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In silico analysis of *Cucumis pubescens* Willd. fruit extract phytocompounds and its activity against anti-diabetic targets

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Abstract

Diabetes mellitus is a chronic disease which causes complications in a large population worldwide. Traditionally, medicinal plants possess numerous bioactive compounds to treat chronic diseases. In this study, the bioactive compounds in *Cucumis pubescens* Willd. were screened computationally to treat diabetes. Ten ligand molecules from *C. pubescens* Willd. plant fruit were selected from GC-MS analysis. The target proteins like Lipase-related Protein 2 (2OXE), α – amylase (2QV4) and β – Glucosidase (2ZOX) were retrieved from the PDB databank. The proteins have been selected based on their role in anti-diabetic activity. All ten ligands were docked with all three proteins to identify the suitable ligand possessing high binding energy. The ligand (4H-Pyran-4-one, 2,3-Dihydro 3,5-Dihydroxy-6-Methyl) had a maximum binding energy of -4.3 kcal/mol and -4.4 kcal/mol with the targets 2OXE and 2ZOX respectively. The ligand molecule 1,4-dimethyl-2,3-diazabicyclo[2.2.1]hept-2-ene produced the highest binding energy of -4.7 kcal/mol with 2QV4. The predicted active phytochemicals can be used as natural drug molecules to treat diabetes mellitus in future.

Keywords: α – amylase; β – glucosidase; anti-diabetic; auto dock Vina; *Cucumis pubescens* Willd; lipase-related Protein 2; molecular docking; phytocompounds

Introduction

Diabetes mellitus is a severe metabolic activity illness that causes high rates of morbidity and loss of life globally. It is currently rated eighth among all diseases globally and is responsible for up to 1.4 million (2.6%) deaths in 2011 (Ma *et al.,* 2003). The International Diabetes Federation (IDF) projects predict that between 2013 and 2035, the number of persons with diabetes will raise from 382 million to 592 million, indicating that the frequency of diabetes in emerging nations has reached epidemic percentage (Florencia and Alex, 2014).

Diabetes is caused primarily by insulin deficiency either by dysfunction in beta cells or insulin resistance externally (Khodakhah *et al*., 2024). Elevated plasma glucose concentrations are a hallmark of diabetes, which can be brought on by abnormalities in different metabolic pathways as well as inadequate insulin, insulin resistance, or both (Patel *et al.,* 2011). Further complications of diabetes include diseases like kidney disorders,

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heart and nervous system damage (Agbo *et al.,* 2024). As the condition worsens, consequences include peripheral vascular diseases, ischemic heart disease, neuropathy, nephropathy, stroke, retinopathy and a host of other heterogeneous disorders, of which type 2 diabetes accounts for about 95% (Punthakee *et al.,* 2018). It arises from pancreatic β-cell malfunction and insulin resistance, leading to hyperglycemia (Lacroix *et al.,* 2014).

In general, plants have natural products especially secondary metabolites which are commonly used for the treatment of different human ailments including cancer and diabetes (Brown and Wright, 2016; Singh *et al.,* 2018). Medicinal plants and their parts are used to extract various phytocompounds and utilized as a potential source for antibiotics, antioxidants, anticancer, antidiabetic and as heart-protective agents (Chen *et al.,* 2015; Gorlenko *et al.,* 2020). Medicinal plant resources involve more than 25% of drugs, 60% of total anticancer drugs and 60% of antitumors are commonly known as natural products and its derivatives (Newman and Cragg, 2012; Boucher *et al.,* 2017).

It is well known that a variety of plant natural products and secondary metabolites can mitigate the harmful effects of pathogenic agents both *in vivo* and *in vitro*. However, in, advances in computer engineering have made a lot of data accessible, and quick molecular docking techniques and software have frequently enhanced molecular simulations and are helpful for screening and discovery of drugs. The protein-ligand interactions through molecular docking have become a valuable tool in drug designing and metabolites screening processes (Vilar *et al.,* 2017; Gurung *et al.,* 2021). This type of research enlightens the applicability of some medicinal plant metabolites utilizing molecular docking studies especially virulent factors of diseases that are relevant targets in clinical trials to treat various diseases (Kumar *et al.,* 2022).

Cucurbitaceae are under 130 genera and 800 species in angiosperms and economically significant family in plants (Ali and Pandey, 2006; Ali *et al.,* 2009; Jeffrey, 2005). Cucurbits are generally involved in the biosynthesis of bitter phytocompounds (Chen *et al.,* 2005; Wang *et al.,* 2015; Zhou *et al.,* 2016).

