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Phylogenetics And Molecular Docking Studies On Glycogen Phosphorylase Inhibitors As Anti-Diabetic Agents

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ABSTRACT

Glycogen phosphorylase (GP) is a key enzyme involved in glycogen breakdown, making it a key focus for managing blood sugar levels, especially in relation to diabetes mellitus. This study combines phylogenetic analysis and molecular docking techniques to identify potential inhibitors of GP as anti-diabetic agents. Phylogenetic analysis was conducted to explore the evolutionary relationships of liver GP from Homo sapiens, highlighting conserved regions that may be crucial for inhibitor binding. The three-dimensional structure of the GP enzyme (1FA9) was retrieved from the Protein Data Bank and used for molecular docking studies with a range of plant-derived compounds. Seventeen ligands were evaluated, and docking results revealed that several compounds displayed strong binding affinities at the active site with its native ligands Pyridoxal Phosphate and Adenosine monophosphate; with the average binding energy reaching around -8 kcal/mol. Kaempferol, Momordenol, and Loganic Acid may have stronger interactions due to the higher number of residues they interact with. Lys574, Thr676, and Gly677 appear to be critical residues in the protein's active or binding site, as they interact with multiple compounds. These findings suggest that the selected plant-based compounds may serve as effective GP inhibitors and offer potential as lead compounds for the development of anti-diabetic drugs. This study underscores the value of integrating phylogenetics and molecular docking in uncovering new therapeutic compounds for diabetes treatment.

Keywords: Diabetes mellitus, Glycogen phosphorylase, anti-diabetic, docking, Herbal Drug

INTRODUCTION

Diabetes mellitus is a complex condition involving disruptions in glucose and protein metabolism, alongside impaired insulin function [1]. As per the International Diabetes Federation, the global prevalence of diabetes in 2021 was estimated at 10.5% (536.6 million individuals), with projections indicating an increase to 12.2% (783.2 million) by 2045. This signifies that over half a billion adults worldwide are affected by diabetes, representing more than 10.5% of the global adult population [2]. The disease is polygenic and is characterized by hyperglycemia, defective pancreatic insulin secretion, and insulin resistance in key tissues such as the liver, adipose tissue, and skeletal muscle. The relative roles of

insulin resistance and pancreatic insulin secretion dysfunction in the development of type 2 diabetes remain actively debated [1,3]. A crucial target for therapeutic intervention is the liver isoform of human glycogen phosphorylase, which plays a significant role in regulating blood glucose levels. Inhibiting this enzyme presents a promising strategy for managing type 2 diabetes [4].

In this study, glycogen phosphorylase (GP) was chosen as a target for type 2 diabetes inhibitors due to its specific role in humans and other non-human mammals. GP is expressed in three isoforms, each encoded by distinct genes located on human chromosomes 11, 14, and 20, corresponding to the liver, brain, and skeletal muscle, respectively [5, 6]. This enzyme operates in two functional states: the catalytically active GP (a) form and the less active GP (b) form. The transition from GP (b) to GP (a) occurs through the phosphorylation of Ser14 by phosphorylase kinase, a process triggered by cAMP, while enzyme activity is further regulated by allosteric effectors that stabilize either the active or inactive conformation [7]. This regulation involves a dynamic balance between phosphorylation, dephosphorylation, and allosteric ligand binding, allowing interconversion between the active 'R' state and inactive 'T' state. Numerous chemical compounds have been identified as allosteric inhibitors, capable of reducing GP activity by binding to the Adenosine monophosphate (AMP) site [8, 9].

The World Health Organization (WHO) advocates for the use of plant-based medicines, and the growing emphasis on natural therapies and alternative medicine has sparked significant interest among researchers in studying plant-derived compounds for diabetes treatment. This interest stems from their affordability, perceived efficacy, and reduced risk of side effects [10, 11, 12]. Advances in bioinformatics and chemoinformatics have enabled the use of computational tools to predict evolutionary relationships and model disease-related proteins. These tools are particularly valuable for molecular docking studies, which help predict the preferred binding orientations of small molecules with target proteins. The increasing demand for novel therapeutic agents has further driven the adoption of computational approaches like molecular docking to explore the interactions between ligands and proteins [13]. In this research, structural models of plant-derived ligands interacting with the binding sites of glycogen phosphorylase (GP) were examined and compared to GP's native ligands. The goal was to identify promising GP inhibitors that could potentially serve as effective treatments for diabetes.

