

Computational Screening of Phytochemicals from *Trigonellafoenum-graecumas* Potential Inhibitor of Alpha-Amylase and Maltase-Glucoamylase in Treatment of Type 2 Diabetes

ABSTRACT

Introduction: Diabetes mellitus is a major illness suffered by several individuals, a metabolic dysfunction of glucose that leads to life-threatening complications. Type 2 diabetes is a combination of insulin action resistance, insufficient insulin production, and excessive glucagon secretion

Aim: Numerous medications have been developed to manage diabetes mellitus by inhibiting various glucose metabolism enzymes and transporter proteins. However, the several adverse effects and high cost of treatment cannot be ignored. Thus, **discovering and designing a small-molecule inhibitor with minimal side effects targeting vital proteins linked to glucose metabolism is essential.**

Methodology: This study utilizes a computer-aided drug design approach to identify bioactive compounds from *Trigonellafoenum-graecum* with inhibitory potential against alpha-amylase and maltase-glucoamylase, which are glucose-metabolizing proteins. The compounds retrieved from the PubChem database were screened against the protein retrieved from the Protein Data Base using **molecular docking analysis, binding energy study, and ADME Toxicity Screening.**

Result: Inositol, Isovitexin, Luteolin, Miglitol, Mimosine, Quercetin, Riboflavin, and Vitexin were identified to have high inhibitory potentials. These compounds showed impressive binding to the target proteins and admirable double-action inhibition of the proteins. The ADME Toxicity screening of the compounds also revealed that they are good drug candidates.

Conclusion: The lead compounds are potential inhibitors of Alpha-Amylase and Maltase-Glucoamylase. Further preclinical investigation is advised to validate this study.

Keywords: Type 2 Diabetes, Small-Molecule Inhibitor, *Trigonellafoenum-graecum*, Computer-Aided Drug Design, Alpha-amylase, Maltase-glucoamylase.

1. INTRODUCTION

Diabetes has been a serious metabolic disorder of glucose affecting people's lives for thousands of years. It is a prevalent disease and a primary worldwide health concern (Standlet *et al.*, 2019). Diabetes complications from patients living with diabetes have been reported to be one of the leading causes of mortality (Tancredi *et al.*, 2015). Diabetes mellitus is a metabolic disease characterized by dysregulation in glucose, proteins, and lipids metabolism brought on by deficiencies in insulin production or action. Symptoms of diabetes include polyuria (excessive urination), polydipsia (excessive thirst), polyphagia (Increased appetite), weight loss, fatigue, blurred vision, and several complicated symptoms (Dwivedi and Pandey., 2020.; Pawaret *et al.*, 2017). Type 2 diabetes **mellitus is a group of dysfunctions marked by elevated blood glucose levels, a combination of insulin action resistance, insufficient insulin production, and excessive glucagon secretion causes this (Olimjonovna., 2024).** Plant compounds are important in medicine discovery, as evidenced by the number of medications licensed in the last few decades (Najmi *et al.*, 2022). Through the inhibition of many enzymes involved in glucose metabolism, plant chemicals have been demonstrated to offer some protection against the pathophysiology of diabetes mellitus as well as management of the condition (Alam *et al.*, 2019). Natural products and their active molecules are a considerable alternative for treating type 2 diabetes and its complications. **Several potent medicinal plants and their naturally occurring bioactive compounds have been shown to have therapeutic effects against type 2 diabetes (Okoduwa *et al.*, 2024., Unuofin and Lebelo., 2020).**