Diabetes mellitus is a metabolic disorder characterized by raising sugar levels in the blood at the bloodstream. It is brought on by malfunctioning pancreatic beta cells. It is impacted by deficiencies in insulin production or biosynthesis as well as an unfinished business with the breakdown of proteins, lipids and carbohydrates. The results reveal that in 2040, approximately 640 million people will suffer from diabetes and its related diseases such as nephropathy, cardiovascular diseases and retinopathy (Gopalasatheeskumar *et al.,* 2020; Ogurtsova *et al.,* 2017; Perera *et al.,* 2019; Semeralo *et al.,* 2015). Lifelong medication is required for this disease and hence there is an emerging need to identify novel and natural medicinal compounds as an alternative to treat diabetes diseases.

To distinguish between the phytocompounds under test and their action against digestive enzymes, molecular docking analysis was employed. Prior studies have documented the ability of flavonoids and phenolic compounds to bind to the pockets of pancreatic lipase, β-glucosidase and α-amylase, thereby generating complexes of enzyme inhibitors (Sundari and Kavitha, 2024a) To validate and rank the most promising compounds from *C. pubescens* for additional research (Sundari *et al.,* 2024 b,c). The current study employed an *in silico* approach to perform molecular docking analysis and screening of ADME/drug-likeness features of the potential active compounds from fruits of *C. pubescens.*

Materials and Methods

Target selection

Protein targets were identified from the literature survey and α-amylase, β-glucosidase and pancreatic lipase were selected whose PDBIDs are 2OXE, 2QV4 and 2ZOX respectively.

Ligand

Ligands were obtained from the GC-MS analysis of *C. pubescens* top 85 percent similarity compounds were selected for the present study and screened for ADME property and Lipinski rule of five. All ten detected phytocompounds' structural data were collected from Pub Chem and refined by using Chemdraw and Argus lab.

Molecular docking

Protein preparation

Preparation of protein was carried out using Biovia Discovery Studio tools 1.5.7 following the usual procedure. Cofactors and water molecules were selected for removal. After the previously linked ligands were eliminated, polar hydrogens and Kollmans charges were added using Auto Prep to create the protein.

Ligand preparation

Chemsketch 16.0 is used to sketch the two-dimensional structures of the ligands that have been derived from literature. The programme Chem 3D was used for the optimization process and every parameter was chosen to produce a stable structure using the least amount of energy. The title chemical's global lowest energy was estimated using the structural optimization method. Using optimized structure, the 3D coordinates (PDB) of each molecule were found.

ADME and drug-likeness prediction

The free online tool Swiss ADME, created by the Swiss Institute of Bioinformatics and publicly accessible (http: www.swissadme.ch), was used for *in silico* ADME screening and drug-likeness assessment (Daina *et al.,* 2017). Compounds having a high necessary power score were subjected to this stage of the selection process. Along with molecular weight (MW), atom counts, molecular refractivity (MR) and polar surface area (PSA), basic physicochemical parameters were determined. OpenBabel, version 2.3.0, was used to accomplish this (O'Boyle *et al.,* 2011). Lipinski (2001), Ghose (1999), Veber (2002), Egan *et al*. (2000), and Muegge (2001) rules of 5 (RO5) screening were used to screen for drug-likeness.

The purpose of the bioavailability score was to represent the probability that a molecule would have at least 10% oral bioavailability based on Lipinsk's filter violation, TPSA and total charge. Lipophilicity was implemented using the iLOGP, XLOGP3, WLOGP, MLOGP, and SILICOS-IT models, and a log Po/w consensus was found (Daina *et al.,* 2017). Three alternative models were used to calculate the solubility (log S) of the chosen ligands: ESOL (Delaney, 2004; Ali *et al.,* 2012), and SILICOS-IT (Daina *et al.,* 2017).

Autodock Vina analysis

The graphical user interface Auto Dock Vina was utilized for Ligand Protein docking interactions**.** Molecular docking study was conducted using Auto Dock tools (ADT), a free image user interface (GUI) or the Auto Dock Vina software. Dimensions 126,126,26 pointing in the x, y and z axis were used to form the grid box. The grid box located in the centre of 1C7N was -30.239, - 2.658,1.925 using Auto Dock Vina methods, nine replacement confirmations were generated for each ligand and these were ranked according to their binding energies.

