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# *In Silico* Modeling Studies of the Constituents of *Gymnema Sylvestre* R.Br.

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## ABSTRACT

*Gymnema sylvestre* has been extensively evaluated for its i*n vitro* and *in vivo* activity against diabetes mellitus. The leaves of the shrub *Gymnema sylvestre* contain a complex of pentacyclic triterpenes known as gymnemic acids that have been reported as potential therapeutic agents in the treatment of diabetes. The present study reveals the relationship between the structure and function of the medicinally important constituents of this plant. To understand the binding mechanisms of these active constituents, molecular modelling studies has been performed with dipeptidyl peptidase-4, protein tyrosine phosphatase 1B, sodium potassium ATPase, aldose reductase and glycogen synthase kinase-3β as target proteins using XP docking program of Glide, version 9.2, Schrödinger suit. These constituents showed favourable interactions with the amino acid residues at the active site, their by substantiating their proven efficacy as antidiabetic agents. The present study also gives an insight to the probable herb-drug interaction and reason for sudden hypoglycemic shocks that may occur on concurrent administration of Gymnema extract and synthetic drug, Glimepiride, for the treatment of diabetes.

**Keywords:** diabetes mellitus, docking, *Gymnema sylvestre*, gymnemagenin, glimepiride, Glide, *in silico* herb-drug interaction.

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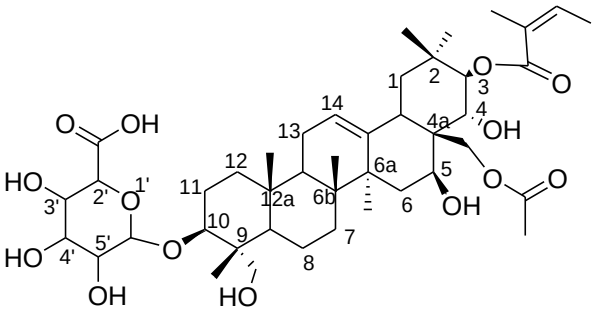
## INTRODUCTION

Type 2 diabetes mellitus (T2DM) is genetically heterogeneous and polygenic disease characterized by altered expression of many genes and tissue types [1, 2.] Various enzymes and proteins have been considered as the potential targets if inhibited, lead to the control over T2DM. There have been many reports on the inhibition of individual targets by various synthetic, semi-synthetic molecules and herbal extracts [3, 4]. One such herb used extensively in the management of T2DM, even before the synthetic era, is *Gymnema sylvestre* (G.S.) (Family: Asclepiadaceae). Various pre-clinical and clinical studies on aqueous and hydro-alcoholic extracts of G.S. leaves have shown its antidiabetic properties in T2DM [5, 6]. Globally marketed formulations such Diabecon and Meshashringi (Himalaya Drugs company, Bangalore, India) also contain G.S. extract as one of the ingredients. The leaves of G.S. consist of triterpene saponins belonging to oleanane and dammarene classes. Oleanane saponins are gymnemic acids and gymnemasaponins where as dammarene saponins are gymnemosides (Figure **1**) [7-9.] These acidic glycosides have been identified as the active constituents responsible for its antidiabetic potential. There are various mechanisms proposed for its mode of action including, regeneration of β-islet cells, increased secretion of insulin, inhibition of glucose uptake and utilisation of glucose via enhanced activity of enzymes responsible for insulin dependent pathways [10]. Further, G.S. when concurrently administered with oral synthetic antihyperglycemics may also lead to herb-drug interactions and sudden hypoglycemia [11]. To the best of our knowledge, the exact mechanisms by which these saponins act have not been demonstrated *in silico*. The present study reveals the relationship between the structure and function of the medicinally important constituents of this plant. To understand the binding mechanisms of these active constituents, molecular modelling studies has been performed with dipeptidyl peptidase-4 (DPP-IV), protein tyrosine phosphatase 1B (PTP-1β), sodium potassium ATPase (NaKATPase), aldose reductase (AR) and glycogen synthase kinase-3β (GSK-3β) as target proteins using XP docking program of Glide, version 9.2, Schrödinger software. The present study also gives an insight to the probable herb-drug interaction and reason for sudden hypoglycemic shocks that may occur on concurrent administration of G.S. and synthetic drug Glimepiride, for the treatment of diabetes.



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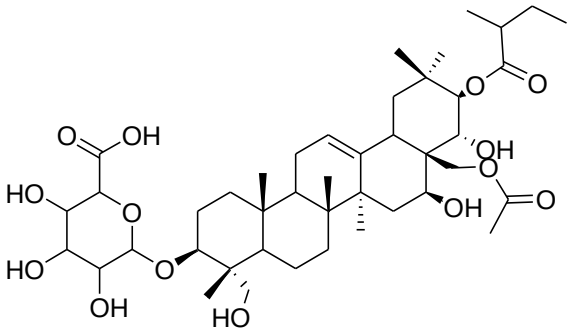

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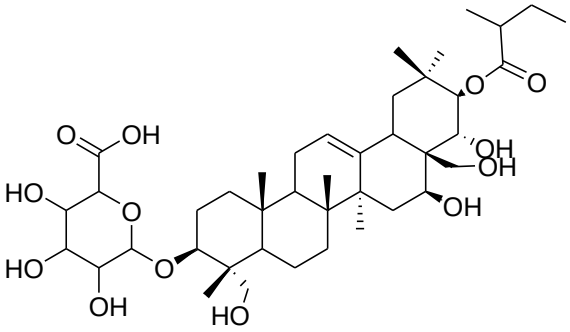

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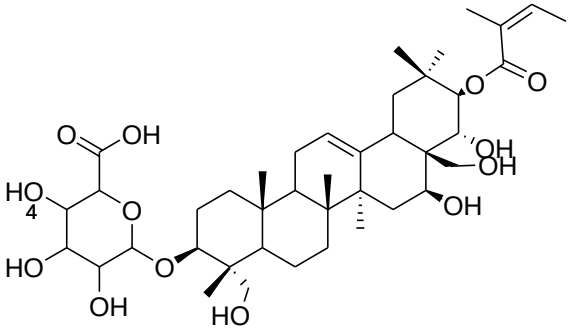

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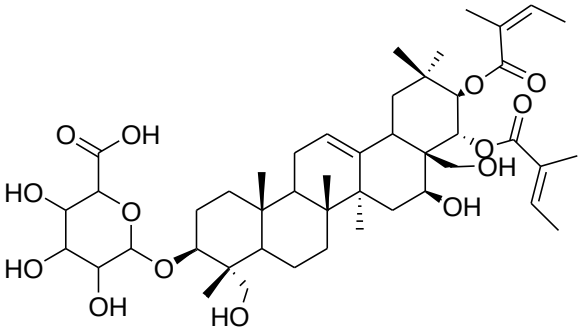

