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Molecular Docking Analysis of Potential Dipeptidyl peptidase - 4 (DPP-4) Inhibitors from Siddha formulation *Pungampoo Chooranam* for treating Diabetes mellitus

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Abstract

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia or increased blood glucose levels with disturbances in carbohydrate, fat and protein metabolism resulting from absolute or relative lack of insulin secretion. The important pathogenic factors responsible for the development of metabolic disorder, insulin resistance, β-cells dysfunction, and finally diabetes are oxidative stress and tissue specific systemic inflammation. It was proven that drug which selectively inhibit the enzyme Dipeptidyl peptidase-4 (DPP-4) benefit the insulin resistance diabetes. As the DPP-4 highly involved in the enzymatic degradation of GLP-1 and GIP required for insulin secretion. Due to increased adverse event caused by conventional anti-diabetic agents researchers are at constant need of exploring alternate therapeutic strategy for clinical management of diabetes mellitus. Siddha system of traditional medicine through its valuable phytocomponents therapy provides highly beneficial effects in treating metabolic disorders such as diabetes since several centuries. The main aim of the resent investigation is to screen the seven bioactive phytocomponents such as Beta Sitosterol, Glabrin, Kanjone, Pongol, Sterolin, Pinnantin, and Quercetin present in the formulation *Pungampoo Chooranam* (PPC) against target protein Dipeptidyl peptidase-4 with PDB code 2P8S along with the standard sitagliptine using computational docking analysis. The results of the present investigation clearly shows that all the seven compound screened Insilco has tendency to binding with the most significant active site 205 GLU,206 GLU,209 SER,357 PHE,358 ARG,547 TYR, 710 ASN,711VALand 740 HIS present on the target protein DDP-4 which may be responsible for degradation of GLP-1 and GIPand hence from this it was concluded that the bioactive phytocomponents present in the formulation *Pungampoo Chooranam* (PPC) has significant DDP-4 enzyme inhibition activity and there by promising anti-diabetic activity and may also be effective in clinical management of diabetes mellitus.

Keywords: Siddha system, Diabetes mellitus, DDP-4 enzyme *Pungampoo Chooranam,* Phytocomponents, Anti-diabetic activity

1. Introduction

It is currently estimated that number of people with diabetes in India will reach 80 million by the year 2025. The incidence of diabetes especially in the young and active population is having negative impact in the economic up-growth of India and is hence a serious problem requiring immediate solution.

Diabetes is a chronic pathological condition in which the pancreas is unable to secrete sufficient insulin. It is characterized by the failure of tissues to respond to a normal concentration of glucose available in the blood, resulting in reduced glucose intake into the peripheral tissue. It was evident through recent

research that selective inhibition of the enzyme Dipeptidyl peptidase 4 (DPP-4) in insulin-resistant skeletal muscle causes improvements in insulin-stimulated glucose transport activity. Based on the literature survey about 8 biologically active phytocomponents were selected for DPP-4 enzyme inhibition analysis by molecular docking study [1].

There are some metabolic hormones known as incretins which includes intestinal peptides glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (also known as: glucose-dependent insulinotropic polypeptide or GIP) both GLP-1 and GIP generally regulates and decreases the blood glucose level. The most important fascinating role played by these GLP-1 and GIP is to stimulate the release of insulin from pancreatic beta cells of the islets of Langerhans in pancreas by a blood glucose-dependent mechanism. According to the literature incretins further inhibit glucagon release from the alpha cells of the islets of Langerhans [2].

Dipeptidyl peptidase-4 (DPP-4) is an enzyme mainly involved in rapid degradation of both GLP-1 and GIP. Hence it was a proven fact the inhibition of this enzyme DPP-4 prolongs the action of both GLP-1 and GIP hence it directly increases the level of insulin releases and thereby decreases the blood glucose level by insulin mediated cell glucose transport mechanism [3].

Drugs currently used for the treatment of diabetes include metformin, sulphonylureas, thiazolidinediones, meglinitides etc., whose long term usage is known to cause side effects like heart failure, myocardial infarction, anxiety, nervousness, seizures, palpitation, and depression. Hence there is a need for alternative therapies that can overcome the limitations of conventional anti-hyperglycemic medications. Siddha system of traditional medicine has novel preparation comprises of herbs which has tendency to limit the activity of crucial enzymes like DDP4. One such novel formulation is *Pungampoo Chooranam* (PPC) majorly comproses of the herb *Pongamia pinnata* and cow ghee which acts synergistically.

