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AN *IN-SILICO* APPROACH ON ANTIDIABETIC ACTIVITY OF COMPOUNDS IN *MOMORDICA CHARANTIA* LINN.

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Keywords:

Alpha-amylase, Diabetes mellitus, *In-silico* study, Interaction fingerprint, *Momordica charantia*, Molecular docking

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ABSTRACT: The use of natural compounds has become more preferable for reducing the severe complications of diabetes mellitus due to having a minimum cost and the least side effects. The recent study is aimed to diagnose *in-vitro* α-amylase inhibition activity and the molecular interactions of various phytocompounds like momordicoside A, momordicoside B, momordicoside C, momordicoside D, momor-dicoside E, gallic acid, β-sitosterol, chlorogenic acid, vicine, gentisic acid, epicatechin, apiole, campesterol, cycloeucalenol, lophenol, cisdihydrocarveol, transnerolidol, daucosterol, arachidic acid, lauric acid, palmitic acid, momordenol, obtusifoliol, γ-linoleic acid, steric acid, oleic acid, capric acid and myristic acid present in seeds of *Momordica charantia* Linn. The molecular docking of the 3D structure of these compounds has been studied with target diabetic protein *i.e.*, α-amylase. 2D interactions were also studied by using Ligand Interaction Diagram. The interaction fingerprinting was generated using the Interaction fingerprint tool available in Maestro (version 12.5) of Schrodinger Suite version 2020-3. The results demonstrated that bioactive compounds *i.e*. momordicoside A, momordicoside C, momordicoside D and momordicoside B, with a higher binding affinity (>9 Kcal/mol) to diabetic target protein were found. The inhibition activity of α-amylase indicated that the seed extract was found with potent antidiabetic potential. These bioactive compounds can be further isolated to explore their *in-vivo* study as potent antidiabetic agents.

INTRODUCTION: According to Aroma World Reports in the year 2000, 61.3 million people in the age group 20-79 in the population had diabetes in India and it is suspected to be doubled by the years 2030 [1]. According to the "International Diabetes Federation" report in the year 2019, Diabetes mellitus is a disorder that is affecting 463 million people in the world and 10% of global health expenditure *i.e.* USD 760 billionspent on diabetes [2].

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The frequency of diabetes mellitus type-2 is progressively increasing in urban areas, approximately six times greater than in rural populations. An increase in weight, decrease in exercise, increased tension, malnutrition, changed diet and consumption of alcohol are the major causes of diabetes mellitus in the previous twenty years [3].

Various strategies are being implemented for the management of type II diabetes mellitus. However many allopathic antidiabetic medicines have many adverse effects like sulfonylurea (hypoglycemia), meglitinides (abdominal pain, vomiting, hypoglycemia), thiazolidinediones (cardiovascular diseases), α-glucosidase, inhibitors (abdominal

pain), glucagon-like peptide (pancreatitis) and dipeptidyl peptidase-4 (angioedema, hemolysis, rheumatoid arthritis) [4, 5]. As natural compounds are given a choice of a wide variety of chemical scaffolds, so they represent a cornerstone of the pharmaceutical industry. According to the Report of literature survey (1981-2014), US food and drug administration approved greater than 50% of medicines derived from natural compounds [6]. *Momordica charantia* Linn. (Bitter gourd, Balsam pear, Balsam apple) is a fruit consumed by the population in India, South America, East America, and Asia. It contains cucurbitane-type triterpenoids and glycosides [7]. *Momordica charantia* Linn. is used as antidiabetic, antiviral, antipyretic, abortifacient, anti-allergic, appetizing, stomachic, anticancer, laxative, antioxidant and immunomodulatory [8]. Bio-informatics tools are becoming very attractive for pinpointing the drug targets for different ligands [9]. In the present *in-silico* analysis, targeted protein *i.e.* α-amylase has been taken to act as a drug target for the prevention of type 2 diabetes mellitus [10]. α-amylase is responsible for breaking the long chain of carbohydrates. The inhibitors of α-amylase reduce the assimilation of carbohydrates in the intestine and are used to manage type 2 diabetes mellitus [11]. The recent study is to evaluate the *in-silico* analysis of bioactive compounds from seeds of *Momordica charantia* Linn. into the binding pockets of above-mentioned target protein in order to find out their role in hypoglycemia.

