

## IN SILICO ANALYSIS OF ACTIVE CONSTITUENTS OF SILYMARIN AS ALPHA-GLUCOSIDASE ENZYME INHIBITORS IN TYPE 2 DIABETES MELLITUS

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### ABSTRACT

**Objective:** Type 2 diabetes mellitus (T2DM) is an acute metabolic disorder, in which the vogue is increasing persistently globally. The maltase-glucoamylase/alpha-glucosidase inhibitor is an oral antidiabetic drug collectively, which is utilizing for regulating carbohydrates that ordinarily transformed into simple sugars and absorbed by the intestine. Researchers need to constantly explore alternative therapeutic strategies for the clinical management of DM due to the increased adverse event caused by conservative antidiabetic agents. The present study proposes a substitute drug to examine the seven bioactive phytoconstituents of *Silybum marianum* (milk thistle) that can regulate the hyperglycemia by downregulating alpha-glucosidase and its activity.

**Methods:** Different integrated web-based *in silico* tools and techniques were used to model the enzyme (receptor) as well as to determine the druggability of different active constituents of silymarin and their pharmacokinetics were predicted. Further, the active site of the enzyme was predicted followed by molecular docking method.

**Results:** The results show silychristin A and silydianin having less carcinogenicity and strong interaction to the target protein (alpha-glucosidase) compare to the reference drugs (acarbose and miglitol) and these two molecules can be used for the best drug molecules in T2DM.

**Conclusion:** In the proposed study, the *in silico* analysis helps researchers to utilize these compounds for clinical applications. The conclusion also suggests that synthetically and semi-synthetically, nucleus and peripheral modifications, either in the form of skeletal rearrangements or partial degradations as well as functional group addition and replacement of the active molecules present in silymarin giving access to new structural motifs, which can be used in future as a lead compounds for antagonising the alpha-glucosidase in the treatment of diabetes mellitus.

**Keywords:** Type 2 diabetes mellitus, Alpha-glucosidase, Silymarin, Silychristin A, Silydianin, Acarbose, Miglitol, Molecular docking, Milk thistle.

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### INTRODUCTION

Diabetes mellitus (DM) is undoubtedly one of the human's oldest known diseases. It was first reported about 3000 years ago in the Egyptian manuscript [1]. A perfect variance among T1 and Type 2 DM (T2DM) was undoubtedly defined [2,3]. T2DM was previously illustrated in 1988 as a component of metabolic syndrome [4]. The prompts of T2DM are complex, multifaceted and comprise both genetic and environmental features as well as behavioral risk factors that affect beta-cell obligation and tissue insulin sensitivity [5-7]. Persons with T2DM are more susceptible to various forms of short-term and long-term medical difficulties, which occasionally lead to premature death. This has been seen in patients with T2DM mostly due to the pervasiveness of this type of DM. As the prevalence of this metabolic disorder, it is promptly rising and standard treatment refuses to steady the disease in most patients, prevention would be perceived as a key objective in the relatively proximate future. Persons who experience T2DM go through a phase of impaired glucose tolerance (IGT). Defects in the action or even secretion of insulin seem to be the two main complications ultimately leading to the development of glucose intolerance. Any interference in the phase of IGT that decreases insulin resistance protects beta-cells or both should also prevent or delay progression to diabetes.

Several potential drugs such as sulfonylureas, meglitinides, biguanides, and thiazolidinediones and alpha-glucosidase inhibitors can be used in medical treatments to regulate high blood glucose problems (hyperglycemia). In general, alpha-glucosidase is concomitant through the breakdown of polysaccharide and disaccharides into

monosaccharide glucose [8,9]. Alpha-glucosidase inhibitors are oral antidiabetic medications which administered to alter the breakdown of carbohydrates that are transformed into simple sugars and absorbed by the intestines [10]. Alpha-glucosidase inhibitors appear to have a greater affinity of 10,000–100,000 times with carbohydrate-binding sites than oligosaccharides and polysaccharides. Oligosaccharides and polysaccharides cannot be converted into simple sugars that the body can absorb by inhibition of alpha-glucosidase enzyme [11]. Hereafter, alpha-glucosidase inhibitors are crucial medications to prevent the absorption of carbohydrates in the intestine and can be used to treat T2DM and IGT [12]. Thus, these hypoglycemic agents are applied to patients with early diabetes or combined with other drugs [13]. Medicinal plants and herbs are noticeable in traditional medicine and have appealing pharmacological activities [14] and the most significant properties of medicinal plants are, either they show lower side effects or they are without any side effects. During the past decade, alpha-glucosidase inhibitors from natural resources have been extensively established as highly purposeful foods or lead compounds to modulate diabetes.