Pongamia pinnata (Fabaceae) is popularly known as Indian beech in English [4]. Commonly known by its vernacularnames karanj (Hindi), honge/karajata (Kannada), pungai (Tamil). As per the literature the extract of stem bark of *P. pinnata* (L.) showed antihyperglycaemic activity in diabetic mice [5]. Further, reports available that concomitant administration of synthetic oral hypoglycemic drugs along with *P. pinnata* produced synergistic effect in diabetic mice [6]. The preliminary phytochemical analysis showed the presence of alkaloids, terpenoids, triterpenes, flavonoids, steroids, and volatile oils [7]. It has been identified that Cycloart-23-ene-3*β*, 25-diol (B2) isolated from the stem bark of *P. pinnata* possesses antidiabetic activity in diabetic animals [8]. Cycloart-23-ene-3*β*, 25-diol improved the abnormalities of diabetic conditions in diabetic mice due to increased glucagon-like peptide 1 (GLP-1) insulin secretion [9] and has a protective effect on vital organs like heart and kidney [10].

The main aim of the present investigation is to screen the anti-diabetic potential of phytocomponents such as Beta Sitosterol, Glabrin, Kanjone, Pongol, Sterolin, Pinnantin and Quercetin present in the formulation *Pungampoo Chooranam* (PPC) against target protein Dipeptidyl peptidase-4 (DPP-4) receptor with PDB code 2P8S along with the standard Sitagliptine using auto-dock computational docking analysis.

2. Materials and Methods

2.1. Source of raw drugs

The herb is collected from southern zone of Tamil Nadu, and other required ingredient is procured from a well reputed indigenous drug shop from Parrys corner, Chennai, Tamil Nadu, India .Herb were authenticated by the Pharmacognosist, SCRI Chennai, Tamil Nadu, India.

2.2. Ingredients

The siddha formulation *Pungampoo Chooranam* (PPC) comprises of two main ingredients as listed below

1. Pungam flowers (*Pongamia pinnata*)
2. Cow's Ghee

2.3. Preparation [11]

The shade dried flowers of *Pongamia pinnata* were roasted slowly by adding little bit of cow's ghee. Then it is powdered and sieved using cloth.

Dosage	: 2 gm twice a day
Adjuvant	: Warm water
Duration	: 48 Days

2.4. Software's required

Several docking tools were been used in recent times which works behind structure-based drug design strategies one among which is auto dock a componential software tools used to analyze the protein Dipeptidyl peptidase-4 (DPP-4) and to study the binding energy properties with the following lead component such as Beta Sitosterol, Glabrin, Kanjone, Pongol, Sterolin, Pinnantin and Quercetin along with standard Sitagliptine. Dipeptidyl peptidase-4 (DPP-4) enzyme with PDB code 2P8S was obtained from protein data bank (www.pdb.org/pdb/). To get insight the intermolecular interactions, the molecular docking studies were done for the above mentioned phytoconstituents along with standard at the active site 3D space of enzyme of interest Dipeptidyl peptidase-4 using auto dock – docking tool module.

2.5. Ligand preparation

The ligands such as Beta Sitosterol, Glabrin, Kanjone, Pongol, Sterolin, Pinnantin and Quercetin along with standard Sitagliptine built using Chemsketch and optimized using Docking server online web tool as shown in Figure 1 and 2 for docking studies by using Geometry optimization method MMFF94 and charge calculation was carried out based on Gasteiger method at pH 7 as shown in Table 1.

Table 1: Ligand Properties of the selected Lead

S.No	Name of the Compounds	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds	Log P
1	Beta Sitosterol	414.718 g/mol	$C_{29}H_{50}O$	1	1	6	9.3
2	Glabrin	175.184 g/mol	$C_7H_{13}NO_4$	3	5	1	-3.5
3	Kanjone	292.29 g/mol	$C_{18}H_{12}O_4$	0	4	2	3.6
4	Pongol	292.29 g/mol	$C_{18}H_{12}O_4$	0	4	2	3.6
5	Sterolin	576.859 g/mol	$C_{35}H_{60}O_6$	4	6	9	7.7
6	Pinnantin	292.29 g/mol	$C_{18}H_{12}O_4$	0	4	2	3.6
7	Quercetin	302.238 g/mol	$C_{15}H_{10}O_7$	5	7	1	1.5
8	Sitagliptine	407.32 g/mol	$C_{16}H_{15}F_6N_5O$	1	10	4	0.7

Fig 1: 2D Structure of lead 1.Beta Sitosterol 2.Glabrin 3.Kanjone 4. Pongol 5. Sterolin 6. Pinnantin 7. Quercetin and 8.Sitagliptine