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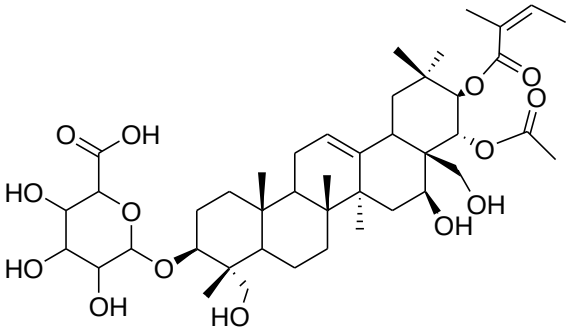
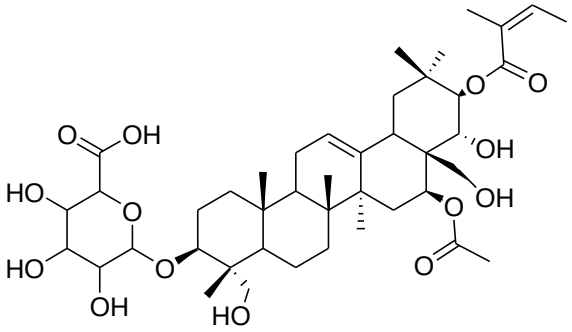
**Code: E**



**Gymnemoside A**
**Code: F**



**Gymnemoside B**
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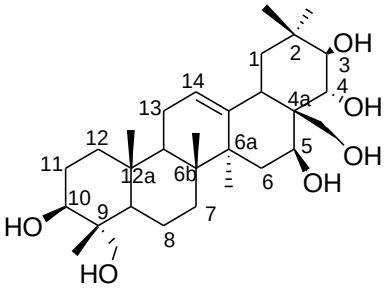


**Gymnemagenin**
**Code: H**

**Figure 1 Active principle components of *Gymnema sylvestre***

## MATERIALS AND METHODS

Docking studies were performed for gymnemic acids (I-V, Gymnemoside A, Gymnemoside B) and the aglycone moiety Gymnemagenin with target proteins by Glide version 9.2 of Schrödinger suit on windows 2007 workstation as the operating system. The X-ray crystal structure of proteins, DPP-4 (PDB ID: 3NOX), PTP-1B (PDB ID: 1C83), NaKATPase (PDB ID: 3A3Y), AR (PDB ID: 3G5E) and GSK-3β (PDB ID: 3F7Z) were obtained from RCSB Protein Data Bank (http://www.rcsb.org/pdb). The proteins were prepared using protein preparation wizard of Schrödinger suit. The proteins were pre-processed separately by deleting the substrate cofactor, optimizing hydrogen bonds and addition of the missing side chain atoms to the protein residues. After assigning charge and protonation state, energy minimization with root mean square deviation (RMSD) of 0.3Å was done using OPLS2005 force field. The prepared proteins were employed to build energy Grids using the default value of protein atom with in a cubic box, centered on the centroid of the X-ray ligand pose. Further, the structures of the ligands were generated in the CDX format using the tool Chem Draw ultra version 8.0. These ligands were then converted to the mol format and prepared using the LigPrep module of Maestro in the Schrödinger suite. Hydrogen atoms were added to the ligand molecules as they had implicit hydrogen atoms. The bonds orders of these ligands were fixed. Most probable tautomers and all possible stereo isomers were generated to study the activity of individual stereotypes of each ligand. In the final stage of LigPrep, compounds were minimized with OPLS-2005 force field.

Docking studies were done for all the prepared proteins separately. All the ligands were docked with the individual proteins using Glide, version 9.2, Schrödinger software in extra precision mode (XP) which uses MCSA (Monte Carlo Simulated Algorithm) based minimization. A standard inhibitor, Glimepiride, of potassium ATP channel and NaKATPase was also included in the study to evaluate its binding affinity and possible herb-drug interaction *in silico* between the plant extract and synthetic molecule if co-administered. The best docked poses (with lowest glide score value) obtained from Glide were analysed. The binding energy was calculated by Liaison module (version 9.2) [12-15].

## RESULTS AND DISCUSSION

Docking the constituents of G.S. showed excellent hydrogen bond interactions at the receptor sites of the targeted proteins especially DPP-IV, PTP-1B, NaKATPase and GSK-3β.

**Dipeptidyl Peptidase-IV**

DPP-IV inhibition increases the endogenous levels of active Glucagon like peptide-1 (GLP-1)

and consequently improves both β-cell insulin secretion and glycaemic control. Based on this concept, the covalent inhibitor Vildagliptin (Galvus®, Novartis), and the non-covalent inhibitor Sitagliptin (Januvia®, Merck) for the treatment of diabetes has been approved. The docking study of Gymnemic acids at the active site of chain A of DPP-IV indicated that these acids interact with one or all of the possible sites S2, S1 and S1' pocket of the enzyme. These components demonstrated hydrogen bond interactions at the important amino acids [16-18] of the site namely GLU 205, GLU 206, ASP 545, TYR 547, CYS 551, LYS 554, ASN 562, SER 630 and TYR 752 with Glide score (docking score) ranging between -7.23 & -7.9 and Glide energy (Free binding energy in Kcal/mol) -42 & -54 Kcal/mol. With these interactions it can be concluded that the components of G.S. may be good inhibitors of DPP-IV enzyme. The aglycone moiety, Gymnemagenin, is reported to be obtained upon hydrolysis of these gymnemic acids. Gymnemagenin also showed good hydrogen bond interactions at the active site of DPP-IV with the glide score and glide energy -6.49 & -34.1754 Kcal/mol respectively (Table **1**, Figure **2**). It has been reported that the fate of gymnemagenin has not been known till date and has only been identified as a biomarker in the identification of these gymnemic acids [19]. With the docking results, it can be concluded that even gymnemagenin may also be an active component of *Gymnema sylvestre* inhibiting the enzyme DPP-IV.

**Table 1 Docking parameters of examined molecules on DPP-IV (Pdb: 3NOX)**

| Ligand Code | Glide Score | Glide Energy (Kcal/mol) | H-Bond Interaction | | |
|---|---|---|---|---|---|
| | | | H –bond Acceptor | H -bond Donor | H –bond Length (Å) |
| Gymnemic acid I A | -7.55 | -54.353061 | (Gym I) 3-O-C=**O** | N**H** (ARG 358) | 1.950 |
| | | | (TYR 752) C-O | **H**O-4'C (Gym I) | 2.101 |
| | | | (Gym I) 5'-C-**O**H | **H**O-C (TYR 48) | 2.491 |
| | | | (Gym I) 2'-C-**O** | N**H** (LYS 554) | 2.094 |
| | | | (Gym I) 2'-C=**O** | N**H** (ASN 562) | 1.991 |
| | | | (ASP 545) C-**O** | **H**O-3'C (Gym I) | 2.414 |
| Gymnemic acid II B | -7.89 | -47.1768 | (GLU 205) C=**O** | **H**O-3'C (Gym II) | 1.942 |
| | | | (GLU 205) C=**O** | **H**O-4'C (Gym II) | 1.883 |
| | | | (SER 209) C-**O** | **H**O-5'C (Gym II) | 2.145 |
| | | | (Gym II) 4'-C-**O** | N**H** (ARG 358) | 2.306 |
| | | | (Gym II) 5'-C-**O** | N**H** (ARG 358) | 1.840 |
| | | | (Gym II) 5-C-**O** | N**H** (LYS 554) | 1.861 |
| | | | (Gym II) 4-C-**O** | N**H** (LYS 554) | 2.107 |
| Gymnemic acid III C | -7.84 | -45.8888 | (Gym III) 5-C-**O** | N**H** (ARG 125) | 2.555 |
| | | | (Gym III) 5-C-**O** | N**H** (ARG 125) | 2.506 |
| | | | (Gym III) 4a-C-**O** | **H**O-C (TYR 547) | 1.644 |
| | | | (Gym III) 2'-C-**O** | N**H** (LYS 554) | 2.079 |
| | | | (Gym III) 2'-C=**O** | N**H** (ASN 562) | 2.020 |

