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RESEARCH ARTICLE

Virtual Screening and Analysis of Bioactive Compounds of *Momordica charantia* against Diabetes using Computational Approaches

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ABSTRACT:

Diabetes is one of the most serious metabolic disorders across the world which affects all age groups. World Health Organization (WHO) ranked the top diabetic countries in which India was ranked among the top three which diagnosed 62 million people. So there is an urgent need for the discovery of novel natural anti-diabetic drugs without any side effects. On analyzing the anti-diabetic plants, *Momordica charantia* exhibited better pharmacological properties and its bioactive compounds, which was found to possess good anti-diabetic activity were selected through literature for *in silico* studies. There were 51 bioactive compounds found in *Momordica charantia*, out of which only 13 compounds were carried out for the next step of analysis whose molecular properties satisfies to be a new lead for drug molecules. Further literature studies on diabetes showed that the enzyme glucokinase involved in the metabolic pathway for the production of energy were mostly responsible for causing diabetes in humans. For docking analysis, Arguslab software was used for screening the binding affinity between the inhibitors and target protein. The result of docking analysis revealed that compound nerolidol from *Momordica charantia* exhibited best binding interaction with diabetic protein. The nerolidol compound predicted from this research could be carried for *in vivo* analysis in future for the designing of novel potential drugs in the treatment of diabetes.

KEYWORDS: Diabetes, *Momordica charantia*, Bioactive compounds, Screening, Molecular Docking.

INTRODUCTION:

Diabetes mellitus is an increasing metabolic disorder which affects both macro vessels and micro vessels of the human body[1]. Diabetes mellitus is the most common and predominant group of endocrinological disorder affecting people worldwide and thus has been a subject of extensive research for development of number of anti-diabetic treatments[2,3]. It occurs throughout the world, especially more in the developed countries like India and China[4]. The presence of diabetes mellitus confers increased risk of many devastating complications such as cardiovascular diseases, coronary artery disease, stroke, neuropathy, renal failure, retinopathy amputations and blindness[5].

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It has been estimated that up to one-third of patients with diabetes use some form of complementary and alternative medicine. One plant that has received the most attention for its diabetic properties is *Momordica charantia*, commonly referred to as bitter gourd, karela and balsam pear. Its fruit is also used for the treatment of diabetes and related conditions amongst the indigenous populations of Asia, South America and East Africa[6]. The *in vitro* studies on *Momordica charantia* reported that the vegetable contains bioactive substances with antioxidants, anti-diabetic, antiviral and antineoplastic activities[7,8]. Recent research on *Momordica charantia* determined that it has more potential to become a leading component for new diabetic drug molecules[9].

Plant-based medicine has been used for cost effective treatment and even no adverse effects have reported during the treatment of diabetes. In fact, many parts of the poor countries in the world practices only plant-

based medicinal therapy to treat diabetic patients. Ayurveda and other traditional medicinal systems used a number of plant materials for the preparation of herbal drugs to treat diabetes. Around the globe, the most common traditional plant *Momordica charantia* is used in all forms of plant based medicine to treat diabetes in different countries without knowing the actual biocompound responsible for treatment. Based on the multitude of medical conditions that bitter melon can treat, scientists are more interested in studying its bioactive compounds and its action in the body[10]. Through phosphorylation, glucokinase is able to increase the metabolism of glucose. In the liver it increases the synthesis of glycogen and it is the first step in glycolysis, the main producer of ATP in the body[11]. Insulin and oral hypoglycemic agents like sulphonylureas and biguanides still play a major role in the development of more effective anti-diabetic agents. A study showed that hypoglycemic activity was shown by the compounds of bitter melon and it revealed significant lower blood glucose levels[12].

Application of cheminformatic techniques has become imperative in the field of drug discovery and development. These techniques enable efficient management and analysis of enormous data generated during drug discovery which covers wide chemical and biological space. Cheminformatic solutions offered by Evolvus are crucially important for hit identification, lead identification and lead optimisation[13].