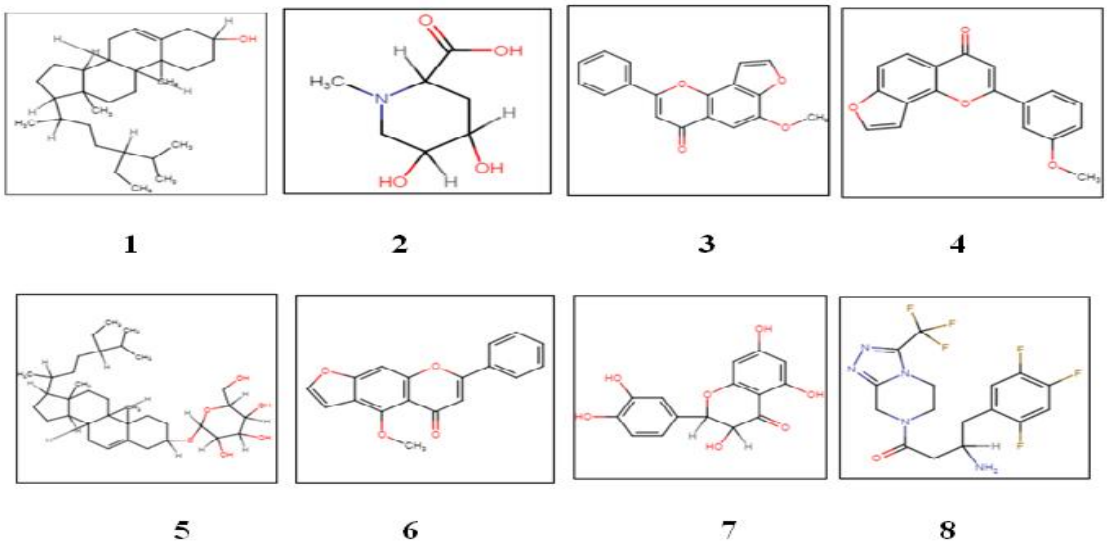
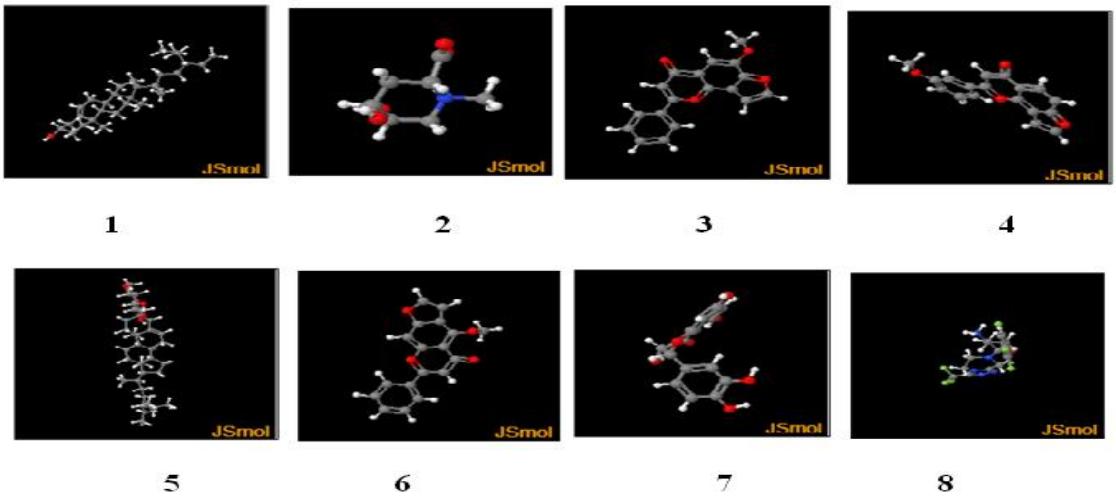


Fig 2: 3D Structure of lead 1.Beta Sitosterol 2.Glabrin 3.Kanjone 4. Pongol 5.Sterolin 6. Pinnantin 7. Quercetin and 8.Sitagliptine



2.6. Active Site Prediction

Active site of enzyme was obtained by LIGSITE web server by using the automatic identification of pockets on protein surface given 3D coordinates of protein. The potential ligand binding sites in 2P8S target protein is identified using grid space of 1 and probe of radius 5.0 angstrom [12]. Ligand site prediction was performed by using online tool GHECOM and the respective pockets calculations [13,14].

