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In Silico Analysis of Bioactive Compound Gymnemagenin: in Diabetes Mellitus

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Abstract:-

➢ ***Background***

***Gymnema sylvestre*, a member of the Asclepiadaceae family and commonly known as Gurmar, thrives in the tropical woodlands of southern and central India as well as Sri Lanka. Celebrated for its manifold medicinal attributes, *Gymnema sylvestre* leaves have earned recognition for their roles as anti-diabetic, hypolipidemic, stomachic, diuretic, refrigerant, astringent, and tonic agents. The primary bioactive components found in *G. sylvestre* are a complex array of triterpenoid glycosides collectively referred to as gymnemic acids, with gymnemagenin as the shared aglycone. Refined gymnemic acids have demonstrated their effectiveness in combating hyperglycaemia, maintaining normal blood glucose levels, and reducing hyperlipidemia in various in-vitro experiments. The mechanism of action of gymnemic acids involves stimulating the regeneration of pancreatic cells, promoting insulin secretion, and inhibiting the absorption of glucose. Gymnemic acid, a well-known constituent sourced from *Gymnema Sylvestre* leaves, plays an integral role in numerous polyherbal formulations designed to manage Diabetes Mellitus. It is important to note that gymnemagenin does not exist independently but serves as a common aglycone within gymnemic acids, attainable through processes involving both acidic and basic hydrolysis. Accurate determination of gymnemic acids poses a formidable challenge due to their intricate composition, comprising closely related compounds, and their scarcity as commercially available reference substances. The ongoing research endeavor is dedicated to devising and validating a rapid and exquisitely sensitive methodology for precisely quantifying this constituent.**

➢ ***Method***

Gymnemagenin, a bioactive compound, possesses the unique capability of triggering the secretion of insulin by the beta-cells of Langerhans within the human body. This intriguing phenomenon has been substantiated through meticulous in-silico analysis. We retrieved the Dipeptidyl peptidases (1NU6) protein structure from the Protein Data Bank website and meticulously identified the active site residues of this protein based on an extensive review of the existing scientific literature [1]. Furthermore, our investigation led us to select gymnemagenin as the bioactive compound, which we sourced from *G. sylvestre* via the PubChem website. Following this, we meticulously prepared the lead molecule for docking studies using the powerful Open Babel software.

The extracted gymnemagenin product is evaluated and formed into tablets in further study, and aimed to study the G. *sylvestre* extracts in the pharmacy field either treating the product with the cell lines or the animal models.

➢ ***Results***

The extracted phytochemicals have shown the presence of several secondary metabolites obtained by phytochemical screening *Gymnema sylvestre* leaves shown positive results for tannins, saponin, terpenoids, flavonoids. The interaction between the protein and ligands were analysed using docking score. Therefore, the protein-ligand complex was further subjected to optimization by MD simulations using WebGRO from simlabs, also MD simulation trajectories have also been adopted as inputs for MMPBSA calculations of ligand binding free energies and analysis of their binding process.

➢ ***Conclusion***

Utilizing gymnemagenin as a therapeutic approach for diabetes mellitus presents an exciting opportunity to enhance insulin production effectively. This potential stems from its ability to modulate multiple signalling transduction pathways that play pivotal roles in diabetes management. The core objective of this study is to gauge the effectiveness of phytochemicals in the treatment of diabetes mellitus through rigorous in-silico analysis. Furthermore, these bioactive compounds can be subjected to in-depth examinations in both laboratory and living systems to comprehensively assess their collective impact.

Keywords:- *In Silico, Molecular Docking, Molecular Dynamics, Diabetes Mellitus.*

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I. INTRODUCTION

Gymnema sylvestre, a member of the Asclepiadaceae family commonly known as Gurmar, is an herb indigenous to the lush tropical woodlands of southern and central India, as well as Sri Lanka. Renowned for its diverse medicinal properties, *G. sylvestre* leaves serve as remedies for various ailments, including diabetes, lipid imbalances, stomach discomfort, diuretic needs, cooling effects, astringency, and toning. The primary bioactive components within *G. sylvestre* are a group of triterpenoid glycosides collectively referred to as gymnemic acids, with gymnemagenin as their shared aglycone. Purified gymnemic acids have demonstrated their therapeutic potential in managing hyperglycemia, maintaining normal blood sugar levels, and mitigating hyperlipidemia in laboratory studies. These gymnemic acids elicit their antihyperglycemic effect by stimulating the regeneration of pancreatic cells, facilitating insulin secretion, and impeding the absorption of glucose. Notably, gymnemic acid, a triterpenic glycoside extracted from *G. sylvestre* leaves, is a well-established constituent utilized in polyherbal formulations for the effective management of Diabetes Mellitus. It's essential to underscore that gymnemagenin does not exist in isolation but rather serves as a common aglycone within gymnemic acids, achieved through processes involving both acidic and basic hydrolysis. Precisely quantifying gymnemic acids poses a considerable challenge due to their complex composition, comprising an amalgamation of closely related compounds, and their limited availability as commercially sourced reference materials. This ongoing research endeavors to devise and validate a rapid and exceptionally sensitive method for accurately estimating this crucial constituent. Furthermore, it's imperative to acknowledge that *G. sylvestre* is categorized as a vulnerable species characterized by a sluggish growth rate, warranting conservation efforts.

Diabetes stands as a persistent metabolic disorder marked by elevated blood sugar levels, stemming from a complex interplay of inadequate insulin production and the body's resistance to insulin. Insulin, a hormone synthesized by the pancreatic beta cells, holds a crucial role in governing glucose metabolism. In diabetes, either the pancreas falters in producing a sufficient quantity of insulin, or the insulin produced falls short or faces challenges in its effectiveness due to cellular resistance. Type 1 diabetes, an autoimmune condition, leads to the destruction of the pancreas's beta cells, resulting in a total deficit of insulin. In contrast, type 2 diabetes emerges from a combination of insulin resistance and impaired insulin secretion. Prolonged elevation of glucose levels in the bloodstream can inflict enduring damage on organs like the eyes, kidneys, nerves, and the cardiovascular system, giving rise to complications such as neuropathy, retinopathy, nephropathy, and cardiovascular diseases.

The effective management of diabetes necessitates a multifaceted approach, encompassing lifestyle modifications such as regular exercise and dietary adjustments, as well as the utilization of oral hypoglycemic agents and insulin therapy. Diligent monitoring of blood glucose levels holds paramount importance in achieving glycemic control and forestalling complications. In summary, diabetes represents an enduring health challenge that poses significant hurdles for individuals, healthcare providers, and society as a whole. A comprehensive strategy that addresses the varied pathophysiological mechanisms underlying diabetes remains essential in mitigating the enduring adverse effects of this condition.