Virtual screening which is also called *in-silico* screening is a new branch of medicinal chemistry that represents a fast and cost effective tool for computationally screening database in search for the novel drug leads. The routes for the virtual screening go back to the structure-based drug design[14]. In virtual screening**,** large libraries of drug-like compounds that are commercially available are computationally screened against targets of known structure, and those that are predicted to bind well are experimentally tested[15].

The bioactivity plays a very important role for the following drug targets: GPCR ligands, kinase inhibitors, ion channel modulators, enzymes and nuclear receptors. G-protein-coupled receptors (GPCRs) are the largest and most diverse group of membrane receptors in eukaryotes. These cell surface receptors act like an inbox for messages in the form of light energy, peptides, lipids, sugars, and proteins[16]. Nuclear receptors are a family of ligand-regulated transcription factors that are activated by steroid hormones[17]. Kinase inhibitors are small molecules that interact with multiple members of the protein kinase family achieving selective inhibition of specific protein kinases[18]. Ion channels are well recognized as important therapeutic targets for treating a number of different pathophysiologies however, development of drugs targeting this protein class has been difficult[19].

Hopkins proposed a concept of desirability for the drug database - quantitative estimate of drug-likeness based on Lipinski's rule of five and results were generated by fitting the distributions of eight properties of the compound[20]. QED helps in understanding the underlying features of drug-likeness. The method can also be applied to control sets other than oral drugs, such as lead-like molecules, compounds that belong to specific target and therapeutic classes[21]. Lipinski's Rule of Five has thus provided medicinal chemists with a simple mnemonic for identifying compounds with medicinally relevant physical chemical properties[22]. Lipinski set the most famous drug-likeness filter, such as Molecular weight ≤400, *Log p* ≤ 5, Hydrogen bond donor ≤3, Hydrogen bond acceptor ≤7 which provides four rules to determine whether a molecule could be orally absorbed or not[23].

The three main properties like mutagenicity, carcinogenicity, toxicity play a vital role in drug designing and development. Mutagenicity refers to the induction of permanent transmissible changes in the structure of the genetic material of cells or organisms; Carcinogenicity uses toxicological end points posing considerable concern for human health whereas toxicity is the degree to which a substance can damage an organism[24].

Docking technique is one of the most important and frequently used methods in structural-based drug designing, which predict the binding affinity of small molecules to their applicable target binding sites there by inhibiting the target functions. Computational approaches that 'dock' small molecules into the structures of macromolecular targets and 'score' their potential complementarities to binding sites are widely used in hit identification and lead optimization. The specific features of small-molecule–protein docking methods highlight selected applications and discuss recent advances that aim to address the acknowledged limitations of established approaches[25]. Docking can execute the outcome and suggest structural hypotheses of how target is repressed by ligand that is vital in lead optimization[26].

MATERIALS AND METHODS:

In Silico Pharmacokinetic Study of anti-diabetic inhibitors:

Pubchem:

The Canonical smiles of 51 phytochemical compounds were identified from the plant *Momordica charantia*, were retrieved by using the Pubchem database. The two

dimensional structure of the best inhibitors were retrieved and saved in.sdf format for further analysis[27].

Molinspiration:
Molinspiration server predicts the bioactivity score for GPCR ligands, kinase inhibitors, ion channel modulators, enzymes and nuclear receptors which was consider being the most important drug targets. Drug likeliness properties of 51 phytochemical were tested against above targets. The bioactivity score of phytochemical lies between 0.50 to 0.00, and then the phytochemical considered possessing good biological activities. If the score was between -0.50 to 0.00, the phytochemicals expected to possess moderate active and if score was more than -0.50, + 0.50 then the phytochemicals predicted to be inactive[28].

Quantitative Estimation of Drug Likeness (QED):
Using QED Database, drug likeness test was carried out based on the Lipinski Rule of Five. The rule predicts that the selected phytochemicals could be used as the oral drug. The rule states that the phytochemical should not > 1 rules out of four rules. The Lipinski Rule of Five rules were molecular weight ≤ 500, logP, number of hydrogen bond donors should be less than 5, and finally number of hydrogen bond acceptors ≤ 10. Apart from these rule QED database predict other 4 properties also they are PSA (polar surface area), number of alerts number of aromatic rings and last properties were number of rotatable bonds[29].