MATERIAL AND METHODS:

Material: Acarbose, 3, 5-dinitrosalicylic acid and α-amylase were procured from Sigma Aldrich, Germany. Analytical grade chemicals were used.

Collection and Authentication of Plant Material: Seeds of *Momordica charantia* Linn. were collected from the Khari Baoli market of Old Delhi and identified as dried seeds of *Momordica charantia* Linn. (family Cucurbitaceae), by Emeritus Scientist named Dr. Sunita Garg, CSIR-NISCAIR i.e., National Institute of Science Communication and Information Resources, New Delhi and Mr. R. S. Jayasomu Senior Principal Scientist, Head Raw Material Herbarium and Museum, Delhi (RHMD) under reference number NISCAIR/RHMD/consult/2021/3745-46, Pusa, New Delhi-110012.

Preparation of Seed Extract: First, wash the gathered seeds with the help of distilled water, followed by drying in the shade. Dried seeds were finely powdered with the help of a mechanical blender. The pulverized seeds were allowed to macerate for a time duration of 72 h with stirring and occasional shaking. The whole mixture was filtered through Whatman filter paper (460mm×510mm). The crude extract was concentrated by using a rotary evaporator at a temperature of 20°C. The extract was subjected to lyophilize and kept in the refrigerator for continued use.

Measurement of α-amylase Inhibitory Activity: The prepared extract was measured for the activity of α-amylase inhibition depending upon colorimetric methodology [12].

Preparation of Stock Solution: 0.25 g of potato starch was mixed with 50 ml of phosphate buffer (20 mM) and heated for 15 min. 1 mg of the α-amylase solution was added to 100 ml of 20 mM solution of phosphate buffer with pH 6.9. Test sample was prepared in different concentration 25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml in DMSO. The colour reagent solution was made by taking 20 ml of sodium potassium tartrate (5.31 mM) with 3, 5 di-nitrosalicylic acid (96 mM) in 12 ml of deionized water and 8 ml of sodium hydroxide (2 mM).

Screening of Antidiabetic Potential of Seed Extract of *Momordica charantia* Linn: 1 ml of prepared seed extract was mixed with 1 ml of the solution of α-amylase and kept in an incubator at 25 °C for 10 min. 1 ml of this mixture was added to 1 ml of the solution of potato starch and incubated at 25°C for 10 min. After adding 1 ml color reagent, the tube was closed and put in the water bath for 15 min at 85 °C. When the reaction mixture cooled, then diluted with distilled water (9 ml). Absorbance was obtained at 540 nm with the help of a UV spectrophotometer. Acarbose was used as standard. The formula used for the calculation of percentage inhibition of α-amylase was

$$\text{Percentage inhibition} = A_{control} - A_{sample} / A_{control}^{x} \times 100$$

Where $A_{control}$ = absorbance of control

A_{sample} = absorbance of test sample

Software used: The study was performed using Schrodinger Software Suite. Maestro (version 12.5) of Schrodinger Suite version 2020-3 was operated by using various tools like Protein Preparation Wizard, Ligprep, Sitemap tool, Grid generation, Glide XP (Xtra precision) Dock and Ligand Interaction. After retrieving ligands from Pub Chem, they were changed into the three-dimensional structure in the Schrodinger Suite. The structure of the protein molecule was retrieved from Protein Data Bank.

Preparation of Protein: The target protein α-amylase was selected for the study. The protein was prepared with the help of the Protein Preparation Wizard tool of Schrodinger Suiteversion 2020-3. The missing side chains and missing loops were updated. Molecules of water were removed to assign bond order, and then further optimization of hydrogen bonding was done. The energy minimization of the optimized structure was performed by using OPLS3e.

Validation of Binding Site: For molecular docking, validation of the binding site is an important parameter. Analysis of site validation shows site scores, locations of ligand-binding, and amino acid residues. We used the Site map tool in Maestro (version 12.5) of the Schrodinger suite for validation of binding site by the generation of a site map of the complete protein molecule. After the generation of the site map, one binding site (dependent on site score) was selected. The binding site with the largest volume was selected for the generation of the grid.

Receptor Grid Generation: The target site of the protein molecule can be fixed by using Receptor Grid Generation. Grid-based ligand docking was used for docking of a ligand with the target site in the protein. For this purpose, a grid box was generated at the centroid of the target site [13].

