RESEARCH ARTICLE

In-vitro and *In-silico* Docking Studies of Active Constituents of *Momordica charantia* and *Emblica officinalis* as Potential Alpha-Amylase Inhibitors

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ABSTRACT

Introduction: Commercially available oral hypoglycemic drugs are known to be potential alpha-amylase inhibitors that reduce postprandial hyperglycemia. Natural drugs are nowadays very popular in the treatment of diabetes.

Aim and Objectives: This study investigated *in-vitro* alpha-amylase inhibition of *Momordica charantia* and *Emblica officinalis* and studied the interaction between active phytoconstituents and alpha-amylase enzyme.

Method: Active constituents of both fruits were identified, docking studies were performed using Autodock Vina, and interactions were studied using PyMOL and Discovery Studio. The alpha-amylase inhibitory potentials of the fresh juice were investigated at the concentration of the fresh juice with alpha amylase enzyme and starch solution at 565 nm was observed.

Result: With the docking studies, it was observed that momoridicin I and II showed better interaction with alpha-amylase enzyme (PDB ID: 1B2Y) and showed binding energies at -8.2 and -8.4 Kcal/mol, respectively. The fresh juice of both fruits showed the most effective alpha-amylase inhibition and IC_{50} values found at 440 and 312 µL/mL, respectively.

Summary and Conclusion: The attempt to study *in-silico* docking studies and *in-vitro* alpha-amylase enzyme inhibition were successfully performed. Comparatively, *M. charantia* showed more enzyme inhibition at low concentrations.

Keywords: *Momordica charantia, Emblica officinalis,* Alpha-amylase activity, IC_{50}, Oral hyperglycemic agents, Docking Studies.

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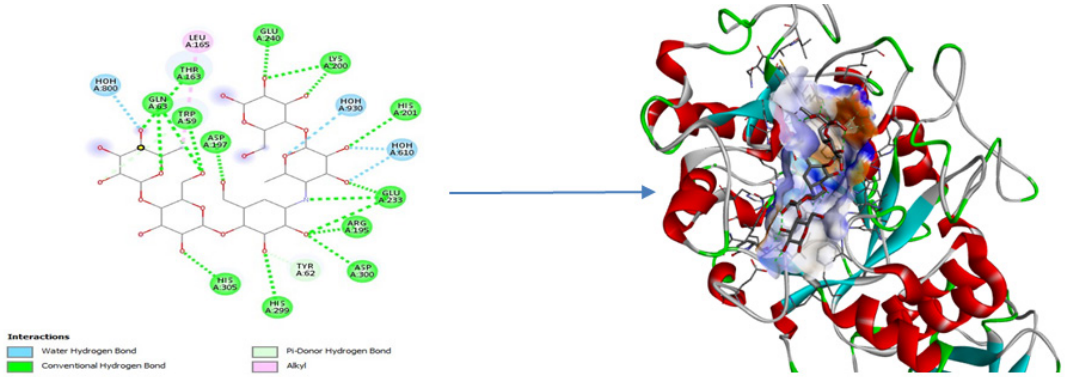

Graphical Abstract

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INTRODUCTION

Hyperglycemia, an endocrine condition, impacts how water, electrolytes, proteins, lipids, and carbs are metabolized. When there is hyperglycemia, the pancreas either produces insufficient insulin or the cells do not react to the insulin that is produced. This results in elevated blood sugar levels.[1] Thus, blocking the action of enzymes that hydrolyze carbohydrates, such as alpha-amylase and alpha-glucosidase, can lower blood sugar levels and treat hyperglycemia, natural ingredients have been used to treat hyperglycemia for a very long time.[2] Plant foods high in polyphenols have been shown to have effects on glucose utilization that are comparable to those of insulin and to be effective inhibitors of enzymes such as alpha-amylase and alpha-glucosidase.[3,4] Additionally, research has demonstrated a connection between plant-based polyphenols' antioxidant activity and potential hypoglycemic qualities and their bioactivity. [5] According to a global survey, 80% of people sought primary care through traditional medicine when it was appropriate.[6] Medicinal herbs are commonly used to treat various illnesses in underdeveloped nations.[7]

