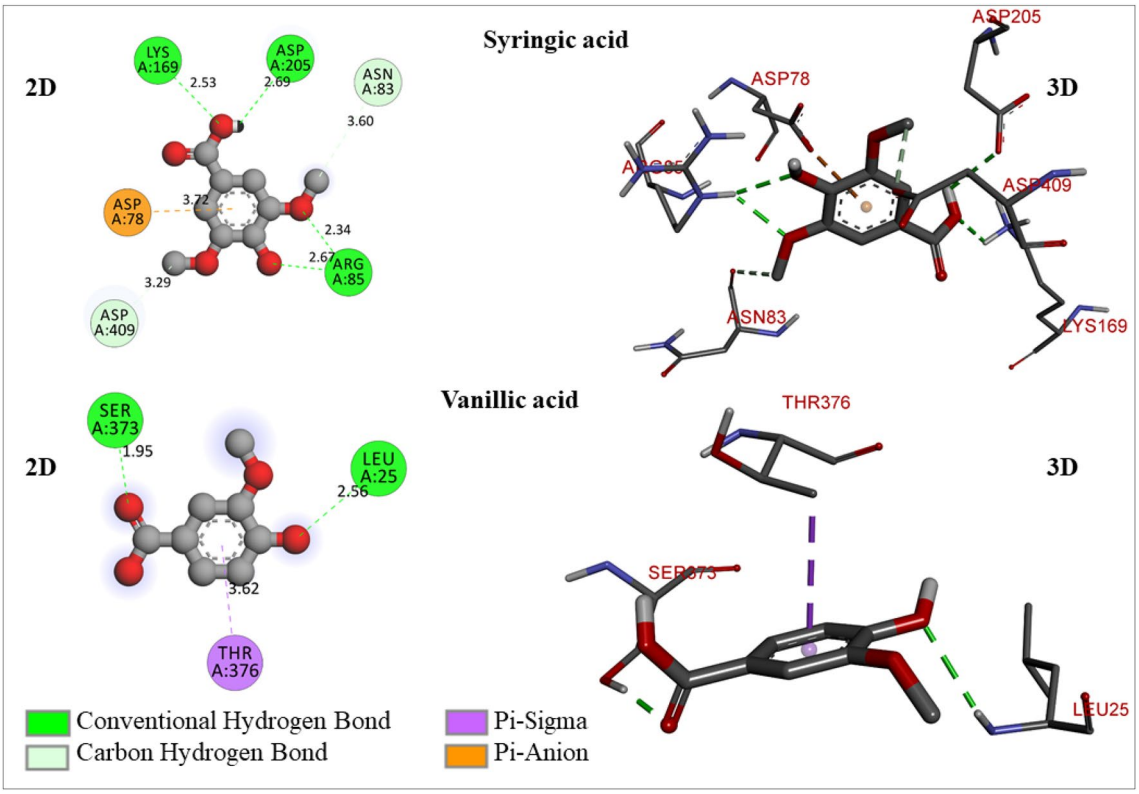

Fig. 6 The 2D- and 3D-molecular interaction poses of syringic acid and vanillic acid with the glucokinase enzyme

MD is a computational research-based technique for exploring possible binding interfaces through the docking of proteins and drugs. A total of 9 conformers were generated through MD for each molecule. The conformer with zero rmsd/ub and rsmd/lb values has been treated as the best fit model for activating the glucokinase enzyme. The binding free energy and binding mode of the native ligand in the allosteric site of the enzyme have been considered the reference for the other molecules' validation. The native ligand has exhibited -7.2 kcal/mol binding free energy with useful binding mode into the enzyme's allosteric site, whereas; it has formed four hydrogen bonds with THR-228, LYS-169, ASP-78, and GLY-81. Based on these findings, the interactions of phytoconstituents have been justified. Apigenin, genkwanin, and swertiamarin exhibited − 8.7, − 7.5, and − 8.3 kcal/mol binding free energy, respectively, which indicates better enzyme activation than the native ligand. Swertiamarin has formed 08, whereas syringic acid exhibited − 5.7 kcal/mol binding affinity but has formed 06 hydrogen bonds with allosteric amino acid residues, which confirms the excellent enzyme activation by these phytoconstituents. Many antidiabetic Ayurvedic formulations contain *E. littorale* extract, which is already known to have therapeutic effects in diabetic patients. We identified and reported the lead phytoconstituent responsible for the antidiabetic potential. We have concluded that if we can isolate and consume the exact active phytoconstituents (glucokinase activators) from this plant, we can use them effectively to treat T2DM and by considering them as a natural lead compound, we can develop and validate more glucokinase activators.