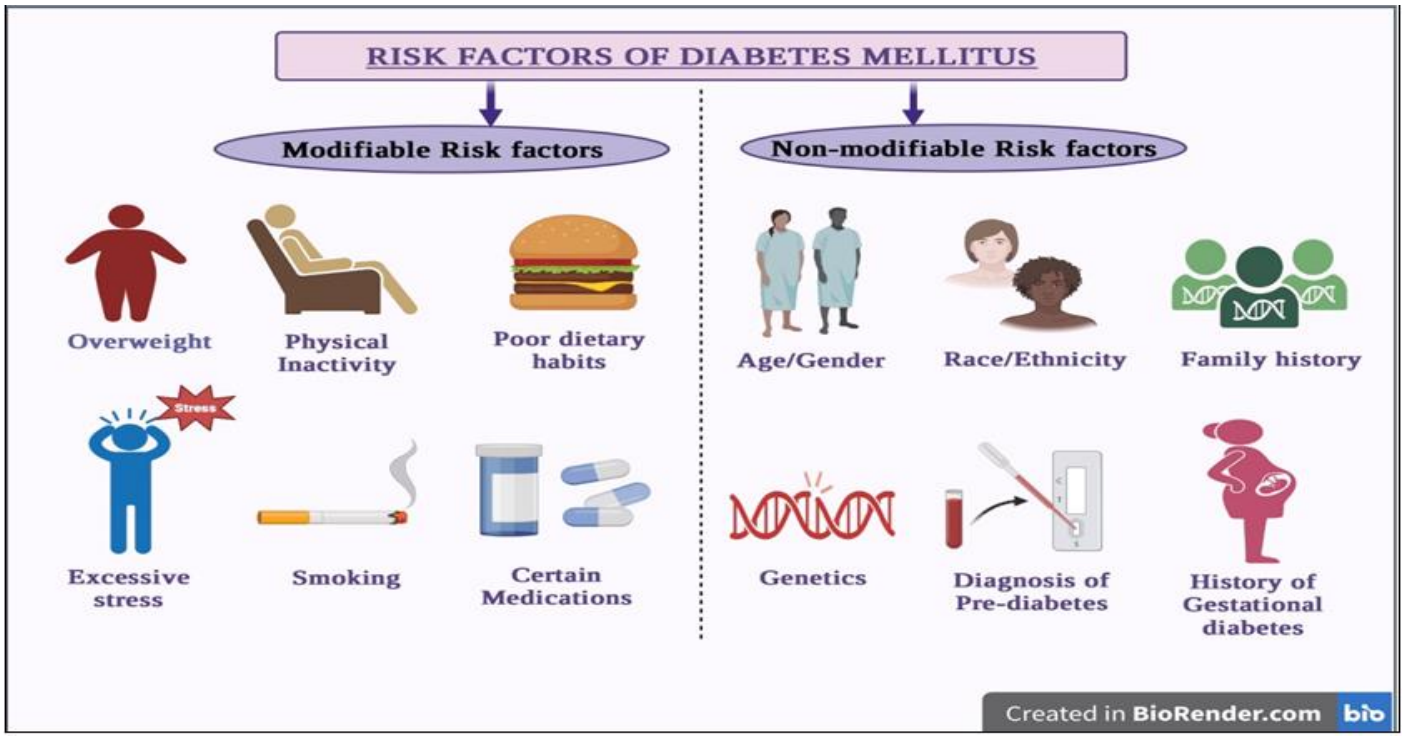

Fig 1 Risk Factors of Diabetes Mellitus

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Studies suggest that, *G. sylvestre* is best known for its anti-diabetic properties. The ethanolic extract derived from this plant exhibited a noteworthy 46% reduction in glucose levels, while the aqueous extracts displayed a 26% decline, and the methanol extract showed a 12% decrease [2,3,4,5]. Notably, the aqueous extract of this botanical has demonstrated its effectiveness in rectifying disrupted glucose, insulin, and lipid profiles in rats afflicted with insulin resistance induced by dexamethasone [6]. Furthermore, in a diabetic animal model, this plant's extract led to decreases in insulin, protein, triglycerides, cholesterol, and blood glucose levels. It also resulted in diminished body weight and improved hepatic histopathology [7].

In another study featuring alloxan-induced diabetic rats, the extract derived from *G. sylvestre* significantly ($p < 0.05$) lowered fasting blood glucose, total cholesterol, and serum triglycerides, while concurrently elevating HDL cholesterol levels. This extract also exerted a significant impact ($p < 0.05$) in reducing heightened levels of urea, uric acid, and creatinine in diabetic rats [8,9]. Furthermore, an investigation centred on the methanolic extract of *G. sylvestre* indicated that both acute and chronic administration in Wister rats resulted in reduced blood glucose levels. Treatment with this plant's extract led to significant reductions ($p < 0.05$) in elevated blood glucose, ALT, AST, triglycerides, total cholesterol, LDL-cholesterol, and malondialdehyde levels in diabetic rats. Additionally, it significantly ($p < 0.05$) increased insulin, HDL-cholesterol, and erythrocyte superoxide dismutase levels in diabetic rats [10,11].