A condition known as postprandial hyperglycaemia (PPG), which is defined as unusually elevated glucose levels in the postprandial period, is a common feature found in patients living with type 2 diabetes mellitus, which is frequently co-occurring with cardiovascular disease (Solomon *et al.*, 2018., Hiyoshi *et al.*, 2019). Postprandial hyperglycaemia is characterised by delayed insulin release following meal consumption and insufficient glucagon secretion reduction (Kuntarattanakoo *et al.*, 2018). This leads to abnormal glucose synthesis in the liver and kidneys, ineffective glucose absorption, and an elevated plasma glucose level of more than 7.8 mmol/L (140 mg/dL) (Hiyoshi *et al.*, 2019). One way to manage such incidents in diabetic patients is the inhibition of enzymes responsible for the metabolism of carbohydrates and the subsequent release of glucose into the bloodstream. Alpha-amylase is a low molecular weight enzyme that cleaves the alpha-D-(1-4) glycosidic bond between starch and glycogen, pancreatic amylase is secreted into the small intestine by the pancreas, and salivary glands in humans and many other species also create an alpha-amylase known as ptyalin in their digestive tracts (Tiwari *et al.*, 2015, Williams, 2019). Glucosidase is another digestive enzyme responsible for the hydrolytic degradation of carbohydrates such as starch, glycogen, and disaccharides into monosaccharides. An example of Glucosidase is Maltase-Glucoamylase located on the brush border of the small intestine and responsible for the absorption of glucose (Rose *et al.*, 2018).

For the management of type 2 Diabetes Mellitus where the insulin produced is not enough to lower the blood glucose, miglitol is an antihyperglycemic drug that works as an oral active inhibitor of carbohydrate metabolism enzymes (Bhatnagar and Mishra., 2022). It inhibits the activity of membrane-bound α -glucosidases, including intestinal glucoamylase, sucrase, maltase, and isomaltase, as well as pancreatic α -amylase (Sugimoto *et al.*, 2015). Miglitol drugs are costly and frequently have gastrointestinal adverse effects, among other issues (Fisher, 2022). Thus, a different approach to managing hyperglycaemia is the development of inhibitors from plants with fewer or no adverse effects. *Trigonella foenum-graecum* commonly known as Fenugreek is a traditional herbal medicine that has been reported to exhibit therapeutic effects on diabetes (Geberemeske *et al.*, 2019) (Baset *et al.*, 2020). It is administered to diabetes patients in unrefined formulations, which leaves a gap in our knowledge of the function of specific phytoconstituents that may improve our ability to identify new drugs derived from these powerful antidiabetic plants. This study aims to investigate the inhibitory potential of various phytochemicals present in *Trigonella foenum-graecum* against alpha-amylase and maltase-glucoamylase protein using a computational approach.

2. MATERIALS AND METHOD

2.1. LIGAND PREPARATION

The 118 compounds used in this study were derived from the Indian Medicinal Plants, Phytochemistry and Therapeutics database (IMPPAT 2.0). The library of compounds was downloaded in 2d SDF format from the NCIB PubChem database (<https://www.ncbi.nlm.nih.gov/pccompound>) along with the FDA-approved standard drug Miglitol (Guo *et al.*, 2024). The compounds were imported into Maestro 13.4 and prepared using the LigPrep module of the Schrodinger suite. They were converted from 2D to 3D structures suitable for molecular docking, The ionization and tautomeric states were generated between pH values of 7.0 + 2.0 using the Epik module. The LigPrep module utilized the Optimized Potentials for Liquid Simulations (OPLS3) force field to minimize the compounds (Chahal *et al.*, 2020).

2.2. PROTEIN PREPARATION

Similarly, the 3D structure of the target proteins (Alpha-amylase PDB ID: 3BAJ, and Maltase-glucoamylase PDB ID: 2QMJ) were retrieved from the Protein Data Base (<https://www.rcsb.org/>). They were prepared using the protein preparation wizard after being imported into the Maestro Schrodinger Suite. Protein structures were revised in terms of bond order, topologies, incomplete and terminal amide groups, missing side chains, formal charges and missing hydrogen atoms. Water molecules Beyond 5Å⁰ were eliminated. Side-chain hydroxyl groups and probable steric conflicts were reoriented by minimizing the protein structure using the OPLS3 force field (Kalirajan *et al.*, 2017). The binding pocket of the proteins in which ligands and proteins interact was generated using the Receptor Grid Generation tool of the Schrodinger software, the coordinates x,y, and z were selected as (10.19, 15.83, 41.07) and (-20.85, -6.68, -5.18) for Alpha-amylase and Maltase-glucoamylase respectively.