| | | | | | |
|---|---|---|---|---|---|
| | | | (SER 630) C-**O** | **H**O-4aC (Gym III) | 2.460 |
| | | | (TYR 48) C-**O** | **H**O-5'C (Gym III) | 2.597 |
| | | | (TYR 752) C-**O** | **H**O-4'C (Gym III) | 2.095 |
| | | | (ASP 545) C-**O** | **H**O-3'C (Gym III) | 2.410 |
| | | | (Gym III) 5'-C-**O** | O**H**-C (TYR 48) | 2.503 |
| Gymnemic acid IV | -7.23 | -43.8062 | (Gym IV) 4a-C-**O** | **H**O-C (TYR 585) | 2.138 |
| | | | (Gym IV) 2'-C-**O** | N**H** (ASN 562) | 1.994 |
| D | | | (Gym IV) 3'-C-**O** | N**H** (ASN 562) | 2.393 |
| | | | (Gym IV) 5'-C-**O** | **H**O-C (TYR 752) | 2.314 |
| | | | (CYS 551) C=**O** | **H**O-4aC (Gym IV) | 2.523 |
| | | | (TYR 752) C-**O** | **H**O-5'C (Gym IV) | 2.190 |
| | | | (TYR 752) C-**O** | **H**O-4'C (Gym IV) | 1.699 |
| Gymnemic acid V | -7.51 | -51.9911 | (Gym V) 5'-C-**O** | N**H** (ARG 669) | 2.335 |
| E | | | (Gym V) 3'-C-**O** | N**H** (ARG 358) | 2.053 |
| | | | (Gym V) 4'-C-**O** | N**H** (ARG 358) | 2.022 |
| | | | (Gym V) 3-C=**O** | N**H** (ASN 562) | 1.844 |
| | | | (SER 209) C-**O** | **H**O-3'C (Gym V) | 2.215 |
| | | | (GLU 205) C=**O** | **H**O-4'C (Gym V) | 1.791 |
| | | | (GLU 206) C=**O** | **H**O-5'C (Gym V) | 1.769 |
| Gymnemoside A | -7.49 | -42.0991 | (Gym A) 4'-C-**O** | N**H** (ARG 358) | 2.143 |
| F | | | (Gym A) 5'-C-**O** | N**H** (ARG 358) | 1.739 |
| | | | (Gym A) 5-C-**O** | N**H** (LYS 554) | 2.534 |
| | | | (Gym A) 3-C=**O** | N**H** (LYS 554) | 1.868 |
| | | | (SER 209) C-**O** | **H**O-5'C (Gym A) | 2.159 |
| | | | (GLU 205) C=**O** | **H**O-4'C (Gym A) | 1.817 |
| | | | (GLU 205) C=**O** | **H**O-3'C (Gym A) | 1.706 |
| Gymnemoside B | -7.98 | -48.2349 | (GLU 205) C=**O** | **H**O-3'C (Gym B) | 1.759 |
| G | | | (GLU 205) C=**O** | **H**O-4'C (Gym B) | 1.845 |
| | | | (Gym B) 5-O-C=**O** | N**H** (LYS 554) | 1.782 |
| | | | (Gym B) 3-C=**O** | N**H** (LYS 554) | 2.084 |
| | | | (Gym B) 4'-C-**O** | N**H** (ARG 358) | 2.310 |
| | | | (Gym B) 5'-C-**O** | N**H** (ARG 358) | 1.836 |
| Gymnemagenin | -6.49 | -34.1754 | (CYS 551) C=**O** | 4a-**H**O (Gymgen) | 2.046 |
| H | | | (GLU 205) C-**O** | 10-**H**O (Gymgen) | 1.672 |
| | | | (GLU 205) C-**O** 4a-H**O**-C (Gymgen) | 9-**H**O (Gymgen) **H**O (TYR 585) | 2.324 1.859 |
| | | | (GLN 553) C=**O** | 3-**H**O (Gymgen) | 1.947 |

