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A Computational approach of phytochemicals from *Bacopa monnieri* in contrast to DPP-4 and peroxisome proliferator-activated receptors gamma as a Target for type-1 diabetes

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Abstract

Affecting the vast majority of the population, diabetes has become one of the major causes of death in India and the world. With the growing number of diabetic people in India, one in six people in the world with diabetes is from India. *Bacopa monnieri* is a wonder herb having many pharmacological applicaions. Traditionally, Brahmi is used to improve memory and concentration. Asthma, bronchitis, gastric ulcers, irritable bowel syndrome, anxiety, etc. DDP-4 inhibitors cause antihyperglycemic effect and regulates blood glucose. PPARs effectively regulates blood glucose levels and reduce triglycerides. The isoform of PPARs i.e., PPARγ plays a vital role in insulin sensitivity. The role of these protein receptors in treating T1DM is under research. Though few instances prove their effectiveness to treat T1DM, its efficacy is questionable and requires more research input. Branched chain and aromatic amino acids like tyrosine and glutamine have their functional role in regulating blood glucose level and onset of diabetic conditions. Bioactive ligands like Jujubogenin, bacogenin and luteolin are selected based on their interactions and binding affinity. T1DM mainly affects children and prediabetic period before the onset of T1DM provides us the golden opportunity to treat or suppress the condition through medical interventions. Thus, the ligand-macromolecule interaction and their efficacy in controlling Type-1 diabetes mellitus is the basis of this study.

Keywords: *Bacopa monnieri*; Phytochemical; Type-1 diabetes melitus; Insulin

1. Introduction

World Health Organization (WHO) states “a medicinal plant is a plant in which, one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis”. The WHO estimates that around 65%-80% of the people from developing countries depend on traditional medicines to heal their illness. Out of 300,000 plant species that are present in the world only 15% of them have been evaluated for their pharmacological applications [1]. The most common bioactive compounds found in a plant include alkaloids, tannins, phenolic compounds, and flavonoids. Knowing the chemical constituents of the plant is important in discovering its therapeutic applications. The majority of modern medicines are derived from natural sources. Hence, these traditionally used medicinal plants have wide applications [2].

Bacopa monnieri L. (Scrophulariaceae) is a creepy, succulent herb with multiple branches, purple flowers, and fleshy leaves that are generally known as ‘Brahmi’. It thrives near water bodies, wet and marshy areas. It is prominently used in Ayurveda, the Indian traditional medicine system. The phytochemical analysis of *Bacopa monnieri* Linn. contains tetracyclic triterpenoids, alkaloid brahmine, saponins A, B, and C, phytosterols, bacoside A and B, hersaponin,

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flavonoids, luteolin-7-glucoside, steroids, anthraquinone, nicotine, stigmastanol, stigmasterol, and carbohydrates. The medicinal applications of *Bacopa monnieri* are wide and diverse. Bacosides aid in the propagation of impulses. Traditionally, Brahmi is used to improve memory and concentration [3]. Asthma, bronchitis, gastric ulcers, irritable bowel syndrome, anxiety, and other conditions are also treated using *Bacopa monnieri*.

Diabetes is a metabolic disorder that affects the vast majority of people. It has an effect on carbohydrates, fats, and protein metabolism. Hypoglycemic agents and insulin are used to treat diabetes, but these compounds have severe side effects. Therefore, there is an increasing demand for herbal anti-diabetic medication with fewer side effects. Type-1 diabetes mellitus (T1DM) shows a 3-5% yearly increase in India. Genetic factors, environmental factors, and immune regulatory mechanism dysfunction are the main causes of T1DM. A combination of these factors results in autoimmune disorder which damages the beta cells of the pancreas causing a genetic lack of insulin. Insulin replacement, immune therapy, and islet transplantation are desired treatment plans for T1DM [4].

Inhibitors of the enzyme dipeptidyl peptidase-4 (DPP-4) are commonly prescribed for people with type 2 diabetics. DPP-4 enzymes destroy the gastrointestinal hormone incretins which stimulate the production of insulin. DPP-4 inhibitors protect incretins and regulate blood glucose levels. DPP-4 is also effective in treating T1DM. DPP-4 inhibitors increase incretin levels (GLP-1: Glucagon-Like Peptide 1 and GIP- Glucose-dependent Insulinotropic Polypeptide). GLP-1 increases insulin secretion and decreases glucagon secretion from β and α cells respectively [5]. This inhibits the production of hepatic glucose causing the antihyperglycemic effect.