Protein structure for docking

The Protein Data Bank (*http://www.rcsb.og/pdb*) provided the three-dimensional structures of α-amylase (PDB ID 2QV4), β-glucosidase (PDB ID 2ZOX) and lipase related Protein 2 (PDB ID 2OXE). The chosen 2OXE, 2QV4 and 2ZOX were fixed with the force field CHARMM (Chemistry at Harvard Macromolecular Mechanics) in Discovery Studio 2.5 (DS, Accelrys Software, San Diego, CA) to add up the

hydrogen atoms, partial charges and missing residues that could be used properly for the processes of molecular docking. Table 1 shows the list of proteins used in this study (Jhong *et al.,* 2015).

Selection of ligands

The ligands were selected from GC-MS analysis of *C. pubescens* fruit extract. Ten active ligand phytocompounds were selected and analyzed chemically through Pubchem. All ten compounds have been listed in the list of ligands/compounds used for molecular docking.

Molecular docking

The putative binding sites of 2OXE, 2QV4 and 2ZOX targets retrieved from the PDB data base was predicted using model tools in Discovery Studio 2.5. Ten phytocompounds were docked with the 2OXE, 2QV4, and 2ZOX in Autodock vina and the enzyme pockets were visualized using the Ligand Fit programme of Discovery Studio 2.5. This programme offers an accurate shape-based method for docking ligands into enzyme pockets. The binding affinity and position of the covalent hydrogen bond were then used to analyse the docked postures (Venkatachalam *et al.,* 2003).

Results

Selection of target

The X-ray crystal structure data of selected proteins were obtained from the RCBS database in .sdf file format. The Protein data bank ID (PDB ID) of the selected target was provided in the Table 1 and its structure is depicted in Figure 1.

Table 1. List of Proteins selected from PDB

S.No	Protein	Source	Classification of Protein	PDB id
1.	Lipase-related Protein 2	Human pancreas	Hydrolase	2OXE
2.	α - amylase	Human pancreas	Hydrolase	2QV4
3.	β - Glucosidase	Human	Hydrolase	2ZOX

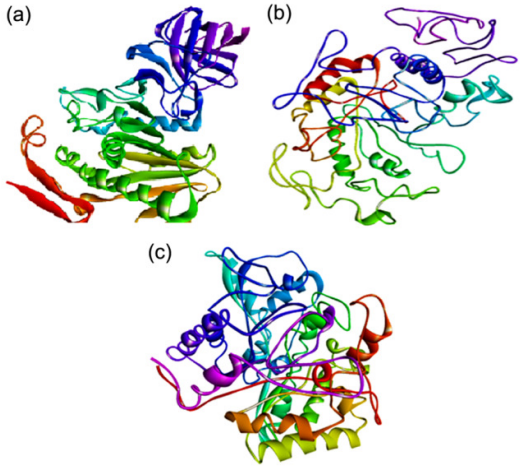
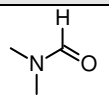
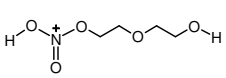
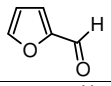
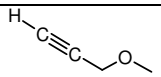
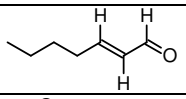
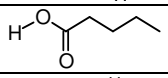
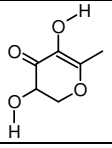
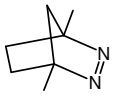
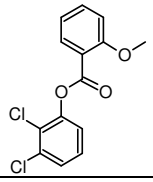


Figure 1. Protein structures of a) 2OXE, b) 2QV4 and c) 2ZOX from PDB database

Physicochemical properties of the selected ligands

Tables 2 list the fundamental physicochemical characteristics of the compounds that showed promise based on the docking result. The chosen compounds' molecular weights varied from 70.09 to 297.13 g/mol. The compounds that were chosen had consensus LogP values ranging from -5.82 to 3.03 for lipophilicity, except (2,3-dichlorophenyl) 2-methoxybenzoate, which displayed a substantially lower and negative lipophilicity (-5.82). All the chosen compounds were soluble in water, however their solubility varied, according to the calculated water solubility index.

Table 2. List of ligands / phytocompounds used for molecular docking studies obtained from GC-MS results of *C. pubescens* fruit extract

