In-silico screening of Alpha Amylase Enzyme Inhibitors from Siddha Formulation Pungampoo Chooranam by Molecular docking analysis for the management of Type II Diabetes mellitus

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Abstract
Diabetes Mellitus is a major health care problem in India with prevalence of about 66.8 million and it was expected to reach 80 million by the year 2025. Type II diabetes (T2DM) accounts for the majority of all diagnosed cases of diabetes in the world with its prevalence increasing sharply in recent decade. The inhibition of alpha-amylase an enzyme involved in the digestion of carbohydrates, can significantly reduce the post-prandial increase of blood glucose and therefore can be an important strategy in the management of blood glucose level in type II diabetic and borderline patients. The main aim of the present investigation is to screen the alpha amylase enzyme inhibition potential of novel phytocomponents such as Beta Sitosterol, Glabrin, Isolonchocarpin, Kanjone, Pongol, Sterolin, Pinnatin and Quercetin present in the formulation Pungampoo Chooranam (PPC) against target protein alpha amylase receptor with PDB code 1HNY along with the standard acarbose using computational docking analysis. The results of the study indicate that the lead pinnatin has shown highest inhibition property similar to that of the standard acarbose. The second highest docking interaction possessed by beta sitosterol followed by this Isolonchocarpin, kanjone , pongol and quercetin. The leads such as glabrin and sterolin has no interaction with any of the significant amino acid residues on the target alpha amylase. Based on the results of the In-silico screening analysis it was concluded that the compound’s such as pinnatin, beta sitosterol , Isolonchocarpin, kanjone ,pongol and quercetin present in the siddha formulation PPC may possess significant anti-diabetic property by inhibition of target enzyme alpha amylase.

Keywords: Diabetes Mellitus, Pungampoo Chooranam, alpha amylase, anti-diabetic, Beta Sitosterol, Glabrin, Isolonchocarpin, Kanjone, Pongol, Sterolin, Pinnatin, Quercetin
1. Introduction

Diabetes is multifactorial and has been associated with various disorders including obesity, dyslipidemia, thrombosis, infarction, hypertension, endothelial dysfunction, and coronary artery disease. The global prevalence of diabetes mellitus is forecast to reach 300 million by 2025, and over three quarters of the deaths amongst this population will be expected to result from cardiovascular disease [1]. Individuals with diabetes are at a significantly greater risk of developing both micro- and macrovascular disease and have a cardiac mortality equivalent to that in nondiabetic patients with confirmed heart disease [2].

Type II diabetes is one of the primary threats to human health due to increasing prevalence, chronic course and disabling complications [3]. Many diverse therapeutic strategies for the treatment of Type II diabetes are in use. The conventional available therapies for diabetes include stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues, oral hypoglycemic agents, such as biguanids and sulfonylureas and the inhibition of degradation of dietary starch by glycosidases such as α-amylase and α-glucosidase [4].

Pancreatic α-amylase inhibitors offer an effective strategy to lower the levels of post-prandial hyperglycemia via control of starch breakdown. Pancreatic α-amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to a mixture of smaller oligosaccharides consisting of maltose, maltotriose, and a number of α-(1-6) and α-(1 - 4) oligoglucans. These are then acted on by α-glucosidases and further degraded to glucose which on absorption enters the blood-stream.

Indian medicinal plants used in the siddha system of traditional medicine to treat diabetes become a potential source of novel anti-diabetic agents. The primary healthcare benefits of using plant-derived siddha formulations are relatively safer when compared to allopathic drugs and offer profound therapeutic benefits [5]. Single and polyherbal preparations have diverse range of bioactive molecules and play a dominant role in the maintenance of human health since ancient times [6]. More than 1500 herbal preparations are sold as dietary supplements or ethnic traditional medicines [7].

*Pongamia pinnata* (Fabaceae) is popularly known as Indian beech in English [8]. Commonly known by its vernacular names 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 [9]. Further, reports available that concomitant administration of synthetic oral hypoglycemic drugs along with *P. pinnata* produced synergistic effect in diabetic mice[10]. The preliminary phytochemical analysis showed the presence of alkaloids, terpenoids, triterpenes, flavonoids, steroids, and volatile oils [11]. It has been identified that Cycloart-23-ene-3β, 25-diol (B2) isolated from the stem bark of *P. pinnata* possesses antidiabetic activity in diabetic animals [12, 13]. Cycloart-23-ene-3β, 25-diol improved the abnormalities of diabetic conditions in diabetic mice due to increased glucagon-like peptide 1 (GLP-1) insulin secretion [14] and has a protective effect on vital organs like heart and kidney [15].