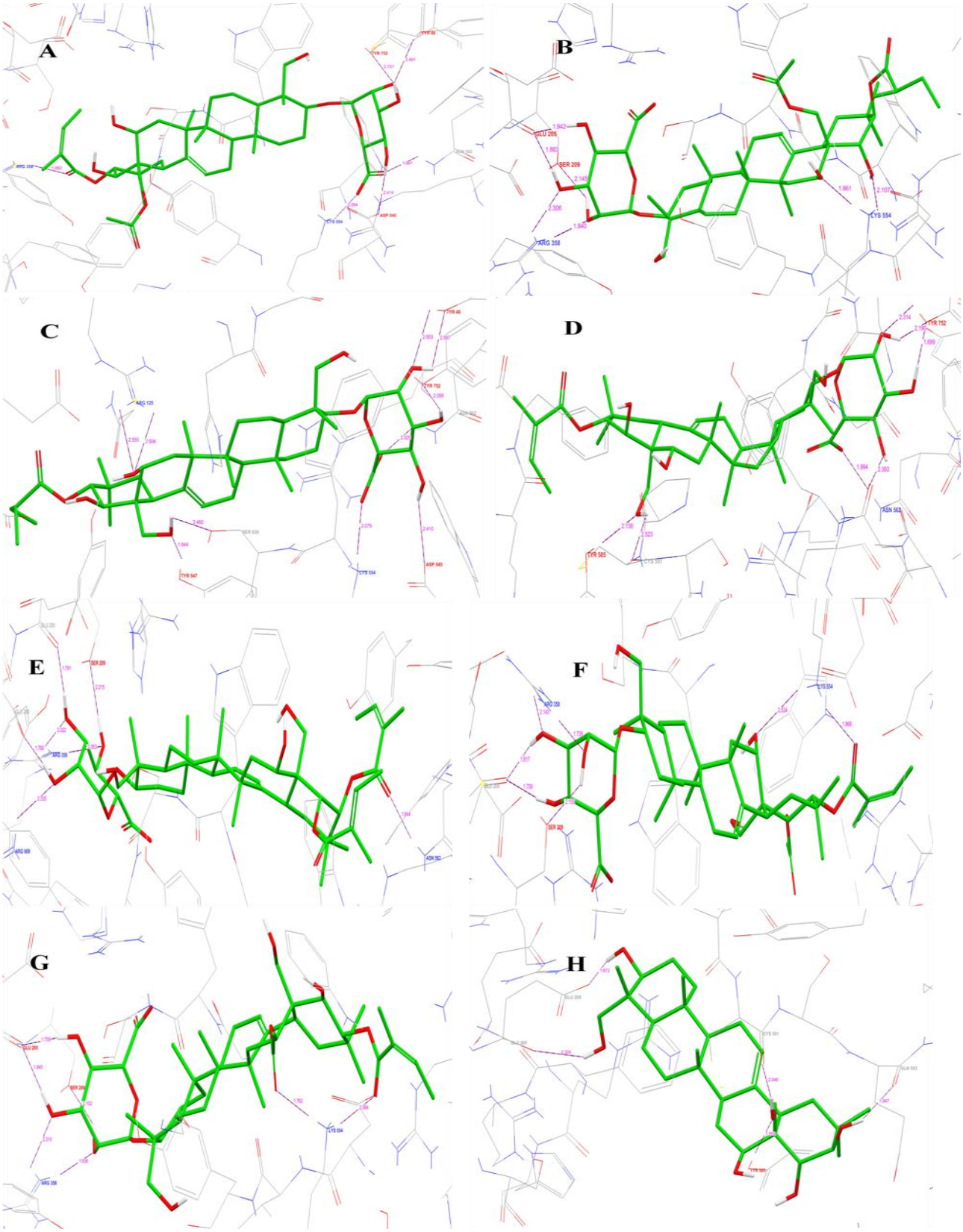



**Figure 2 Docked poses of Gymnemic acids (I-V (A-E), Gymnemoside A (F), Gymnemoside B (G) & Gymnemagenin (H)) at the active site of DPP-4 (Pdb: 3NOX)**

**Protein Tyrosin Phosphatase-1B**

PTP-1B has recently been proved as a key regulator of insulin receptor activity and downstream signaling pathways. It has been reported that the lack of PTP-1B enzyme activates the insulin receptors, improve sensitivity to insulin, and stimulate the glucose uptake [20]. Therefore, to evaluate the potential of G.S., we also performed the *in silico* screening of its components at the catalytic site of the chain B of PTP-1B. The observed results were comparable to the proved PTP-1B inhibitors and suggested that gymnemic acids and gymnemagenin both interacts with all the known amino acids [21], ARG 24, ASP 48, ARG 254, GLN 262, and LSY 120 at the catalytic site of protein by hydrogen bond interactions with Glide score ranging between -6.46 & -7.65 and Glide energy between -34.2836 & -37.8929 Kcal/mol. (Table **2**, Figure **3**). Hence, it can be concluded that G.S. may also be acting as antidiabetic due to its synergistic property as a PTP-1B inhibitor.

**Table 2 Docking parameters of examined molecules on protein tyrosin phosphatase 1B (Pdb: 1C83)**

| Ligands Code | Glide Score | Glide Energy (Kcal/mol) | H-Bond Interaction | | |
|---|---|---|---|---|---|
| | | | H –bond Acceptor | H -bond Donor | H -bond Length (Å) |
| Gymnemic acid I A | -7.0272 | -33.5006 | (Gym I) 2'-C-**O** | N**H** (ARG 24) | 1.808 |
| | | | (Gym I) 2'-C-**O** | N**H** (ARG 24) | 2.209 |
| | | | (Gym I) 2'-C=**O** | N**H** (ARG 24) | 2.258 |
| | | | (Gym I) 2'-C=**O** | N**H** (ARG 254) | 2.283 |
| | | | (Gym I) 2'-C=**O** | N**H** (ARG 254) | 2.317 |
| | | | (Gym I) 2'-C-**O** | N**H** (GLN 262) | 2.531 |
| | | | (Gym I) 1'-C-**O**-C | N**H** (GLN 262) | 1.947 |
| | | | (ASP 48) C-**O** | **H**O-9C (Gym I) | 2.000 |
| Gymnemic acid II B | -7.4198 | -45.2232 | (Gym II) 2'-C=**O** | N**H** (ARG 24) | 2.280 |
| | | | (Gym II) 2'-C=**O** | N**H** (ARG 24) | 2.086 |
| | | | (Gym II) 2'-C-**O** | N**H** (ARG 254) | 2.463 |
| | | | (Gym II) 2'-C=**O** | N**H** (GLN 262) | 2.603 |
| | | | (Gym II) 1'-C-**O**-C | N**H** (GLN 262) | 1.702 |
| | | | (ASP 48) C-**O** | **H**O-5C (Gym II) | 2.329 |
| | | | (ASP 48) C-**O** | **H**O-4C (Gym II) | 2.078 |
| | | | (ASP 48) C-**O** | **H**O-10C (Gym II) | 1.688 |
| | | | (Gym II) 4a-C=**O** | N**H** (LYS 120) | 1.868 |
| | | | (Gym II) 3-C=**O** | N**H** (LYS 120) | 1.793 |
| Gymnemic acid III C | -7.1828 | -35.1233 | (Gym III) 2'-C-**O** | N**H** (ARG 24) | 1.810 |
| | | | (Gym III) 2'-C-**O** | N**H** (ARG 24) | 2.185 |
| | | | (Gym III) 2'-C=**O** | N**H** (ARG 24) | 2.345 |
| | | | (Gym III) 2'-C=**O** | N**H** (ARG 254) | 2.369 |
| | | | (Gym III) 2'-C=**O** | N**H** (ARG 254) | 2.308 |
| | | | (Gym III) 2'-C-**O** | N**H** (GLN 262) | 2.538 |

| | | | | | |
|---|---|---|---|---|---|
| | | | (Gym III) 1'-C-**O**-C (ASP 48) C-**O** | N**H** (GLN 262) **H**O-10C (Gym III) | 1.885 2.069 |
| Gymnemic acid IV | -6.4671 | -34.2836 | (Gym IV) 2'-C-**O** | N**H** (ARG 24) | 2.017 |
| | | | (Gym IV) 2'-C-**O** | N**H** (ARG 24) | 2.321 |
| D | | | (Gym IV) 2'-C-**O** | N**H** (GLN 262) | 2.573 |
| | | | (Gym IV) 1'-C-**O**-C (ASP 48) C-**O** | N**H** (GLN 262) **H**O-5'C (Gym IV) | 2.001 1.633 |
| | | | (Gym IV) 4a-C-**O** | N**H** (LYS 120) | 2.033 |
| Gymnemoside B G | -7.6503 | -37.8929 | (Gym B) 2'-C=**O** | N**H** (ARG 24) | 2.020 |
| | | | (Gym B) 2'-C=**O** | N**H** (ARG 24) | 2.096 |
| | | | (Gym B) 2'-C-**O** | N**H** (ARG 24) | 2.627 |
| | | | (Gym B) 2'-C-**O** | N**H** (ARG 254) | 2.163 |
| | | | (Gym B) 1'-C-**O**-C | N**H** (GLN 262) | 1.816 |
| | | | (Gym B) 2'-C=**O** | N**H** (GLN 262) | 2.565 |
| | | | (ASP 48) C-**O** | **H**O-5'C (Gym B) | 2.030 |
| | | | (ASP 48) C-**O** | **H**O-5'C (Gym B) | 1.750 |
| | | | (Gym B) 3-C=**O** | N**H** (LYS 120) | 1.911 |
| Gymnemagenin H | -2.6798 | -22.9036 | (ASP 48) C-**O** | **H**O-9C (Gymgen) | 2.379 |
| | | | (Gymgen) 10-C-**O** | N**H** GLN 262 | 2.042 |

