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Molecular docking analysis of α-amylase with phytocompounds from *Enicostemma littorale*

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Abstract

Diabetes mellitus (DM) is one of the deadliest metabolic diseases in the world, with a high mortality rate. Diabetes mellitus is characterised by insulin resistance and inadequate insulin production from pancreatic -cells, as is well known. Due to the adverse reactions of the current therapy, there is an urgent need for the development of new effective anti-diabetic pharmaceuticals, particularly alpha-amylase inhibitors with fewer side effects. In addition, natural products are well-known sources for the discovery of novel bioactive compounds which may function as scaffolds for drug discovery, including the discovery of new antidiabetic medications. Using an in silico approach, the current investigation sought to identify novel drug-like molecules as anti-diabetic compounds in Enicostemma littorale. Enicostemma littorale blume (E. littorale), a perennial herb belonging to the Gentianaceae family, is widespread in India. Observed intermolecular interactions between target proteins and various anti-diabetic compounds derived from Enicostemma littorale. Four compounds with the highest docking scores (vanillic acid, p-coumaric acid, protocatechuic acid, and hydroxybenzoic acid) were chosen for further interaction analysis based on the results of docking studies. For experimental substantiation of the antidiabetic activity of these compounds, testing is necessary.

Key words: Diabetes mellitus, *Enicostemma littorale blume*, Molecular docking,alpha-amylase.

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INTRODUCTION

The metabolic syndrome known as diabetes mellitus (DM) is characterised by hyperglycemia as a result of the body's failure to produce enough insulin, irregularities in its production, or tissue resistance to its action [1, 2]. Defects in the metabolic procedures responsible for digesting carbs, proteins, and lipids can also lead to hyperglycemia in DM [3, 4]. Some traditional symptoms, such as polyuria, polydipsia, and polyphagia, develop as a result [5]. These metabolic anomalies are brought on by low insulin levels or by target tissues' resistance to insulin at the level of signal transduction, insulin receptors, genes, or effector enzymes (adipose tissue, skeletal muscles, and liver) [6].A variety of acute or chronic problems in DM are linked to long-term blood glucose elevation [7]. According to estimates, the prevalence of diabetes will rise from 4% in the world in 1995 to 5.4% by 2025 [8]. According to the International Diabetes Federation (IDF), the global prevalence climbed to 366 million individuals in 2011 and is predicted to reach 552 million people by the year 2030 [9]. Additionally, it has been reported that 450 million people worldwide have DM, and that number is anticipated to rise to 690 million by the year 2044 [10].

Targeting the -amylase enzyme, which is of high pharmacological interest and is used to control elevated glucose levels in T2DM, is one of the crucial targets when it comes to treating type-2 diabetes because it causes the metabolic breakdown of complex dietary carbohydrates into simple sugars, which are then absorbed [11].

Oral hypoglycemic drugs such as metformin, sulfonylureas, thiazolidinediones [10], biguanides, meglitinides, dipeptidyl peptidase-IV inhibitors [11], etc., have been developed to treat type 2 DM; however, prolonged use of these medicinal products may cause severe side effects, including liver complication, hypoglycemia, and diarrhoea [12]. Now, it is crucial to create novel inhibitors in significant effectiveness and low risk. Due to their reduced toxicity, specificity, target affinity, and abundance, herbal medicinal plants can be a superior source for the production of novel drugs under these conditions [13]. Various botanicals have been shown to have effective medicinal properties for lowering blood glucose levels [14].

In poor nations, the use of natural remedies and medicinal plants continues to be a common form of therapy [13–15]. The process of identifying, isolating, and characterising target molecules has been made easier by the development of current analytical techniques [16–18]. There are more than 400 plants known to have antidiabetic properties, but only a small number of these plants have been studied in terms of medicine and science [19]. Different microorganisms and plants produce a large number of -amylase inhibitors to control the activities of these enzymes [20].