Virtual evaluation of chemical properties and toxicity (VEGA):
VEGA database performs computer-based models to evaluate chemical properties and biological activity of the phytochemical. The model VEGA includes three main properties mutagenicity, carcinogenicity, and developmental toxicity. The predicted result enables research to evaluate the reliability of query phytochemicals and also judge whether the phytochemical have the ability to pass through the animal experiments[30].

Open Babel:
Open Babel conversion software, helps in converting one chemical format into a different format. The researchers can use open babel as programming libraries to handle chemical files in the field of drug designing, cheminformatics, computational chemistry and material science[31].

***In Silico* Molecular docking studies:**
Protein Data bank (PDB):
Glucokinase is a monomeric enzyme which regulates the glucose levels in the liver and pancreas and consider to be an important target for diabetics. The three dimensional crystalline structure of glucokinase with PDB ID of 1V4S was retrieved by Protein Data Bank. The structure was determined in 2004 through X-Ray Diffraction with Resolution of 2.3 Å, R-Value Free: 0.273, R-Value Work: 0.232 and length of protein determined as 455 in single chain[32].

Metapocket:
Metapocket database is used to predict the protein–ligand binding sites on protein structure which helps in the functions of protein. Metapocket predicts the pocket depending on the size of protein structure and determine the name, position of active site present in the chain with available methods: pass11, ligsitecs, q_sitefinder, ghecom, pocasa, fpocket, surfnet, concavity[33].

ArgusLab:
ArgusLab is a free docking software that runs under all windows. It was first developed by Mark Thompson were the ligands were docked with the receptor to inhibit the function of the receptor there by curing the disease. In ArgusLab, the selected 3D structure of the receptor was loaded in pdb format and energy minimization were executed to remove water molecules present in the protein. The 2D structures of ligand were loaded in mol format and hydrogen atoms were added. Docking calculation parameter was set as shape-based search algorithm and AScore scoring function. The grid box was developed between ligand and receptor and scoring function evaluate the binding interaction between the ligand and the receptors. According to the lowest AScore generated the best docking model was predicted by ArgusLab and the binding conformation between the ligand and receptor near the binding site were analyzed by its hydrogen interaction[34].

Binding interaction visualization:
PyMol:
PyMoL is visualization software which used to visualize molecules and protein. The predicted best docking interactions were analyzed using PyMoL. The hydrogen bonding between the ligands and the receptor can be clearly viewed and the distance of hydrogen formation revealed the stability of inhibition of ligands which was measured in $Å^5$.

RESULTS AND DISCUSSION:

***In Silico* Pharmacokinetic Study of anti-diabetic inhibitors:**
The Canonical smiles were retrieved for 51 phytochemical compounds which was identified from plant *Momordica charantia* through literature survey. Using Molinspiration server, the above compounds were screened for its biological properties Table1. The server predicted that only 13 compounds: (Ascorbigen, Cucurbitin, Diosgenin, Goyaglycoside C, Goyasaponins,

Lauric acid, Nerolidol, Pentadecane, Trans-zeatin, Uracil, L-serine, L-alanine and Verbacoside*)* satisfies all biological properties and also form the physiological actions by interacting with important drug targets with the predicted score which lies between -0.50 to +0.50. Further these 13 compounds were tested for its drug likeliness properties using QED database. The result of QED database showed that only 4 compounds (Ascorbigen, Diosgenin, Nerolidol, Trans-zeatin) cleared the test of drug likeliness based on Lipinski rule of five Table 2. The 4 compounds were further verified for its mutagenicity, carcinogenicity and toxicity properties using VEGA software Table 3. The software identified that out of 4 compounds, Nerolidol and Trans-zeatin were found to exhibit non mutagenic, non carcinogenic and non toxic properties.