**Sodium potassium ATPase**

NaKATPase is a ubiquitous enzyme that allows the exchange of three sodium and two potassium ions across the cellular membrane. Interaction of NaKATPase and Potassium ATP channel has already been established suggesting that ATP serves as the link between the two moieties. Inhibition of NaKATPase activity, leads to the elevation of ATP which in turn blocks the Potassium ATP channel and efflux of potassium ions from the resting β-cells. This results in opening of the voltage-dependent calcium channels causing stimulation of insulin release [22]. G.S. and sulfonyl ureas like Glimepiride have been reported to bind with NaKATPase and Potassium ATP channel respectively [23, 24]. G.S has also been reported to have a suppressive effect on the high potassium induced contraction on guinea-pig ileum muscle and blood glucose level [25] which means G.S. may also be directly inhibiting potassium ATP channel. Direct or indirect inhibition of NaKATPase / potassium ATP channel by G.S. will block the efflux of potassium ions from the resting β-cells resulting in opening of the voltage-dependent calcium channels and stimulation of insulin release. The docking study of Gymnemic acids at the active site of NaKATPase indicated that these acids interacted very prominently with the NaKATPase. These components demonstrated hydrogen bond interactions at the important amino acids [26] of the active site namely, ARG 893, ASP 891, ARG 887, ALA 330, ILE 322, GLU 319, GLU 310, ASN 129, ASP 128 and GLN 118 with Glide score ranging between -8.17 & -9.15 and Glide energy between -37.2659 & -45.9689 Kcal/mol. Gymnemagenin also showed good hydrogen bond interactions at the active site of NaKATPase with the glide score and glide energy -8.49 & -

37.6606 Kcal/mol respectively. With these docking results, it may be concluded that NaKATPase inhibition is one of the major pathways for the mode of action of Gymnemic acids and Gymnemagenin as antidiabetic agents (Table **3**, Figure **4**). Further, these docking results also gave an insight about the possible pharmacokinetic and pharmacodynamic herb-drug interaction of G.S. constituents with glimepiride (sulphonyl ureas) if co-administered.

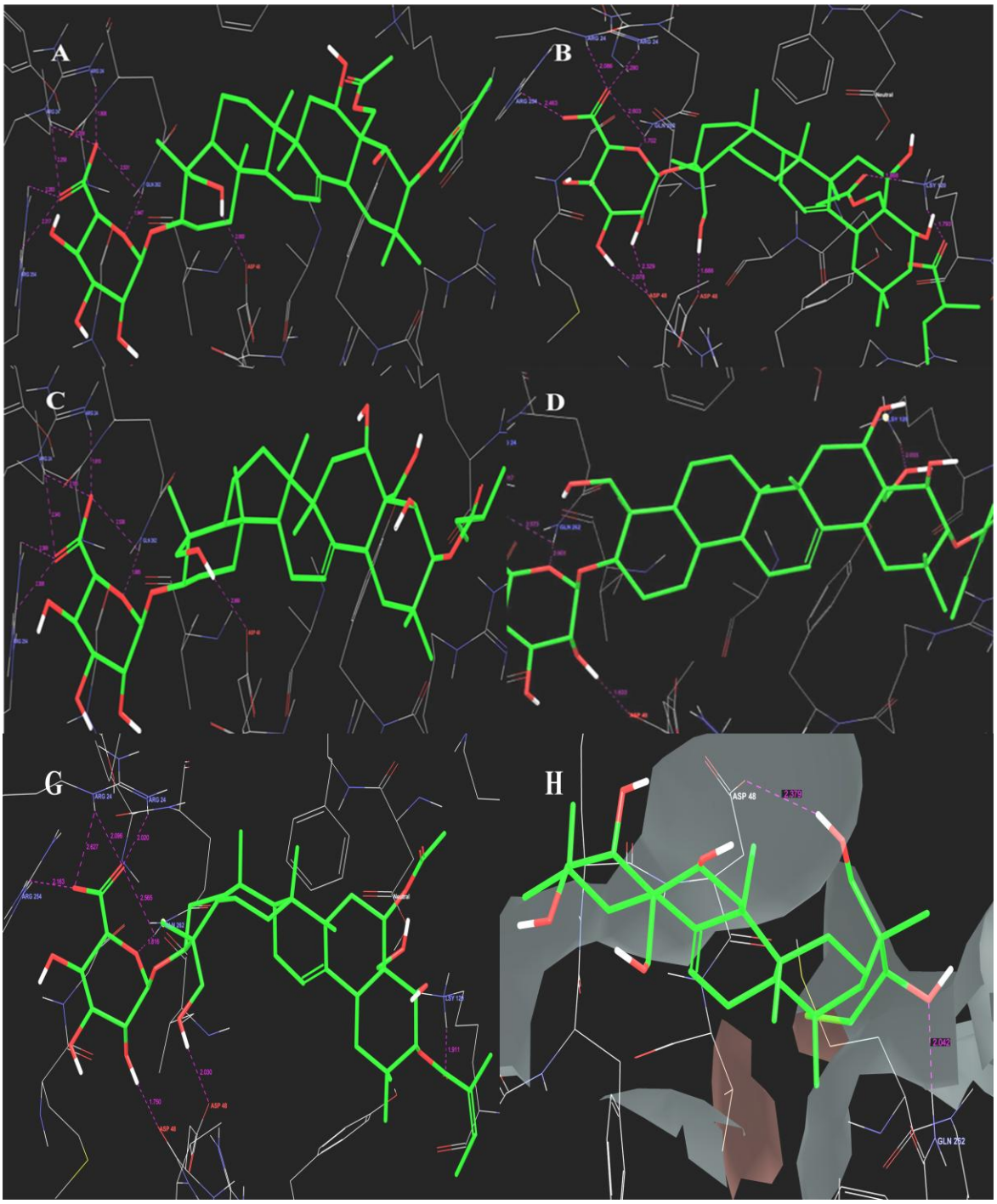



**Figure 3 Docked poses of Gymnemic acids (I-IV (A-D), Gymnemoside B (G) & Gymnemagenin (H))at the active site of protein tyrosin phosphatase 1B (Pdb: 1C83)**