Computer aided drug discovery attains greater importance mainly because of the reliability in the results and also paves a new way for the research focus towards the alternative animal models. Molecular Docking continues to hold great promise in the field of Computer based drug design that screens small molecules by orienting and scoring them in the binding site of a protein. As a result novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions. Dock score was used to estimate the ligand-binding energies. Apart from these, other input parameters for docking are also considered for evaluating the compounds inhibition efficacy. It is estimated that docking programs currently dock 70 – 80% of ligands correctly [16]. The main aim of the present investigation is to screen the anti-diabetic potential of phytocomponents
such as Beta Sitosterol, Glabrin, Isolonchocarpin, Kanjone, Pongol, Sterolin, Pinnatin and Quercetin present in the formulation Pungampoo Chooranam (PPC) against target protein alpha amylase receptor with PDB code 1HNY along with the standard acarbose 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 [17]

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 1HNY and to study the binding energy properties with the following lead component such as Beta Sitosterol, Glabrin, Isolonchocarpin, Kanjone, Pongol, Sterolin, Pinnatin and Quercetin along with standard Acarbose. Human alpha amylase receptor with PDB code 1HNY 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 alpha amylase using online DOCKING SERVER web tool module.

2.5. Ligand preparation

The ligands such as Beta Sitosterol, Glabrin, Isolonchocarpin, Kanjone, Pongol, Sterolin, Pinnatin and Quercetin along with standard Acarbose 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

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the Compounds</th>
<th>Molar weight g/mol</th>
<th>Molecular Formula</th>
<th>H Bond Donor</th>
<th>H Bond Acceptor</th>
<th>Rotatable bonds</th>
<th>Log P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Beta Sitosterol</td>
<td>414.718 g/mol</td>
<td>C29H50O</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>9.3</td>
</tr>
<tr>
<td>2</td>
<td>Acarbose</td>
<td>645.608 g/mol</td>
<td>C25H43NO18</td>
<td>14</td>
<td>19</td>
<td>9</td>
<td>-8.5</td>
</tr>
<tr>
<td>3</td>
<td>Glabrin</td>
<td>175.184 g/mol</td>
<td>C7H13NO4</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>-3.5</td>
</tr>
<tr>
<td>4</td>
<td>Isolonchocarpin</td>
<td>306.361 g/mol</td>
<td>C20H18O3</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>3.8</td>
</tr>
<tr>
<td>5</td>
<td>Kanjone</td>
<td>292.29 g/mol</td>
<td>C18H12O4</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>3.6</td>
</tr>
<tr>
<td>6</td>
<td>Pongol</td>
<td>292.29 g/mol</td>
<td>C18H12O4</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>3.6</td>
</tr>
<tr>
<td>7</td>
<td>Sterolin</td>
<td>576.859 g/mol</td>
<td>C35H60O6</td>
<td>4</td>
<td>6</td>
<td>9</td>
<td>7.7</td>
</tr>
<tr>
<td>8</td>
<td>Pinnatin</td>
<td>292.29 g/mol</td>
<td>C18H12O4</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>3.6</td>
</tr>
<tr>
<td>9</td>
<td>Quercetin</td>
<td>302.238 g/mol</td>
<td>C15H10O7</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>1.5</td>
</tr>
</tbody>
</table>
2.5. 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 1HNY target protein is identified using grid space of 1 and probe of radius 5.0 angstrom [18]. Ligand site prediction was performed by using online tool GHECOM and the respective pockets calculations [19,20].

2.6. Docking Methodology

Docking calculations were carried out using Docking Server [21,22]. 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, Isolonchocarpin, Kanjone, Pongol, Sterolin, Pinnatin and Quercetin along with standard Acarbose and their binding affinity towards the target protein with PDB 1HNY 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 [23]. 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 [24].