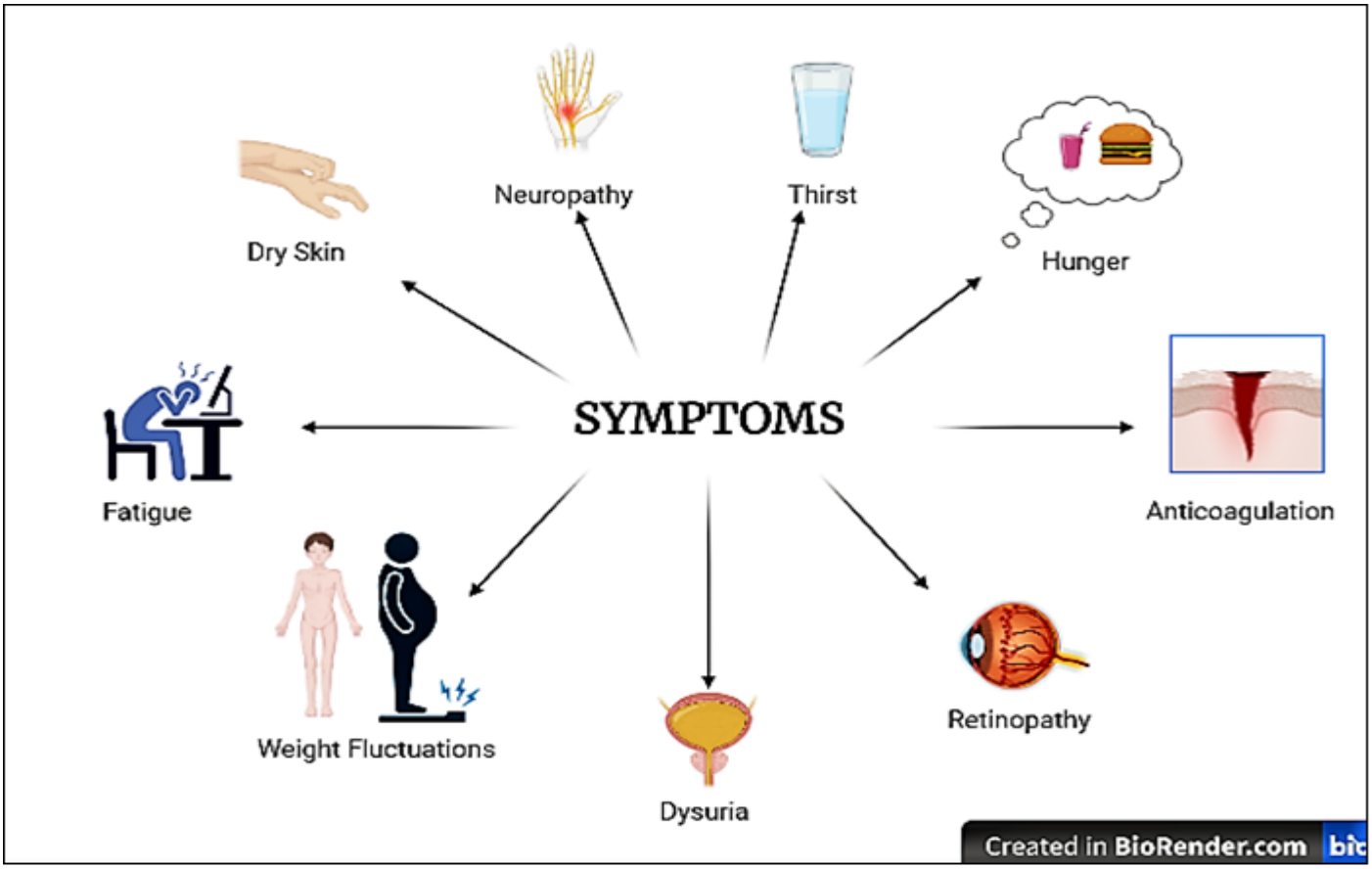

Fig 2 Symptoms of Diabetes Mellitus

The bioactive constituents extracted from *G. sylvestre*, known as gymnemic acids and belonging to the triterpene saponin group, stand in contrast to glibenclamide. Gymnemic acid IV, administered at a dose of 3.4/13.4 mg/kg over a 6-hour period, exhibited a significant reduction in blood glucose levels, ranging from 14.0% to 60.0%. Intriguingly, when given at a concentration of 13.4 mg/kg, gymnemic acid IV elevated plasma insulin levels in mice with STZ-induced diabetes [12]. In a separate investigation, oral administration of minimal doses (0.2 g/kg) of this plant resulted in the mitigation of elevated blood sugar levels induced by sucrose. The primary aim of this study was to assess the chemical and analytical methods employed in characterizing the hydrolysed product gymnemagenin.

- *Active Constituents:*

The G.*sylvestre* leaves contain the highest phenolic compounds, the bioactive compounds like polyphenols are also associated with the G.*sylvestre* leaves. It also exhibits superoxide-free radicals. Rich in gymnemagenin and gymnemic acids. Gymnemic acids contain the main active constituents are triterpenes and saponins.

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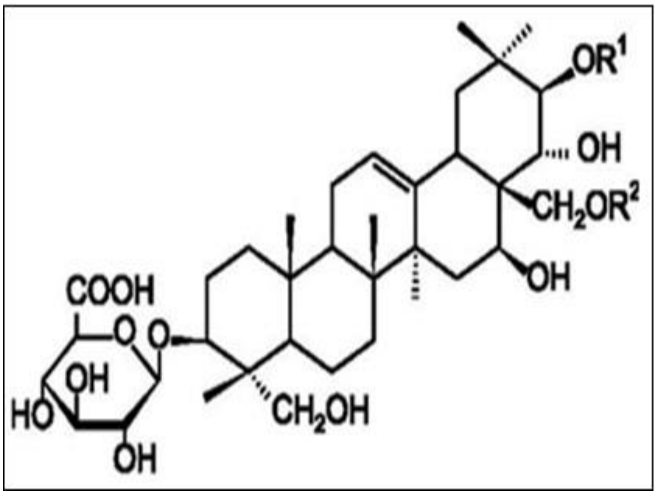

Fig 3 2D Image of Gymnemic acid found in *G. Sylvestre*

➢ *1NU6 Protein*

In the pursuit of combatting type II diabetes, researchers have turned their attention to the inhibition of dipeptidyl peptidase IV (DPP-IV), which stands as the primary enzyme responsible for degrading glucagon-like peptide 1 (GLP1). Our study involved the successful expression and purification of the ectodomain of human DPP-IV, accomplished using the Pichia pastoris system. Subsequently, we conducted an intricate X-ray analysis, yielding a high-resolution structure at 2.1A. This enzyme is composed of two distinct domains: the catalytic domain, characterized by its alpha/beta hydrolase fold, and the beta propeller domain, featuring a remarkable 8-fold repetition of a four-strand beta sheet motif. Notably, the beta propeller domain possesses dual functionalities that distinguish it from similar structural configurations. It contributes an additional beta sheet motif, which plays a pivotal role in the dimerization interface. Furthermore, it boasts an extra short helix housing a unique double Glu sequence motif. Of particular significance, this Glu motif serves as the linchpin for substrate recognition and binding. Our investigation shed light on this crucial function through an analysis of the complex structure involving Di protein A, a substrate with low turnover. Within this complex, Di protein A becomes ensnared in the tetrahedral intermediate of the reaction, unravelling the intricate molecular interactions within the crystalline environment.

➢ *Molecular Docking and Molecular Dynamics*

Molecular docking proves itself as a valuable and adept technique, gaining ever-increasing significance in the realm of rational drug development. This computational approach focuses on the quest for a compatible ligand that harmonizes energetically and geometrically with a protein's binding site. In essence, it entails a comprehensive analysis of the interactions unfolding between two or more molecules, be it a ligand and a protein. The docking process itself unfolds through a two-step procedure: firstly, it involves the exploration of diverse ligand conformations within the active site of the protein, followed by an evaluation of these conformations via a scoring function. In an ideal scenario, the sampling algorithms would possess the capacity to faithfully replicate the experimental binding mode, while the scoring function would award the highest score to this particular conformation among the myriad generated [13]. Molecular dynamics simulation (MDs) emerges as an indispensable instrument for delving deep into the intricate nuances of biomolecular processes. MD and similar approaches are on the verge of becoming standard statistical instruments for drug development. Their primary benefit is that they directly address systemic stability and entropic consequences. As improved algorithms and hardware designs expand their use, this makes for a more precise approximation of the thermodynamics and kinetics involved with drug target identification and binding [14].

II. METHODS AND MATERIALS

➢ *Plant Extract Preparation:*

- *Soxhlet Extraction:*

To commence the extraction process, 30 grams of *G. sylvestre* leaf powder was meticulously weighed and subsequently deposited into a Soxhlet apparatus. This extraction endeavour was executed utilizing 250 millilitres of ethanol as the solvent of choice. After six hours of diligent extraction, the resulting suspension underwent filtration and was subsequently subjected to concentration via a rotary evaporator [7]. The concentrated extract was then meticulously collected using a spatula and preserved within an Eppendorf's tube, securely stored within the confines of a deep freezer for subsequent experimentation.