**Table 3 Docking parameters of examined molecules on sodium potassium ATPase (Pdb: 3A3Y)**

| Ligands Code | Glide Score | Glide Energy (Kcal/mol) | H-Bond Interaction | | |
|---|---|---|---|---|---|
| | | | H –bond Acceptor | H -bond Donor | H -bond Length (Å) |
| Gymnemic acid I A | -9.15 | -43.3838 | (Gym I) 2'-C=**O** | N**H** (ARG 887) | 1.823 1.912 |
| | | | (Gym I) 2'-C=**O** | N**H** (ARG 887) | 2.025 2.401 |
| | | | (Gym I) 5'-C=**O** | N**H** (ARG 887) | 2.323 2.461 |
| | | | (Gym I) 4'-C=**O** | **H**-O (GLU 319) | 2.087 2.593 |
| | | | (Gym I) 4-C=**O** | N**H** (ASN 129) | |
| | | | (Gym I) 5-C=**O** | N**H** (ASN 129) | |
| | | | (Gym I) 4-C=**O** | **H**-O (THR 804) | |
| | | | (THR 804) C-**O** | **H**O-4-C (Gym I) | |
| Gymnemic acid II B | -9.31 | -41.6457 | (ASP 891) C=**O** | **H**O-5'C (Gym II) | 1.920 1.899 |
| | | | (ASP 891) C=**O** | **H**O-4'C (Gym II) | 2.070 2.402 |
| | | | (Gym II) 2'-C=**O** | N**H** (TRP 894) | |
| | | | (Gym II) 3-C=**O** | **H**-O (THR 804) | |
| Gymnemic acid III C | -8.39 | -44.9726 | (Gym III) 5'-C=**O** | N**H** (ARG 893) | 2.612 2.055 |
| | | | (Gym III) 2'-C=**O** | N**H** (ARG 887) | 1.968 2.511 |
| | | | (ASP 891) C=**O** | **H**O-5'C (Gym III) | |
| | | | (ILE 322) C=**O** | **H**O-4aC (Gym III) | |
| Gymnemic acid IV D | -9.84 | -38.0348 | (ASP 891) C=**O** | **H**O-5'C (Gym IV) | 2.265 1.942 |
| | | | (ASP 891) C=**O** | **H**O-4'C (Gym IV) | 2.434 2.035 |
| | | | (Gym IV) 2'-C-**O** | N**H** (ARG 887) | |
| | | | (Gym IV) 2'-C=**O** | N**H** (ARG 887) | |
| Gymnemic acid V E | -9.74 | -45.9689 | (Gym V) 3'-C=**O** | N**H** (ARG 893) | 2.146 2.080 |
| | | | (ASP 891) C=**O** | **H**O-3'C (Gym V) | 2.035 |
| | | | (Gym V) 4-C=**O** | **H**-O (THR 804) | |
| Gymnemoside A F | -8.17 | -40.6072 | (Gym A) 4-C=**O** | N**H** (ASN 129) | 2.494 1.772 |
| | | | (Gym A) 5-C-**O** | N**H** (ASN 129) | 2.577 1.834 |
| | | | (GLU 310) C=**O** | **H**O-3'C (Gym A) | 2.023 1.915 |
| | | | (GLU 310) C-**O** | **H**O-3'C (Gym A) | 1.904 |
| | | | (Gym A) 2'-C=**O** | N**H** (ARG 887) | |
| | | | (Gym A) 2'-C=**O** | N**H** (ARG 887) | |
| | | | (Gym A) 9-C-**O** | N**H** (ARG 887) | |
| Gymnemoside B G | -9.12 | -37.2659 | (Gym B) 4a-C-**O** | **H**-O (THR 804) | 1.760 1.967 |
| | | | (Gym B) 2'-C-**O** | N**H** (ASN 129) | 2.386 1.941 |
| | | | (Gym B) 2'-C=**O** | N**H** (GLN 118) | 2.054 |
| | | | (ASP 128) C-**O** | **H**O-3'C (Gym B) | |
| | | | (ASP 128) C-**O** | **H**O-4'C (Gym B) | |
| Gymnemagenin H | -8.49 | -37.6606 | Gymgen 4a-C-**O** | N**H** (ASN 129) | 2.046 1.850 |
| | | | (GLY 326) C=**O** | **H**O-9C (Gymgen) | 1.849 2.420 |
| | | | (GLU 786) C=**O** | **H**O-10C Gymgen) | |
| | | | Gymgen 9-C-**O** | N**H** (ALA 330) | |
| Glemipiride (Standard) | -6.32 | -46.7852 | (Glm) C=**O** | **H**-O (THR 804) | 2.217 1.988 |
| | | | (Glm) C=**O** | N**H** (ASN 129) | |

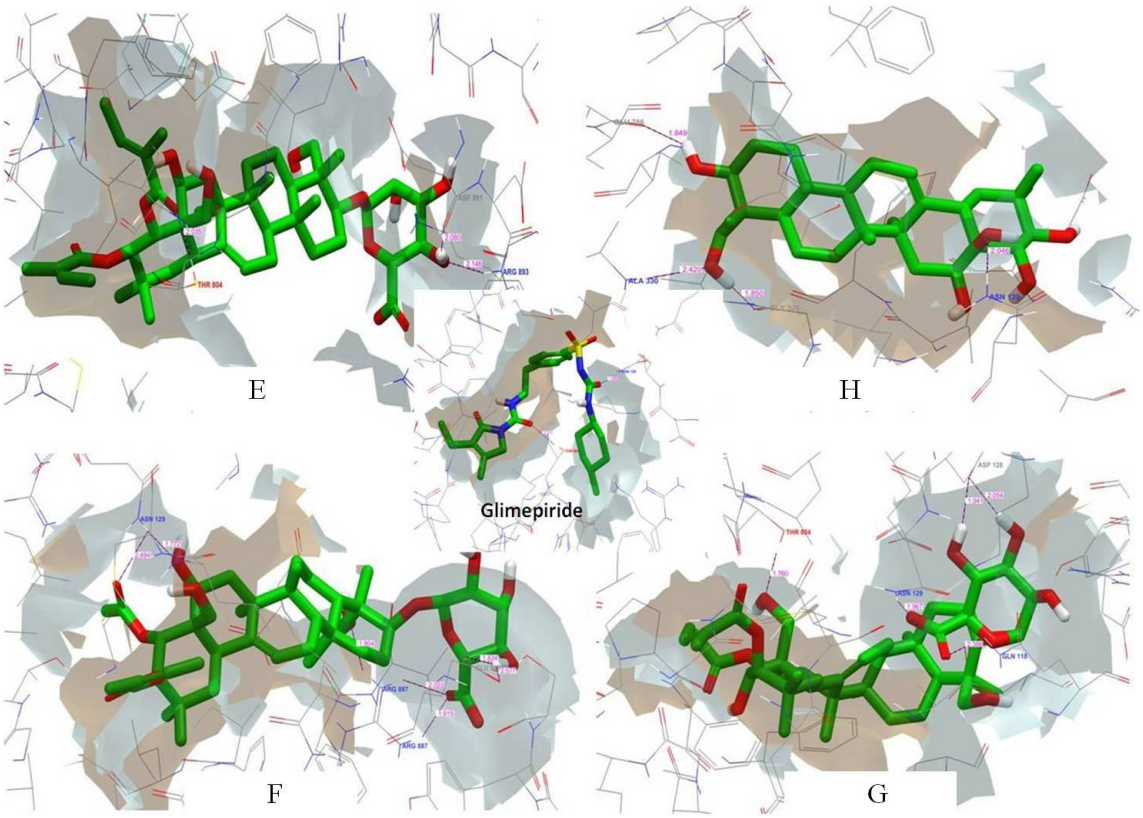



**Figure 4 Docked poses and hydrophobic (brown) -hydrophilic (blue) map of Gymnemic acids (I-V (A-E), Gymnemoside A (F), Gymnemoside B (G) & Gymnemagenin (H)) at the active site of Sodium potassium ATPase (Pdb: 3A3Y)**

**Aldose Reductase**

A.R. is an NADPH-dependent enzyme in the development of long-term diabetic complications and has been targeted for design and development of antidiabetic agents. NADPH coenzyme is located in the deep hydrophobic cleft of the enzyme which is surrounded by the hydrophilic (anionic) residues of the catalytic site. The standard inhibitors of A.R., binds to this anionic site at the amino acid residues TYR 48, HIS110 and TRP 111 [27]. The docking study on this enzyme with gymnemic acids was a failure because the process died with all the gymnemic acids. The most important fact that we could visualise in this study was the hydrogen bond interactions of gymnemagenin docked at the active catalytic site involving amino acid residues TYR 48, HIS110 and TRP 111 (Figure **5**) with the glide score and glide energy -8.02 & -23.044 Kcal/mol respectively. This suggested that the aglycone moiety of gymnema acids "Gymnemagenin" may also function as an inhibitor of aldose reductase and a cure for long-term diabetic complications.