MATERIALS AND METHODS

Protein Sequence Retrieval and Phylogenetic Analysis: The reference glycogen phosphorylase (GP) enzyme sequence was obtained from UniProt (www.uniprot.org) using the accession number P06737, which corresponds to the human liver GP with established molecular functions. This sequence was utilized as a query for database searches in human protein repositories. BLASTP [14] was employed to perform searches against the Protein Data Bank (PDB) (www.rcsb.org/pdb) and the GenBank non-redundant (NR) database (www.ncbi.nlm.nih.gov/genbank). The identified protein sequences were analysed to predict corresponding PDB codes. Multiple sequence alignments were conducted using ClustalX 2.0 (www.clustal.org), where proteins with over 30% identity to any of the reference sequences were considered homologous and functionally similar. These proteins were assigned the same nomenclature as the reference proteins. The ClustalX-generated alignments [15] served as the basis for phylogenetic analysis. Phylogenetic trees were constructed using the neighbor-joining method in MEGA software (version 16). The protein 1FA9 was selected for molecular docking studies, as it binds allosterically to glycogen phosphorylase at one of its binding sites, alongside its native ligand, adenosine monophosphate [8].

Protein and Ligand Docking: Molecular docking examines how two molecules interact and fit together within a 3D space, making it a valuable technique in computer-aided drug design and structural biology [13]. Ligand molecules were sourced from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), a freely accessible repository of small molecules that provides detailed information on their structures, physicochemical properties, and query retrieval methods [17]. The PyMOL software (www.pymol.org) was used to visualize and manipulate the ligands. Ligand structures were converted from two-dimensional to three-dimensional formats, with their physicochemical properties analyzed to

enhance activity. Docking studies were conducted using AutoDock Vina [18], a widely used docking program, to identify the most effective molecular interactions and inhibitors. The interactions between the ligands and the active sites of the target protein were examined, focusing on the type of interactions and bond distances. These were analyzed using LIGPLOT [19] and Discovery Studio Visualizer [20]. To ensure the validity of the docking results, the co-crystallized ligand within the 1FA9 protein structure was redocked, and the root-mean-square deviation (RMSD) between the experimental and predicted poses was calculated. An RMSD value of less than 2 Å was deemed acceptable, confirming the reliability of the docking methodology.

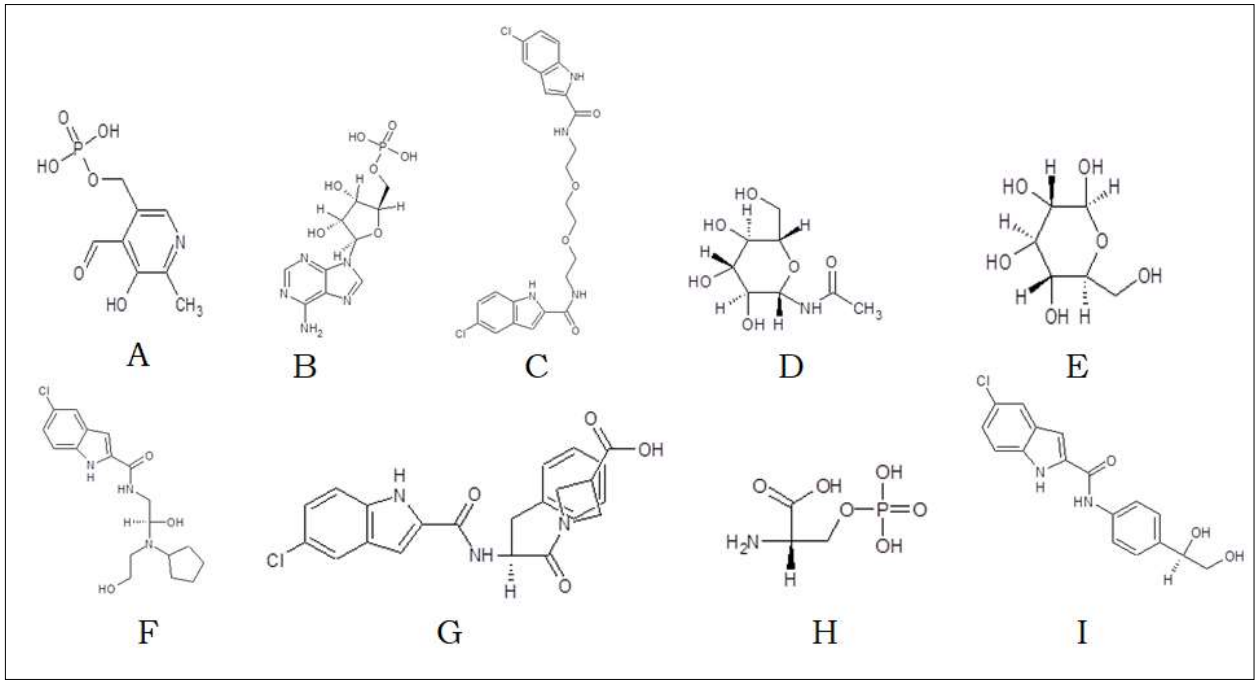
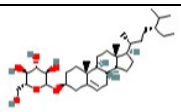
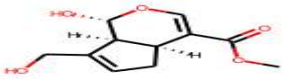
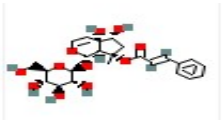
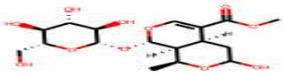
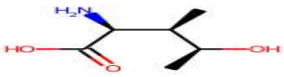
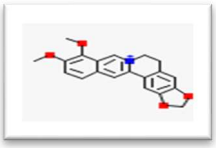
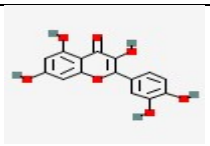