2.3. ADMET SCREENING

The qikprop module of the Maestro Schrodinger suite was used to screen the library of compounds according to their Adsorption, Distribution, Metabolism, Excretion and Toxicity properties. The various compounds which violate more than one of the Lipinski rules of five were eliminated and the remaining compounds were used in further analysis.

2.4. MOLECULAR DOCKING

The glide tool of the Maestro Schrodinger suite was used to carry out the molecular docking analysis, the ligands were treated as flexible and docked into the binding pocket of the protein via the receptor grid generated. By default, the partial charge cutoff (0.15) and vdW radius scaling factor (0.80) were used and the output was limited to write out at most one pose per ligand. The docking was performed at two different levels of precision, the standard precision was initially used to score and rank the compounds. The top-scoring performing compounds were subjected to extra precision, a more accurate bind-scoring function. The protein-ligand complexes' binding positions were examined more thoroughly, and the docking scores were exported.

2.5. PRIME MM/GBSA

The free energy of binding (ΔG_{bind}) of the hit compounds and reference ligands' protein-ligand complexes was calculated using the Prime-MM-GBSA module of the Maestro Schrodinger suite. The solvation model was set as VSGB, force field OPLS was used, and the sampling method was minimized. The binding free energy is calculated as:

$$\Delta G_{\text{bind}} = E_{\text{complex}(\text{minimized})} - (E_{\text{protein}(\text{unbound, minimized})} + E_{\text{ligand}(\text{unbound, minimized})})$$

The ΔG_{bind} is the calculated binding free energy, $E_{\text{ligand}(\text{unbound, minimized})}$ is the MM-GBSA energy of the ligand after releasing it from the crystal complex, $E_{\text{protein}(\text{unbound, minimized})}$ is the MM-GBSA energy of the minimized protein after releasing it from its bound ligand, and $E_{\text{complex}(\text{minimized})}$ is the MM-GBSA energy of the minimized complex (Mulakala and Viswanadhan., 2013).

2.6. PHARMACOKINETICS AND DRUG-LIKENESS

The hit compounds from the molecular docking analysis of *Trigonella foenum-graecum* against alpha-amylase and maltase-glucoamylase were made to undergo ADMET assessment using the online SWISSADME tool (<http://www.swissadme.ch/>). The isomeric smiles of the compounds were retrieved from the NCIB database (<https://pubchem.ncbi.nlm.nih.gov/>), the isomeric smiles were supplied to the SWISSADME online server to evaluate further their physicochemical properties, lipophilicity, hydrophilicity, pharmacokinetics, and drug-likes characteristics.

3. RESULTS

Table 1: Docking Score and MM/GBSA of Hit Compounds from *Trigonellafoenum-graecum* Against Alpha-Amylase Protein (3BAJ).

PubChem ID	Compound Name	Docking Score (Kcal/mol)	MMGBSA(ΔG_{Bind})
5280445	Luteolin	-8.077	-37.13
162350	Isovitexin	-7.99	-34.46
5280441	Vitexin	-7.907	-28.25
5280343	Quercetin	-7.653	-36.26
493570	Riboflavin	-7.497	-47.51
440473	Mimosine	-7.134	-14.84
892	Inositol	-7.133	-23.04
441314	Miglitol	-6.983	-17.53

Table 2: Docking Score and MM/GBSA of Hit Compounds from *Trigonellafoenum-graecum* Against Maltase-glucoamylase Protein (2QMJ).