PPARs (peroxisome proliferator-activated receptors) are important in glucose and lipid metabolism as they can effectively decrease triglycerides and blood glucose levels. They are members of the ligand-activated transcription factors family. They are expressed in beta cells of the pancreas and in the immune cells to regulate insulin secretion and T-cell differentiation. Hyperlipidemia and type-2 diabetes are treated with PPARγ isoform. Beta-cell death/ dysfunction is the major cause of T1DM. PPARs isoforms are the possible targets to restore beta cell function. PPARα causes the glucose-dependent upregulation of insulin. PPARβ/δ is the common isoform and is essential for the development and differentiation of the pancreatic cells. Insulin secretion is negatively regulated by PPARβ/δ. PPARγ agonist enhances beta-cell function and is involved in the regulation of insulin sensitivity [6].

The current research is focused on treating T1DM using bioactive compounds derived from natural sources such as medicinal plants. These ligand molecules interact with DPP-4 and PPARs. Thus, the ligand-macromolecule interaction and their efficacy in controlling Type-1 diabetes mellitus is the basis of this study.

2. Material and methods

2.1. Active binding site

The 3D structures of Human Dipeptidyl Peptidase IV (DPP-4: PDB ID-1J2E) and Human peroxisome proliferator-activated receptor-gamma ligand-binding domain (PDB ID-2ZK0) were built using SWISS-MODEL. FT site server and ProBiS server are used to identify protein binding sites. The recognition of binding sites has a broad range of applications, including functional protein relationships, structure-based predictions, and drug design. Thus, the FT server emerged as a tool to identify specific protein bindings.

2.2. Macromolecule structure retrieval

RCSB PDB is a Protein Data Bank that has information about the 3-dimensional structures of proteins, nucleic acids, and complex assemblage of molecules. The crystal structure of Human Dipeptidyl Peptidase IV (DPP-4: PDB ID-1J2E) and Human peroxisome proliferator-activated receptor-gamma ligand-binding domain (PDB ID-2ZK0) were obtained from the PDB databases. Both 1J2E and 2ZK0 are homodimers. Of the two chains (A chain and B chain) only A chain was used for docking studies, the other chain and water molecules were removed. Dassault Systems Biovia Discovery Studio Visualizer was used to perform molecular graphics.

2.3. Preparation of ligands

Phytochemicals such as Bacogenin A, Bacoside B, Betulinic acid, Jujubogenin, Luteolin, and Wogonin were selected based on their medicinal and therapeutic application for the ligand-protein docking studies. The PubChem database was used to obtain the SDF (Structure Data Format). Online SMILES translator was used for converting SDF files to PDB format. The ligand's physicochemical properties were investigated using PubChem databases.

2.4. Drug likeness of ligands

ADME (Absorption, Distribution, Metabolism, and Excretion) analysis is carried out before in-vivo studies and synthesis. The Insilico ADME model studies are carried out using SWISS ADME predictor, a drug discovery tool used to determine drug-likeness, pharmacokinetics, BBB (Blood Brain Barrier) penetration parameters, water-solubility, glycoprotein permeability, and GI (Gastrointestinal) absorption.

Implemented from the Ghose (Amgen), Veber (GSK), Egan (Pharmacia), and Muegge (Bayer) method, the Lipinski (Pfizer) filter uses the rule of five for drug compounds. Lower log P value for high lipophilicity, water solubility values, molar refractivity, and the number of Hydrogen bond donors and acceptors are the important parameters considered for Lipinski analysis.

2.5. Molecular docking studies

Molecular docking aims to assume or predict the ligand's binding properties and to determine the molecule's orientation concerning the active site. The inhibitory activity or the interaction of the ligand with the targeted protein is analyzed using molecular docking studies. The docking score determines all of the molecules' binding pores within the enzyme's catalytic site which results in the proper interaction between the molecules.

For the molecular docking studies Patchdock, PyRx (A Virtual Docking Tool), and IMOD (For Tomographic and 3D reconstruction) were used to determine the phytocompounds' inhibitory activity, which contributes to binding affinity and docking score.