E. littorale has a long history of usage in India as a stomachic, bitter tonic, fever reducer, and appetite stimulant [21, 22]. E. littorale is an herb that is sometimes taken alongside other herbs in Indian ayurveda treatment, particularly for diabetes. Since E. littorale plays a significant role in lowering blood sugar, raising serum insulin levels, and significantly improving kidney function, lipid profiles, systolic and diastolic blood pressure, and pulse rate, it is given as an ayurvedic pill for the treatment of type 2 diabetes [*23]*.The goal of this research is to find a new class of -amylase inhibitors that can not only control existing diabetes but also have therapeutic value in the insulin resistance stage of prediabetes, where the onset of the ailments may either be completely avoided or greatly postponed.

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Materials and Method

Ligand preparation

From the literature, 15(Table 1) E. *littorale* compounds were chosen. The 2D structures of these crystallised compounds were obtained in structure file format (SDF) from the PubChem database (www.pubchem.ncbi.nlm.nih.gov). Then, using Open Babel, the chemical structures were translated into mol2 format. Then, while the non-polar hydrogen molecules were combined with the carbon atoms, polar hydrogen charges of the Gasteiger type had been assigned to the atoms. The ligands were then translated to the PDBQT format for molecular docking simulation using AutoDock Tools.

Table 1: List of compound selected from *Enicostemmalittorale*

S.NO	COMPOUND NAME
1	Apigenin
2	Enicoflavine
3	Ferulic acid
4	Genkwanin
5	P-Coumaric Acid
6	P-Hydroxy Benzoic Acid
7	Protocatechuic Acid
8	Saponarin
9	Swertiamarin
10	Swertisin
11	Syringic Acid
12	Vanillic Acid
13	Threonine
14	Methionine
15	L-Glutamic Acid

Table 2: Molecular interaction of selected compounds with target protein

S.NO	COMPOUND NAME	BINDING ENERGY KCAL/MOL	INTERACTING AMINO ACIDS
1	Vanillic acid	-6.5	ARG-252 ARG-398 PRO-332
2	p-coumaric_acid	-6	ARG-398 ARG-421 ASP-402

3	protocatechuic_acid	-5.5	ARG-252 ARG-398 ASP-402
4	hydroxy_benzoic_acid	-5.1	ARG-252