2.7. Docking Methodology

Docking calculations were carried out using Docking Server [15,16]. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out based on the binding free energy on the following compounds like Beta Sitosterol, Glabrin, Kanjone, Pongol, Sterolin, Pinnantin and Quercetin along with standard Sitagliptine and their binding affinity towards the target protein with PDB 2P8S as shown in figure 3. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools. Affinity (grid) maps of Å grid points and 0.375 Å spacing were generated using the Autogrid program. Auto Dock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method [17]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied [18].

Fig 3:Target protein Dipeptidyl peptidase-4 (DPP-4) with PDB code 2P8S

3. Results

3.1.Dock scores

The result of binding interactions of the ligand with Dipeptidyl peptidase-4 has revealed that out of seven compounds docked against PDB 2P8S. The lead Beta Sitosterol has shown highest inhibition property similar to that of the standard Sitagliptine. The second highest docking interaction possessed by quercetin followed by this pinnantin, pongol, glabrin, kanjone and sterolin with most significant amino acid residues on the target Dipeptidyl peptidase-4. The binding free energy of Beta Sitosterol was found to be -7.71Kcal/mol and for Pinnantin it was -6.24 followed by this Pongol with -5.34 , Glabrin and Kanjone with -4.6. The docking score with respect to Binding Free energy,Inhibition constant including Total Interaction Surface were listed in table 2.

Table 2: Summary of the molecular docking studies of compounds against Dipeptidyl peptidase-4 receptor

S.No	Name of the Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)(**nm)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
1	Beta Sitosterol	-7.71	2.24	-0.04	-9.74	841.81
2	Glabrin	-4.6	423.66	-1.82	-5.57	388.33
3	Kanjone	-4.6	425.58	-1.84	-5.56	388.25
4	Pongol	-5.34	122.21	-0.15	-5.92	643.02
5	Sterolin	-3.9	1.39*	-0.34	-7.88	896.31
6	Pinnantin	-6.24	26.76	-0.27	-6.65	670.99
7	Quercetin	-3.55	2.49*	-0.36	-5.03	634.53
8	Sitagliptine	-9.57	95.93**	-3.09	-11.39	707.94

Fig 4 : Possible ligand binding pockets on the surface of target Dipeptidyl peptidase-4 with PDB code 2P8S. Pockets calculated by GHECOM. 1.Beta Sitosterol 2.Glabrin 3.Kanjone 4. Pongol 5.Sterolin 6. Pinnantin 7. Quercetin and 8.Sitagliptine

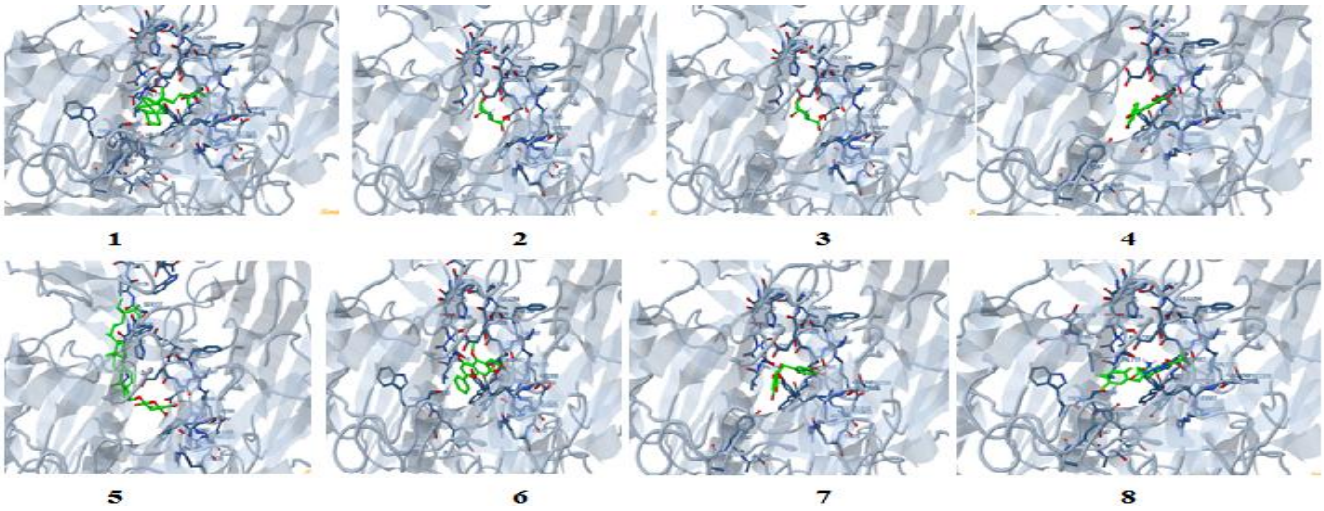


Table 3: Interaction of lead compounds with active site amino acid residue of Dipeptidyl peptidase-4 Receptor