Fig 4 *G Sylvestre* Ethanolic Extract

➢ *Qualitative Analysis:*

- *Phytochemical Analysis:*

It is the process of screening and identification of bio-active constituents which are present in the plant source. The bioactive constituents which can be derived from plant sources are alkaloids, carbohydrates, terpenoids, flavonoids, saponins, tannins, steroids and phenolic compounds.

➢ *Molecular Docking*

- *Ligands:*

In this study the bio-active compounds present in a plant source were regarded as ligands and they were downloaded using PubChem. These ligands were prepared using Open Babel by adding hydrogen and converting the .sdf file into. pdbqt format.

PreADMET, a web-based platform that predicts the absorption, distribution, metabolism, and elimination/ excretion (ADME) characteristics of chemicals and produces a drug-like library using an in-silico approach, was used to assess the toxicity of the bioactive compounds. This analysis was performed using .sdf format of the ligands and after the submission process, results were obtained which has shown a good range of ADME, non-toxic and drug-likeliness properties of the ligands.

➢ *Preparation of Protein:*

RCSB Protein Data Bank was used to obtain the protein crystal structure. (PDB ID: 1NU6).

Within the AutoDock Software, we meticulously processed the protein structure by introducing polar hydrogen atoms and applying Kollman charges, rectifying bond orders, substituting missing atoms and residues, removing any extraneous ions, and thoroughly assessing and adjusting the protonation states of side chains [14]. To establish our docking framework, we forged a three-dimensional grid box with dimensions of 22 x 27 x 22, adhering to the default parameters while imposing no restrictive constraints. Additionally, we delineated an inner box, measuring 10 x 15 x 10, with its focal point residing at specific X, Y, Z coordinates of -23.75, 48.00, and 4.0 [15]. This grid was meticulously designed to encompass the pivotal active site region of the protein. Subsequently, we embarked on molecular docking of the protein, meticulously conducting the procedure within a Linux environment using AutoDock Vina, wherein Gymnemagenin served as the ligand of interest. The resultant outcome exhibited a highly favorable docking score, affirming the robust interaction between the ligand and the protein. Consequently, we undertook a comprehensive analysis of the protein-ligand complexes utilizing PyMOL, and these docking scores were securely retained for subsequent reference [The PyMOL Molecular Graphics System, Version 1.5.0.4, Schrödinger, LLC].

➢ *Molecular Dynamics*

Molecular Dynamics was performed using WebGroweb-based simulation tool [16]. The forcefield used for simulation is GROMOS96. The water model surrounding the protein and the ligand to mimic the environment is SPC water model. Energy minimization parameters help in representing the minimum energy conformation for the structure. The algorithm used during simulation is Steepest Descent is used. NaCl is used neutralizing and for equilibration of the environment NVT is used that is where N represents constant number, V represents volume and T represents temperature.

For Molecular Dynamics, Leap-frog algorithm is used. The frames per simulation was 1000 and total time interval is 50ns [15]. Root Mean Standard Deviation (RMSD) is a quantitative measure of resemblance between two or more protein structures that is commonly used. RMSD graphs which revealed stability of the protein complexes throughout the simulation time interval. The RMSF analysis was instrumental in scrutinizing the dynamic fluctuations that transpired within each residue throughout the simulation. Our specific focus honed in on discerning the oscillations occurring within the residues strategically positioned within the protein complex's active site during their interaction with ligands throughout the simulation timeframe [17].

Moreover, we harnessed the Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) approach, a well-established and respected methodology renowned for its adeptness in modelling molecular recognition phenomena, particularly the intricate interplay between proteins and ligands. This approach is celebrated for its precision and efficacy as a tool for simulating free energy dynamics [13,18]. Our study entailed meticulous calculations of the binding energy, specifically during the latter 20 nanoseconds of the simulation, encompassing the time interval spanning from 30 to 50 nanoseconds, to illuminate the intricacies of ligand-protein interactions.

➢ *ADME Property Prediction:*

We conducted a comprehensive assessment of the absorption, distribution, metabolism, and excretion (ADME) attributes pertaining to our target compounds. Employing the QikProp tool within Schrödinger software, we accurately predicted various physiologically significant ADME property descriptors and pertinent pharmaceutical properties associated with the selected ligand [19, 20].

- *Toxicity Prediction:*

The selected lead compound underwent a toxicity assessment to determine its potential for harmful effects. This evaluation utilized the Property Tox Checker [21], a valuable tool for identifying any adverse characteristics exhibited by our molecules. The prediction process involved comparing the functional groups within our query molecules to the in vitro and in vivo data present in the database.

- *Bioactivity Prediction:*

The online server known as PASS (Prediction of Activity Spectra for Substances) employs structural-activity relationships to gauge the comprehensive biological potential of a compound. Utilizing this tool, predictions are generated and subsequently compiled into a catalogue of potential biological activities, with each activity accompanied by its respective probability of being active (Pa) and probability of being inactive (Pi).

- *DFT Analysis:*

The lead compound underwent examination through density functional theory (DFT). For this assessment, the Schrodinger software's Jaguar module [22] was utilized to determine the energies of both the Lowest Unoccupied Molecular Orbital (LUMO) and the Highest Occupied Molecular Orbital (HOMO).

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III. RESULT AND DISCUSSION

➢ *Phytochemical Extract Yield:*

Table 1 Summary of Plant extraction

Sample	Sample weight taken in grams	Solvent (250ml)	Yield in grams
Gymnema *sylvestre*	30g	Ethanol	2.891g

➢ *Phytochemical Analysis Results:*

A phytochemical screening test was done on *G. sylvestre* leaf extract to evaluate for the presence of phytochemicals. Table 2 describes the presence and absence of phytochemicals in the extract. *G. sylvestre* shows positive results for tannins, saponin, triterpenoids, alkaloid, steroid, flavonoids and negative results for glycosides.

Table 2 Phytochemical Screening of Gymnemagenin Extract for Bioactive Compound

Compound	Ethanolic Extract
Alkaloids	+
Triterpenoids	+
Glycosides	-
Saponins	+
Tannins phenols	+
Flavonoids	+
Steroids	+

- *Note: +: Positive, -: Negative*

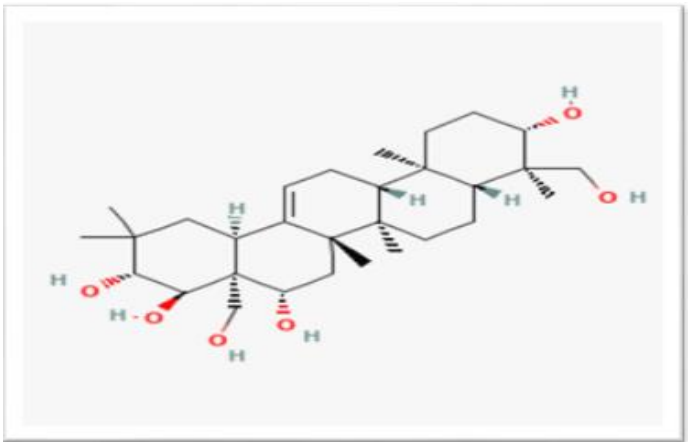

Fig 5 Structure of Ligand Downloaded from PubChem.