3. Results

3.1. Active binding sites of protein molecules

The active site of the target proteins (DPP-4 and Human peroxisome proliferator-activated receptor-gamma ligand-binding domain) were predicted using the FT server in this analysis.

Ligand preparation

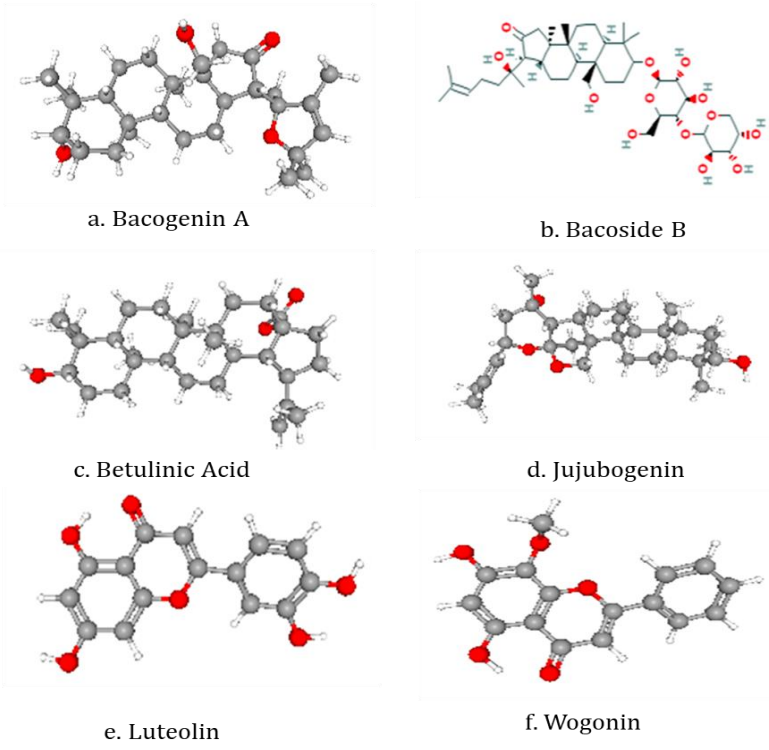


Figure 1 3-D Structures of all selected phytochemicals

3.2. Ligands

3.2.1. Drug likeness analysis

The rigidity of all compounds to be considered for structure-based drug design is explained using Lipinski filter analysis. The properties of the compounds with respect to their usage as a drug were listed out using ADME analysis.

Physico-chemical properties of ligand

Ligands	Molecular Formula	Molecular Weight	Monoisotropic Mass	Heavy Atom Count	Topological Polar Surface Area
Bacogenin A	C30H48O4	472.7 g/mol	472.35526 g/mol	34	66.8 Å²
Bacoside B	C41H68O13	769 g/mol	768.465992 g/mol	54	216 Å²
Betulinic Acid	C30H48O3	456.7 g/mol	456.360345 g/mol	33	57.5 Å²
Jujubogenin	C30H48O4	472.7 g/mol	472.35526 g/mol	34	58.9 Å²
Luteolin	C15H10O6	286.24 g/mol	286.047738 g/mol	21	107 Å²
Wogonin	C16H12O5	284.26 g/mol	284.068473 g/mol	21	76 Å²

Lipinski filter analysis

Ligand	Molecular Formula	Hydrogen Bond Donor	Hydrogen Bond Acceptor	cLogP	Molar Refractivity
Bacogenin A	C30H48O4	2	4	4.94	137.92
Bacoside B	C41H68O13	8	13	2.35	198.88
Betulinic Acid	C30H48O3	2	3	6.11	136.91
Jujubogenin	C30H48O4	2	4	5.29	136.70
Luteolin	C15H10O6	4	6	1.73	76.01
Wogonin	C16H12O5	2	5	2.54	78.46

Criteria for Lipinski filter: H bond donor's ≤ 5, H bond acceptors ≤10, and molecular weight should be in the range of 150-500g/mol. Except for Bacoside B, all the other phytochemicals in the table pass the Lipinski filter analysis and shows potential drug properties as the values were noted to be in the acceptable range for human use.