Fig. 1. Structures of native ligands present along with IFA9 protein
A) Pyridoxal Phosphate B) Adenosine monophosphate C) CP-526423 D) 1-N-Acetyl-Beta-D-Glucosamine E) Beta-D-Glucose F) 5-Chloro-1h-Indole-2-Carboxylic Acid{[Cyclopentyl-(2-Hydroxy-Ethyl)-Carbamoyl]-Methyl}-Amide G) Cp403700,(S)-1-{2-[(5-Chloro-1h-Indole-2-Carbonyl)-Amino]-3-Phenyl-Propionyl}-Azetidine-3-Carboxylate H) Phosphonoserine I) 5-chloro-N-{4-[(1R)-1,2-dihydroxyethyl]phenyl}-1H-indole-2-carboxamide.

In this study, several plant-based compounds were considered to know the inhibitory effect of representative compounds against glycogen phosphorylase (Table 1). The Auto Dock vina [18] was used for the molecular docking analysis as it is an integrated platform for predicting the protein-ligand interactions. Also, it handles all aspects of the development, from drawing the molecules to determining the potential binding site of the target protein and predicting the binding mode of the ligand. It offers high-quality docking based on a novel optimization technique combined with a user interface experience focusing on usability and productivity.

Table 1. List of selected plant-based compounds used as inhibitors for diabetes understudy

S.NO	CHEMICAL NAME	CHEMICAL STRUCTURE	SOURCE
1	Charantin		*Momordica charantia*
2	Momordenol		*Momordica charantia*
3	Momordicilin		*Momordica charantia*
4	Aucubin		*Plantago asiatica*
5	Genipin		*Gardenia jasminoides*
6	Harpagoside B		*Scrophularia deserti*
7	Loganic acid		*Corni fructus*
8	Loganin		*Corni fructus*
9	Morroniside		*CorniFructus*
10	Swertiamarin		*Ennicostema littorale*
11	Kaempferol		*Brassica oleracea var. italica*
12	Naringenin		*Citrus × paradisi*

13	Marsupsin		*Pterocarpus marsupium*
14	Pterostilbene		*Pterocarpus marsupium*
15	Hydroxyisoleucine		*Trigonellafoenum-graecum*
16	Berberine		*Berberis vulgaris*
17	Quercetin		*Vitis vinifera*

RESULTS AND DISCUSSION

With the availability of NCBI data, it was possible to construct an overview of GP (Accession no.P06737) of the Human liver, which has positive molecular implications, was chosen for analysis. Taking these proteins as query sequences, searching was carried out for orthologous sequences in GenBank using BLASTP. After conducting BLASTP searches in the databanks, protein sequences belonging to the relevant family were selected. Redundant sequences within each family were excluded, and the remaining sequences were analyzed to confirm the presence of signature motifs corresponding to glycogen phosphorylase (GP). Phylogenetic analysis of the sequences with liver GP revealed that the relation with other GP was divergent, showing branches in tree view (Fig. 2). The phylogenetic analysis revealed multiple branches, signifying diverse origins, which were represented by PDB codes for the sequences. A total of eighteen glycogen phosphorylases (GPs) were classified into five distinct groups. The placement of 1FA9 on this tree suggests that it shares a close evolutionary relationship with proteins labeled 3DDS and 2QLL, as evidenced by their proximity in the tree. The bootstrap values, such as "577" and "543," provide confidence levels for the branching points; higher values indicate stronger evolutionary support for the grouping of these protein sequences.