No	Name of the compound	IUPAC name	CID	Formula and molecular weight	Structure
1.	N,N-Dimethyl-formamide	N,N-Dimethyl-formamide	6228	C_3H_7NO 73.09	
2.	2-(2-Hydroxyethoxy)-ethanol 1-nitrate	Hydroxy-[2-(2-hydroxyethoxy)ethoxy]-oxoazanium	102239817	$C_4H_{10}NO_5$+ 152.13	
3.	Furfural	Furan-2-carbaldehyde	7362	$C_5H_4O_2$ 96.08	
4.	2-Hydroxy-2-cyclopentenone	2-Hydroxycyclopent-2-en-1-one	82674	$C_5H_6O_2$ 98.1	
5.	Methyl propargyl ether	3-Methoxyprop-1-yne	69393	C_4H_6O 70.09	
6.	2-Heptenal	(*E*)-Hept-2-enal	5283316	$C_7H_{12}O$ 112.17	
7.	Valeric acid	Pentanoic acid	7991	$C_5H_{10}O_2$ 102.13	
8.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4H-pyran-4-one	119838	$C_6H_8O_4$ 144.13	
9.	1,4-Dimethyl-2,3-diazabicyclo[2.2.1]-hept-2-ene	1,4-Dimethyl-2,3-diazabicyclo[2.2.1]-hept-2-ene	556835	$C_7H_{12}N_2$ 124.18	
10.	2-Methoxybenzoic acid, 2,3-dichlorophenyl ester	(2,3-Dichlorophenyl)-2-methoxybenzoate	91714954	$C_{14}H_{10}Cl_2O_3$ 297.13	

ADMET and drug-likeness properties

The calculated ADMET features of these drugs include gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeation, inhibition of the cytochrome P450 system, and permeability glycoprotein (P-gp) substrate. All the compounds, apart from compounds A and E, had substantial gastrointestinal absorption as

per the results. For chemicals C, D, F, G, I, and J, predicted BBB permeability potential was made. Compound B demonstrated potential as a P-gp substrate. Compounds A (for three isoforms), G, H, D, and J (for two isoforms), and I (for one isoform) were found to have the ability to inhibit cytochrome P450 (CYP) isoforms. It was anticipated that compounds E, F, and L would not exhibit any inhibitory activity towards any of the CYP isoforms.

Interaction with 2OXE

In agreement with the *in vitro* studies, the docking outcome of the result indicated that phytocompounds (1-10) bind to the enzyme pockets of Lipase-related Protein 2 (2OXE). Figure 2 shows Molecular docking interactions of isolated compounds (1-10) with Lipase-related Protein 2 (2OXE). The protein produced the binding energies of −2.8, −3.4, −3.7, −4.4, -2.5, -3.5, -3, -4.3, -3.9 and −5 kcal/mol respectively. The docking score of ligands with 2OXE is given in Table 3.