Author contributions All the authors have contributed equally.

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Availability of data and materials The properties of all the phytoconstituents were calculated from SwissADME online tool (http://www.swissadme.ch/index.php). The structures of all the phytoconstituents and native ligand (.sdf File format) were downloaded from the National Center for Biotechnology Information PubChem (https://pubchem.ncbi.nlm.nih.gov/). A crystalline structure of human glucokinase was obtained from RCSB's Protein Data Bank (PDB) as entry 1V4S (https://www.rcsb.org/structure/1V4S).

Code availability Not applicable.

Declarations

Conflict of interest Declared none.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

Abirami P, Gomathinayagam M (2011) A review on *Enicostemma littorale*. Pharmacologyonline 1:75–83

Agrawal M, Kharkar P, Moghe S, Mahajan T, Deka V, Thakkar C, Nair A, Mehta C, Bose J, Kulkarni-Almeida A, Bhedi D, Vishwakarma RA (2013) Discovery of thiazolyl-phthalazinone acetamides as potent glucose uptake activators via high-throughput screening. Bioorganic Med Chem Lett 23:5740–5743. https://doi.org/10.1016/j.bmcl.2013.07.067

Alam P, Ali M, Singh R, Shakeel F (2011) A new HPTLC densitometric method for analysis of swertiamarin in *Enicostemma littorale* and commercial formulations. Nat Prod Res 25:17–25. https://doi.org/10.1080/14786411003754348

Ali F, Rahul NF, Jyoti S, Siddique YH (2017) Health functionality of apigenin: a review. Int J Food Prop. https://doi.org/10.1080/10942912.2016.1207188

Babu PS, Prince PSM (2004) Antihyperglycaemic and antioxidant effect of hyponidd, an ayurvedic herbomineral formulation in streptozotocin-induced diabetic rats. J Pharm Pharmacol 56:1435–1442. https://doi.org/10.1211/0022357044607

Baumann S (2008) Apigenin. Ski Allergy News 39:32. https://doi.org/10.1016/s0037-6337(08)70149-9

Castelhano AL, Dong H, Fyfe MCT, Gardner LS, Kamikozawa Y, Kurabayashi S, Nawano M, Ohashi R, Procter MJ, Qiu L, Rasamison CM, Schofield KL, Shah VK, Ueta K, Williams GM, Witter D, Yasuda K (2005) Glucokinase-activating ureas. Bioorganic Med Chem Lett 15:1501–1504. https://doi.org/10.1016/j.bmcl.2004.12.083

Charaya N, Pandita D, Grewal AS, Lather V (2018) Design, synthesis and biological evaluation of novel thiazol-2-yl benzamide derivatives as glucokinase activators. Comput Biol Chem 73:221–229. https://doi.org/10.1016/j.compbiolchem.2018.02.018

Chaudhari RN, Khan SL, Chaudhary RS, Jain SP, Siddiqui FA (2020) B-Sitosterol: isolation from muntingia calabura linn bark extract, structural elucidation and molecular docking studies as potential inhibitor of SARS-CoV-2 Mpro (COVID-19). Asian J Pharm Clin Res 13:204–209. https://doi.org/10.22159/ajpcr.2020.v13i5.37909

Dallakyan S, Olson AJ (2015) Small-molecule library screening by docking with PyRx. Methods Mol Biol 1263:243–250. https://doi.org/10.1007/978-1-4939-2269-7_19