Emblica officinalis and *Momordica charantia* were well-known plants used to treat various diseases such as antioxidants, diabetes, anticough, neuroprotective, immunomodulator, chemo preventive, nephroprotective, gastroprotective, anti-inflammatory, anthelmintic, anti-fungal, anti-bacterial, anti-parasitic, anti-viral, anti-fertility, anti-tumorous, hypoglycemic, and anti-carcinogenic properties.[8-10]

E. officinalis (Amla) main active constituents are vitamin C (Ascorbic acid), gallic acid and phenols, alkaloids, tannins, and emblicanin A and B.[11] *M. charantia Linn*. (Karela or Bitter melon or Bitter gourd) the main active constituents are lipids, phenolic compounds, and alkaloids momoridicin I and II and charantin.[12-14]

Natural alpha-amylase inhibitors derived from dietary plants can treat hyperglycemia with less adverse effects, unlike the currently employed synthetic enzyme inhibitors with GIT side effects. The present study was carried out to investigate *in-silico* molecular docking studies on selected active constituents of both *E. officinalis* and *M. charantia* on alpha amylase enzyme and carried out *in-vitro* alpha-amylase inhibition activity.

MATERIAL AND METHODS

Following methods and chemical are used for performing the research work.

Software Required for Docking Studies

Discovery Studio, Pubchem and Pubmed Database, MGL (Molecular Graphic Laboratory) tool, Autodock 4.2, and Autodock Vina were used to perform *in-silico* docking studies.

Insilco Docking Studies using Autodock Vina[15]

The crystal structure of the alpha-amylase enzyme (PDB ID: 1B2Y) complex with acarbose was downloaded from RSCB Protein Data Bank. The structure has a 3.20 Å resolution (single chain). The water molecules, heteroatoms, and undesired

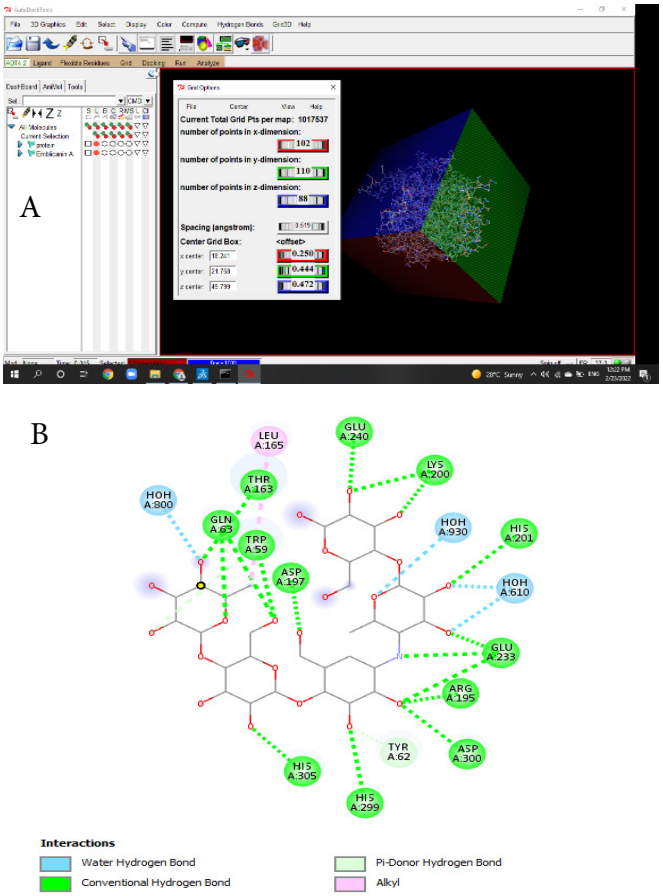


Figure 1: A Grid box for the alpha-amylase enzyme (PDB ID: 1B2Y) with the standard ligand. B. PDB sum's ligplot results for 1B2Y show all amino acid residues

ligands were removed from the alpha-amylase enzyme (PDB ID: 1B2Y) using Discovery Studio. The polar hydrogen was added to the crystal structure to stabilize the charge, and the PDB file was converted into PDBQT using Autodock 4.2. All *E. officinalis* and *M. charantia* bioactive compounds were downloaded from Pub CHEM public database. All bioactive constituents are subjected to minimize the energy, and the 3D structure of ligands is converted into PDBQT. The grid box for the alpha-amylase enzyme (PDB ID: 1B2Y) was generated with the help of Autodock 4.2 with dimensions X= 102, Y = 110, Z = 88 Å) and the center grid box was set as 18.2 x 21.7 x 49.7 And PDB sum's ligplot' results for 1B2Y showing all amino acid residues of active pocket shown in Figure 1 (A and B).