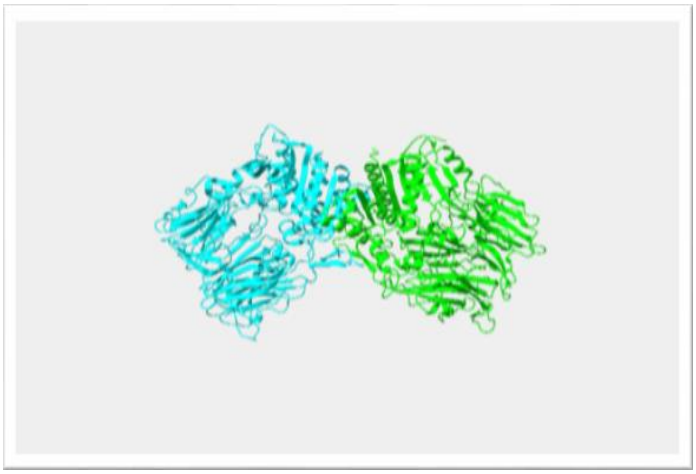

Fig 6 3D Structure of the Protein 1nu6

- *Green Colour: Chain A*
- *Blue Colour: Chain B*

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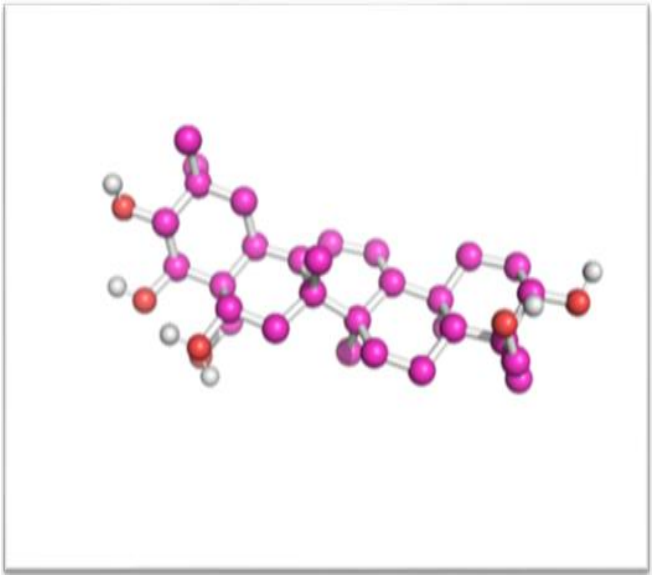

Fig 7 3D Structure of the Ligand Gymnemagenin

➢ *Molecular Docking*

The proteins were prepared for docking by removing bound water molecules, added non-polar hydrogens and Kollman charges.

Molecular Docking interactions of 1NU6 protein binds with the receptor gymnemagenin were visualized using PyMOL software [14]. The Protein-Ligand complex having great docking score were screened and the bonded interactions were highlighted as yellow broken lines, the bonded and non-bonded residues of the protein were represented as green sticks (fig 6), and the Ligands were represented as ball and stick model having magenta colour as shown in Fig 7.

The 2D interaction plot of Protein and Ligands were prepared using LigPLOT software and 3D interaction images were prepared using the PyMOL software (table 3).

Table 3 Docking Table

Sl. No	Compound	Diabetic Protein	Docking score (kcal/mol)	Bonded residues	Non bonded residues
1.	Gymnemagenin	1NU6	-8.7	Glu-205(2.80,2.89,2.77), GLN-553(2.87) Glu-206(2.89)	Lys554, Phe357, Arg125, Ser209, Tyr547, Ser552.

The docking score of gymnemagenin is-**8.7 kcal/mol,** Molecular docking interaction results, Following that, the ligand interacted with the active site residues, Gln553(2.87), Glu205(2.80,2.89,2.77), Glu206(2.89) and the non-bonded active site residues are Lys554, Phe357, Arg125, Ser209, Tyr547, Ser552.

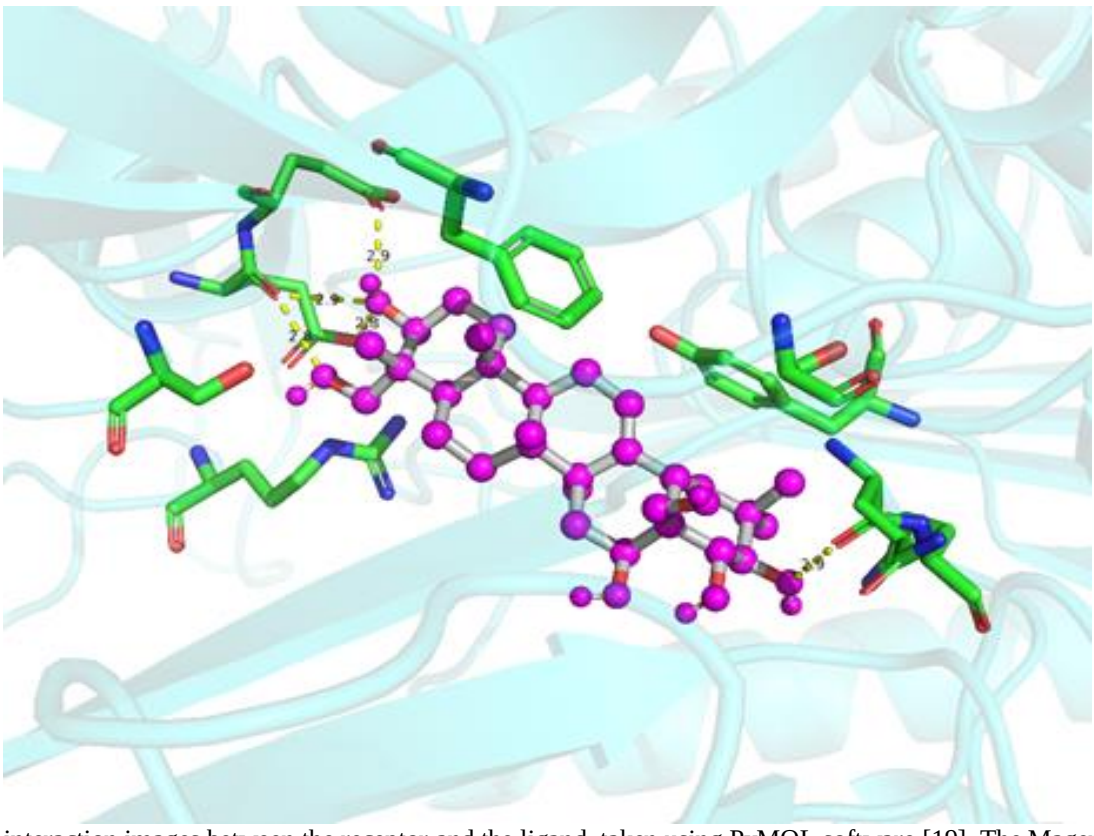

Fig 8 The 3D interaction images between the receptor and the ligand, taken using PyMOL software [19]. The Magenta colour ball and stick model in the images represents the respective drug molecule and green colour sticks are the interacting residues (bonded and non-bonded) and the bonded interactions are shown in yellow broken lines with the distance mentioned in Angstroms.