Admet analysis

Ligand	Blood-Brain Barrier	GI Absorption	Permeability Glycoprotein Substrate	Log S(SILICOS-IT) (scale Insoluble < -10<Poorly<-6< Moderately <-4<Soluble<-2Very<0< Highly)[Water solubility]
Bacogenin A	No	High	No	-6.07(poorly soluble)
Bacoside B	No	Low	Yes	-2.27(soluble)
Betulinic Acid	No	Low	No	-5.70(moderately soluble)
Jujubogenin	No	High	No	-5.23(moderately soluble)
Luteolin	No	High	No	-3.82(soluble)
Wogonin	No	High	No	-5.10(moderately soluble)

Molecular docking analysis

Human Dipeptidyl Peptidase 4 (DPP-4: PDB ID-1J2E) Docking Score with selected ligands.

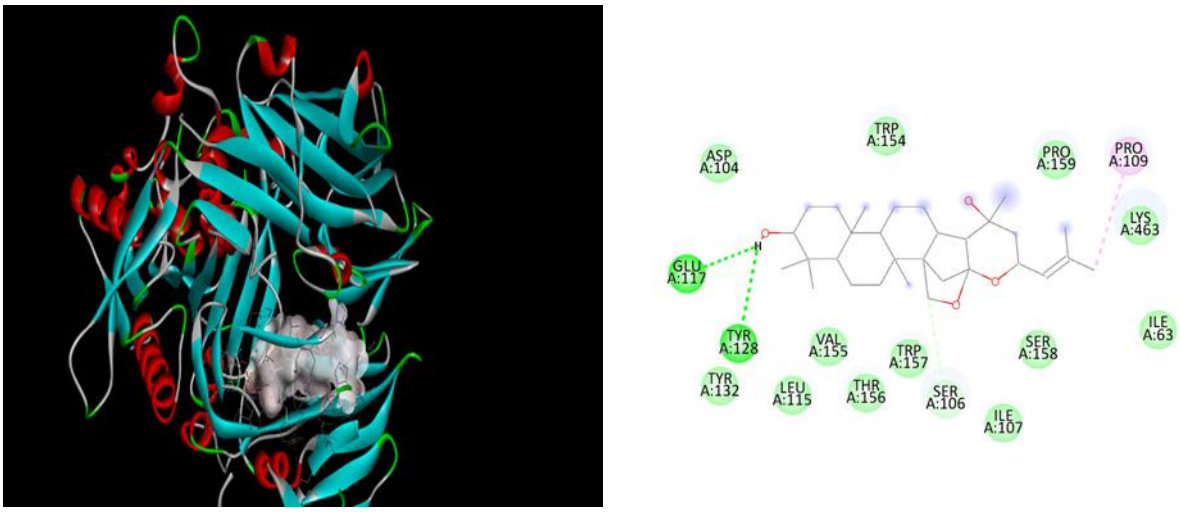
Ligand	Binding Affinity
1j2e_bacogenin_A_uff_E=929.17	-9.3
1j2e_bacoside_b_uff_E=1191.79	-8.4
1j2e_betulinic_acid_uff_E=790.90	-8.8
1j2e_jujubogenin_uff_E=992.09	-9.5
1j2e_luteolin_uff_E=241.50	-9.1
1j2e_wogonin_uff_E=315.80	-7.6

The ligands jujubogenin and bacogenin had a higher binding affinity of -9.5 and -9.3respectively with DPP-4 and hence these ligands were selected based on their binding affinity.

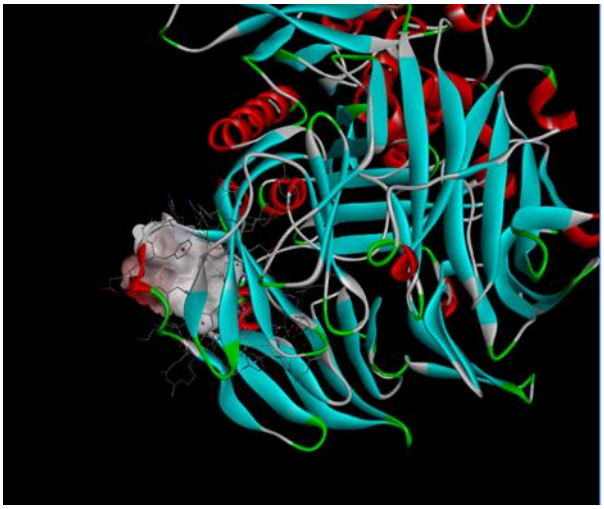
Human peroxisome proliferator-activated receptor-gamma ligand-binding domain (PDB ID-2ZK0) Docking Score with selected Ligands.