To obtain different docked conformations, Auto Dock Vina was run multiple times. The estimated docking energy was then analyzed. Auto Dock Tools offer multiple ways to examine the outcomes of docking simulations, including RMSD of the alpha-amylase enzyme with all active constituent chemicals as indicated in Table 1, conformational similarity, and visualization of the binding site and its binding energy.

Plant Material and Identification

Fresh fruits of *M. charantia* and *E. officinalis* were purchased from the local market. The fresh fruits were positively

Table 1: Binding affinity, RMSD and amino acid Interactions calculations using AutoDock Vina

S. No.	*Name of compound*	*Binding affinity (kcal/mol)*	*RMSD (Å)*	*Hydrogen bonds formed*
1.	Standard Ligand Acarbose	-3.2	0.021	THRA163, HISA299, GLUA233
2.	Standard drug Metformin	-3.3	5.870	ARGA161, LEUA162, GLYA164
3.	Ascorbic acid	-5.4	1.719	TRP88, ASP300, ILU33, ARG305
4.	Gallic Acid	-4.8	7.366	GLN347, PRO345
5.	Chebulinic Acid	-7.5	2.038	ARG306. ASP881, ILU231
6.	Emblicanin A	-7.4	1.428	HISA305, LEU165, TYRA62, GLUA233
7.	Emblicanin B	-7.9	2.420	GLUA233, ASPA300. HISA305, ALA198
8.	Momoridicin I	-8.2	2.021	ARG138, GLU233, ASP300, GLN347, PRO345
9.	Momoridicin II	-8.4	2.585	PRO345, GLUA233, ARG138, ASP300

identified and confirmed by Pharmacogonist (Dr. Dishant Gupta, Professor, Department of Pharmacognosy, Oriental University, Indore, Madhya Pradesh).

Preparation of Fresh Juice and Phytochemical Screening

Freshly cut 50 gm fruits of *M. charantia* and *E. officinalis* into small slice and ground well using a grinder, then filtered the fresh juice to obtain a clear solution of both fruits which was subjected to phytochemical screening and *in-vitro* activity.

Phytochemical screening of *M. charantia* and *E. officinalis* were performed by using the standard phytochemical procedures for phlobatannins, carbohydrates, alkaloids, glycosides, tannin, saponin, steroids, flavonoids and terpenoids.[16] The presence of these phytoconstituents is shown in Table 2.

Method For Determination of *In-vitro* Alpha-Amylase Inhibition[17]

Alpha-amylase is an enzyme that hydrolyses alpha-bonds of large alpha-linked polysaccharides such as glycogen and starch to yield glucose and maltose.

Requirements

Phosphate buffer (0.1M, pH 7.2):- Potassium dihydrogen phosphate 26.22 gm and sodium carbonate 7.78 gm added to sufficient water to produce 1000 mL.

Potato starch solution (1%w/v)

Add one gram of potato starch solution to 100 mL of distilled water.

Iodine iodide indicator

Add 635 mg of iodine and 1-gm of potassium iodide in 250 mL of distilled water.

Standard drug

Metformin was used as the reference drug against which all test compounds were compared.

Test sample preparation

The standard drug and extract were diluted with phosphate buffer (pH 7.2), then we prepared the different concentrations of test solutions 100, 200, 300, 400, 500 µL/mL of both fresh juice and similarly in comparison method 1:1 ratio of each juice was taken.

Procedure

The reaction mixture consists of 1-mL of starch solution, 1-mL of amylase solution, 2 mL of phosphate buffer (pH 7.2), and 1-mL of different concentration test solutions. All test solutions were incubated at 37ºC in a BOD incubator for 60 minutes. Then the reaction mixture was measured at 565 nm (SHIMADZU UV 1800)

Control

Reaction mixture of control does not include any drug.

