

***In silico* docking studies of alpha amylase inhibitory activity of some commercially available flavonoids**

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Abstract

Objective: The objective of this study was to evaluate the α -amylase inhibitory activity of certain flavonoids using *in silico* docking studies. In this perspective, 4-methyl esculetin, Genistein and Herbacetin were prepared for the docking evaluation.

Methods: *In silico* docking studies were carried out using recent version of AutoDock 4.2, which has the basic principle of Lamarckian genetic algorithm.

Results: The results showed that the selected flavonoids showed binding energy ranging between -6.00 kcal/mol to -5.62 kcal/mol when compared with that of the standard (-1.97 kcal/mol). Inhibition constant (39.96 μ M to 75.71 μ M) and intermolecular energy (-6.60 kcal/mol to -7.46 kcal/mol) of the ligands also coincide with the binding energy.

Conclusions: The selected compounds contributed excellent α -amylase inhibitory activity than the standard because of its structural parameters. These *in silico* approach analyses of the selected flavonoids could lead to the further development to find the potent α -amylase inhibitors for the treatment of diabetes.

Keywords: Genistein, 4-methyl esculetin, Binding interactions, Docking, Herbacetin

1. Introduction

Diabetes mellitus is a metabolic disease characterized by hyperglycemia resulting from fault in insulin secretion, insulin action or both. Most effective treatment for type II diabetes is the control of postprandial hyperglycemia after a meal. Stabilization of blood glucose is necessary for diabetic patients, because it prevents hyperglycemia and the complexity associated with diabetes [1]. The properties of diabetes mellitus consist of long-term injury, dysfunction and failure of various organs [2]. At the current situation it is predicted that 150 million people, universally have diabetes and that this will increase to 300 million by 2050 [3].

Modern medicines such as sulfonylureas, biguanides, and thiozolidinediones are intended for the treatment of diabetes. Though, they also have undesired effects related with their uses [4]. The natural products or medicinal plants reduce the absorption of glucose by reduce the carbohydrate hydrolyzing enzymes, such as pancreatic amylase. The inhibition of this enzyme slow down the carbohydrate digestion and extend the overall carbohydrate digestion time, ensuing in the decrease in glucose absorption rate and as a result reduce the postprandial plasma glucose rise. Several indigenous medicinal plants have a great potential in the α -amylase inhibition [5].

Flavonoids are a different group of metabolite derivative familiar for having a range of human health-promoting actions [6]. Several researches of flavonoids gives the details about their valuable biological activities, such as anti-allergenic, anti-cancer, anti-inflammatory, anti-oxidant, anti-viral, and vasodilating properties [7-9]. However, the *in silico* approach of the flavonoids on the α -amylase were not been characterized. The current study is to predict the *in silico* evaluation of α -amylase inhibitory activity and the stereochemistry binding of the flavonoids on α -amylase has been carried out, which may helpful in the development of potent α -amylase inhibitors.

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2. Materials and Methods

2.1 Software required

Python 2.7 - language was downloaded from www.python.com, Cygwin was downloaded from www.cygwin.com, Molecular graphics laboratory (MGL) tools and AutoDock4.2 was downloaded from www.scripps.edu, ChemSketch was downloaded from www.acdlabs.com, Discovery studio visualizer 2.5.5 was downloaded from www.accelrys.com. Online smiles translation was carried out using cactus.nci.nih.gov/translate/.

2.2 Docking Methodology:

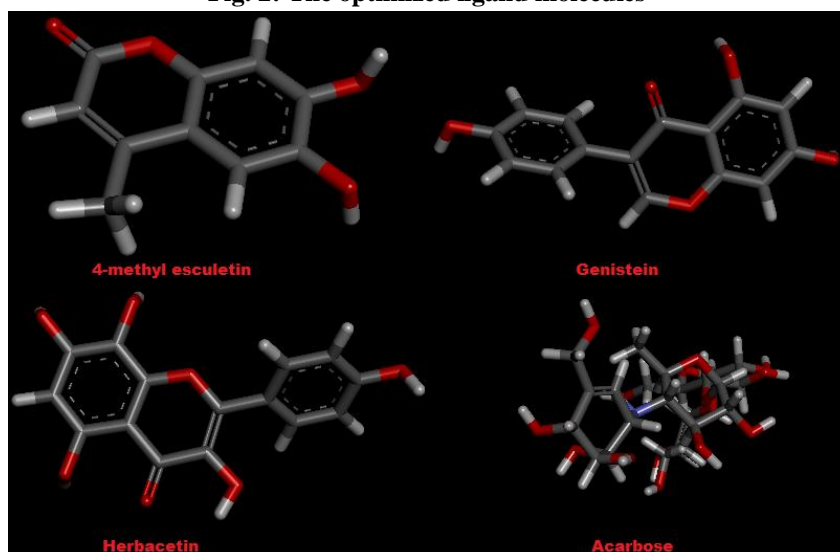
Crystal structure of target enzyme α -amylase (1HNY) was obtained from the RCSB protein data bank (**Fig.1**). The preparation of the target enzyme with the AutoDock Tools involved in the addition of hydrogen atoms to the target enzyme, which is a necessary step for the computation of partial atomic charges. Gasteiger charges were considered for each atom present in the target in AutoDock 4.2. Three-dimensional affinity grids of size $277 \times 277 \times 277 \text{ \AA}$ with 0.6 \AA spacing on the geometric center of the target and were considered for each of the following atom types: HD, C, A, N, OA, and SA, representing all probable atom types in the target enzyme. In addition, a desolvation map and an electrostatic map were also calculated [10].