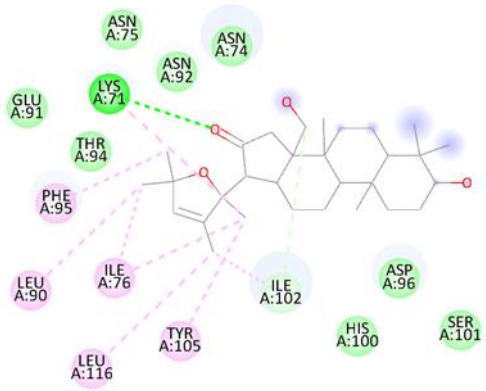
Ligand	Binding Affinity
2zk0_bacogenin_A_uff_E=929.17	-8.1
2zk0_bacoside_b_uff_E=1191.79	-7.7
2zk0_betulinic_acid_uff_E=790.90	-8
2zk0_jujubogenin_uff_E=992.09	-8.9
2zk0_luteolin_uff_E=241.50	-8.9
2zk0_wogonin_uff_E=315.80	-9.1

The docking of PPAR's with ligands wogonin, jujubogenin, and luteolin had a binding affinity of -9.1, -8.9, and -8.9 respectively. The ligands jujubogenin and luteolin were selected based on their interaction with PPARs.

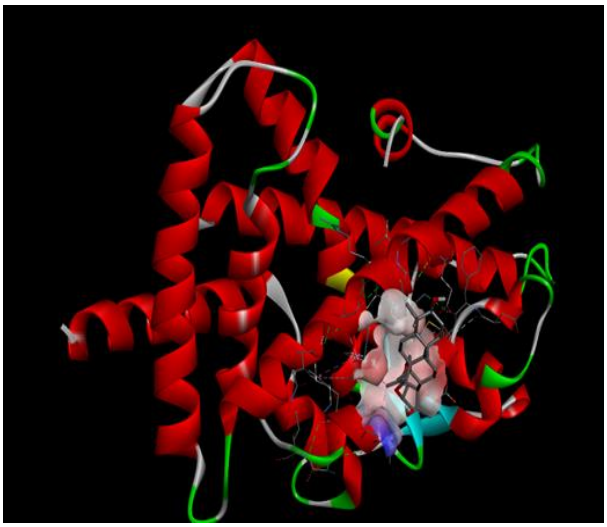


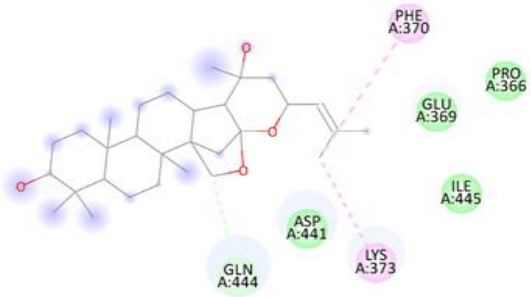
a. DPP4-JUJUBOGENIN



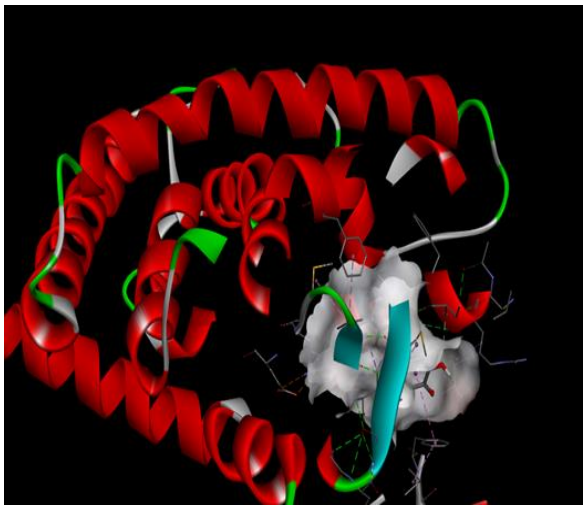


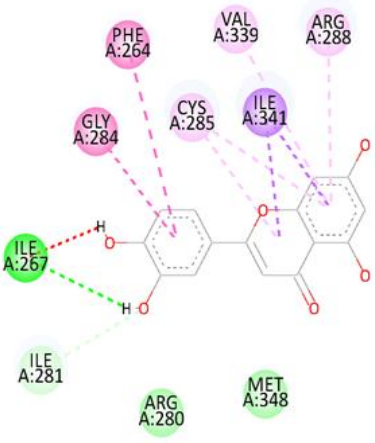
b. DPP4-BACOGENIN A





c. 2ZK0-JUJUBOGENIN





d. 2ZK0-LUTEOLIN

4. Discussion

Brahmi aka *Bacopa monnieri* is the magical memory booster plant that is used in traditional medicine for more than 3000 years. In ayurvedic medicine, they were used orally as tonics and the concentration of which varied depending upon age and severity of the disease. They were employed to improve digestion, concentration, memory, and learning, to treat the nervous system and disorders associated with it like Alzheimer's, insanity, anxiety, epilepsy, repair of damaged neurons, neuronal synthesis, to improve brain function, etc. Asthma, bronchitis, and several skin disorders or allergies were also treated using this herb. Neuroprotective, hepatoprotective, anti-inflammatory, antimicrobial, antilipidemic, analgesic, antidiabetic, antipyretic, anticancer, antiarthritic, antihypertensive, gastrointestinal, endocrine, muscle-relaxing effects, antianxiety, antioxidant, antidepressant sedative, and memory enhancers are some of the pharmacological applications of the herb [7].

Although they have a wide range of medical uses, the medications are limited to enhance memory functions [8-10]. There are no FDA-approved drugs of *Bacopa monnieri* to treat Alzheimer's disease, blood pressure, anxiety, and hypoglycemia. Hence drug development using this herb has research scope and future market.