From this result, it is evident that the 1FA9 protein is part of a well-supported clade (branch) that includes proteins with similar structural or functional characteristics. This supports the hypothesis that the liver isoform of GP, as represented by 1FA9, shares conserved structural features with other GP proteins, which may influence its role in glucose metabolism and its suitability as a drug target for diabetes. The clustering of related proteins suggests that inhibitors targeting 1FA9 might also have effects on other similar isoforms, providing insights into potential off-target interactions. This phylogenetic relationship is important for drug design as it helps in understanding the evolutionary conservation of the active site, which could guide the selection of broad-spectrum or isoform-specific inhibitors.

Molecular docking analysis using Auto Dock Vina resulted and revealed that docked orientations were saved, and the resultant molecules are shown in Table 2 and 3. The average binding affinity of the native ligands was -8.0 kcal/mol. Therefore, given the average score of experimentally docked compounds, criteria have been adopted to filter those compounds from all the plant-based compounds under study, which exhibited a docking score higher than -8.0 kcal/mol.

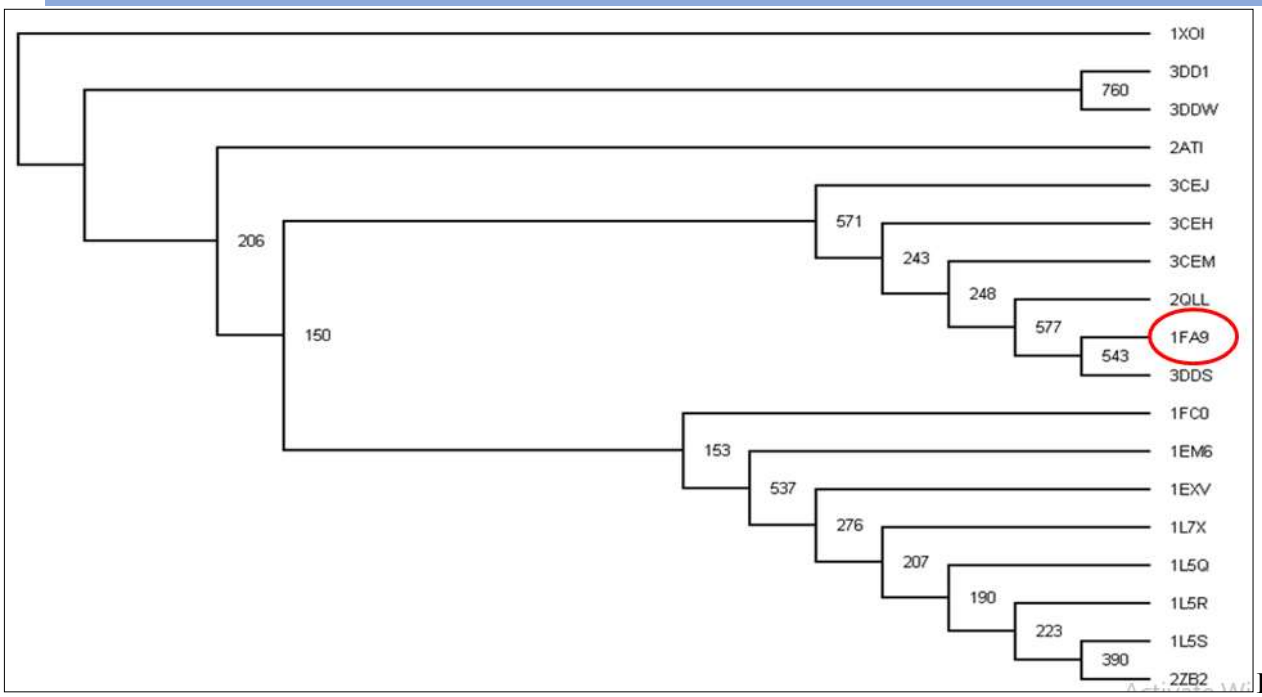


Fig.2. Phylogenetic tree of sequences identified from PDB databank for liver Glycogen phosphorylase from Homo sapiens.