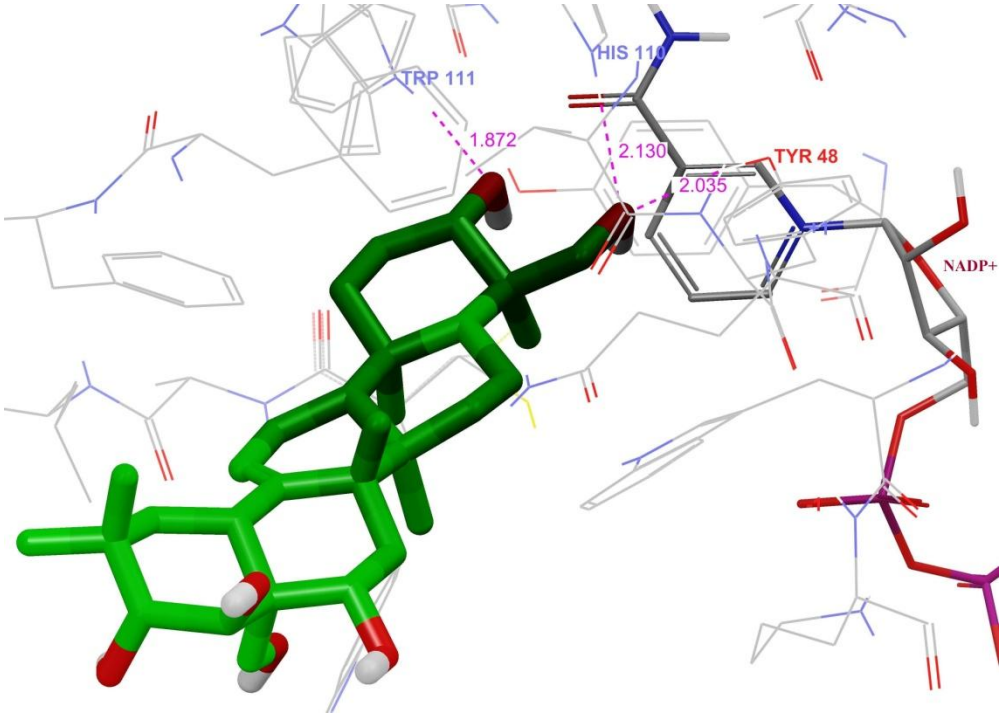


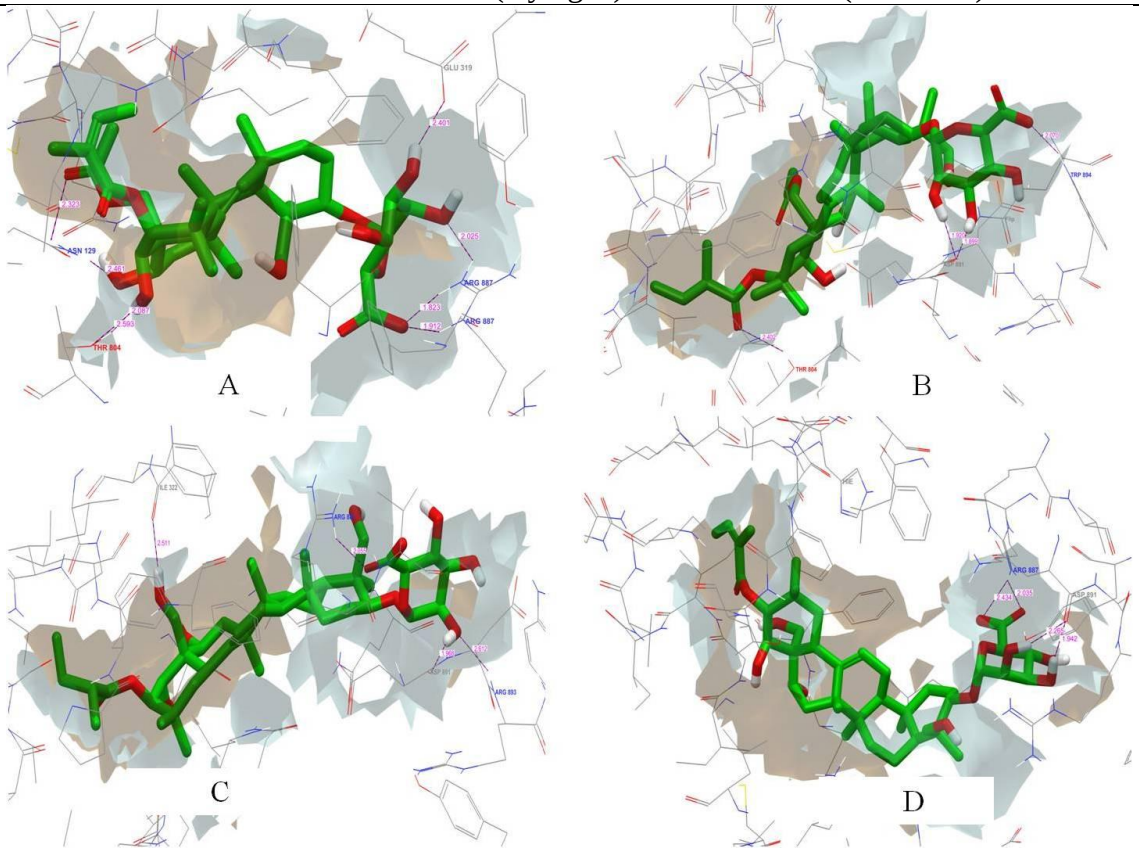

**Figure 5 Docked pose of Gymnemagenin (H) at the active site of Aldose reductase (Pdb: 3G5E)**

**Glycogen Synthase Kinase-3β**

GSK-3β plays a crucial role in glucose homeostasis. The inhibition of GSK-3β dependent phosphorylation activates insulin-dependent glycogen synthesis which mimics the action of insulin to lower plasma glucose. Thus, GSK-3β inhibitors present a novel mode for the treatment of type II diabetes. The docking study of Gymnemic acids at the active site of GSK-3β indicated that only four components namely, Gymnemic acids II, III, Gymnemoside B and Gymnemagenin had hydrogen bond interactions at the important amino acids [28, 29] of the active site namely, LYS 60, LYS 85, TYR 134, VAL 135, PRO 136, ARG 141, LYS 183, ASN 186, ASP 200 and SER 66 with Glide score ranging between -6.02 & -7.06 and Glide energy between -38.76 & -45.6876 Kcal/mol. With these docking results also, it was clear that Gymnemic acids II, III, Gymnemoside B and Gymnemagenin interact at the active site of GSK-3β and its inhibition can be one of the pathways for the mode of action of Gymnemic acids and Gymnemagenin as antidiabetic agents (Table **4**, Figure **6**).