Observation

Absorbance and percentage of inhibition was calculated in respect of control. The percentage inhibition of alpha-amylase was calculated by using the following formula:

$$\% \text{ of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test compound}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

It is discussed into two parts, docking analysis and alpha-amylase activity

Docking Analysis

Molecular docking is a frequently used tool in computer-aided structure-based drug design and can be defined by the *"Lock and key"* mechanism. It can be defined as an optimization problem, which would be described the best-fit orientation of the ligand to a particular protein. [18]

The best conformation was selected with the lowest docked energy was selected. The average binding affinity was shown in Table 1. The interactions of the complex between alpha-amylase enzyme (PDB ID: 1B2Y) and ligand conformation, including hydrogen bond and bond length, were analyzed by Discovery Studio and PyMOL as shown in Figures 2 and 3.

In Figure 2, the docked pose of the alpha-amylase enzyme (PDB ID: 1B2Y) with the standard ligand acarbose showed characteristic binding positions between the enzyme and ligand, with dotted lines showing the polar interaction with the active sites of the enzyme.

Docked poses of all selected active phytoconstituents with the alpha-amylase enzyme (PDB ID: 1B2Y) are shown in Figure 3

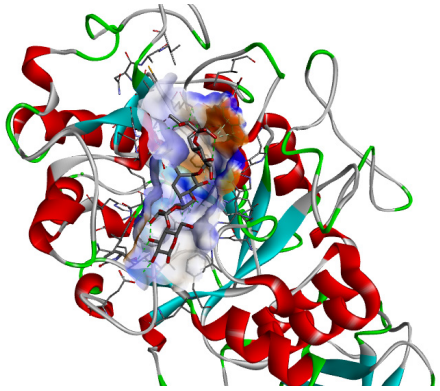


Figure 2: Docked pose of the alpha-amylase enzyme (PDB ID: 1B2Y) with the standard ligand acarbose

Table 2: Results obtained phytochemical screening of *M. charantia* and *E. officinalis*

S. No.	*Tests performed*	*Observation of phytoconstituents*	
		M. charantia	*E. officinalis*
1	Alkaloids	(+)	(+)
2	Glycosides	(+)	(+)
3	Carbohydrates	(+)	(+)
4	Saponins	(+)	(+)
5	Amino Acids	(+)	(+)
6	Flavonoids	(+)	(+)
7	Proteins	(+)	(+)
8	Terpenoids	(+)	(+)
9	Phlotannins	(+)	(-)
10	Steroids	(+)	(+)
11	Cardiac Glycosides	(+)	(+)