Table 2. Binding energies of native ligands understudy

S.no	Inhibitor	Affinity (Kcal/mol)
1	Beta-D-Glucose	-5.8
2	5-Chloro-1h-Indole-2-Carboxylic Acid{[Cyclopentyl-(2-Hydroxy-Ethyl)-Carbamoyl]-Methyl}-Amide	-8.4
3	5-chloro-N-{4-[(1R)-1,2-dihydroxyethyl]phenyl}-1H-indole-2-carboxamide	-8.7
4	Pyridoxal Phosphate	-8.8
5	CP-526423	-8.6
6	1-N-Acetyl-Beta-D-Glucosamine	-6.3
7	Adenosine monophosphate	-8.6
8	Cp403700, (S)-1-{2-[(5-Chloro-1h-Indole-2-Carbonyl)-Amino]-3-Phenyl-Propionyl}-Azetidine-3-Carboxylate	-8.3
9	Phosphonoserine	-6.0

The table 2 summarizes the molecular docking results, showing the binding affinities (in kcal/mol) of different native inhibitors to glycogen phosphorylase (GP). These binding affinities provide insights into the strength of interaction between each inhibitor and the GP active site, with more negative values indicating stronger binding. Among the native inhibitors tested, Pyridoxal Phosphate exhibited the strongest binding affinity, with a binding energy of -8.8 kcal/mol, indicating its potential as a highly effective inhibitor of glycogen phosphorylase. This is closely followed by 5-chloro-N- {4-[(1R)-1,2-di hydroxyl ethyl] phenyl} -1H-indole-2-carboxamide with a binding energy of -8.7 kcal/mol, and both CP-526423 and Adenosine monophosphate, each with -8.6 kcal/mol. These results suggest that these compounds have a strong potential for inhibiting GP activity, which could be beneficial for controlling glycogen breakdown and managing blood glucose levels in diabetic patients.

Among the compounds tested, Charantin exhibited the strongest binding affinity to GP, with a docking score of -9.7 kcal/mol, closely followed by Momordicilin with -9.6 kcal/mol and Berberine with -9.3 kcal/mol. These highly negative

docking scores indicate that these compounds have significant potential as GP inhibitors, suggesting strong interactions with the enzyme's active site, which could be useful for regulating glycogen breakdown in diabetic patients. Other notable compounds include Genipin (-8.9 kcal/mol), Momordenol, Kaempferol, and Quercetin (all -8.8 kcal/mol), which also demonstrated strong binding affinities, making them promising candidates for further investigation as anti-diabetic agents. These compounds could inhibit GP activity, helping to control blood glucose levels by reducing glycogenolysis. Compounds like Marsupsin and Morroniside, with relatively weaker docking scores of -8.0 kcal/mol and -8.1 kcal/mol, may have lower inhibitory potential compared to the top candidates, but they still show moderate binding affinity, suggesting that they could be explored further, perhaps in combination with other inhibitors. Overall, the docking results highlight several compounds, particularly Charantin, Momordicilin, and Berberine, as strong candidates for further in vitro and in vivo testing. Their significant binding affinities suggest they may effectively inhibit GP, offering potential for the development of natural anti-diabetic therapies.

SNO	CHEMICAL NAME	Docking Score (Kcal/Mol)
1	Charantin	-9.7
2	Momordenol	-8.8
3	Momordicilin	-9.6
4	Aucubin	-8.7
5	Genipin	-8.9
6	Harpagoside B	-8.2
7	Loganic acid	-8.7
8	Loganin	-8.2
9	Morroniside	-8.1
10	Swertiamarin	-8.4
11	Kaempferol	-8.8
12	Naringenin	-8.3
13	Marsupsin	-8.0
14	Pterostilbene	-8.5
15	Hydroxyisoleucine	-8.6
16	Berberine	-9.3
17	Quercetin	-8.8