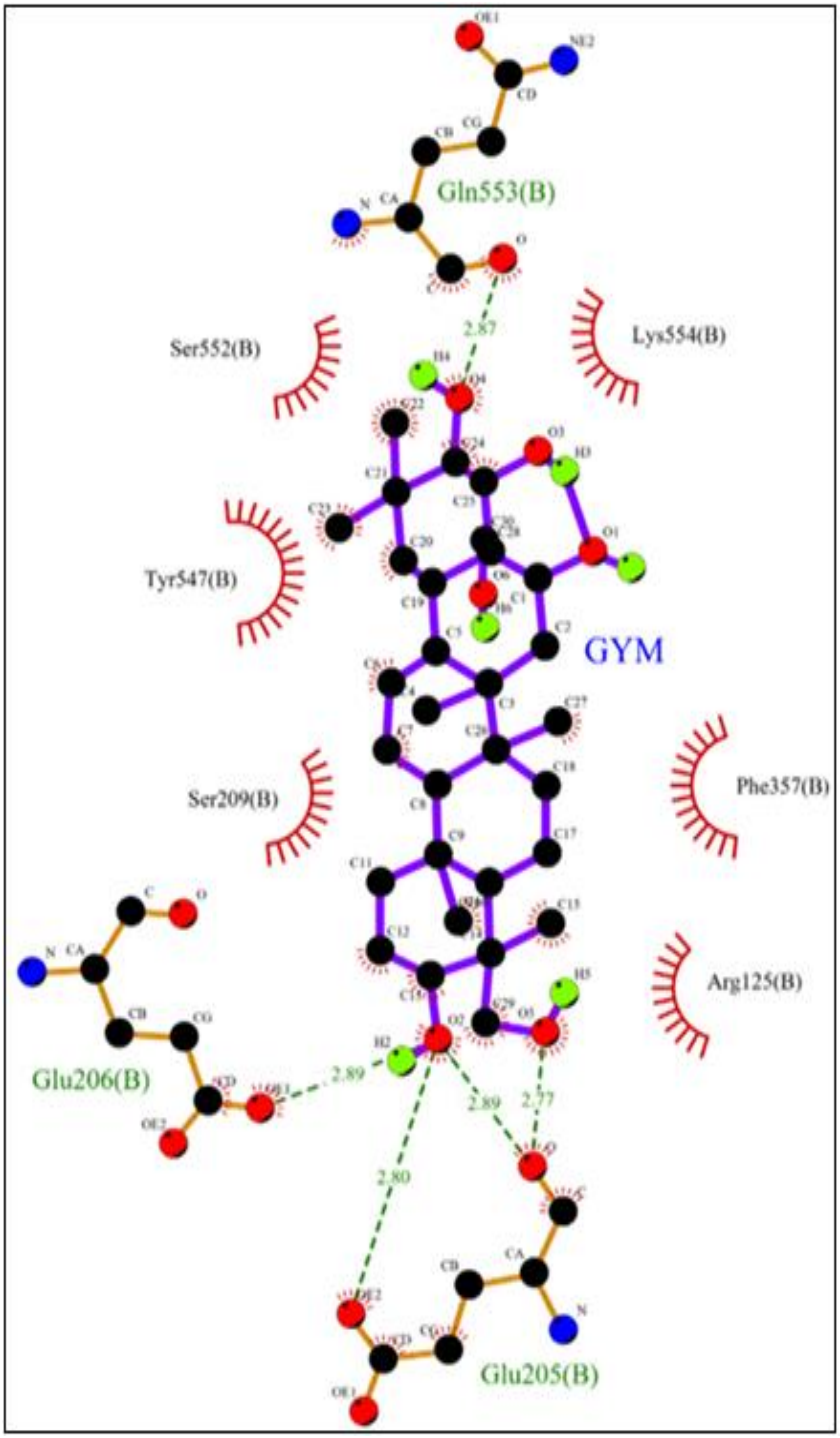

Fig 9 The above images are prepared using LigPLOT software. This image shows the bonded and non-bonded interactions between the ligand and protein 1NU6.1NU6_GYM image demonstrates the interaction of the 1NU6 protein receptor with the gymnemagenin (GYM) ligand.

➢ *Molecular Dynamics*

The model protein systems' structural comparisons are conducted through the calculation of the Root Mean Square Deviation (RMSD), a widely used metric. This analysis involves aligning the instantaneous coordinates of structures with a reference structure, maximizing overlap after rotation. As shown in Fig.10, the RMSD graphically represents the overall structural dynamics, with the X-axis denoting time intervals in nanoseconds and the Y-axis depicting deviations in nanometres.

For a closer examination of specific residue stability, assessing how individual residues fluctuate during a simulation, we employ Root Mean Square Fluctuation (RMSF). RMSF offers insights into residue fluctuations throughout the simulation. In Fig.11, you'll find the RMSF values plotted against amino acid residue numbers, shedding light on which amino acids within the protein contribute most significantly to structural molecular motion. Here, the X-axis represents amino acid residues, while the Y-axis quantifies fluctuations in nanometres.