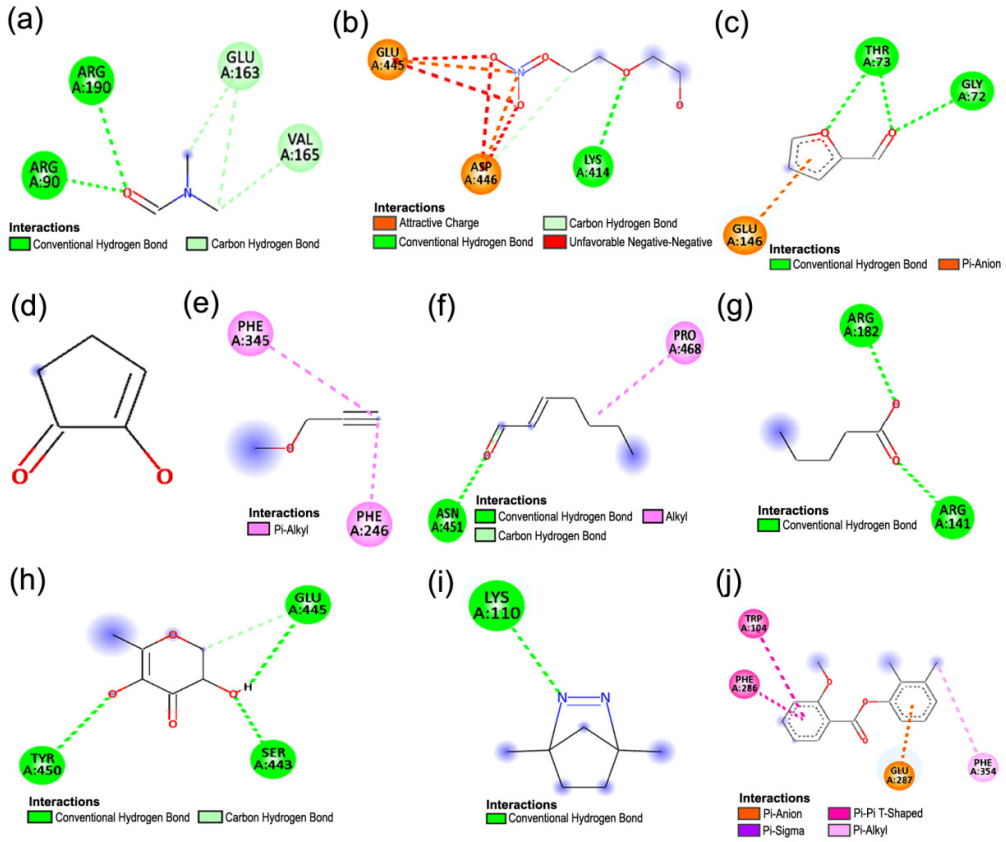


Figure 2. Molecular docking interactions of isolated compounds (a–j) with 2OXE (Lipase-related Protein and Ligands (a-j) a) 2OXE with Ligand 1 b) 2OXE with Ligand 2 c) 2OXE with Ligand 3 d) 2OXE with Ligand 4 e) 2OXE with Ligand 5 f) 2OXE with Ligand 6 g) 2OXE with Ligand 7 h) 2OXE with Ligand 8 i) 2OXE with Ligand 9 j) 2OXE with Ligand 10. Binding interactions of isolated phytocompounds from *C. pubescens* fruit extract

Table 3. The docking score of ligands with 2OXE (Lipase-related Protein 2) with ten selected phytocompounds from *C. pubescens* fruit extract

S. No.	Ligands	Target protein - 2OXE		
		Binding energy (kcal/mol)	No. of covalent hydrogen bond	Position of covalent hydrogen bond
1.	N,N-Dimethyl Formamide	-2.8	2	ARG190 and ARG90
2.	2-(2-Hydroxy ethoxy) Ethanol 1 – Nitrate	-3.4	1	LYS414
3.	Furfural	-3.7	3	THR73, GLU146 and GLY72
4.	2- Hydroxy -2-Cyclopentenone	-4.4	-	-
5.	Methyl Propargyl Ether	-2.5	-	-
6.	2-Heptenal	-3.5	1	ASN451
7.	Valeric acid	-3	2	ARG141 and ARG182
8.	4H – Pyran-4-one, 2,3-Dihydro 3,5- Dihydroxy – 6 - Methyl	-4.3	3	SER443, GLU445 and TYR450
9.	1,4-dimethyl-2,3-diazabicyclo[2.2.1]hept-2-ene	-3.9	1	LYS110
10.	2-Methoxy Benzoic acid, 2,3-Dichloro Phenyl Ester	-5	-	-

Interactions with 2QV4

Confirming the *in vitro* results, the docking results showed all ten compounds bind with α – amylase (2QV4) and it has been given as Figure 3. It produced the binding scores of -2.8, -3.6, -3.6, -4, -2.9, -3.7, -3.5, -5.1, -4.7, and −5 kcal/mol respectively. Table 4 shows the binding scores of the ligands with 2QV4.

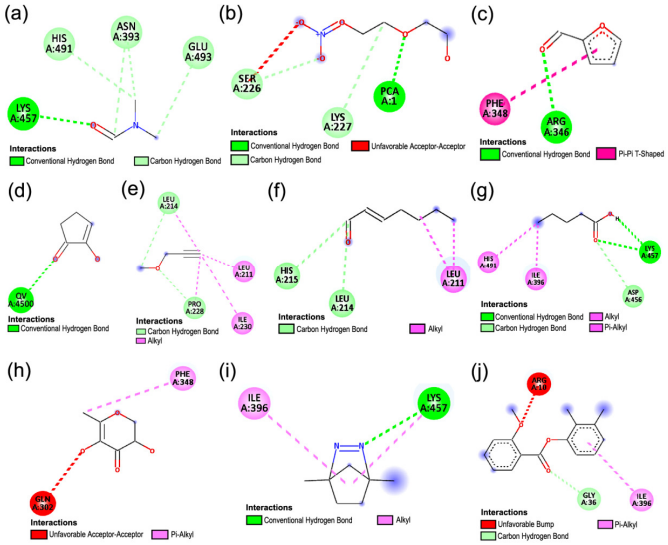


Figure 3. Molecular docking interactions of isolated compounds (a–j) with 2QV4 (α- amylase and Ligands: a–j) a) 2QV4 with Ligand 1 b) 2QV4 with Ligand 2 c) 2QV4 with Ligand 3 d) 2QV4 with Ligand 4 e) 2QV4 with Ligand 5 f) 2QV4 with Ligand 6 g) 2QV4 with Ligand 7 h) 2QV4 with Ligand 8 i) 2QV4 with Ligand 9 j) 2QV4 with Ligand 10 Binding interactions of isolated phytocompounds from C. pubescens fruit extract