Bacosides are nootropic, in diabetic nephropathy, betulinic acid exhibits a protective effect. Betulinic acid offers better insulin resistance than metformin. Bacosides are complex mixtures of structurally similar compounds, such as jujubogenin or pseudojujubogenin glycosides. Luteolin is a flavonoid with potential anticancer, antioxidant, and anti-inflammatory activities. It improves nerve conduction, blood flow in the nerves and prevents the destruction of renal tissues and diabetic nephropathy [11]. Wogonin has neuroprotective, antioxidant, anticancer, anti-inflammatory, and anti-hepatitic activity. Wogonin is an effective modulator to express PPARγ. They also have a significant effect on lipid and glucose metabolism and can be used as a therapeutic agent to treat diabetes. It is remarkable to note that these compounds are effective to treat complications caused due to diabetes, than the disease itself. Their efficacy in protecting from renal damage, hepatic damage, nerve cell damage, conducting nerve impulse, initiating adipogenesis, initiating the differentiation and maturation of pancreatic cells is worth noting. Hence, research focusing on individual complications caused due by diabetes and developing and designing drugs to treat these conditions are required. Controlling the complications is equally important as controlling the disease. Research focussing on nerve cell damage is of high importance, as this damage is not reversible [12,13].

The herb is rich in more than 52 bioactive compounds. In this study, 6 bioactive compounds were selected based on their pharmacological applications. The ligands jujubogenin and bacogenin had a higher binding affinity of -9.5 and -9.3 respectively with DPP-4. The docking of PPAR's with ligands wogonin, jujubogenin, and luteolin had a binding affinity of -9.1, -8.9, and -8.9 respectively. The ligands jujubogenin and bacogenin A were selected for DPP-4 whereas, jujubogenin and luteolin for PPARs are based on their docking score and binding interactions. These interactions were visualized using Dassault Systems Biovia Discovery Studio. The visualization of the docked structures proved that Jujubogenin had better binding with the receptors [14].

The 2D diagram for the binding of Jujubogenin and DPP-4 shows the involvement of some important amino acids like GLU, ASP, TYR, LEU, VAL, THR, TRP, SER, ILE, LYS, ASP, TRP, etc. The amino acids like GLU and TYR are bounded by conventional hydrogen bonds. Amino acids like ASP, THR, TRP, SER, ILE, and PRO binds to jujubogenin through van der Waals's forces of attraction. Alkyl group binding is seen with amino acids like TYR, TRP, VAL, LEU, LYS, and PRO.