Table 1 : Predicted score for biological properties of compounds using Molinspiration

S. no	Compound	Canonical smiles	GPCR ligand	Ion channel modu-lator	Kinase in-hibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1.	Cryptoxanthin	CC1=C(C(CCC1)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2=C(CC(CC2(C)C)O)C)C)C	-0.03	-0.26	-0.20	0.49	0.02	0.24
2.	Cucurbitin	C1CNCC1(C(=O)O)N	-0.8	-0.50	-1.75	-2.83	-0.71	-0.96
3.	Cucurbitacin	CC(=O)OC(C)(C)C=CC(=O)C(C)(C1C(CC2(C1(CC(=O)C3(C2CC=C4C3CC(C(=O)C4(C)C)O)C)C)C)O)O	0.15	-0.21	-0.43	0.35	-0.04	0.52
4.	Cycloartenol	CC(CCC=C(C)C)C1CCC2(C1(CCC34C2CCC5C3(C4)CCC(C5(C)C)O)C)C	0.21	0.10	-0.40	0.86	0.14	0.66
5.	Diosgenin	CC1CCC2(C(C3C(O2)CC4C3(CCC5C4CC=C6C5(CCC(C6)O)C)C)C)OC1	0.05	-0.14	-0.57	0.58	-0.06	0.61
6.	Erythrodiol	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1)C)CO)C	0.19	-0.13	-0.30	0.71	0.10	0.55
7.	Galacturonic Acid	C1(C(C(OC(C1O)O)C(=O)O)O)O	-0.29	0.03	-1.03	-0.36	-0.38	0.46
8.	Goyaglyco-side C	CC(CC=CC(C)(C)OC)C1CCC2(C1(CCC34C2C=CC5(C3CCC(C5(C)C)OC6C(C(C(C(O6)CO)O)O)O)OC4OC)C)C	0.05	-0.81	-0.81	-0.25	0.00	-0.01
9.	Goyasaponins	CC1C(C(C(C(O1)OC2C(C(C(OC2OC3C(C(C(OC3OC4CC5(C(C4(C)CO)CCC6(C5CC=C7C6(CCC8(C7CC(CC8O)(C)C)C)C)C)C(=O)O)O)O)CO)O)O)O)O)O	-3.93	-3.98	-4.00	-3.96	-3.90	-3.90
10.	Gypsogenin	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C=O)O)C)C)C2C1)C)C(=O)O)C	0.2	-0.03	-0.38	0.72	0.27	0.65
11.	Karounidiol	CC1(C(CCC2(C1CC=C3C2=CCC4(C3(CCC5(C4CC(CC5)(C)CO)C)C)C)C)O)C	0.1	-0.14	-0.22	0.76	0.04	0.58
12.	Lanosterol	CC(CCC=C(C)C)C1CCC2(C1(CCC3=C2CCC4C3(CCC(C4(C)C)O)C)C)C	0.18	-0.05	-0.39	0.82	0.06	0.64
13.	Lauric Acid	CCCCCCCCCCCC(=O)O	-0.27	-0.04	-0.75	-0.24	-0.36	0.04
14.	Linoleic Acid	CCCCCC=CCC=CCCCCCCCC(=O)O	0.29	0.17	-0.16	0.31	0.12	0.38
15.	Linolenic Acid	CC/C=C\C/C=C\C/C=C\CCCCCCCC(=O)O	0.33	0.23	-0.19	0.35	0.13	0.42
16.	Momordenol	CCC(CCC(C)C1C(=O)C=C2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	0.10	-0.08	-0.50	0.89	0.08	0.56
17.	Momordicilin	CCC=CC(C)(O)OCC1(C2CCC3(C(C2(CCC1=O)C)CCC4C3(CCC5(C4C(C(CC5)C)C)C)C)C)C	0.08	-0.06	-0.43	0.54	0.07	0.40
18.	Multifloreno	CC1(CCC2(CCC3(C4=CCC5C(C(CCC5(C4CCC3(C2C1)C)C)O)(C)C)C)C)C	0.22	-0.05	-0.31	0.67	0.11	0.56
19.	Myristic Acid	CCCCCCCCCCCCCC(=O)O	-0.11	0.03	-0.51	-0.06	-0.19	0.13
20.	Nerolidol	CC(=CCCC(=CCCC(C)(C=C)O)C)C	-0.17	0.21	-0.64	0.42	-0.43	0.39
21.	Oleanolic Acid	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1)C)C(=O)O)C	0.28	-0.06	-0.40	0.77	0.15	0.65
22.	Oleic Acid	CCCCCCCCC=CCCCCCCCC(=O)O	0.17	0.07	-0.22	0.23	0.07	0.27
23.	Oxalic Acid	C(=O)(C(=O)O)O	-3.58	-3.54	-3.73	-3.46	-3.38	-3.31
24.	Pentadecane	CCCCCCCCCCCCCCC	-0.38	-0.07	-0.53	-0.45	-0.50	-0.13
25.	Petroselinic Acid	CCCCCCCCCCCC=CCCCCC(=O)O	0.17	0.07	-0.22	0.23	0.07	0.27
26.	Rosmarinic Acid	C1=CC(=C(C=C1CC(C(=O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O	0.17	-0.08	-0.18	0.57	0.15	0.24