**Table 4 Docking parameters of examined molecules on Glycogen synthase kinase-3β (Pdb: 3F7Z)**

| Ligands Code | Glide Score | Glide Energy (Kcal/mol) | H-Bond Interaction | | |
|---|---|---|---|---|---|
| | | | H –bond Acceptor | H -bond Donor | H -bond Length (Å) |
| Gymnemic acid II B | -6.0358 | -45.6876 | (Gym II) 3-C=**O** | N**H** (LYS 60) | 2.077 |
| | | | (Gym II) 5-C-**O** | N**H** (ARG 141) | 1.832 |
| | | | (ASN 186) C=**O** | **H**O-5'C (Gym II) | 1.702 |
| | | | (ASP 200) C-**O** | **H**O-3'C (Gym II) | 2.591 |
| | | | (Gym II) 2'-C-**O** | N**H** (LYS 183) | 1.538 |
| Gymnemic acid III C | -4.9992 | -56.7853 | (VAL 135) C=**O** | **H**O-5C (Gym III) | 1.956 |
| | | | (PRO 136) C=**O** | **H**O-4C (Gym III) | 1.823 |
| | | | (TYR 134) C-**O** | **H**O-4aC (Gym III) | 2.447 |
| | | | (Gym III) 2'-C-**O** | N**H** (SER 66) | 1.959 |
| | | | (ASN 186) C=**O** | **H**O-9C (Gym III) | 1.964 |
| Gymnemoside B G | -6.6898 | -43.3165 | (Gym B) 2'-C=**O** | N**H** (LYS 60) | 2.128 |
| | | | (ASN 186) C=**O** | **H**O-4C (Gym B) | 1.829 |
| | | | (Gym B) 5-C=**O** | N**H** (LYS 183) | 2.065 |
| | | | (Gym B) 4a-C-**O** | N**H** (ASN 64) | 2.251 |
| Gymnemagenin H | -7.0661 | -38.761 | (ASN 186) C=**O** | **H**O-5C (Gymgen) | 1.995 |
| | | | (Gymgen) 3-C-**O** | N**H** (LYS 85) | 2.382 |
| | | | (Gymgen) 10-C-**O** | N**H** (ARG 141) | 2.081 |



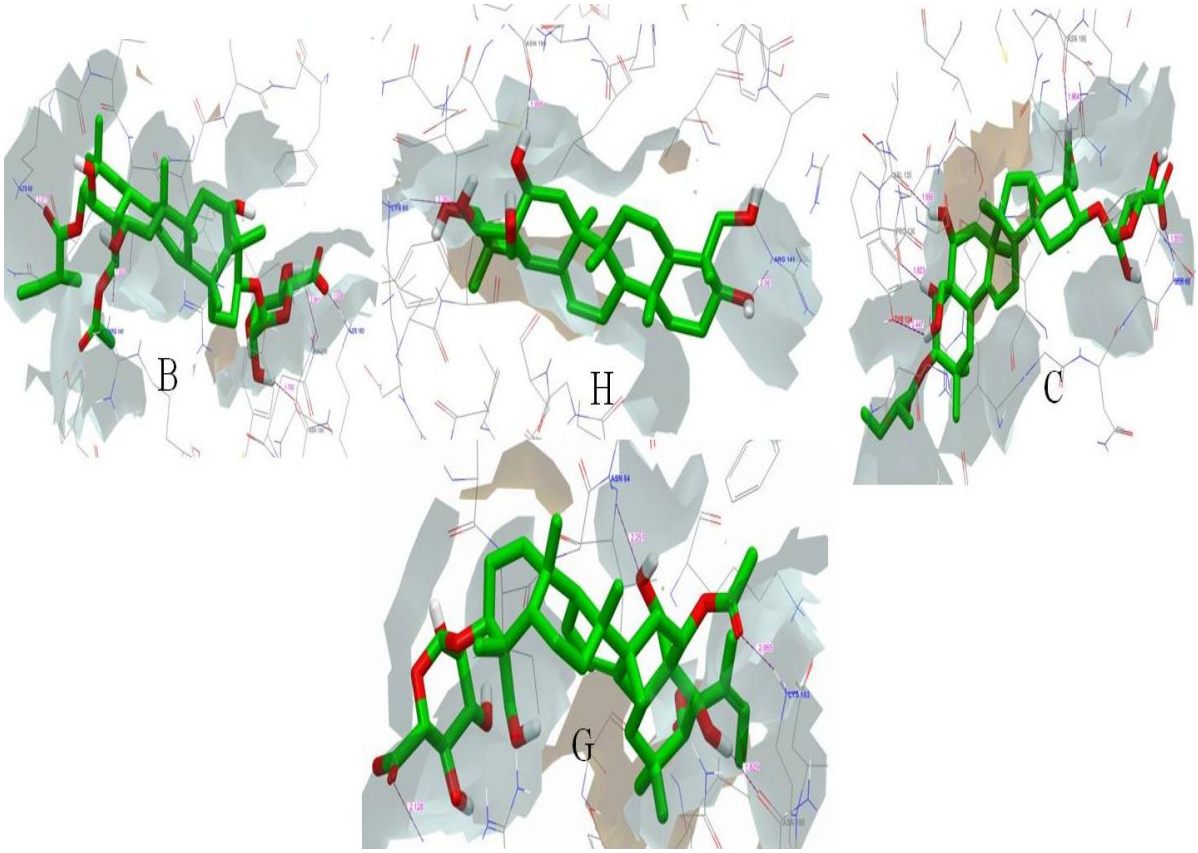



**Figure 6 Docked poses and hydrophobic (brown) -hydrophilic (blue) map of Gymnemic acids (II, III (B, C), Gymnemoside B (G) & Gymnemagenin (H)) at the active site of Glycogen synthase kinase 3β (Pdb: 3F7Z)**

## CONCLUSION

The antidiabetic potential of G.S. is known worldwide since ancient times but the relationship between the structural interaction and function of medicinally important constituents of this plant with various targets involved in the pathophysiology of diabetes has never been explored *in silico*. To understand the binding mechanisms of these active constituents, molecular modelling studies was performed with DPP-IV, PTP-1B, NaKATPase, A.R. and GSK-3β as target proteins using XP docking program of Glide, version 9.2, Schrödinger suit. These constituents showed favourable interactions with the amino acid residues at the active sites of the proteins explored, their by substantiating their proven efficacy as antidiabetic agents. This *in silico* study also evaluated the fate of gymnemagenin as a potential inhibitor of proteins involved in the pathogenesis of diabetes. With the explored results we conclude that the *in vitro* and *in vivo* studies on Gymnemagenin must be performed to evaluate its exact potential in diabetes. The present study also gives an insight to the probable herb-drug interaction and reason for sudden hypoglycemic shocks that may occur on concurrent administration of G.S. extract and synthetic

drug, Glimepiride, in the treatment of diabetes. It may also alter the pharmacokinetic and pharmacodynamic profile of Glimepiride. These observations motivated us to perform antidiabetic studies on the isolated gymnemagenin and *in vivo* herb-drug interaction studies on the perspectives of pharmacokinetics. The studies are underway and the results will be published elsewhere.

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