The 2D diagram for the binding of Jujubogenin and PPAR's shows the involvement of some important amino acids like GLU, GLN, PRO, ASP, ILE, and PHE which are bound by van der Waals forces of interaction. LYS binds to the alkyl group of jujubogenin.

Lower concentrations of glutamine help to reduce blood glucose levels. The current study proves that jujubogenin has a better binding affinity with glutamine. Intake of glutamine supplements helps to increase insulin levels and reduce blood glucose concentrations. Therefore, glutamine supplementation can be considered for patients with Type-1 diabetes who have no insulin secretion. Insulin sensitivity is also affected by glutamine as it is involved in many metabolic pathways like gluconeogenesis, synthesis of peptides, antioxidants, nitric oxide, etc. These pathways impact glucose concentration in the body.

People with increased levels of aromatic amino acids like phenylalanine, tyrosine, and tryptophan and branched-chain amino acids like leucine, isoline, and valine are at higher risk to develop diabetes at later stages of their life. Hence it is very important to focus on reducing levels of these amino acids in the blood. This research shows that the ligand jujubogenin has a better binding affinity with amino acids like glutamine and tyrosine, lowering their levels helps to control blood glucose levels [15,16].

Jujubogenin is also a component of Bacoside A, a major phytocompound isolated from *Bacopa monnieri.* The major pharmacological and therapeutical application of the herbs is due to the presence of bacosides. The flavonoid, Luteolin present in the herb plays a major role in immunity and inflammation. Diabetes-associated nerve damage, memory loss, and renal damage are common among patients. The cues passed down from traditional knowledge and practice are used so far in the treatment. This herb is a treasure of bioactive phytochemicals but lacks clinical evidence to support the claims hence it requires experimental and research evidence.

In this study, DPP-4 and PPARs were taken as they are already proved to be effective in treating type 2 diabetes mellitus (T2DM). DPP-4 (Gliptins) are not prescribed as primary medications. Gliptins are prescribed as the second or third line of medications if T2DM is not controlled using conventional drugs (metformin, sulphonylureas). PPARs play a vital role in treating T2DM. The isoform PPARγ improves insulin sensitivity. Thiazolidinediones (TZDs) are antidiabetic oral drugs that activate adipocyte differentiation and adipogenesis. The role of these protein receptors in treating T1DM is under research. Though few instances prove their effectiveness to treat T1DM, its efficacy is questionable and requires more research input [17].

Affecting the vast majority of the population, diabetes has become one of the major causes of death in India and the world. Lifestyle and disease management also attributes to the severity of the disease. Physical inactivity, obesity, and overweight linked to diabetes may cause further complications. Increased consumption of fats, sugars calories, physical inactivity, and increased stress affects insulin sensitivity and obesity and this is the major reason for the shift in the age of onset diabetes. T2DM in India is caused mainly due to lifestyle changes and environmental factors. According to WHO, 2% of all deaths are caused due to diabetes in India. With the growing number of diabetic people in India, one in six people in the world with diabetes is from India. With these alarming numbers, it is important to know that India harbors more T1DM people than any other western country. T1DM mainly affects children and is caused due to genetic, environmental, or immune reasons. The prediabetic period before the onset of TIDM provides us the golden opportunity to treat or suppress the condition through medical interventions including immunosuppressants, cyclosporins, and steroids. Once the disease has progressed, maintenance of normal blood glucose is mandatory for the long-term management of the disease. Therefore, a proper medical intervention before the onset of the disease during the prediabetic phase can control and reduce the severity of the disease. Hence the new drug development should target on focusing this phase of the disease.

5. Conclusion

The herb is rich in more than 52 bioactive compounds. In this study, 6 bioactive compounds were selected based on their pharmacological applications. The ligands jujubogenin and bacogenin had a higher binding affinity of -9.5 and -9.3 respectively with DPP-4. The docking of PPAR's with ligands wogonin, jujubogenin, and luteolin had a binding affinity of -9.1, -8.9, and -8.9 respectively. The ligands jujubogenin and bacogenin A were selected for DPP-4 whereas, jujubogenin and luteolin for PPARs are based on their docking score and binding interactions. These interactions were visualized using Dassault Systems Biovia Discovery Studio. The visualization of the docked structures proved that Jujubogenin had better binding with the receptors.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

If two or more authors have contributed in the manuscript, the conflict of interest statement must be inserted here.

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