Filipski KJ, Futatsugi K, Pfefferkorn JA, Stevens BD (2012) Glucokinase activators. Pharm Pat Anal. https://doi.org/10.4155/ppa.12.26

Fyfe MCT, Procter MJ (2009) Glucokinase activators as potential antidiabetic agents possessing superior glucose-lowering efficacy. Drugs Future. https://doi.org/10.1358/dof.2009.034.08.1394557

Ghosal S, Singh AK, Sharma PV, Chaudhuri RK (1974) Chemical constituents of gentianaceae IX: natural occurrence of erythrocentaurin in *Enicostemma hyssopifolium* and *Swertia lawii*. J Pharm Sci. https://doi.org/10.1002/jps.2600630632

Grewal A, Sekhon B, Lather V (2014) Recent updates on glucokinase activators for the treatment of type 2 diabetes mellitus. Mini-Rev Med Chem 14:585–602. https://doi.org/10.2174/1389557514666140722082713

Grewal AS, Lather V, Pandita D, Bhayana G (2017) Synthesis, docking and evaluation of phenylacetic acid and trifluoro-methylphenyl substituted benzamide derivatives as potential PPARδ agonists. Lett Drug Des Discov. https://doi.org/10.2174/1570180814666170327164443

Grewal AS, Sharma K, Singh S, Singh V, Pandita D, Lather V (2018) Design, synthesis and antidiabetic activity of novel sulfamoyl benzamide derivatives as glucokinase activators. J Pharm Technol Res Manag 6:115–124. https://doi.org/10.15415/jptrm.2018.62008

Grewal AS, Kharb R, Prasad DN, Dua JS, Lather V (2019) N-pyridin-2-yl benzamide analogues as allosteric activators of glucokinase: Design, synthesis, in vitro, in silico and in vivo evaluation. Chem Biol Drug Des 93:364–372. https://doi.org/10.1111/cbdd.13423

Grewal AS, Lather V, Charaya N, Sharma N, Singh S, Kairys V (2020) Recent developments in medicinal chemistry of allosteric activators of human glucokinase for type 2 diabetes mellitus therapeutics. Curr Pharm Des 26:2510–2552. https://doi.org/10.2174/1381612826666200414163148

Grimsby J, Sarabu R, Corbett WL, Haynes NE, Bizzarro FT, Coffey JW, Guertin KR, Hilliard DW, Kester RF, Mahaney PE, Marcus L, Qi L, Spence CL, Tengi J, Magnuson MA, Chu CA, Dvorozniak MT, Matschinsky FM, Grippo JF (2003) Allosteric activators of glucokinase: Potential role in diabetes therapy. Science (80-) 301:370–373. https://doi.org/10.1126/science.1084073

Houze JB, Dransfield P, Pattaropong V, Du X, Fu Z, Lai S, Park J, Jiao X, Kohn TJ, Aicher TD, Boyd SA, Bencsik J, Condroski KR, Hinklin RJ, Kraser CF, Pratt S, Singh A, Wenglowsky SM, Boys ML, Chicarelli MJ, Mohr PJ, Cardozo MG (2013) Urea compounds as GKa activators and their preparation. PCT Int Appl

Indumathi C, Durgadevi G, Nithyavani S, Gayathri PK (2014) Estimation of terpenoid content and its antimicrobial property in Enicostemma litorrale. Int J ChemTech Res 6:4264–4267

Ishikawa M, Nonoshita K, Ogino Y, Nagae Y, Tsukahara D, Hosaka H, Maruki H, Ohyama S, Yoshimoto R, Sasaki K, Nagata Y, Eiki J, Nishimura T (2009) Discovery of novel 2-(pyridine-2-yl)-1H-benzimidazole derivatives as potent glucokinase activators. Bioorganic Med Chem Lett 19:4450–4454. https://doi.org/10.1016/j.bmcl.2009.05.038

Jahan E, Perveen S, Malik A (2009) Verticilliside, a new flavone C-glucoside from *Enicostemma* verticillatum. J Asian Nat Prod Res 11:257–260. https://doi.org/10.1080/10286020802675019