Fig. 1: α -amylase enzyme from RCSB protein data bank (1HNY)



The ligands such as 4-methyl esculetin, genistein, herbacetin and the standard acarbose were built using ChemSketch and optimized using “Prepare Ligands” in the AutoDock 4.2 (**Fig.2**). The optimized ligand molecules were docked into refined α -amylase enzyme using “LigandFit” in the AutoDock 4.2.

Fig. 2: The optimized ligand molecules



Rapid energy evaluation was attained by precalculating the atomic resemblance potentials for each atom in the selected flavonoids. In the AutoGrid process, the target was enclosed on a three dimensional grid point and the energy of interface of the each atom in the flavonoids were encountered. The following docking factors were chosen for the Lamarckian genetic algorithm as follows: population size of 150 individuals, 2.5 million energy evaluations, maximum of 27000 generations, and number of top individuals to automatically survive to next generation of 1, mutation rate of 0.02, crossover rate of 0.8, 10 docking runs, and random initial positions and conformations. The probability of performing local search on a single compound in the population was set to 0.06. AutoDock was run various times to obtain various docked conformations, and used to calculate the predicted binding energy [11].

3. Results

The docking poses were obtained according to their docking parameters and their corresponding binding pockets. This evaluation of the flavonoids were based upon their binding parameters with the target enzyme.

In **Fig. 3**, docked pose of α -amylase enzyme with the ligands 4-methyl esculetin, genistein, herbacetin and acarbose clearly demonstrated the binding positions of the ligand with the target. The potential binding sites of the 4-methyl esculetin (**Fig. 3a**) was found to be ALA 198, LYS 200, HIS 201, GLU233, ILE 235 and the genistein (**Fig. 3b**) was found that, TRP 58, TRP 59, TYR 62, ASP 157, LEU 162, HIS 299, ASP 300.

The potential binding sites of the herbacetin (**Fig. 3c**) was found to be, TRP 58, TYR 62, ARG 195, ASP 197, HIS 201, ILE 285, HIS 299, ASP 300, HIS 305, GLY 306. The binding sites of the standard acarbose (**Fig. 3d**) was found to be ASP 197, ALA 198, HIS 305, ALA 307. This proves that the effective binding sites are present in the selected flavonoids when compared with the standard. It proves that the ability of inhibiting the α -amylase enzyme by the selected ligands.

Fig. 3: Docked pose of α -amylase enzyme with the ligands and standard (a 4-methyl esculetin, b genistein c herbacetin, and d acarbose)