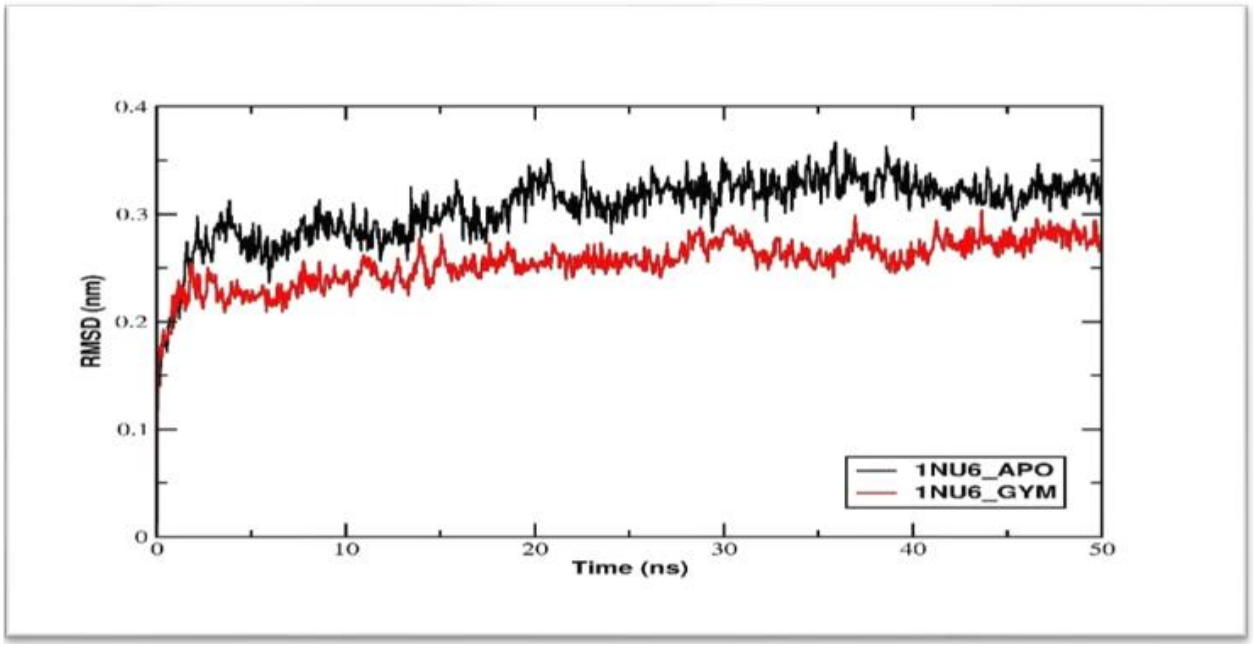

Fig 10 RMSD Graph showing overall structure

- *Note: X-axis Represents Time Interval in Nano Seconds and Y-axis Represents Deviation in Nanometers.*

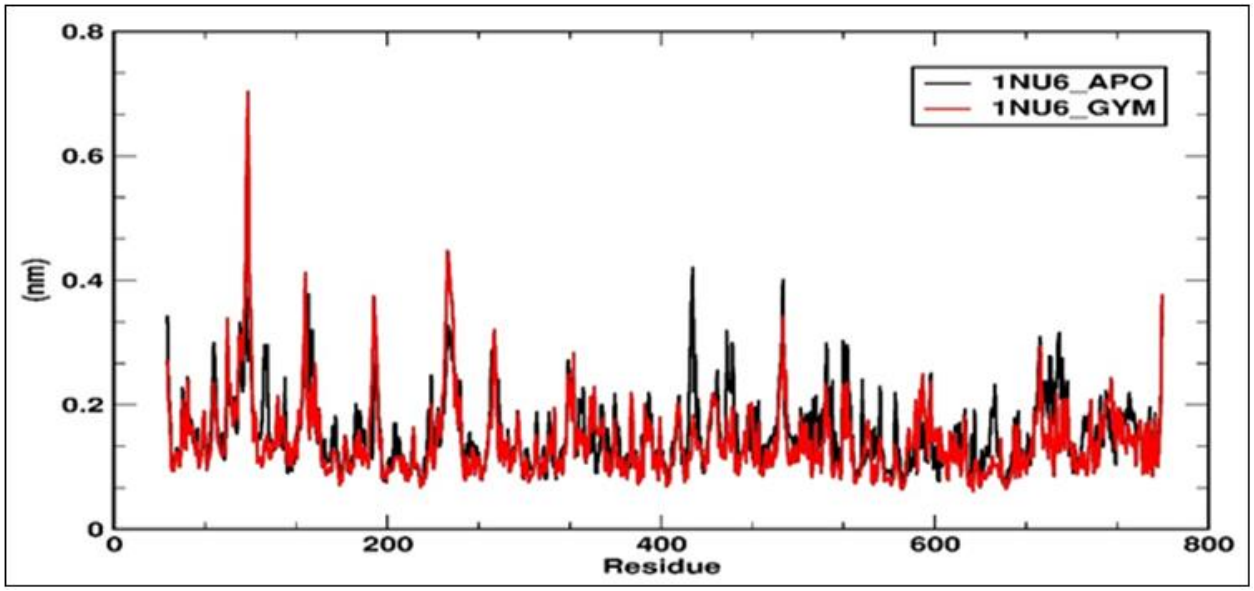

Fig 11: RMSF Graph showing Fluctuation of Residues throughout the Simulation

- *Note: X-axis is the protein sequence and Y-axis is the fluctuation*
- *Unit: X-axis=amino acids, Y-axis= nm*

➢ *The Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) calculation Tables:*

The Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) analysis yielded the free energy simulation results for 1NU6-GYM during the final 20 nanoseconds of the simulation, specifically spanning from 30ns to 50ns. This approach, being a free energy simulation method, is instrumental in comprehending the binding affinity between the protein and ligand. The binding energy calculated for gymnemagenin is -107.337 kJ/mol, signifying a strong affinity of the inhibitor for 1NU6, as detailed in Table 4.

Table 4 MMPBSA Calculation for Gymnemagenin

Gymnemagenin	**Binding energy = -107.337 +/- 0.000 kJ/mol**

- ***Note:*** *Once the protein and the ligand come together, the score reflects the possible energy change. This means that a least binding score showed a good binding, whereas positive value shows a weak or non-existent binding.*

This method allows for a comparative assessment of the binding free energy across various complex trajectories. It involves a thorough analysis and interpretation to probe how non-alanine mutations, post-translational modifications, and non-natural amino acids impact the binding free energy within the system being studied. Additionally, it can leverage molecular dynamics (MD) simulations of proteins in conjunction with different ligands to quantify and rank their relative affinities for the same receptor. These calculations are conducted separately for each component of the simulated complex, including the protein and ligand. Based on the insights garnered from the MM-PBSA calculations, gymnemagenin has exhibited a binding energy of 107.337 kJ/mol, indicative of a notably higher affinity for 1NU6 as an inhibitor.

➢ *ADME Property Prediction:*

We conducted ADME predictions on the lead compound to gain deeper insights into its pharmacokinetic profile. These characteristics are of paramount importance in the drug development journey, as any shortcomings in meeting these criteria could lead to significant delays or even the ultimate failure of a drug candidate to reach the market. The examined pharmacokinetic properties encompass factors such as molecular weight, adherence to Lipinski's rule of five, human oral absorption, predicted octanol/water partition coefficient, projected aqueous solubility, and star properties. You'll find a comprehensive breakdown of these criteria in Table 5, shedding light on the compound's potential as a drug candidate.

Table 5 ADME Properties of Lead Compound

Compound	MW	HOA	HB Acceptor	HB Donor	Qplog $P_{o/w}$	$QPlog_{HERG}$	QPPcaco	QPP_{MDCK}	Rule of five
Gymnemagenin	506.721	48.829	10.200	6.000	2.161	-3.885	91.970	37.512	2

➢ *Toxicity Prediction:*

The lead compound showed less toxicity, as evidenced from the results obtained through toxicity prediction server - ProTox-II are shown in (Table 6). Toxicity misinterpretation can lead to drug failure during development. As a result, in silico toxicity prediction is critical for predicting the biological effect of a chemical before subjecting it to experimental testing.