Table 4. The docking score of ligands with 2QV4 (α – amylase) with ten selected phytocompounds from *C. pubescens* fruit extract

No	Compounds	Target protein - 2QV4		
		Binding energy (kcal/mol)	No. of covalent hydrogen bond	Position of covalent hydrogen bond
1.	N,N-Dimethyl Formamide	-2.8	1	LYS457
2.	2-(2-Hydroxy ethoxy) Ethanol 1 – Nitrate	-3.6	1	PCA1
3.	Furfural	-3.6	1	ARG346
4.	2- Hydroxy -2- Cyclopentenone	-4	1	QV4500
5.	Methyl Propargyl Ether	-2.9	-	-
6.	2-Heptenal	-3.7	-	-
7.	Valeric acid	-3.5	2	LYS457
8.	4H – Pyran-4-one, 2,3- Dihydro 3,5- Dihydroxy – 6 - Methyl	-5.1	-	-
9.	1,4-dimethyl-2,3-diazabicyclo[2.2.1]hept-2-ene	-4.7	1	LYS457
10.	2-Methoxy Benzoic acid, 2,3-Dichloro Phenyl Ester	-5	-	-

Interactions with 2ZOX

The docking results showed that all compounds have the ability to bind with the target protein as shown in Figure 4. The binding scores of the interactions are -3.2, -3, -3.3, -4.1, -2.5, -3.1, -3.2, -4.4, -4.4, and -6.1 kcal/mol respectively. Table 5 shows the binding scores of the ligands with 2ZOX.

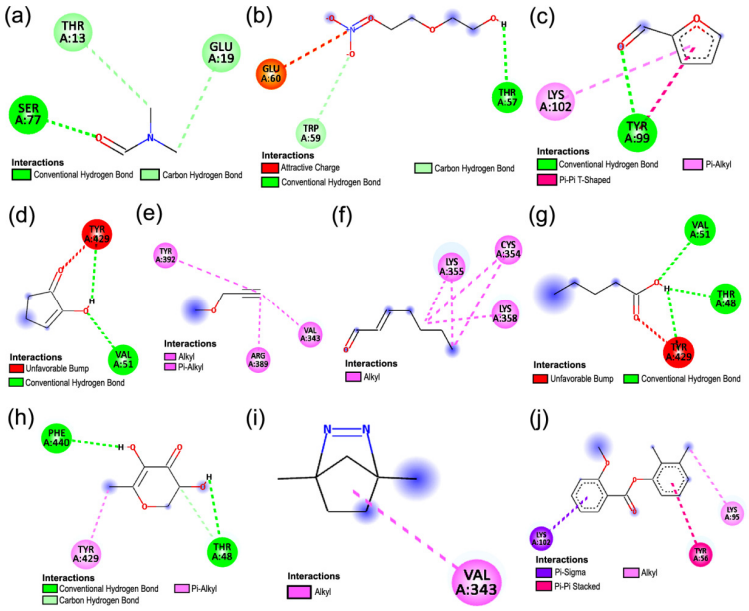


Figure 4. Molecular docking interactions of isolated compounds (a–j) with 2ZOX (β-glucosidase and Ligands a–j) a) 2ZOX with Ligand 1 b) 2ZOX with Ligand 2 c) 2ZOX with Ligand 3 d) 2ZOX with Ligand 4 e) 2ZOX with Ligand 5 f) 2ZOX with Ligand 6 g) 2ZOX with Ligand 7 h) 2ZOX with Ligand 8 i) 2ZOX with Ligand 9 j) 2ZOX with Ligand 10 Binding interactions of isolated phytocompounds from C. pubescens fruit extract

Table 5. The docking score of ligands with 2ZOX (β-glucosidase) with ten selected phytocompounds from *C. pubescens* fruit extract

S. No.	Compounds	Target protein - 2ZOX		
		Binding energy (kcal/mol)	No. of covalent hydrogen bond	Position of covalent hydrogen bond
1.	N,N-Dimethyl Formamide	-3.2	1	SER77
2.	2-(2-Hydroxy ethoxy) Ethanol 1 – Nitrate	-3	1	THR57
3.	Furfural	-3.3	1	TYR99
4.	2- Hydroxy -2- Cyclopentenone	-4.1	2	VAL51 and TYR429
5.	Methyl Propargyl Ether	-2.5	-	-
6.	2-Heptenal	-3.1	-	-
7.	Valeric acid	-3.2	3	THR48,VAL51 and TYR429
8.	4H – Pyran-4-one, 2,3- Dihydro 3,5-Dihydroxy – 6 - Methyl	-4.4	2	THR48 and PHE440
9.	1,4-dimethyl-2,3-diazabicyclo[2.2.1]hept-2-ene	-4.4	-	-
10.	2-Methoxy Benzoic acid, 2,3-Dichloro Phenyl Ester	-6.1	-	-