Ligand Preparation: The ligands *i.e.* momordicoside A, momordicoside B, momordicoside C, momordicoside D, momordicoside E, gallic acid, β-sitosterol, chlorogenic acid, vicine, gentisic acid, epicatechin, apiole, campesterol, cycloeucalenol, lophenol, cisdihydrocarveol, transnerolidol, daucosterol, arachidic acid, lauric acid, palmitic acid, momordenol, obtusifoliol, γ-linoleic acid, steric acid, oleic acid, capric acid and myristic acid, present in seed extract of *Momordica charantia* Linn. were used for evaluating their interaction against target protein. The 3D structures of ligands were obtained from the Pub Chem database. We use the Ligprep tool of Schrodinger Suite for the generation of 3D geometries with an OPLS3e force field. Epik was used for the generation of ionization state at pH 7.0±2.0. A maximum of 32 possible stereoisomers per ligand were generated.

Molecular Docking: To investigate the binding affinity and ligand-receptor interactions, molecular docking was performed by Ligand docking using Glide Xtra precision (XP) in Maestro (version 12.5) of Schrodinger Suite [14]. Two-dimensional interaction of protein-ligand was studied by using the Ligand interaction Diagram tool. The structural interaction fingerprint was generated by using the Interaction fingerprint tool. Interaction fingerprint provides structural binding information of protein-ligand complex and translates into one-dimensional binary string. The structural interaction profile obtained from the interaction fingerprint is further utilized to analyze, organize and visualize the encoded information of protein-ligand complex [15].

RESULTS AND DISCUSSION:

IC_{50} Values of Seed Extract and Acarbose (Standard): The *in-vitro* antidiabetic activity showed that the seed extract has potent antidiabetic activity with an IC_{50} value of 17.08 µg/ml as compared to standard. The graphical representation of percentage inhibition with respect to concentration is depicted in **Fig. 1.** The IC_{50} values are reported in **Table1.**

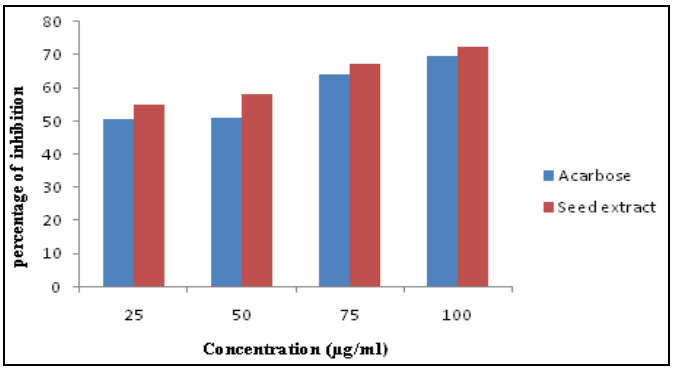


FIG. 1: PERCENTAGE OF α-AMYLASE INHIBITORY ACTIVITY OF ACARBOSE AND SEED EXTRACT OF *MOMORDICA CHARANTIA* LINN

TABLE 1: TABLE 1: IC_{50} VALUE OF ACARBOSE AND SEED EXTRACT OF *MOMORDICA CHARANTIA* LINN

Drug	IC_{50} value (µg/ml)
Acarbose	17.12
Seed extract	17.08

Validation of Protein Model and Binding Site: The validation of the protein model was carried out by the generation of the Ramachandran plot **Fig. 2.** The binding sites in the receptor protein can be predicted using the Sitemap Tool. If the site score is greater than 1, then it concludes as a binding site. If the site score is 0.80, it suggests a borderline for differentiating binding and non-binding sites. The site scores of 4 diabetic target proteins are listed in **Table 2.**