PubChem ID	Compound Name	Docking Score (Kcal/mol)	MMGBSA(ΔG_{Bind})
441314	Miglitol	-9.637	-46.87
5280343	Quercetin	-7.582	-40.85
5280441	Vitexin	-7.549	-32.7
892	Inositol	-7.221	-12.04
162350	Isovitexin	-7.148	-39.31
440473	Mimosine	-6.495	-13.44
5280445	Luteolin	-5.967	-36.09
493570	Riboflavin	-5.913	-21.03

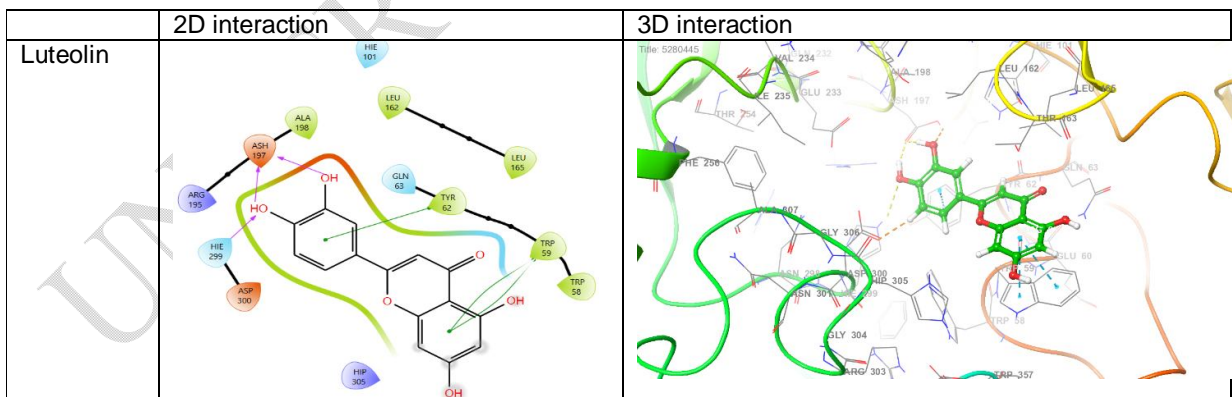


Table 3: Pharmacokinetics properties of the hit compounds from *Trigonellafoenum-graecum*

Compound Name	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)
Inositol	Low	No	Yes	No	No	No	No	No	-10.03
Isovitexin	Low	No	No	No	No	No	No	No	-8.79
Luteolin	High	No	No	Yes	No	No	Yes	Yes	-6.25
Miglitol	Low	No	Yes	No	No	No	No	No	-9.39
Mimosine	High	No	No	No	No	No	No	No	-10.6
Quercetin	High	No	No	Yes	No	No	Yes	Yes	-7.05
Riboflavin	Low	No	No	No	No	No	No	No	-9.63
Vitexin	Low	No	No	No	No	No	No	No	-8.79

graecum

Table 4: Drug-likeness properties of the hit compounds from *Trigonellafoenum-graecum*

Compound Name	Formula	Molecular Weight	Lipinski Violations	Ghose Violations	Veber Violations	Egan Violations	Bioavailability Score
Inositol	C6H12O6	180.16	1	2	0	0	0.55
Isovitexin	C21H20O10	432.38	1	0	1	1	0.55
Luteolin	C15H10O6	286.24	0	0	0	0	0.55
Miglitol	C8H17NO5	207.22	0	1	0	0	0.55
Mimosine	C8H10N2O4	198.18	0	1	0	0	0.55
Quercetin	C15H10O7	302.24	0	0	0	0	0.55
Riboflavin	C17H20N4O6	376.36	0	1	1	1	0.55
Vitexin	C21H20O10	432.38	1	0	1	1	0.55