Table 6 Toxicity of Lead Compound

S. No	Compound	Predicted LD 50 (mg/kg)	Hepato toxicity	Carcino genicity	Immuno Toxicity	Cytotoxicity	Mutagenicity
1	Gymnemagenin	1190	Active (mild)	Inactive (mild)	Active	Inactive	Inactive

➢ *Bioactivity Prediction:*

The PASS prediction method was used to predict the bioactivity of phytochemical compounds. The projected impacts included modes of action and pharmacological activities, as well as calculated probabilities for activity above the probability edge (Pa > Pi) (Table 7).

Table 7 Bioactivity of Lead Compound

S. No	Compound	Pa	Pi	Activity
1	Gymnemagenin	0.950	0.004	Antineoplastic
		0.927	0.002	Hepatoprotectant
		0.712	0.005	Antiviral
		0.687	0.005	Antiulcerative
		0.300	0.023	Antioxidant
		0.318	0.073	Antidiabetic
		0.259	0.082	Antitoxic

➢ *DFT Analysis:*

HOMO and LUMO orbital energies at the frontier were used to study the electronic characteristics of the lead molecule. The electron affinity is indicated by the term LUMO, while the ionisation potential is indicated by the term HOMO. For the selected lead compound, the distributions of HOMO, LUMO, and energy gap were determined (Fig 12). The energy gap between HOMO and LUMO controlled the chemical stability and reactivity of the lead molecule.A low HOMO-LUMO gap indicates that the lead molecule is highly reactive, unstable, and polarizable. The difference in energy between HOMO and LUMO energies ranged -0.247eV (Table 8). HOMO and LUMO have tiny gap values demonstrate that rapid electron transfer and exchange makes the hit particularly reactive.

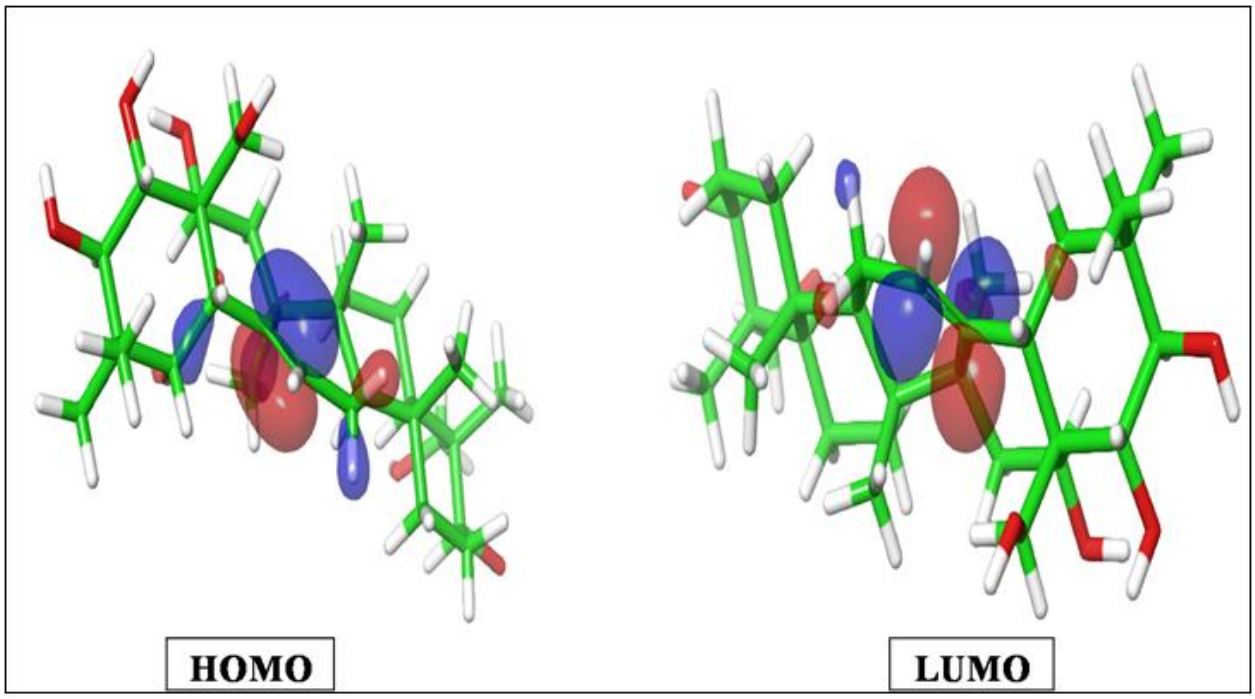

Fig 12 The Lead Compound's HOMO and LUMO Profiles

Table 8 Electronic Characteristics of Lead Compound

S. No	Compound	HOMO E-Value	LUMO E-Value	HLG (Homo Lumo Gap) eV
1	Gymnemagenin	-0.223574	0.024165	-0.247739

IV. SUMMARY AND CONCLUSION

Gymnema sylvestre, a deeply studied medicinal plant steeped in historical usage dating back to ancient civilizations, has been the subject of comprehensive in vitro and in vivo investigations. These research endeavours have sought to categorize the multitude of compounds extracted from this botanical wonder, scrutinizing their structural, functional, and pharmaceutical properties. This paper serves as a blueprint for establishing standardized testing protocols dedicated to assessing *G. sylvestre's* anti-stress and antiallergic capabilities. Among the intriguing compounds contributing to these desirable effects, gymnemagenin stands out. These discoveries are pivotal in the ongoing pursuit of more potent and safer pharmaceutical options. The findings emerging from diverse in vivo experiments continue to underscore the remarkable therapeutic potential harboured by this plant, capable of addressing a wide spectrum of diseases. This indicates that, the bioactive constituent is extracted from plant sources. In this study, In Silico analysis was carried out with INU6 protein having the PDB ID: INU6. The ligand was downloaded from PubChem and processed it in Open BABEL. The bioactive compound has qualified in drug likeliness properties using PreADMET tool. The interaction between the protein and ligand was determined using docking score of ligand in which gymnemagenin shown good inhibition towards1NU6 protein in Docking study. After docking, the 2D and 3D docking images are constructed using LigPLOT and PyMOL.

MMPBSA Calculation in Molecular Dynamics has shown that the Binding affinity of gymnemagenin with the protein where gymnemagenin has maximum binding energy, this makes gymnemagenin as a good inhibitor of 1NU6 protein in dynamic studies. Also, RMSD showed that the structure is stable and RMSF shown that the fluctuation in active site is less. By analysing these data obtained by In Silico study, it is seen that these bioactive compounds act as ligands and get binds to the protein which says that these bioactive compounds can be considered for production of insulin.

The present study elucidates the possible roles of gymnemagenin as ligand binding with potential protein targets associated with glucose metabolism. The study should also encourage further pharmacological investigations to confirm the in-silico findings in animal models and serve to validate the use of this very important medicinal herb as well. Our study underscores the importance of gymnemagenin which could be developed as a potent anti-diabetic drug.

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- *Conflict of Interest.*

The authors declare that they have no conflict of interest to disclose.

- *Data Availability Statement*

Data sharing not applicable to this article as no datasets were generated or analysed during the current study

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