Table 3. Binding energies of plant-derived chemical constituents against 1FA9

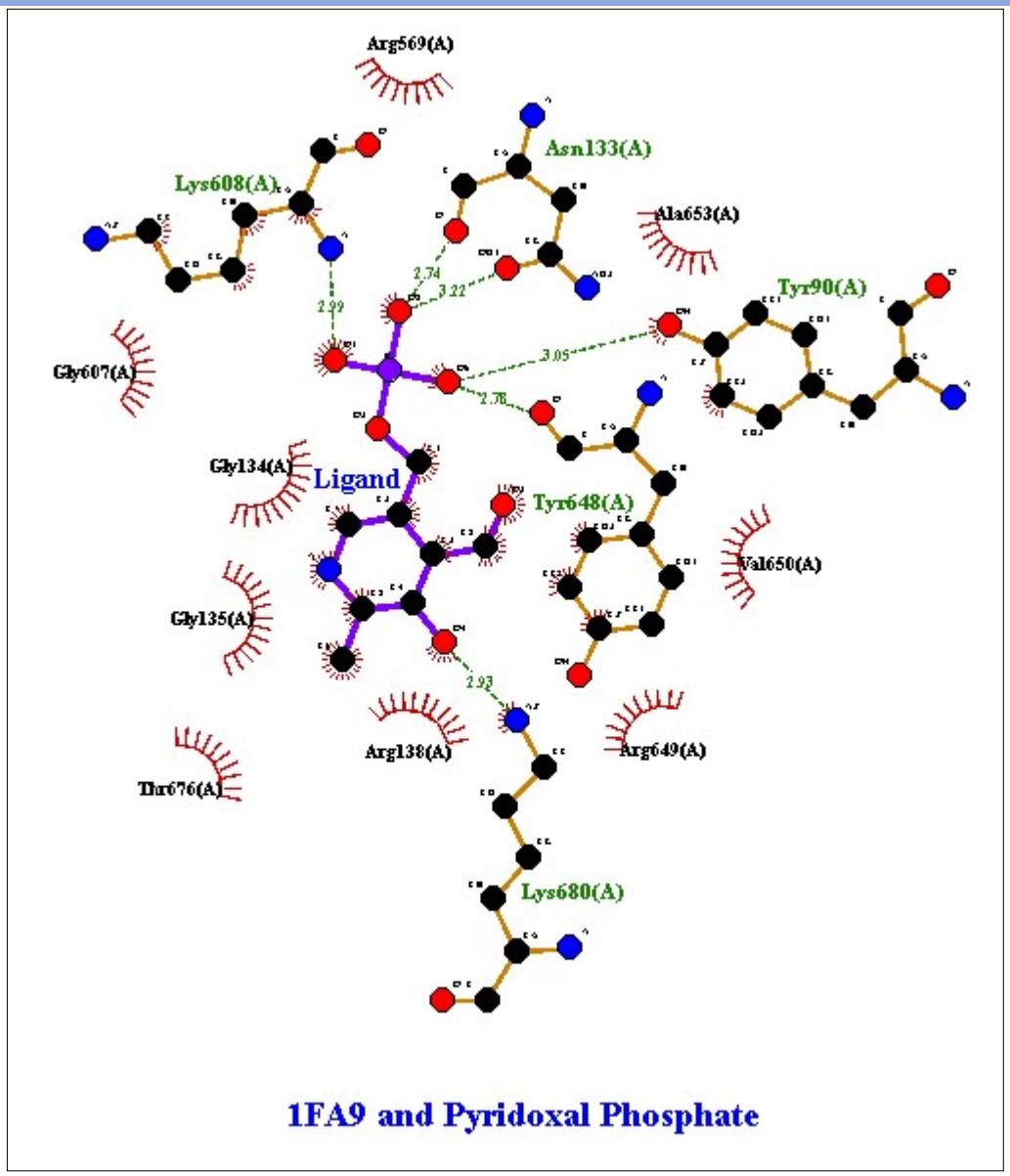


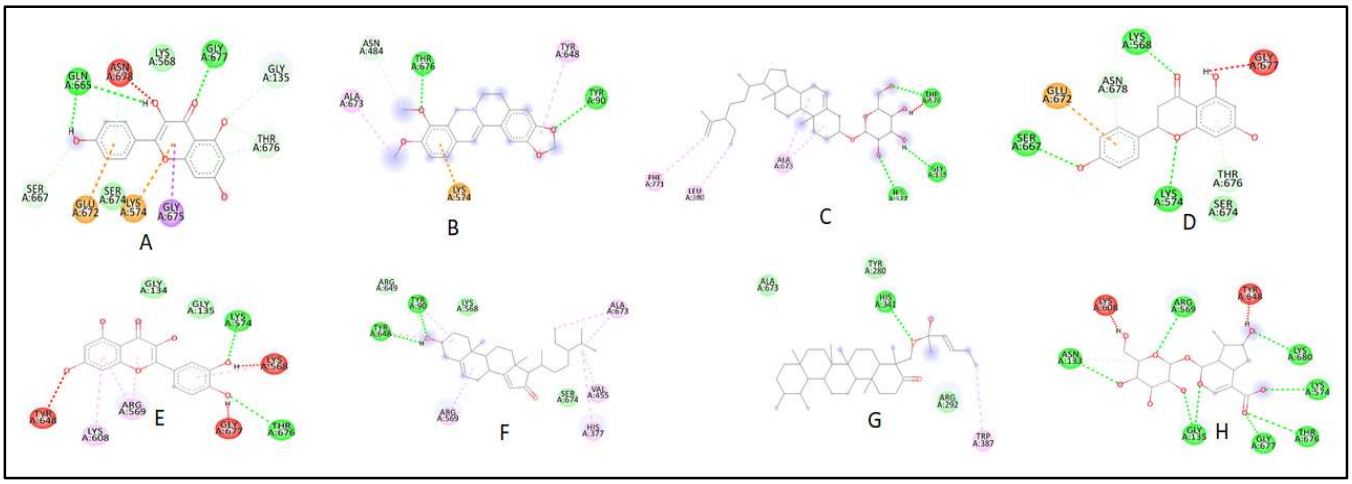
Fig.3. LIGPLOT Interactions of the native ligand, pyridoxal phosphate with 1FA9 active site residues.

Table 4. Interactions of top 8 chemical compounds with 1FA9 active site residues.

Name of the compounds from plant origin	Interacting amino acids	No.of amino acid residues
Kaempferol	Ser667, glu672, lys574, gln665, gly675, asn678, thr676, gly135, gly677	9

Berberine	Tyr648, tyr90, thr676, ala673, lys574, asn484	6
Charantin	Gly135, thr676, his377, ala673, phe771, leu380	6
Naringenin	Asn678, thr676, gly677, glu672, ser667, lys574, lys568	7
Quercetin	Lys574,Lys568,Thr676,Gly677, Arg569,Lys608,Tyr648	7
Momordenol	Ser674,Tyr90,Arg649,Lys568,Tyr648,Arg569,Ala673,Val455 ,His377	9
Momordicilin	His341,Trp387,Ala673,Tyr280,Arg292	5
Loganic acid	Asn133,Arg569,Lys680,Lys574,Thr676,Glu677,Gly135,Tyr6 48,Lys608	9

Kaempferol, Momordenol, and Loganic Acid stand out because they interact with the highest number of amino acid residues (9 each), suggesting potentially stronger or more stable binding interactions with the protein. Berberine, Charantin, and Naringenin interact with 6 or 7 amino acids, indicating moderately strong interactions. Momordicilin has the least