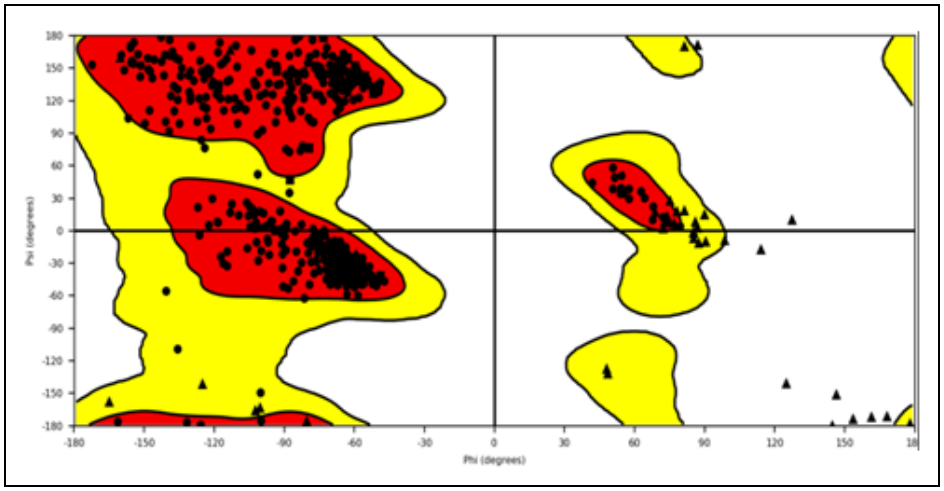


FIG. 2: VALIDATION OF PROTEIN MODEL BY RAMACHANDRAN PLOT REPRESENTING THE RAMACHANDRAN PLOT MODELLED STRUCTURE OF α-AMYLASE

TABLE 2: SITE SCORE AND CONTACT/BINDING SITE VOLUME OF LIGAND IN DIABETIC PROTEIN *i.e.* α-AMYLASE

Protein	Site Score	Binding Site Volume
	4GQR	
SITEMAP-1	0.944	332.024
SITEMAP-2	0.913	239.071
SITEMAP-3	0.719	100.842
SITEMAP-4	0.695	106.330
SITEMAP-5	0.577	53.508

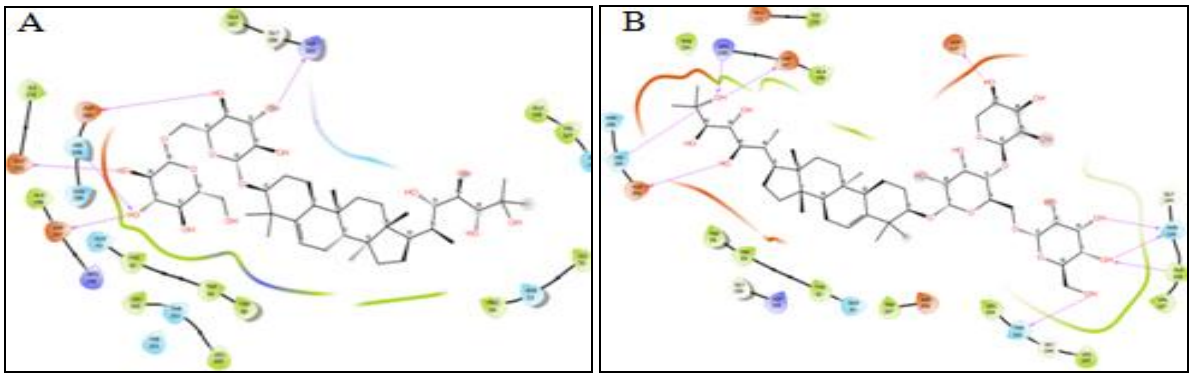
Molecular Docking: Molecular docking analysis was performed for the prediction of interaction between ligand and protein [16]. The effect of ligands on different proteins can be predicted by molecular docking. A specific algorithm is used by molecular docking to find the best ligand fitted in the target protein's active site. This utilizes an energy scoring function demonstrating that the ligand having minimum binding energy has the greatest binding affinity for the target protein. The comparative binding affinity of different ligands is symbolized as Kcal/mol as specified in **Table 3**. The binding affinity of more than or closer to 10 Kcal/mol indicates an efficient binding. A two-dimensional protein-ligand interaction study is shown in **Fig. 3.** Hydrogen bonding plays a significant role in the stabilization of the protein-ligand complex [17]. Hydrogen bond interaction and hydrophobic interaction among docked complexes are depicted in **Fig. 3.** Three-dimensional protein-ligand interaction studies are given in **Fig. 4.** The interaction of the different ligands with amino acid residues of the target protein is depicted in **Table 3.** The ligand molecule selected for target protein 4GQR are momordicoside A, momordicoside C, momordicoside D, momordicoside B with binding energy -9.9 Kcal/mol, -9.8 Kcal/mol, -9.4 Kcal/mol, -9.0 Kcal/mol respectively. It was found that overall, momordicoside A, momordicoside C, momordicoside D, momordicoside B have the binding capacity with the target protein taken for the study. The Interaction fingerprint of ligands for hydrophobic amino acid residues and polar amino acid residues of a target protein is shown in **Fig. 5.**