27.	Rubixanthin	CC1=C(C(CC(C1)O)(C))C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC=C(C)CCC=C(C)C)C)C	-0.02	-0.27	-0.21	0.52	0.01	0.30
28.	Spinasterol	CCC(C=CC(C)C1CCC2C1(CCC3C2=CCC4C3(CCC(C4)O)C)C)C(C)C	0.18	0.05	-0.30	0.68	0.06	0.53
29.	Stigmasterol	CCC(C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	0.12	-0.05	-0.48	0.74	-0.02	0.53
30.	Taraxerol	CC1(CCC2(CC=C3C4(CCC5C(C(CCC5(C4CCC3(C2C1)C)C)O)(C)C)C)C)C	0.21	0.02	-0.20	0.08	-0.01	0.49
31.	Trehalose	C(C1C(C(C(C(O1)OC2C(C(C(C(O2)CO)O)O)O)O)O)O)O	0.19	0.14	0.08	-0.01	0.23	0.45
33.	Uracil	C1=CNC(=O)NC1=O	-3.36	-3.47	-3.60	-3.65	-3.62	-2.68
34.	Vicine	C(C1C(C(C(C(O1)OC2=C(NC(=NC2=O)N)N)O)O)O)O	0.14	0.10	0.16	-0.54	-0.06	0.67
35.	Trans –Zeatin	CC(=CCNC1=NC=NC2=C1NC=N2)CO	0.25	0.34	0.47	-1.62	-0.47	0.70
36.	Zeatin Riboside	CC(=CCNC1=NC=NC2=C1N=CN2C3C(C(C(O3)CO)O)O)CO	0.94	0.41	0.68	-1.00	0.12	1.04
37.	Zeaxanthin	CC1=C(C(CC(C1)O)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2=C(CC(CC2(C)C)O)C)C)C	-0.08	-0.36	-0.24	0.35	0.01	0.13
38.	Zeinoxanthin	CC1=C(C(CC(C1)O)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2C(=CCCC2(C)C)C)C)C	0.02	-0.25	-0.18	0.54	-0.04	0.31
39.	L-Serine	C(C(C(=O)O)N)O	-2.66	-2.54	-3.34	-3.34	-2.36	-2.38
40.	Glutamic Acid	C(CC(=O)O)C(C(=O)O)N	-0.29	0.25	-1.07	-0.96	-0.16	0.23
41.	L-Alanine	CC(C(=O)O)N	-3.20	-3.17	-3.69	-3.54	-2.72	-3.12
42.	Ascorbigen	C1C(C2C(O1)(C(C(=O)O2)(CC3=CNC4=CC=CC=C43)O)O)O	0.52	0.27	0.20	0.40	0.27	0.59
43.	L-Citrulline	C(CC(C(=O)O)N)CNC(=O)N	-0.17	0.14	-0.74	-1.09	0.04	0.23
44.	Elasterol	CCC(CCC(C)C1=CCC2C1(CCC3C2=CCC4C3(CCC(C4)O)C)C)C(=C)C	0.18	0.12	-0.47	0.93	0.14	0.60
45.	Flavochrome	CC1=CCCC(C1C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C2C=C3C(CCCC3(O2)C)(C)C)C)C)(C)C	0.14	-0.08	-0.11	0.46	-0.05	0.33
46.	Lutein	CC1=C(C(CC(C1)O)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2C(=CC(CC2(C)C)O)C)C)C	0.03	-0.28	-0.25	0.47	-0.03	0.28
47.	Lycopene	CC(=CCCC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC=C(C)CCC=C(C)C)C)C)C	0.07	-0.12	-0.06	0.29	-0.06	0.17
48.	Eleostearic Acid	CCCCC=CC=CC=CCCCCCCCC(=O)O	0.20	0.12	-0.18	0.29	0.09	0.31
49.	6droxytryptamines	C1=CC2=C(C=C1O)C(=CN2)CCN	0.14	0.33	0.14	-0.60	-0.36	0.21
50.	Verbacoside	CC1C(C(C(C(O1)OC2C(C(OC(C2OC(=O)C=CC3=CC(=C(C=C3)O)O)CO)OCCC4=CC(=C(C=C4)O)O)O)O)O)O	0.00	-0.54	-0.31	-0.24	0.06	0.00
51.	B-Sitostreol	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	0.14	0.04	-0.51	0.73	0.07	0.51