3. Results

3.1. Dock scores

The result of binding interactions of the ligand with alpha amylase has revealed that out of eight compounds docked against PDB 1HNY. The lead pinnatin has shown highest inhibition property similar to that of the standard acarbose. The second highest docking interaction possessed by beta sitosterol followed by this Isolonchocarpin, kanjone, pongol and quercetin. The leads such as glabrin and sterolin has no interaction with any of the significant amino acid residues on the target alpha amylase as shown in table 2 and fig 4.
Table 2: Summary of the molecular docking studies of compounds against Alpha amylase receptor

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the Compounds</th>
<th>Binding Free energy Kcal/mol</th>
<th>Inhibition constant Ki µM (*mM)</th>
<th>Electrostatic energy Kcal/mol</th>
<th>Intermolecular energy Kcal/mol</th>
<th>Total Interaction Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Beta Sitosterol</td>
<td>-8.18</td>
<td>1.01</td>
<td>-0.01</td>
<td>-9.67</td>
<td>807.19</td>
</tr>
<tr>
<td>2</td>
<td>Acarbose</td>
<td>-9.34</td>
<td>143.4*</td>
<td>-0.05</td>
<td>-11.23</td>
<td>802.44</td>
</tr>
<tr>
<td>3</td>
<td>Glabrin</td>
<td>-4.26</td>
<td>752.7</td>
<td>-0.95</td>
<td>-5.32</td>
<td>393.48</td>
</tr>
<tr>
<td>4</td>
<td>Isolonchocarpin</td>
<td>-6.25</td>
<td>26.27</td>
<td>-0.31</td>
<td>-8.83</td>
<td>753.68</td>
</tr>
<tr>
<td>5</td>
<td>Kanjone</td>
<td>-6.22</td>
<td>27.38</td>
<td>-0.11</td>
<td>-8.95</td>
<td>909.2</td>
</tr>
<tr>
<td>6</td>
<td>Pongol</td>
<td>-5.98</td>
<td>41.69</td>
<td>-6.42</td>
<td>-6.55</td>
<td>570.38</td>
</tr>
<tr>
<td>7</td>
<td>Sterolin</td>
<td>-8.55</td>
<td>540.42*</td>
<td>-0.05</td>
<td>-10.75</td>
<td>1081.66</td>
</tr>
<tr>
<td>9</td>
<td>Pinnantin</td>
<td>-5.73</td>
<td>63.56</td>
<td>-0.13</td>
<td>-6.29</td>
<td>627.76</td>
</tr>
<tr>
<td>10</td>
<td>Quercetin</td>
<td>-6.25</td>
<td>26.20*</td>
<td>-0.38</td>
<td>-6.8</td>
<td>591.56</td>
</tr>
</tbody>
</table>

Fig 4: Possible ligand binding pockets on the surface of target Alpha amylase receptor with PDB code 1HNY. Pockets calculated by GHECOM.

3.2 Amino acid interaction Study

The core amino acids responsible for alpha amylase enzyme activity was found to be Trp 58, Trp 59, Tyr 62, His 101, Leu 162, Arg 195, Asp 197, Ala 198, Ser 199, Lys 200, His 201, Glu 233, Asp 300. It was observed from the present investigation that the lead molecules such as pinnatin, beta sitosterol, Isolonchocarpin, kanjone, pongol and quercetin has a tendency to interact with above mentioned amino acid residues responsible for enzyme inhibition potential of the formulation PPC. The results were shown in table 3.
### Table 3: Interaction of lead compounds with active site amino acid residue of Alpha amylase Receptor