TABLE 3: BINDING ENERGY (Kcal/mol) OF 28 BIOACTIVE COMPOUNDS IN SEEDS OF *MOMORDICA CHARANTIA* LINN TO α-AMYLASE

Ligands	α-amylase (4GQR)
Control	**MYC -8.869**
Momordicoside A	-9.953
Momordicoside B	-9.072
Momordicoside C	-9.844
Momordicoside D	-9.425
Momordicoside E	-7.179
Gallic acid	-4.746
β-sitosterol	-4.054
Chlorogenic acid	-7.211
Vicine	-6.155
Gentisic acid	-5.351
Epicatechin	-8.259
Apiole	-3.895
Campesterol	-3.737
Cycloeucalenol	-4.371
Lophenol	-3.472
Cisdihydrocarveol	-5.331
Transnerolidol	-2.156
Daucosterol	-5.574
Arachidic acid	-0.355
Lauric acid	-1.195
Palmitic acid	-1.303
Momordenol	-4.349
Obtusifoliol	-3.448
γ-linoleic acid	-2.376
Steric acid	-1.922
Oleic acid	-2.252
Capric acid	-1.472
Myristic acid	-1.682

TABLE 4: HYDROGEN BONDING AND HYDROPHOBIC INTERACTION OF DIFFERENT LIGANDS WITH AMINO ACID RESIDUES OF DIABETES TARGET PROTEIN i.e., α-AMYLASE

S. no.	Ligand	PDB	Amino acid interacted through Hydrogen Bonding	Hydrophobic interaction
1	Momordicoside A	4GQR	ASP300,HIE299,GLU233, ASP197,HIP305	ALA307,ALA106,VAL107,ILE51,PRO54,TRP58,TRP59,TYR62,ALA198,ILE235,LEU162,LEU165
2	Momordicoside B	4GQR	ASP197,ARG195,ASP147,HIE299,ASP300,THR163,ALA106,ASN105	ILE235,ALA198,PHE256,TRP58,TRP59,TYR62,TRP357,LEU162,LEU165,VAL107,ALA106
3	Momordicoside C	4GQR	HIP305,ASP300,ASP197,ARG195,GLU233,GLM63	TRP58,TRP59,TRP357,TYR62,ALA106,VAL107,ILE51,PRO54,VAL98,ILE235,PHE256,ALA198,LEU165,LEU162,ALA307
4	Momordicoside D	4GQR	ASP300,ASP197,THR163	ILE51,TYR151,ALA307,ILE235,ALA198,TYR62,TRP59,TRP58,LEU162,LEU165,ALA106,VAL107



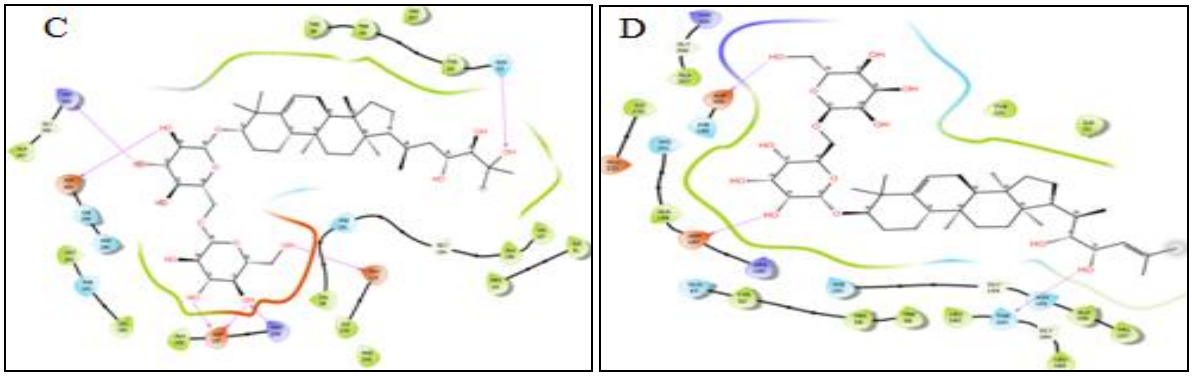


FIG. 3: 2D REPRESENTATION OF LIGAND-RECEPTOR INTERACTION OF - (A) MOMORDICOSIDE A (B) MOMORDICOSIDE B (C) MOMORDICOSIDE C (D) MOMORDICOSIDE D WITH α-AMYLASE (PDB ID 4GQR). AMINO ACIDS ARE DENOTED BY COLORED SPHERES, AND LIGAND-RECEPTOR INTERACTIONS ARE DENOTED BY STRAIGHT LINES