Kakkar S, Bais S (2014) A review on protocatechuic acid and its pharmacological potential. ISRN Pharmacol 2014:1–9. https://doi.org/10.1155/2014/952943

Khan SL, Siddiui FA (2020) Beta-Sitosterol: as immunostimulant, antioxidant and inhibitor of SARS-CoV-2 spike glycoprotein. Arch Pharmacol Ther. https://doi.org/10.33696/pharmacol.2.014

Khan SL, Siddiqui FA, Jain SP, Sonwane GM (2020a) Discovery of potential inhibitors of SARS-CoV-2 (COVID-19) main protease (Mpro) from Nigella Sativa (black seed) by molecular docking study. Coronaviruses 2:384–402. https://doi.org/10.2174/2666796701999200921094103

Khan SL, Sonwane GM, Siddiqui FA, Jain SP, Kale MA, Borkar VS (2020b) Discovery of naturally occurring flavonoids as human cytochrome P450 (CYP3A4) inhibitors with the aid of computational chemistry. Indo Glob J Pharm Sci 10:58–69. https://doi.org/10.35652/igjps.2020.10409

Khan SL, Siddiqui FA, Shaikh MS, Nema NV, Shaikh AA (2021) Discovery of potential inhibitors of the receptor-binding domain (RBD) of pandemic disease-causing SARS-CoV-2 Spike glycoprotein from Triphala through molecular docking. Curr Chinese Chem. https://doi.org/10.2174/2666001601666210322121802

Kohn TJ, Du X, Lai S, Xiong Y, Komorowski R, Veniant M, Fu Z, Jiao X, Pattaropong V, Chow D, Cardozo M, Jin L, Conn M,

DeWolf WE, Kraser CF, Hinklin RJ, Boys ML, Medina JC, Houze J, Dransfield P, Coward P (2016) 5-Alkyl-2-urea-substituted pyridines: identification of efficacious glucokinase activators with improved properties. ACS Med Chem Lett 7:666–670. https://doi.org/10.1021/acsmedchemlett.6b00145

Krzywinski M, Altman N (2013) Points of significance: significance P values and t-tests. Nat Methods 10:1041–1042. https://doi.org/10.1038/nmeth.2698

Lee H, Kim BG, Kim M, Ahn JH (2015) Biosynthesis of two flavones, apigenin and genkwanin, in *Escherichia coli*. J Microbiol Biotechnol 25:1442–1448. https://doi.org/10.4014/jmb.1503.03011

Leong XY, Thanikachalam PV, Pandey M, Ramamurthy S (2016) A systematic review of the protective role of swertiamarin in cardiac and metabolic diseases. Biomed Pharmacother. https://doi.org/10.1016/j.biopha.2016.10.044

Li YQ, Zhang YL, Hu SQ, Wang YL, Song HR, Feng ZQ, Lei L, Liu Q, Shen ZF (2011) Design, synthesis and biological evaluation of novel glucokinase activators. Chinese Chem Lett 22:73–76. https://doi.org/10.1016/j.cclet.2010.07.023

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2012) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. https://doi.org/10.1016/j.addr.2012.09.019

Maroo J, Vasu VT, Aalinkeel R, Gupta S (2002) Glucose lowering effect of aqueous extract of *Enicostemma littorale* Blume in diabetes: a possible mechanism of action. J Ethnopharmacol 81:317–320. https://doi.org/10.1016/S0378-8741(02)00095-8

Maroo J, Vasu VT, Gupta S (2003) Dose dependent hypoglycemic effect of aqueous extract of *Enicostemma littorale* Blume in alloxan induced diabetic rats. Phytomedicine 10:196–199. https://doi.org/10.1078/094471103321659933

Mathew S, Abraham TE (2006) Bioconversions of ferulic acid, an hydroxycinnamic acid. Crit Rev Microbiol. https://doi.org/10.1080/10408410600709628

Matschinsky FM (2004) Glucokinase and glycemic disease: from basics to novel therapeutics. Front Diabetes Front Diabetes 16:169–179