All three proteins were compared with all ten ligands to find the suitable interactions. The ligand-8 (4H-Pyran-4-one, 2,3- Dihydro 3,5- Dihydroxy- 6- Methyl) binds with 2OXE (Lipase related Protein 2) and 2ZOX (β-glucosidase) with higher binding energy -4.3 and -4.4 (kcal/mol) respectively by producing hydrogen bonds. Even, Ligand-10 (2-Methoxy Benzoic acid, 2,3-Dichloro Phenyl Ester) produced -5 and -6.1 (kcal/mol) respectively, but they have not produced hydrogen bonds.

The ligand-8 (4H- Pyran-4-one, 2,3- Dihydro 3,5- Dihydroxy-6-Methyl) produced three hydrogen bonds with 2OXE (Lipase related Protein 2). They are at 443rd position with Serine, 445th position with Glutamic acid and 450th position with Tyrosine. Whereas, the same ligand produced two hydrogen bonds with 2ZOX (β-glucosidase) at 48th position with Threonine and 440th position with Phenylalanine.

Even, Ligand-9 (1,4-dimethyl-2,3-diazabicyclo[2.2.1]hept-2-ene) has the binding energy of -4.7 (kcal/mol) by producing hydrogen bond with 2QV4(α – amylase). It produced single hydrogen bond at 457th position with Lysine. Table 6 shows the comparative analysis of binding energy of ligands with all target proteins.

Hence it is concluded that, Ligand-8 (4H- Pyran-4-one, 2,3- Dihydro 3,5- Dihydroxy- 6-Methyl) is the suitable ligand with 2OXE (Lipase related Protein 2) and 2ZOX (β-glucosidase). Ligand-9 (1,4-dimethyl-2,3-diazabicyclo[2.2.1]hept-2-ene) is suitable for 2QV4(α – amylase).

Table 6. Comparative analysis of binding energy of ligands with all target proteins (2OXE, 2QV4 and 2ZOX) with ten selected phytocompounds from *C. pubescens* fruit extract

S. No.	Compounds	Binding energy with target proteins (kcal/mol)		
		2OXE	2QV4	2ZOX
1.	N,N-Dimethyl Formamide	-2.8	-2.8	-3.2
2.	2-(2-Hydroxy ethoxy) Ethanol 1 – Nitrate	-3.4	-3.6	-3
3.	Furfural	-3.7	-3.6	-3.3
4.	2- Hydroxy -2- Cyclopentenone	-4.4	-4	-4.1
5.	Methyl Propargyl Ether	-2.5	-2.9	-2.5
6.	2-Heptenal	-3.5	-3.7	-3.1
7.	Valeric acid	-3	-3.5	-3.2

8.	4H – Pyran-4-one, 2,3- Dihydro 3,5- Dihydroxy – 6 - Methyl	-4.3	-5.1	-4.4
9.	1,4-dimethyl-2,3-diazabicyclo[2.2.1]hept-2-ene	-3.9	-4.7	-4.4
10.	2-Methoxy Benzoic acid, 2,3-Dichloro Phenyl Ester	-5	-5	-6.1

Discussion

The natural compounds that are extracted from microbes, plants and animals to lower blood sugar have grown in popularity. Molecular docking offers a rapid solution to address the requirement for efficient screening of a large number of natural chemicals. To anticipate the shape of binding, affinity and binding free energy (ΔG) of the ligands with α-amylase, β-glucosidase and Lipase related Protein 2, computational docking studies were carried out in Autodock vina. Because of their quick turn-around times, cost-effectiveness and labor-saving qualities, *in silico* technologies are becoming more and more important in drug discovery and development (Ekins *et al.,* 2007). This is particularly true when compared to their *in vitro* and *in vivo* counterparts, which are used to recognize novel natural phytocompounds as possible targets for drugs with identified biological activity. There are two well-known kinds of *in silico* approaches: structure-based models, which mostly use co-complex structure proteins, to study protein-ligand interactions through docking and analogue-based modelling, which uses pharmacophore and quantitative structure-activity relationship (QSAR) to generate predictive models based only on ligand information. Significantly, high throughput techniques to abbreviate large screens are a natural progression of regulated *in vitro* studies into *in silico* technologies. There are the obvious outcomes of the research scientist's access to an even-greater amount of processing power (Colquitt *et al.,* 2011).