Name of the Compounds	Amino Acid Interaction													
Beta Sitosterol	125 ARG	205 GLU	206 GLU	209 SER	357 PHE	358 ARG	547 TYR	630 SER	656 VAL	662 TYR	666 TYR	669 ARG	710 ASN	711 VAL
Glabrin	125 ARG	126 HIS	205 GLU	206 GLU	209 SER	357 PHE	666 TYR	669 ARG						
Kanjone	125 ARG	126 HIS	205 GLU	206 GLU	209 SER	357 PHE	666 TYR	669 ARG						
Pongol	205 GLU	206 GLU	357 PHE	358 ARG	547 TYR	662 TYR	666 TYR							
Sterolin	125 ARG	126 HIS	128 TYR	154 TRP	205 GLU	206 GLU	357 PHE	666 TYR	669 ARG					
Pinnantin	125 ARG	126 HIS	205 GLU	206 GLU	209 SER	357 PHE	630 SER	662 TYR	666 TYR	669 ARG	710 ASN			
Quercetin	125 ARG	205 GLU	206 GLU	209 SER	357 PHE	358 ARG	547 TYR	662 TYR	669 ARG	710 ASN				
Sitagliptine	125 ARG	205 GLU	206 GLU	207 VAL	209 SER	357 PHE	358 ARG	547 TYR	630 SER	662 TYR	666 TYR	710 ASN	740 HIS	

Based on the results of the In-silico screening analysis it was concluded that the compound's such as Beta Sitosterol, Glabrin, Kanjone, Pongol, Sterolin, Pinnantin and Quercetin present in the siddha formulation PPC may possess significant anti-diabetic property by inhibition of target Dipeptidyl peptidase-4 enzyme as shown in table 3 and fig 4.

4. Discussion

Docking is a modern scientific approach which involves the prediction of valuable lead towards specific drug target. Enzyme being a primary target in the pathology of most dreadful disease either under expression or over expression of this precursor involves change in normal physiology. Docking fundamentally works behind the logic of target (enzyme/protein) lead (drug) interaction. Most of the drug acts either by antagonistic or agonistic action. Both of these mechanisms of drugs rely on binding of functional group present in the drug with the biologically active amino acid present in the target protein. Hence drug likeness is the most important property to predict the mode of bind of drug with that of the receptor [19].

Naturally occurring compounds, known as phytochemicals (phyto means plant in Greek) are thought to be largely responsible for the protective health benefits of these plant-based foods and beverages, beyond those conferred by their vitamin and mineral contents. These phytochemicals, which are part of a large and varied group of chemical compounds, also are responsible for the color, flavor, and odor of plant foods, such as blueberries' dark hue, broccoli's bitter taste, and garlic's pungent odor. Research strongly suggests that consuming foods rich in phytochemicals provides health benefits, but not enough information exists to make specific recommendations for phytochemical intake.

Identification of active site on to the surface of the target seems to be significant step as this predicts the actual docking score of the molecule. In recent time various online tools available to predict the drug likeness, ADMET pathway, BBB crossing including structural activity relationship of the potential of the lead with high accuracy The reason for which the docking is considerable important as it aids in identification of promising lead by involving logical application, active site prediction, mode of drug action and above all it narrow down the research by decreasing the time spent on need less molecules [20].

The result of binding interactions of the ligand with Dipeptidyl peptidase-4 has revealed that out of seven compounds docked against PDB 2P8S. The lead Beta Sitosterol has shown highest inhibition property similar to that of the standard Sitagliptine. The second highest docking interaction possessed by quercetin followed by this pinnantin, pongol, glabrin, kanjone and sterolin with any most significant amino acid residues on the target Dipeptidyl peptidase-4.

5. Conclusion

In recent times docking techniques used widely for screening novel leads to optimize the potential therapeutic leads for validation In-vivo. Docking provides some evidence based data's on binding sites of the leads in target protein from the researcher can arrives the conclusion whether the lead act as agonist or antagonist against the selected target. According to the literature the most active amino acid residue involved in the DDP-4 enzymatic action are Try226, Glu205, Glu206,Try547, Try667, Asn710, Val711, His740, Ser630, Ser209, Arg358, Phe357, and Val207. The results of the present investigation clearly shows that all the seven compound screened Insilco has tendency to binding with these significant active site and hence from this it was concluded that the bioactive phytocomponents present in the formulation *Pungampoo Chooranam* (PPC) possess promising anti-diabetic activity and may also be effective in clinical management of type I and II diabetes.

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