Recently, drug designs used to significantly diminish blood glucose levels and try to maintain blood glucose homeostasis with slightly fewer adverse effects. In the structure-based drug design method, structures of considered target proteins are usually utilizing to perceive novel compounds that are therapeutically applicable. Computational or molecular docking is a simulation strategy that predicts molecules that properly bind to targets including enzymes and receptor proteins. Moreover, the rational methodology in virtual screening is known as the hierarchical method, which seems to be a valuable tool for computing

and screening protein structures to explore new ligands [15]. The ligand must have a high affinity with proteins to assess the binding site of a compound for a given receptor, as well as the property of interactions and leverage based on a scoring entity [16]. For example, the activity of a precise protein in human can be antagonized by the inhibitor discovery and protein-ligand interactions can be chosen mainly as a possible drug development which is called a pharmacophore model that can be utilized to achieve and recognize small molecules in two-dimensional or three-dimensional (3D) (*in silico*) protein structure and function prediction [17]. Therefore, the demand for enhanced pharmacophore modeling is significant to diminish drug costs and new drugs are most likely to bind to the target protein [18,19]. The current study conducted a high-performance molecular docking to screen the potential of alpha-glucosidase from natural compounds and evaluated the analysis of alpha-glucosidase activities.

## METHODS

### Homology modeling

The target sequences of alpha-glucosidase enzyme were compiled from the UniProtKB protein knowledgebase (ID: O43451) and compelled using NCBI PSI-BLAST to identify the template sequences. Further, the 3D protein structures were built using Swiss-Model (2QLY.1.A) and the protein structure was modeled using Swisspdbv viewer [20-23]. The consequential 3D structure of the protein was exposed to quality analysis using SAVES to predict the stereochemical activity of amino acids on the bases of geometry through various parameters such as WHATIF, ERRAT, and PROCHECK [24-27].

### Active site prediction

The geometric and topological possessions of modeled protein 2QLY.1.A (Computed Atlas of Surface Protein Topography [CASTp] ID: J\_5C744E039BB01) exerted to predict active site amino acid, based on surface pockets, interior cavities, cross-channel, and hydrogen bond interactions using CASTp calculation server. CASTp is a web server which provides online services for retrieving, outlining, and quantifying these geometric and topological attributes of protein structures [28]. The basic components of computational geometry exert in CASTp are Delaunay triangulation, alpha shape, and discrete flow [29-33]. The modeled 2QLY.1.A (CASTp ID: J\_5C744E039BB01) active sites were revealed using CASTp web server.

### Ligand preparation and pharmacophore analysis

Silybin A, silybin B, isosilybin A, isosilybin B, silychristin A, silychristin B, and silydianin are active components (phytoconstituents) present in milk thistle that was used as an assessment compounds, and acarbose and miglitol as a standard compound were retrieved from ChEMBL Database using SDF file format (<https://www.ebi.ac.uk/chembl/>). Moreover, the structures of the ligands (active molecules) were converted to Mol. format using Chemscketch software to avoid any unsolicited mistake in the structures. The pharmacophore analysis was performed to determine chemical properties and predict drug-like molecule, by means of molinspiration online server. The rule of 5 shows the considerations which are a set of basic molecular descriptors to predict drug-like molecule which have Log P $\leq$ 5, molecular weight (MW)  $\leq$ 500, hydrogen bond acceptor numbers  $\leq$ 10, and hydrogen bond donor numbers  $\leq$ 5. Those molecules which violate rather than one of these rules might have bioavailability problems [34]. These parameters help to screen the compounds to understand drug-like properties.

### Pharmacokinetic properties

Pharmacokinetic properties of different components of the silymarin were calculated using an admetSAR online server. Calculation of physicochemical parameters of small molecules is valuable to screen computationally of the chemical compounds for their druglikeness and even toxicity potential. The overall of Log P, topological polar surface area (TPSA), H-bond acceptor, H-bond donors, and MW were worked out separately for an individual constituent by means of OpenBabel v2.3.1 [35] that can be utilized for absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction. The incipience of initial