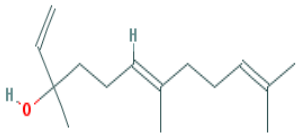
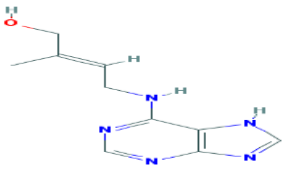
Table 2 : Drug likeliness Prediction of compounds using QED

S.no	Compound	Pubchem ID	MW	LogP	HBA	HBD	PSA	Rotb	Prediction
1.	Ascorbigen	3081416	305.283	1.101	6	4	112.010	2	Drug-like
2.	Cucurbitin	442634	130.145	0.516	2	4	75.350	1	Non-druglike
3.	Diosgenin	99474	414.621	4.293	3	1	4.293	0	Drug-like
4.	Goyaglycoside C	101077715	662.893	4.154	9	4	127.070	9	Non-druglike
5.	Goyasaponins	10654046	1379.487	5.102	31	16	485.650	16	Non-druglike
6.	Lauric acid	3893	200.318	3.499	2	1	37.300	0	Non-druglike
7.	Nerolidol	5284507	222.366	4.211	1	1	20.230	7	Drug-like
8.	Pentadecane	12391	212.41	5.768	0	0	0.000	12	Non-druglike
9.	Trans-zeatin	449093	219.243	1.024	4	3	86.720	4	Drug-like
10.	Uracil	1174	112.087	1.352	2	2	65.720	0	Non-druglike
11.	L-serine	5951	105.093	1.453	3	4	83.550	2	Non-druglike
12.	L-alanne	5950	89.093	1.042	2	3	63.320	1	Non-druglike
13.	Verbacoside	3081416	624.587	1.142	15	9	245.290	11	Non-druglike

Table 3 : VEGA prediction for selected compounds

S.no	Compound	Mutagenicity	Carcinogenicity	Toxicity
1.	Ascorbigen	Non-Mutagenici	Carcinogen	Toxicant
2.	Diosgenin	Non-Mutagenic	Carcinogen	Toxicant
3.	Nerolidol	Non-Mutagenic	Non-Carcinogen	Non-toxicant
4.	Trans-zeatin	Non-Mutagenic	Non-Carcinogen	Non-toxicant