interactions with 5 amino acids, possibly indicating a weaker binding interaction compared to the others. Shared Interactions: Several compounds, such as Naringenin, Quercetin, and Berberine, all interact with Lys574; indicate that this particular residue could be essential for binding for multiple plant-based compounds. Similarly, Thr676 and Gly677 are frequently interacting residues, implying their importance in binding (Table 4 and 5).

Fig.4. Two-dimensional (2D) Interactions of compounds with 1FA9 active site residues through Discovery Studio Visualizer software tool. A)Kaempferol B)Berberine C)Charantin D)Naringenin E)Quercetin F)Momordenol G)Momordicilin H)Loganic acid.

CONCLUSION

This research provides valuable insights into the potential of plant-based compounds as anti-diabetic agents through phylogenetic and molecular docking studies on glycogen phosphorylase (GP) inhibitors. Phylogenetic analysis highlighted the evolutionary conservation of the liver isoform of GP, a key target in glucose metabolism for type 2 diabetes treatments. Promising inhibitors like Kaempferol, Charantin, Momordicilin, and Berberine demonstrated strong binding affinities, suggesting their ability to regulate glycogen breakdown. These findings suggest natural compounds could offer low-cost, effective alternatives to synthetic drugs. However, further in vitro and in vivo studies are needed to confirm these results and assess clinical potential. This study emphasizes the role of bioinformatics in advancing plant-based therapies for diabetes.

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