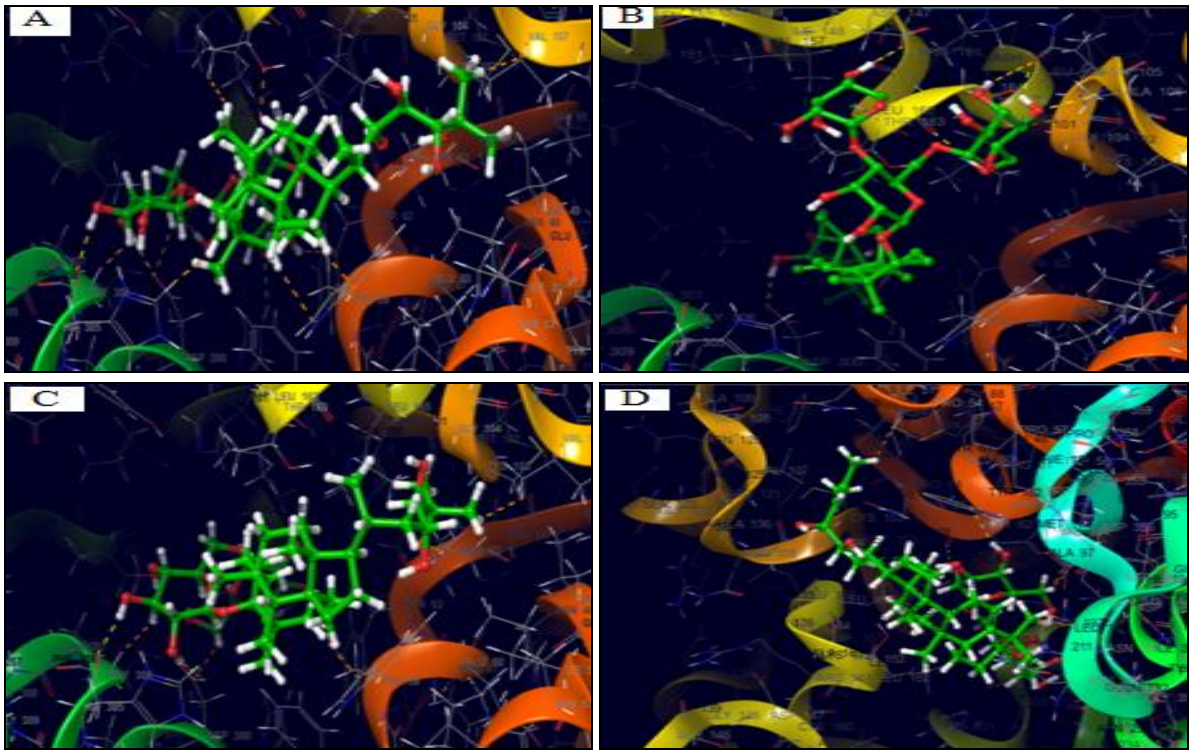
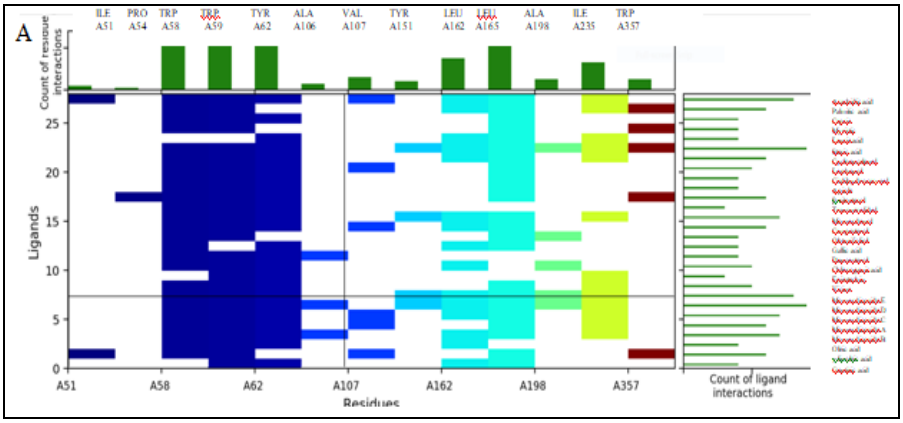