Table 4: Physiological properties retrieved from Pubchem

Physiological properties	Nerolidol	trans-Zeatin
Molecular Formula	$C_{15}H_{26}O$	C10H13N5O
Canonical SMILES	CC(=CCCC(=CCCC(C)(C=C)O)C)C	CC(=CCNC1=NC=NC2=C1NC=N2)CO
Melting Point	< 25 °C	207-208 deg C
Solubility	Not yet predicted	In water, 2.24X10+3 mg/L at 25 deg C (est)
Vapor Pressure	Not yet predicted	2.02X10-11 mm Hg at 25 deg C (est)
2D structure		

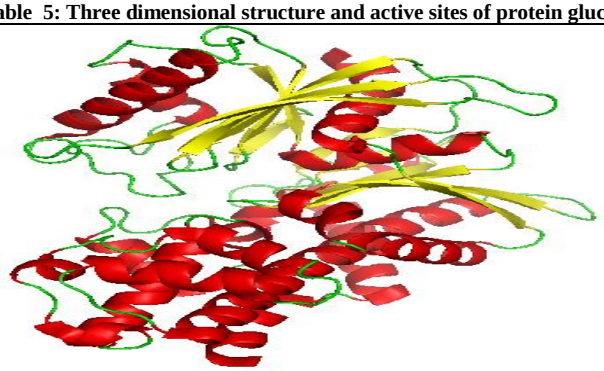
In Silico Molecular docking studies:

Preparation of predicted inhibitors and protein for docking studies:

The two dimensional structure of the inhibitor of Nerolidol and Trans-zeatin were retrieved and saved in.sdf format from Pubchem with other physiological properties Table 4. Further.SDF format of two inhibitors were converted into.mol format using Open Babel and these two inhibitors in.mol format were set for docking analysis.

The three dimensional crystal structure targets protein glucokinase with PDB ID: 1V4S were retrieved from PDB databases was viewed by PyMol (Table 5). The glucokinase protein was determined by X-ray diffraction method with a resolution factor of 1.55 Å, R value 0.186. After procurement, the pdb format of proteins was further processed for docking analysis by removing native ligand and crystalline water structure present in the protein. The prominent active sites of protein glucokinase were evaluated using MetaPocket server with aavailable methods (Table 5). In glucokinase, totally 80 binding pockets were predicted and revealed the position of amino acids involved in configuration of an active site ranging from ARG 63-ARG 447.

Table 5: Three dimensional structure and active sites of protein glucokinase



Three dimensional structure of glucokinase (1V4S)

LEU_A^276^	SER_A^280^	ARG_A^327^	ARG_A^303^	GLU_A^300^
LEU_A^304^	GLU_A^279^	LEU_A^307^	VAL_A^277^	SER_A^281^
ALA_A^282^	GLY_A^328^	LYS_A^296^	TYR_A^297^	ASN_A^283^
GLY_A^170^	LYS_A^169^	PHE_A^330^	GLY_A^299^	GLU_A^331^
ALA_A^329^	THR_A^332^	GLY_A^295^	MET_A^298^	THR_A^82^
THR_A^228^	ARG_A^63^	ASN_A^83^	GLY_A^81^	GLY_A^227^
GLY_A^229^	GLY_A^80^	MET_A^107^	GLY_A^294^	SER_A^411^
PRO_A^153^	THR_A^168^	GLU_A^256^	GLN_A^287^	GLU_A^290^
SER_A^151^	PHE_A^152^	TRP_A^167^	PHE_A^171^	ASP_A^78^
PHE_A^84^	ARG_A^85^	VAL_A^412^	CYS_A^230^	GLY_A^258^
ASP_A^205^	ASN_A^204^	ALA_A^259^	SER_A^336^	LEU_A^415^
ASN_A^231^	ILE_A^225^	PHE_A^150^	VAL_A^335^	GLN_A^337^
GLY_A^410^	ASN_A^254^	THR_A^149^	SER_A^445^	HIS_A^416^
ASP_A^409^	PRO_A^417^	GLU_A^443^	LYS_A^414^	THR_A^209^
GLY_A^444^	LYS_A^102^	GLY_A^446^	MET_A^87^	SER_A^441^
GLU_A^442^	VAL_A^86^	HIS_A^105^	GLN_A^106^	ARG_A^447^