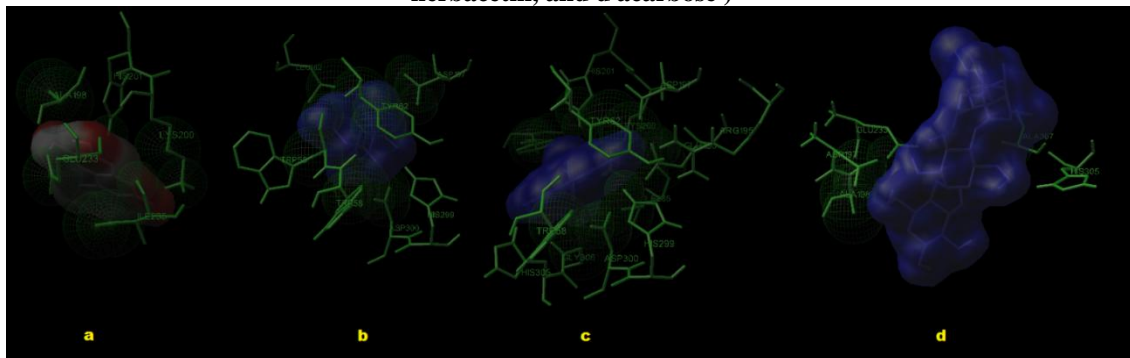


Table 1: Binding energies of the compounds based on their rank

Compounds	Binding energies of the compounds based on their rank (kcal/mol)									
	1	2	3	4	5	6	7	8	9	10
4 Methyl Esculetin	-6.00	-5.98	-5.98	-5.97	-5.97	-5.96	-5.90	-5.51	-5.47	-4.50
Genistein	-5.62	-5.60	-5.57	-5.61	-4.88	-4.74	-4.63	-4.53	-4.53	-4.38
Herbacetin	-5.67	-5.27	-5.66	-5.66	-5.34	-5.32	-5.18	-4.66	-4.99	-4.98
Acarbose	-1.97	-0.71	-0.47	0.14	0.75	0.85	1.36	1.81	1.99	2.10

As shown in **Table 1**, flavonoids showed binding energy ranging between -6.00 kcal/mol to -5.62 kcal/mol. The selected flavonoids had showed excellent binding energy when compared to that of standard acarbose (-1.97 kcal/mol). This proves that flavonoids consists potential α -amylase inhibitory binding sites.

Table 2: Inhibition Constant of the compounds based on their rank

Compounds	Inhibition Constant of the compounds based on their rank (μ M, mM*)									
	1	2	3	4	5	6	7	8	9	10
4-Methyl Esculetin	39.96	41.12	41.19	41.71	42.11	42.48	47.00	91.77	97.33	506.93
Genistein	75.71	78.97	81.97	77.16	264.03	332.75	401.53	477.32	481.90	616.04
Herbacetin	69.40	136.06	70.53	70.91	122.06	126.69	159.85	382.83	218.91	223.32
Acarbose	35.70*	300.71*	452.02*	454.03*	456.14*	472.18*	476.02*	482.13*	488.04*	491.02*

In addition, two other parameters like inhibition constant (K_i) and intermolecular energy were also determined. As shown in **Table 2**, flavonoids showed inhibition constant ranging from 39.96 μ M to 75.71 μ M. The selected compounds had lesser inhibition constant when compared to the standard (35.70 mM). Inhibition constant is directly proportional to binding energy. Thus, the α -amylase inhibitory activity of the selected flavonoids were proved using molecular simulations.

Table 3: Intermolecular energies of the compounds based on their rank

Compounds	Inter molecular energies of the compounds based on their rank (kcal/mol)									
	1	2	3	4	5	6	7	8	9	10
4-Methyl Esculetin	-6.60	-6.58	-6.58	-6.57	-6.57	-6.56	-6.50	-6.10	-6.07	-5.09
Genistein	-6.82	-6.79	-6.77	-6.80	-6.07	-5.94	-5.83	-5.72	-5.72	-5.57
Herbacetin	-7.46	-7.06	-7.45	-7.45	-7.13	-7.11	-6.97	-6.45	-6.78	-6.77
Acarbose	-8.54	-7.27	-7.03	-6.42	-5.82	-5.72	-5.21	-4.75	-4.57	-4.47

As shown in **Table 3**, the selected flavonoids showed intermolecular energy ranging from -6.60 kcal/mol to -7.46 kcal/mol which was lesser when compared to the standard (-8.54 kcal/mol). These result further proved the excellent α -amylase inhibitory activity of the selected flavonoids compared to the standard.

4. Discussion

Several studies carried out with the virtual screening of the compounds, most of which have been presented within the last five years, have necessary to utilize different computational methods to find out potential ligands for target protein of pharmacological/therapeutic significance. The screening methods include pharmacophore search, molecular docking, and fingerprints [12].

The need of reliable and widespread experimental data is definitely a major barrier in the development of accurate computational models. The docking parameters such as hydrogen bond interactions, $\pi - \pi$ interactions, binding energy, inhibition constant, intermolecular energy and orientation of the docked ligand within the active site has been generated for the evaluation [13-15].

Based on the *in silico* evaluation and stereochemistry binding of the flavonoids, the α -amylase inhibitory activity of the selected compounds was found to be decreased in the order of 4-methyl esculetin, herbacetin, genistein and acarbose. On the basis of the above study, the selected flavonoids possess potential α -amylase inhibitory excellent binding sites when compared to that of the standard. This may be attributed due to the differences in the position of the functional groups in the compounds.

In conclusion, the results of the present study clearly demonstrated that, 4-methyl esculetin, herbacetin, genistein has excellent binding sites and interactions with α -amylase compared to the standard. Further investigations on the above compounds and *in vivo* studies are necessary to develop potential chemical entities for the prevention and treatment of diabetes.

Conflict of interest

We declare that we have no conflict of interest.

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