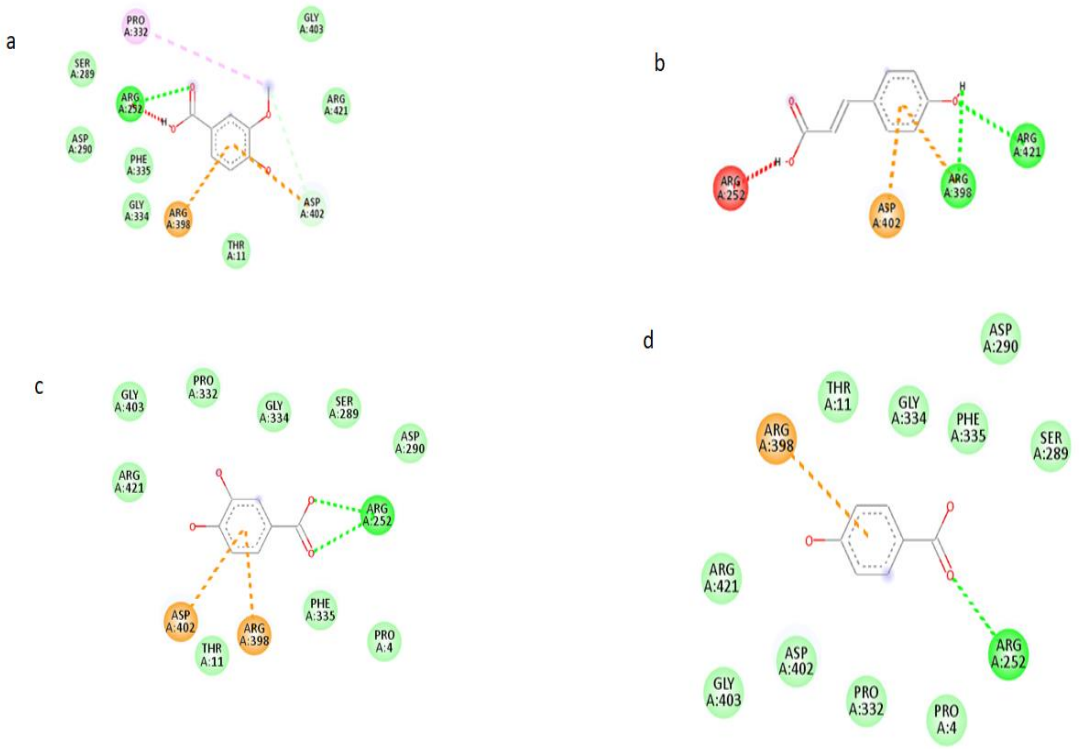

Figure.1: Docking interaction of alpha amylases with a) Vanillic acid b)p-coumaric_acid c) protocatechuic_acid d) hydroxy_benzoic_acid

Retrieval and Preparation of Alpha-Amylase Drug Target

Alpha-amylase's three-dimensional crystal structure was downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) PDB (http://www.rcsb.org/pdb/home/home.do) using the Protein Data Bank (PDB) ID 2QV4 and resolution 1.97. The PyMol tool was used to prepare the proteins. The non-essential water molecules and complicated molecules that were bound to the proteins during their preparation were taken out. Discovery Studio 2017 R2 was also used to remove whole heteroatoms. When seen on PyMol and Discovery Studio 2017 R2 visualizer, the co-crystallized ligand was removed from the protein's active site in order to show the grid coordinate surrounding the binding pocket.

Molecular Docking Using PyRx

After preparing the drug target and ligand, PyRx's Auto Dock Vina option was used to do the molecular docking study. The dimensions of the grid box were left alone. Following the docking study, the best conformation from each compound was chosen for further

examination based on the RMSD and binding energy. Utilising the discovery studio visualizer, a study of molecular interactions was conducted.

Results and Discussion

A computer-based technique called molecular docking predicts the preferred orientation of one molecule to another when they are bonded together to form a stable complex. Predicting how two molecules will interact is the goal of molecular docking. Finding the most stable configuration between the ligand and protein is its ultimate objective.

A successful method for accelerating the drug discovery process is molecular docking. Given that it has already produced a large number of bioactive structures for a variety of targets, it is undoubtedly a successful technique for structure-based virtual screening. To find more potent -amylase inhibitors from the E. littorale plants, molecular docking was done in light of this. The intermolecular interactions between these binding partners affect how strong a protein-ligand complex is. By creating intermolecular contacts with important residues including ARG-252, ARG-398, PRO-332, ARG-421, and ASP-402, the results of docking experiments demonstrated that all the compounds had a high affinity for binding to -amylase. Figure 1 depicts every interaction between these substances and the target protein. The computation of binding energy to fit a ligand in a binding site is the most crucial step in molecular docking. The majority of the compounds displayed good binding energy according to the docking study, thus we chose the top four compounds based on their low binding energy scores of -6.5 to -5.1 kcal/mol.

The molecular docking analysis between these compounds and target protein showed the amino acid residues and interactions responsible for the stable protein–inhibitor complex formation. The results of the binding affinity (kcal/mol) of the compounds to the target protein was shown in Table 2.

Conclusions

The phytochemicals from *E. littorale's* anti-diabetic properties showed that this plant is a rich source of abietane diterpenes with potent alpha-amylase inhibitory properties. The findings of these docking studies imply that the chosen compounds might emerge as noteworthy natural medicinal candidates with antidiabetic efficacy against alpha-amylase enzymes. As a result, substances with exceptional alpha-amylase inhibitory properties may be excellent options for regulating plasma glucose level and associated difficulties in diabetes patients.

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