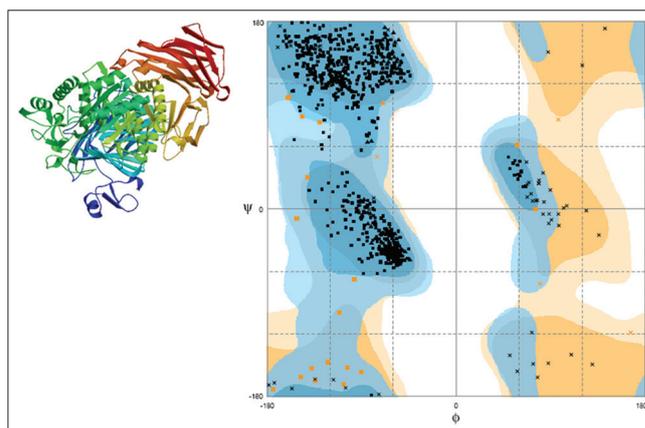
screening for ADMET has augmented for drug nominees incept in the drug discovery phase and decreased the percentage of compounds that failed in clinical trials due to ADMET reasons. The TPSA calculation has relied on the summation of the fragment contributions, which regards the polar fragments centered on O and N. Polar surface area is revealed to be an admirable descriptor for drug absorption such as intestinal absorption, bioavailability, Caco-2 permeability, and blood-brain barrier penetration [36]. The measurement of molecular flexibility is the number of rotatable bonds (Nrotb), which is the ordinary topological parameter. The Nrotb is a good descriptor to measure oral drug bioavailability [37]. Single non-ring bond, bound to the non-terminal heavy atom (i.e., non-hydrogen), is considered to be a rotatable bond. Amide C-N bonds are not considered due to their high rotational energy barrier.

### Molecular docking

Based on the pharmacophore and pharmacokinetic analysis, the designated compounds were docked with the target protein (alpha-glucosidase) using MGL Tools 1.5.6 and AutoDock 4.2; (Autodock, Autogrid, Copyright-1991e2000) from the Scripps Research Institute, <http://www.scripps.edu/mb/olson/doc/autodock> so that to start and run AutoDock, polar hydrogens were added to the protein alongside with the Gasteiger type and the non-polar hydrogen was merged with the carbons. After that, the internal degrees of freedom and torsion were determined to prepare flexible and rigid molecule and to prepare grid maps of different grid points that keep ligands covering binding pockets fully based on active site amino acids within the selected proteins. The size of the grid box was adjusted based on X, Y, and Z axis and 70 $\times$ 70 $\times$ 70 further require to calculate grid parameters using Autogrid. Molecular simulation parameters further to calculate Autodock properties to understand protein-ligand interactions by adding the Lamarckian genetic algorithm and molecular simulation methods to evaluate molecular docking. Though all parameters were considered necessary for molecular docking and therefore, pharmacophore mapping were fixed and used regularly for different compounds [38-40]. Consequently, the output of the docking process was analyzed utilizing EduPymol version 1.7.4.4.

## RESULTS AND DISCUSSION

The selected alpha-glucosidase protein sequences were aligned using PSI-BLAST and modeled using modeler. The resultant protein structure is built using Swisspdb viewer. The results showed alpha-glucosidase protein has 99.77% of similarity to 5KZW\_A protein template with the e-value of 0.0 and total score of 1798. The quality analysis of the modeled protein was predicted using SAVES. The modeled alpha-glucosidase protein has a similar template of 99.77% similar to alpha-glucosidase with e-value of 0.0. We built the modeled structure and observed the Ramachandran plot with amino acids in the allowed region that shows 85.6% of amino acids are in complex prediction (Fig. 1) that it shows the



**Fig. 1:**  $\alpha$ -Glucosidase protein structure is modeled by Swiss-Model and Ramachandran plot was predicted using RAMAPAGE

rigidity of the protein structure. The ligand-binding site was observed using CASTp based on the pocket region of geometrically distributed amino acids and based on interaction energy. Some of the active site amino acids such as TYR385, ASP413, ILE414, MET417, ARG420, ASP422, ILE450, ASP452, PRO453, ALA454, GLU490, VAL491, TRP492,

PRO493, PHE498, TRP527, ASP529, MET530, VAL533, SER534, ASN535, PHE536, ARG612, TRP625, ASP628, ASP657, PHE661, and HIS686 amino acid residues were found to be the best binding sites (Fig. 2). These active sites were considered for molecular docking.

#### Ligand structure preparation and pharmacophore analysis

Chemical library database such as ChEMBL was used to retrieve the bioactive compounds along with reference molecules. The ligand structures were screened to understand drug-like properties (Tables 1-3). ADMET and toxicity were performed employing Molinspiration and admetSAR online tools. The result obtained shows that the standard drugs having the higher predicted value of carcinogenicity, acarbose =1.00 and miglitol =0.9714 in comparison with silychristin A =0.9165 and silydianin =0.9571 with the lower value of carcinogenicity. The molecular simulation techniques were utilized to simulate the chemical structure to predict the electrostatic interaction and potential energy prediction. QSAR properties were predicted to the molecular simulated chemical structures utilizing Hyperchem.