Murali B, Upadhyaya UM, Goyal RK (2002) Effect of chronic treatment with *Enicostemma littorale* in non-insulin-dependent diabetic (NIDDM) rats. J Ethnopharmacol 81:199–204. https://doi.org/10.1016/S0378-8741(02)00077-6

Murray A, Lau J, Jeppesen L, Vedso P, Ankersen M, Lundbeck JM, Kristiansen M, Valcarce-Lopez MC, Polisetti DR, Subramanian G, Andrews RC, Christen DP, Cooper JT, Santhosh KC (2005) Preparation of heteroaryl ureas and their use as glucokinase activators. PCT Int Appl

Nagarathnamma M, Sudarshana MS, Niranjan MH, Pandurangamurthy, (2010) Rapid regeneration of enicostemma littorale blume from leaf and stem cultures. J Plant Interact 5:69–73. https://doi.org/10.1080/17429140903353549

Nasr Bouzaiene N, Chaabane F, Sassi A, Chekir-Ghedira L, Ghedira K (2016) Effect of apigenin-7-glucoside, genkwanin and naringenin on tyrosinase activity and melanin synthesis in B16F10 melanoma cells. Life Sci 144:80–85. https://doi.org/10.1016/j.lfs.2015.11.030

Pal M (2009) Medicinal chemistry approaches for glucokinase activation to treat type 2 diabetes. Curr Med Chem 16:3858–3874. https://doi.org/10.2174/092986709789177993

Park K, Lee BM, Hyun KH, Han T, Lee DH, Choi HH (2015) Design and synthesis of acetylenyl benzamide derivatives as novel glucokinase activators for the treatment of t2dm. ACS Med Chem Lett 6:296–301. https://doi.org/10.1021/ml5004712

Patel SS, Shah RS, Goyal RK (2009) Antihyperglycemic, antihyperlipidemic and antioxidant effects of Dihar, a polyherbal ayurvedic formulation in streptozotocin induced diabetic rats. Indian J Exp Biol 47:564–570

Patel P, Harde P, Pillai J, Darji N, Patel B (2012) Antidiabetic herbal drugs a review. Pharmacophore 3:18–29

Patel TP, Soni S, Parikh P, Gosai J, Chruvattil R, Gupta S (2013) Swertiamarin: an active lead from *Enicostemma littorale* regulates hepatic and adipose tissue gene expression by targeting PPAR-γ and improves insulin sensitivity in experimental niddm rat model. Evid-Based Complement Altern Med. https://doi.org/10.1155/2013/358673

Pei K, Ou J, Huang J, Ou S (2016) p-Coumaric acid and its conjugates: dietary sources, pharmacokinetic properties and biological activities. J Sci Food Agric 96:2952–2962. https://doi.org/10.1002/jsfa.7578

Pfefferkorn JA, Guzman-Perez A, Oates PJ, Litchfield J, Aspnes G, Basak A, Benbow J, Berliner MA, Bian J, Choi C, Freeman-Cook K, Corbett JW, Didiuk M, Dunetz JR, Filipski KJ, Hungerford WM, Jones CS, Karki K, Ling A, Li JC, Patel L, Perreault C, Risley H, Saenz J, Song W, Tu M, Aiello R, Atkinson K, Barucci N, Beebe D, Bourassa P, Bourbounais F, Brodeur AM, Burbey R, Chen J, D'Aquila T, Derksen DR, Haddish-Berhane N, Huang C, Landro J, Lee Lapworth A, MacDougall M, Perregaux D, Pettersen J, Robertson A, Tan B, Treadway JL, Liu S, Qiu X, Knafels J, Ammirati M, Song X, Dasilva-Jardine P, Liras S, Sweet L, Rolph TP (2011) Designing glucokinase activators with reduced hypoglycemia risk: discovery of N, N-dimethyl-5-(2-methyl-6-((5-methylpyrazin-2-yl)-carbamoyl)benzofuran-4- yloxy)pyrimidine-2-carboxamide as a clinical candidate for the treatment of type 2 diabetes mellitus. Medchemcomm 2:828–839. https://doi.org/10.1039/c1md00116g