A number of molecular models selected protein-ligand complexes from the protein data bank data base and ligand Fit, Glide, Gold, MOE Dock, Auto Dock and surflex-Dock were among the programmes used to assess the docking performance (Wilke *et al.,* 2015).Comparing the interactions of compounds with target proteins 2OXE and 2ZOX, the binding affinity of ligand-8 (4H – Pyran-4-one, 2,3- Dihydro 3,5- Dihydroxy- 6- Methyl) is higher and for the protein 2QV4, ligand-9 (1,4-dimethyl-2,3-diazabicyclo[2.2.1]hept-2-ene) has the high binding energy. The ligand-8 (4H- Pyran-4-one, 2,3-Dihydro 3,5-Dihydroxy- 6- Methyl) from the leaves of *Moringa* plant has the binding energy of -5.031 (kcal/mol) by producing three hydrogen bonds with 1FSY (Ampc beta-lactamase protein). They are at 674th position with Asparagine, 489th position with Asparagine and 672nd position with Threonine (Khandelwal *et al.,* 2019). The antioxidant activity of ligand-8 (4H – Pyran-4-one, 2,3- Dihydro 3,5- Dihydroxy – 6 – Methyl) has reported by (Dilek Tepe *et al.,* 2020). Similarly, this ligand-8 (4H- Pyran-4-one, 2,3-Dihydro 3,5- Dihydroxy- 6- Methyl) from *Gardenia gummifera* fruit methanol extract has the binding energy of -3.9 (kcal/mol) by producing three hydrogen bonds with 2XCT (*S. aureus* Gyrase complex with Ciprofloxacin and DNA). They are at 436th position with Glycine, 458th position with Arginine and 1123rd position with Phenylalanine (Kumar *et al.,* 2021).Here, result shows that ligand-8 (4H- Pyran-4-one, 2,3-Dihydro 3,5-Dihydroxy- 6- Methyl) binds with 2OXE (Lipase related Protein 2) and 2ZOX (β-glucosidase) with higher binding energy -4.3 and -4.4 (kcal/mol) respectively by producing three hydrogen bonds with 2OXE (Lipase related Protein 2) at 443rd position with Serine, 445th position with Glutamic acid and 450th position with Tyrosine and produced two hydrogen bonds with 2ZOX (β-glucosidase) at 48th position with Threonine and 440th position with Phenylalanine. Ligand-9 (1,4-dimethyl-2,3-diazabicyclo[2.2.1]hept-2-ene) has a binding energy of -4.7 (kcal/mol) by producing a single hydrogen bond with 2QV4(α – amylase) at 457th position with Lysine. The key residues involved in the docking is due to the hydrogen interaction (Akintemi *et al.,* 2023).

Usman *et al*. (2017) reported that next few decades, medicines from herbal resources most important regime in the medical sciences to manage various human diseases. The development of new and effective computer-aided drug design tools has led to numerous advancements in the drug screening process, making the process of developing therapeutic agents more dependable, affordable and innovative.

Therefore, it has been demonstrated that ligand docking with a protein's known three-dimensional (3D) structure is a crucial technique for the discovery and design of novel treatment regimes. One such tool for pharmaceuticals would be the molecular docking techniques. Medicines against virulent factors for the treatment of several diseases (Shakya, 2016; Kumar *et al.,* 2022).

Conclusions

The plant *C. pubescens* produces several chemical compounds which were identified through GC-MS analysis. Ten ligand molecules were selected based on their pharmacokinetic properties. On the other hand, three protein targets responsible for anti-diabetic activity have been selected from PDB databank. Molecular docking analysis was performed for all ten ligand molecules with three target proteins to find the best dock score. It was performed using Autodock Vina to predict the binding mode, affinity and binding free energy (ΔG) of the ligands with all three proteins. 4H – Pyran-4-one, 2,3- Dihydro 3,5- Dihydroxy – 6 – Methyl (Ligand 8) bound tightly with Lipase Related Protein 2 (2OXE) and β-glucosidase (2ZOX), produced -4.3 and -4.4 (kcal/mol) as binding energy. It has been concluded that 4H – Pyran-4-one, 2,3- Dihydro 3,5- Dihydroxy – 6 – Methyl from *C. pubescens* plant displays high potential against diabetes and it could be employed as an anti-diabetic drug in future for the societal needs.

Authors' Contributions

TS: Data curation, Investigation, Validation, Writing manuscript-Original draft; RK: Conceptualization, Supervision, Validation, Writing, review and editing; MA: Data curation, Review and Writing. All authors read and approved the manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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