80 active sites of protein glucokinase

Binding interaction of inhibitors with glucokinase:

The above prepared inhibitors and receptors were all set for docking analysis using Arguslab. Docking analysis were performed with the grid resolution of 0.400000 Å using GAdock algorithm, Calculation Type – Dock, ligand – Flexible mode. The docked poses with the lowest binding free energy between receptor and inhibitors were evaluated and recorded (Table 6). The best docking score (lowest binding energy) determined the highest inhibitory affinity between ligand and receptor.

Table 6: Molecular Docking score between inhibitors and receptor

S. No.	Docking between Protein and Ligands	Binding Interaction
1.	1V4S-Nerolidol	-13.3575kcal/mol
2.	1V4S-Trans-zeatin	-6.13003kcal/mol

On further analysis of docking result and the pose, it was confirmed that the inhibitor Nerolidol exhibited the best binding interaction with glucokinase (-13.3575 Kcal/mol) when compared with Trans-zeatin (-6.13003kcal/mol). Nerolidol binding interaction with the receptor was explored that the 2 active sites of arginine played a major role in inhibiting the function of glucokinase through 2 hydrogen linkages (Figure1). The first hydrogen bond interaction with 3.14Å was formed between oxygen atom present in the active site ARG63 and Nitrogen 6 atom in the ligand followed by hydroxyl group present in the active site of ARG 447 bound to the Nitrogen 23 atom in the ligand with the hydrogen bond interaction of 2.45 Å.

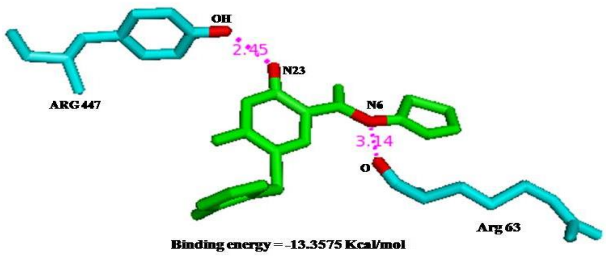


Fig.1: Best docking results between Nerolidol and Glucokinase

- **Indicates the active site of the glucokinase**
- **Indicates the ligand**
- **Indicates the binding area**
- **Indicated the hydrogen diatance in Å**

Thus in the current study, nerolidol exhibited the least binding score against important receptor glucokinase, the former study on the nerolidol compound from the plant *Alpinia calcarata* exhibited high level of *in vitro* anti diabetic properties by controlling blood glucose level in high rate[35]. The new active site ARG 63, ARG 447 was predicted in the protein structure of glucokinase. In early research on the protein glucokinase different active site were predicted by docking process with good binding interaction[36]. On comparing previous studies with the current study, it was confirmed that the compound nerolidol possess best binding interaction with new active sites of 1V4S.

CONCLUSION:

Knowledge on the virtual screening and molecular interactions of natural compounds of *Momordica charantia* with essential diabetic enzyme glucokinase is a potentially useful tool for the identifying, designing and developing new anti-diabetic drugs. The current *in silico* research revealed that out of 51 secondary phytochemical compounds present in the plant, only one phytochemical compound found to exhibit better inhibitory activity over the diabetic enzymes. Virtual screening of 51 phytochemical present in the *Momordica charantia* resulted that only two compounds Nerolidol and Trans-zeatin satisfied all the Pharmacokinetic Studies. Further these two compounds were examined for its diabetic inhibitory activity using Molecular docking. The docking result confirmed that Nerolidol compound exhibited best inhibitory activity over the enzyme with the least score of -13.3575 Kcal/mol. Nerolidol compound is expected to increase a better vision in diabetic inhibitory activity through *in vitro* animal model studies in future which would pursue as novel anti-diabetic drugs.

CONFLICT OF INTEREST:

The authors declare they have no competing interests.

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