4. DISCUSSION

The molecular docking methodology investigates the actions of tiny compounds within a target protein's binding region (De Ruycket *et al.*, 2016). The two main processes in the molecular docking process are the prediction of the conformation of the ligand, which is typically a tiny molecule, as well as its orientation and position within the protein binding site, and the evaluation of the pose's quality using a scoring function (Stanzione *et al.*, 2021). The library of 118 compounds retrieved from an online database along with an FDA-approved diabetes drug Miglitol was studied to evaluate their binding affinities against two different proteins of therapeutic effect on diabetes. To determine the degree of inhibition of the protein by the ligands, these compounds were docked against the identified active regions of the protein targets utilizing the mechanism for scoring. After that, an MM/GBSA technique was applied to the complexes' Glide-generated poses to further assess their binding potential. The compounds with the highest docking scores along with their MM/GBSA calculation result are presented in Table 1. and Table.2. Luteolin, Isovitexin, Vitexin, Quercetin, Riboflavin, Mimosine, and Inositol were all observed to exhibit a higher binding and inhibitory potential on Alpha-amylase protein than the standard drug Miglitol. Luteolin (-8.077), Isovitexin(-7.99), and Vitexin(-7.907) possess the highest binding affinity when compared to other compounds isolated from *Trigonella foenum-graecum*. On the other hand, the standard drug Miglitol (-9.637) had a better docking score when compared to the hit compounds which also manifest a good inhibitory potential against maltase-glucoamylase. Nevertheless, Quercetin (-7.582), Vitexin (-7.549), Inositol (-7.221), and Isovitexin (-7.148) with the highest binding affinity score make them a potential inhibitor of the Maltase-glucoamylase. The 2D and 3D ligand-protein interaction of the compounds and the target proteins alpha-amylase and maltase-glucoamylase are presented in Fig1.1 and Fig1.2 respectively. The protein-ligand complexes were further subjected to the MM/GBSA methodology for rescoring since the MM/GBSA technique computes the binding free energy the quickest among force-field-based methods (Zhang *et al.*, 2017). A shift in Gibbs free energy indicates an associated energy change during the complex's formation, Gibbs free energy is a metric used to quantify the stability and spontaneity of drug-target complexes (Jayaram and Prajapat, 2023). The compounds all exhibited good MM/GBSA scores indicating the feasibility of the ligand-protein binding. The hit ligands bonded properly to both alpha-amylase and maltase-glucoamylase proteins with varying scores, and the pharmacokinetics and drug-likeness properties of the compounds were analysed using the SWISSADME online server tool (<http://www.swissadme.ch/>) to validate further the ligands' drug potentials. The pharmacokinetic properties of the hit compounds are provided in Table3 showing various parameters. Luteolin, Mimosine, and Quercetin possess a high gastrointestinal adsorption value while the remaining compounds possess a relatively low value. Between the blood and the brain's interstitium is a selective semi-permeable membrane known as the blood-brain barrier (BBB), which enables cerebral blood vessels to control the flow of ions and molecules between the two (Bors and Erdő., 2019). All the compounds are negative to the blood-brain barrier which indicates they are impermeable to the barrier. The p-glycoprotein is a membrane protein that inhibits the adsorption and metabolism of compounds that bind to the protein, these compounds are known as pgp substrates (Mollazadeh *et al.*, 2018). Table 3 shows that inositol and miglitol are pgp substrates thus reducing their absorption and bioavailability. Quercetin and luteolin are inhibitors of the cytochrome p450 isoforms CYP1A2 and CYP2D, while the remaining compounds are interestingly not inhibitors of the cytochrome p450 isoforms. The log Kp values of the lead compounds range between 6.25 to 10.60 indicating their low skin penetration ability. Table 4 shows the result of the drug-likeness characteristics of the lead compounds including the chemical formula, Lipinski's rule of five, Ghose's filter rule, Veber's rule and Egan's rule. Impressively, all the compounds have a molecular weight of less than 500 and a 0.55 bioavailability score.

5. CONCLUSION

Fenugreek also known as *Trigonella foenum-graecum* has been reportedly used in the treatment of many health-related issues including diabetes. This computational study predicted the antidiabetic mechanism of compounds isolated from *Trigonella foenum-graecum*, Inositol, Isovitexin, Luteolin, Miglitol, Mimosine, Quercetin, Riboflavin, and Vitexin exhibited double-action inhibitory potential through good binding affinity to the protein. Supplemental ADMET screening complemented the ability of the compounds to reduce the blood glucose level in diabetic patients as a potential drug candidate. Further analysis such as *in vivo* study should be carried out on the compounds to validate their inhibitory potential against alpha-amylase and maltase-glucoamylase proteins.

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