#### Molecular docking and virtual screening

Molecular docking of particular bioactive compounds alongside with reference drug molecules was docked with target protein alpha-glucosidase. On the other hand, the docking results were predicted based on the binding energy (kcal/mol), number of the hydrogen bond, inhibitory constant (Ki), ligand efficiency, and interacting amino acids

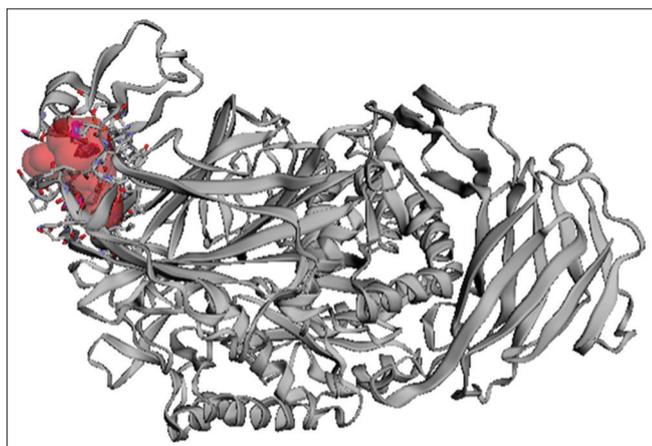


Fig. 2:  $\alpha$ -Glucosidase protein structures are used to predict ligand-binding sites and pockets using CASTp calculation server

Table 1: Pharmacophore analysis of silymarin active compounds predicted using Molinspiration

Ligand	miLogP	TPSA	nAtoms	MW	nON	nOHNH	Nrotb	Volume
Silybin A	1.47	155.15	35	482.44	10	5	4	400.86
Silybin B	1.47	155.15	35	482.44	10	5	4	400.86
Isosilybin A	1.47	155.15	35	482.44	10	5	4	400.86
Isosilybin B	1.47	155.15	35	482.44	10	5	4	400.86
Silychristin A	1.26	166.14	35	482.44	10	6	4	399.89
Silychristin B	1.26	166.14	35	482.44	10	6	4	399.89
Silydianin	1.21	162.98	35	482.44	10	5	3	395.34
Acarbose	-5.51	321.16	44	645.61	19	14	9	544.93
Miglitol	-2.75	104.38	14	207.23	6	5	3	189.18

TPSA: Topological polar surface area, nAtoms: Number of atoms, MW: Molecular weight, nON: Number of hydrogen bond acceptor, nOHNH: Number of hydrogen bond donor, Nrotb: Number of rotatable bond

Table 2: Absorption, distribution, metabolism, excretion, and toxicity analysis of silymarin active compounds predicted using admet SAR tool

Ligand	LogS	CYP2D6	LogP	Carcinogenicity	HIA	AMES	PPB	BBB	Hepatotoxicity
Silybin A	-2.649	0.9231	2.36	0.9022	0.9884	0.6300	0.849	0.6573	0.6000
Silybin B	-2.649	0.9231	2.36	0.9022	0.9884	0.6300	0.849	0.6573	0.6000
Isosilybin A	-2.649	0.9231	2.36	0.9022	0.9884	0.6300	0.843	0.6573	0.6500
Isosilybin B	-2.649	0.9231	2.36	0.9022	0.9884	0.6300	0.843	0.6573	0.6500
Silychristin A	-2.518	0.7995	2.40	0.9165	0.9889	0.5500	0.862	0.3007	0.6750
Silychristin B	-2.518	0.7995	2.40	0.9165	0.9889	0.5500	0.862	0.3007	0.6750
Silydianin	-3.035	0.8457	0.99	0.9571	0.9074	0.6000	0.918	0.2878	0.7750
Acarbose	1.383	0.8974	-8.56	1.0000	0.9664	0.5200	0.193	0.3230	0.6250
Miglitol	-0.222	0.9535	-3.26	0.9714	0.7664	0.6000	-0.02	0.9051	0.8750

LogS: Solubility measured in mol/liter, HIA: Human intestinal absorption, AMES: Chemical mutagenicity, PPB: Plasma protein binding, BBB: Blood-brain barrier

Table 3 Molecular docking of silybin active compounds against alpha-glucosidase protein structure using AutoDock

Ligand	H-bond	Binding energy (kcal/mol)	IC <sub>50</sub> Ki (uM/nM)	Amino acids
Silybin A	2	-8.35	760.85 (nM)	ALA454, TYR691
Silybin B	2	-7.72	2.18 (uM)	ALA454, GLN689
Isosilybin A	4	-8.86	320.68 (nM)	TRP492, GLN689, GLN689, TYR691
Isosilybin B	3	-8.06	1.24 (uM)	TYR385, TRP492, TYR691
Silychristin A	3	-10.00	46.70 (nM)	TRP492, ARG612, TYR691
Silychristin B	3	-9.51	106.26 (nM)	TYR385, ALA454, ASP628
Silydianin	4	-9.80	65.25 (nM)	GLU490, SER534, TRP492, ARG612
Acarbose	3	-7.19	5.41 (uM)	GLN689, GLN689, GLN689
Miglitol	5	-5.60	77.96 (uM)	TRP492, SER534, GLU490, GLU490, SER534



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