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amino Acid Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta Sitosterol</td>
<td>59 PRO 60 PRO 61 ASN 63 ASN 79 PRO 94 PHE 110 VAL 112 VAL 113 VAL 114 ILE 197 TYR 198 MET 201 LEU 202 ILE</td>
</tr>
<tr>
<td>Acarbose</td>
<td>59 PRO 61 ASN 79 PRO 82 TYR 110 VAL 111 ASP 112 VAL 113 VAL 114 ILE 194 ILE 197 TYR 198 MET 201 LEU</td>
</tr>
<tr>
<td>Glabrin</td>
<td>30 HIS 56 GLN 111 ASP 210 ARG 248 GLU 269 THR 271 PHE 310 PHE 313 ASN 314 HIS 315 ASP 352 ARG</td>
</tr>
<tr>
<td>Isolonchocarpin</td>
<td>59 PRO 60 PRO 61 ASN 63 ASN 79 PRO 82 TYR 110 VAL 111 ASP 112 VAL 113 VAL 114 ILE 197 TYR 201 LEU 209 PHE</td>
</tr>
<tr>
<td>Kanjone</td>
<td>30 HIS 32 PHE 56 GLN 58 SER 59 PRO 77 TYR 111 ASP 210 ARG 310 PHE 313 ASN 314 HIS 352 ARG 354 MET</td>
</tr>
<tr>
<td>Pongol</td>
<td>59 PRO 60 PRO 61 ASN 63 ASN 76 ARG 79 PRO 82 TYR 110 VAL 112 VAL 113 VAL 114 ILE 194 ILE 197 TYR</td>
</tr>
<tr>
<td>Steroln</td>
<td>30 HIS 56 GLN 210 ARG 246 TYR 248 GLU 269 THR 271 PHE 310 PHE 311 VAL 312 ASP 313 ASN 314 HIS 315 ASP 336 TYR</td>
</tr>
<tr>
<td>Pinnatin</td>
<td>112 ALA 114 ILE 194 ILE 197 TYR 198 MET 201 LEU 209 PHE 211 LEU 214 SER 217 MET 222 ILE 225 ILE 226 LEU</td>
</tr>
<tr>
<td>Quercetin</td>
<td>17 TYR 198 MET 209 PHE 211 LEU 226 LEU 229 LEU 242 LYS 243 PRO 244 PHE 245 ILE</td>
</tr>
</tbody>
</table>


4. Discussion

Diabetes is multifactorial and has been associated with various disorders including obesity, dyslipidemia, thrombosis, infarction, hypertension, endothelial dysfunction, and coronary artery disease [25]. However, diastolic and systolic dysfunctions are mainly altered apart from traditional cardiac risks parameters such as hypertension, atherosclerosis, and dyslipidemia [26]. Diabetic patients have an increased risk of cardiovascular diseases and these are the major cause of death in them [27, 28]. Cardiomyopathy is a prevalent cause of death in patients with diabetes [29]. The world prevalence of diabetes among adults (aged 20–79 years) will be 6.4%, affecting 285 million adults, in 2010 and will increase to 7.7%, affecting 439 million adults, by 2030 [30,31].

Search of alternate therapy from the natural origin is a never ending process in this view herbs become the predominant source for the supply of valuable therapeutic leads with multiple components and may render versatile pharmacological action against several diseases in recent times. Use of plants as a source of medicine has been inherited and is an important component of the health care system. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world [32]. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [33].

Siddha system of medicine become popular throughout the world in recent days as it claims high curative value with minimum toxicity and have less side effects. It has been estimated that 70-80% of world’s population relies on traditional healthcare. The mode of preparation and plant used in traditional medicine varies from place to place. In addition acceptance of traditional medicines, especially herbal medicines in the developed world is sharply increasing [34].

Molecular docking has become an increasingly important tool for drug discovery. In this review, we present a brief introduction of the available molecular docking methods, and their development and applications in drug discovery. The relevant basic theories, including sampling algorithms and scoring functions, are summarized. The differences in and performance of available docking software are also discussed. Flexible receptor molecular docking approaches, especially those including backbone flexibility in receptors, are a challenge for available docking methods [35].

The result of binding interactions of the ligand with alpha amylase has revealed that out of eight compounds docked against PDB 1HNY. The lead pinnatin has shown highest inhibition property similar to that of the standard acarbose. The second highest docking interaction possessed by beta sitosterol followed by this Isolonchocarpin, kanjone, pongol and quercetin. The leads such as glabrin and sterolin has no interaction with any of the significant amino acid residues on the target alpha amylase.

The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes [36]. The docking process involves two basic steps: prediction of the ligand conformation as well as its position and orientation within these sites (usually referred to as pose) and assessment of the binding affinity. These two steps are related to sampling methods and scoring schemes, respectively, which will be discussed in the theory section.

5. Conclusion

From the results of the In-silico screening analysis it was concluded that the compound’s such as pinnatin, beta sitosterol, Isolonchocarpin, kanjone, pongol and quercetin present in the siddha formulation Pungampoo Chooranam (PPC) may possess significant anti-diabetic property by inhibition of target enzyme alpha amylase. It is evident that effective inhibition of the enzyme pancreatic α-amylase offers an effective strategy...
to lower the levels of post-prandial hyperglycemia via control of starch breakdown. Hence it was concluded that the formulation.

PPC may be used for the clinical management of T2DM because of its biologically significant phytotherapeutics which acts synergistically by inhibiting the key enzymes involved in the regulation of blood glucose level.

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References


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