Polisetti DR, Kodra JT, Lau J, Bloch P, Valcarce-Lopez MC, Blume N, Guzel M, Santhosh KC, Mjalli AMM, Andrews RC, Subramanian G, Ankersen M, Vedso P, Murray A, Jeppesen L (2004) Preparation of thiazolyl aryl ureas as activators of glucokinase. PCT Int Appl

Rappé AK, Casewit CJ, Colwell KS, Goddard WA, Skiff WM (1992) UFF, a full periodic table force field for molecular mechanics and molecular dynamics simulations. J Am Chem Soc 114:10024–10035. https://doi.org/10.1021/ja00051a040

Rathod MC, Dhale DA (2013) Pharmacognostic characterization of *Enicostemma littorale* blume. Int J Res Ayurveda Pharm 4:893–898. https://doi.org/10.7897/2277-4343.04624

Sadique J, Chandra T, Thenmozhi V, Elango V (1987) The anti-inflammatory activity of *Enicostemma littorale* and *Mollugo cerviana*. Biochem Med Metab Biol 37:167–176. https://doi.org/10.1016/0885-4505(87)90023-5

Salehi B, Venditti A, Sharifi-Rad M, Kręgiel D, Sharifi-Rad J, Durazzo A, Lucarini M, Santini A, Souto EB, Novellino E, Antolak H, Azzini E, Setzer WN, Martins N (2019) The therapeutic potential of Apigenin. Int J Mol Sci. https://doi.org/10.3390/ijms20061305

San Diego: Accelrys Software Inc. (2012) Discovery Studio Modeling Environment Release 35. Accelrys Softw Inc., San Diego

Sanmugarajah V (2013) Phyto, physicochemical standardization of medicinal plant *Enicostemma Littorale*. Blume IOSR J Pharm 3:52–58. https://doi.org/10.9790/3013-32205258

Sarabu R, Berthel SJ, Kester RF, Tilley JW (2008) Glucokinase activators as new type 2 diabetes therapeutic agents. Expert Opin Ther Pat 18:759–768. https://doi.org/10.1517/13543776.18.7.759

Sawant LP, Prabhakar BR, Pandita NS (2011) A validated quantitative HPTLC method for analysis of biomarkers in *Enicostemma littorale* Blume. J Planar Chromatogr 24:497–502. https://doi.org/10.1556/JPC.24.2011.6.8

Selvaraj S, Selvaraj S, Chittibabu CV, Janarthanam B (2014) Studies on phytochemical screening, antioxidant activity and extraction of active compound (Swertiamarin) from leaf extract of *Enicostemma littorale*. Asian J Pharm Clin Res 7:240–244

Shukla S, Gupta S (2010) Apigenin: a promising molecule for cancer prevention. Pharm Res. https://doi.org/10.1007/s11095-010-0089-7

Siddiqui FA, Khan SL, Marathe RP, Nema NV (2021) Design, synthesis, and in silico studies of novel N-(2-Aminophenyl)-2,3- diphenylquinoxaline-6-sulfonamide derivatives targeting receptor- binding domain (RBD) of SARS-CoV-2 spike glycoprotein and their evaluation as antimicrobial and antimalarial agents. Lett Drug Des Discov 18:915–931. https://doi.org/10.2174/1570180818666210427095203

Sidduri A, Grimsby JS, Corbett WL, Sarabu R, Grippo JF, Lou J, Kester RF, Dvorozniak M, Marcus L, Spence C, Racha JK, Moore DJ (2010) 2,3-Disubstituted acrylamides as potent glucokinase activators. Bioorganic Med Chem Lett 20:5673–5676. https://doi.org/10.1016/j.bmcl.2010.08.029

Singh R, Lather V, Pandita D, Judge V, Arumugam K, Grewal A (2016) Synthesis, docking and antidiabetic activity of some newer benzamide derivatives as potential glucokinase activators. Lett Drug Des Discov 14:540–553. https://doi.org/10.2174/1570180813666160819125342