FIG. 4: 3D REPRESENTATION OF LIGAND-RECEPTOR INTERACTION OF (A) MOMORDICOSIDE A (B) MOMORDICOSIDE B (C) MOMORDICOSIDE C (D) MOMORDICOSIDE D WITH α-AMYLASE (PDB ID 4GQR). THE GREEN STICK STYLE SYMBOLIZE LIGANDS AND THE RIBBON STYLE SYMBOLIZE RECEPTOR. AMINO ACIDS OF TARGET PROTEIN MOLECULE ARE SKETCHED WITH THREE-LETTER CODE. HYDROGEN BONDS INTERACTIONS SYMBOLIZED BY THE YELLOW DOTTED LINE



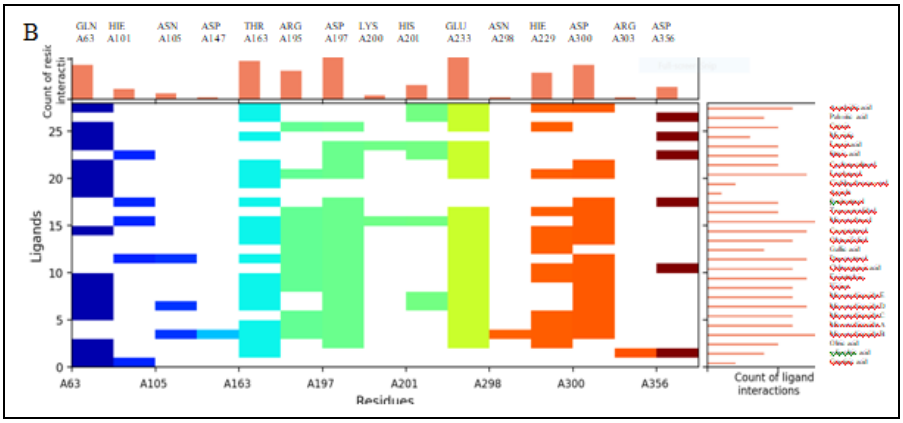


FIG. 5: INTERACTION FINGERPRINT OF BIOACTIVE COMPOUNDS (SEEDS OF *MOMORDICA CHARANTIA* LINN.) WITH α-AMYLASE (A) HYDROPHOBIC RESIDUES (B) POLAR RESIDUES OF α-AMYLASE

CONCLUSION: The present *in-silico* analysis is aimed to predict *in-vitro* antidiabetic potential by α-amylase assay and the interaction of twenty-eight bioactive compounds present in seeds of *Momordica charantia* Linn. *i.e.* momordicoside A, momordicoside B, momordicoside C, momordicoside D, momordicoside E, gallic acid, β-sitosterol, chlorogenic acid, vicine, gentisic acid, epicatechin, apiole, campesterol, cycloeucalenol, lophenol, cisdihydrocarveol, transnerolidol, daucosterol, arachidic acid, lauric acid, palmitic acid, momordenol, obtusifoliol, γ-linoleic acid, steric acid, oleic acid, capric acid and myristic acid with diabetic target protein *i.e.* α-amylase was employed for conducting the molecular docking. The seed extract of *Momordica charantia* Linn. showed a potent α-amylase inhibitory effect as compared to standard *i.e.*, acarbose. The results of *in silico* analysis suggest that the ligand molecules *i.e.*, momordicoside A, momordicoside C, momordicoside D, and momordicoside B with target protein 4GQR, showed significant binding affinity (> 9 Kcal/mol). However, to strengthen the research findings, a further *in-vivo* study is also required. Based on these studies, *in-vitro* and *in vivo* study is going on to further validate the biological activity of polypeptides from *Momordica charantia* Linn.

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CONFLICTS OF INTERESTS: None.

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