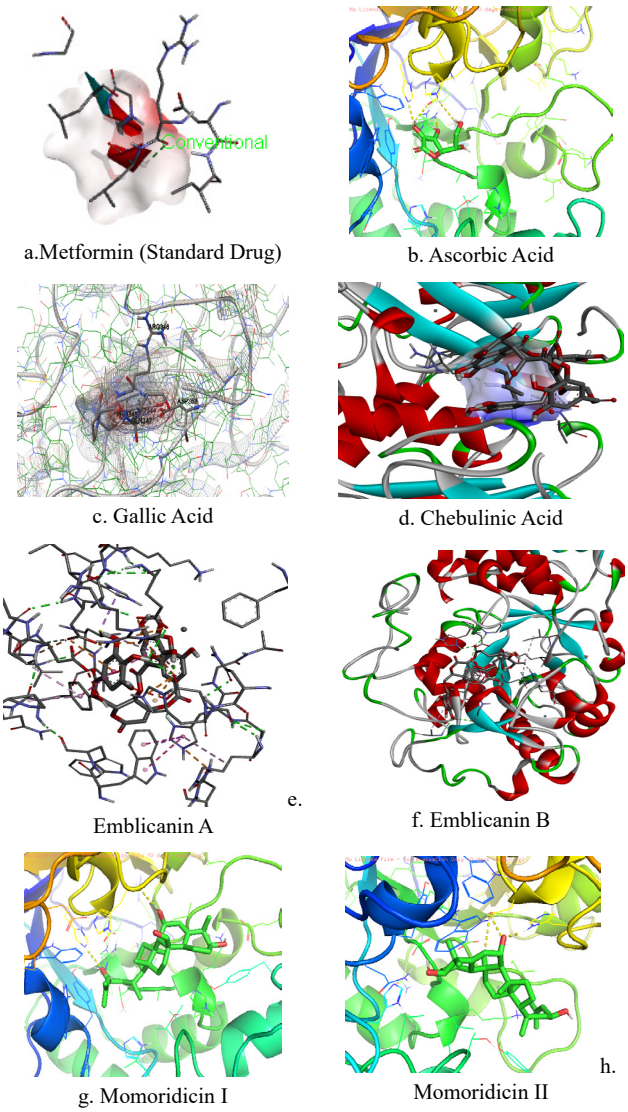
(+) – Positive; (-) Negative

As a rule, both hydrogen bond and hydrophobic interactions between the ligands and enzyme active sites were found to be responsible for biological activity. Docking parameters based on Binding affinity (Kcal/Mol), hydrogen bond interactions, hydrophobic interaction, orientation of the docked compound within the active site, and the root mean square deviation (RMSD) of active site residues.[19]

The potential binding site for the alpha-amylase enzyme (1B2Y) hydrogen bond was found, which was shown in Table 2. Based on the study, the Binding affinity of chebulinic acid, emblicanin A and B, and momoridicin I and II showed better interaction with the alpha-amylase enzyme.

Phytochemical Screening of *M. charantia* and *E. officinalis*

The fresh juices of both fruits were subjected to various qualitative phytochemical tests for the identification of phytoconstituents present in *M. charantia* and *E. officinalis.* The results are shown in Table 2. Phytochemical screening revealed the presence of alkaloids, glycosides, carbohydrates, saponins, amino acids, terpenoid, phalotannin, steroids, and cardiac glycosides.



a.Metformin (Standard Drug) b. Ascorbic Acid

c. Gallic Acid d. Chebulinic Acid

Emblicanin A e. f. Emblicanin B

g. Momoridicin I Momoridicin II h.

Figure 3: Docked the poses of all selected active phytoconstituents with the alpha-amylase enzyme (PDB ID: 1B2Y) a. Standard drug Metformin; b. Ascorbic acid; c. Gallic Acid; d. Chebulinic Acid; e. Emblicanin A; f. Emblicanin B; g. Momoridicin I; h. Momoridicin II

Table 3 displays the outcomes of the *in-vitro* alpha-amylase inhibition *a*ssay that was conducted on the fresh juices of *M. charantia* and *E. officinalis*. The enzyme alpha-amylase breaks down the alpha bonds in big alpha-linked polysaccharides like starch and glycogen to produce glucose and maltose.[20]

Both Juices showed a concentration-dependent increase in percentage inhibitory activity against alpha amylase enzyme. The IC_{50} values of *M. charantia* and *E. officinalis* were found at 312 and 440 µL/mL, respectively, so the antidiabetic potential increased in the combination of fresh juice compared to single use. *M. charantia* exhibited good alpha-amylase inhibitory activity at the mentioned concentration.

It has been proposed that inhibition of the alpha-amylase enzyme would delay the degradation of carbohydrates, which would in turn, cause a decrease in the absorption of glucose and, as a result, the reduction of postprandial hyperglycemia.[21,22]

Table 3: The percent inhibition of alpha amylase by fresh Juices of *M. charantia* and *E. officinalis* at varying concentrations

S. No.	*Concentration (µL/mL)*	*%Inhibition of E. officinalis*	*IC_{50} (µL/mL) E. officinalis*	*% Inhibition of M. charantia*	*IC_{50} (µL/mL) M. charantia*	*Combined juice % of inhibition*	*IC_{50} (µL/mL) of combined juices*
1	Control	-		-		-	
2	100	15		32		40	
3	200	24	440	42	312	54.2	153
4	300	32		48.97		65	
5	400	48		59.18		72.94	
6	500	52		65.64		75	

CONCLUSION

The attempts to study *in-silico* docking studies and *in-vitro* alpha-amylase enzyme inhibition were successfully performed. The results revealed that *M. charantia* and *E. officinalis* Juice*s* showed excellent alpha-amylase enzyme inhibition. Comparatively, *M. charantia* showed more enzyme inhibition at low concentrations. In conclusion, more research is required to develop a potential natural plant product used as an alpha-amylase inhibitors.

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