Sonawane RD, Vishwakarma SL, Lakshmi S, Rajani M, Padh H, Goyal RK (2010) Amelioration of STZ-induced type 1 diabetic nephropathy by aqueous extract of *Enicostemma littorale* Blume and swertiamarin in rats. Mol Cell Biochem 340:1–6. https://doi.org/10.1007/s11010-010-0393-x

Srinivasan M, Padmanabhan M, Prince PSM (2005) Effect of aqueous *Enicostemma littorale* Blume extract on key carbohydrate metabolic enzymes, lipid peroxides and antioxidants in alloxan-induced diabetic rats. J Pharm Pharmacol 57:497–503. https://doi.org/10.1211/0022357055722

Srinivasulu C, Ramgopal M, Ramanjaneyulu G, Anuradha CM, Suresh Kumar C (2018) Syringic acid (SA): a review of its occurrence, biosynthesis, pharmacological and industrial importance. Biomed Pharmacother. https://doi.org/10.1016/j.biopha.2018.09.069

Thirumalai T, Viviyan Therasa S, Elumalai EK, David E (2011) Hypolipidaemic and antioxidant effect of *Enicostemma littorale* Blume. Asian Pac J Trop Biomed 1:381–385. https://doi.org/10.1016/S2221-1691(11)60084-1

Upadhyay UM, Goyal RK (2004) Efficacy of *Enicostemma littorale* in type 2 diabetic patients. Phyther Res. https://doi.org/10.1002/ptr.1434

Vaidya H, Rajani M, Sudarsanam V, Padh H, Goyal R (2009a) Swertiamarin: a lead from *Enicostemma littorale* Blume. For anti-hyperlipidaemic effect. Eur J Pharmacol 617:108–112. https://doi.org/10.1016/j.ejphar.2009.06.053

Vaidya H, Rajani M, Sudarsanam V, Padh H, Goyal R (2009b) Antihyperlipidaemic activity of swertiamarin, a secoiridoid glycoside in poloxamer-407-induced hyperlipidaemic rats. J Nat Med 63:437–442. https://doi.org/10.1007/s11418-009-0350-8

Vasu VT, Modi H, Thaikoottathil JV, Gupta S (2005) Hypolipidaemic and antioxidant effect of *Enicostemma littorale* Blume aqueous extract in cholesterol fed rats. J Ethnopharmacol 101:277–282. https://doi.org/10.1016/j.jep.2005.05.007

Vijayvargia R, Kumar M, Gupta S (2000) Hypoglycemic effect of aqueous extract of *Enicostemma littorale* Blume (*Chhota chirayata*) on alloxan induced diabetes mellitus in rats. Indian J Exp Biol 38:781–784

Vishwakarma SL, Rajani M, Bagul MS, Goyal RK (2004) A rapid method for the isolation of swertiamarin from Enicostemma littorale. Pharm Biol 42:400–403. https://doi.org/10.1080/13880200490885095

Vishwakarma SL, Sonawane RD, Rajani M, Goyal RK (2010) Evaluation of effect of aqueous extract of enicostemma littorale blume in streptozotocin-induced type 1 diabetic rats. Indian J Exp Biol 48:26–30

Wu P, Li L, Du GH (2018) Ferulic acid. Natural small molecule drugs from plants. Springer, Berlin, pp 75–80. https://doi.org/10.1007/978-981-10-8022-7_12

Zelent D, Najafi H, Odili S, Buettger C, Weik-Collins H, Li C, Doliba N, Grimsby J, Matschinsky FM (2005) Glucokinase and glucose homeostasis: Proven concepts and new ideas. Biochem Soc Trans. https://doi.org/10.1042/BST0330306

Zhang L, Hu S, Lei L, Zhang Y, Zhang L, Song H, Shen Z, Feng Z (2016) Design, synthesis and evaluation of novel derivatives of orotic acid amide as potent glucokinase activators. Lett Drug Des Discov 14:252–261. https://doi.org/10.2174/1570180813666161013150056