Aldose reductase inhibitors from the fruits of *Physalis peruviana* Linn.- An In silico Approach

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**ABSTRACT**

**Background:** The fruits of *Physalis peruviana* Linn. have been widely used in the traditional medicine for various ailments. Recently, we have reported that the oral administration of *Physalis peruviana* Linn. fruit extract improves insulin sensitivity and ameliorates hyperglycemia in high-fat diet low dose STZ-induced type 2 diabetic rats. Molecular docking, the technique primarily employed for predicting and analyzing the interactions between protein receptors and ligands, is now an integral aspect in drug discovery and development area. Hence, the present study was aimed to screen the identified phytoconstituents of *Physalis peruviana* L. fruit to identify the potent constituent attributing for its antidiabetic activity using insilico approach. **Methods:** HPLC analysis of the ethanolic extract of fruits was performed using Shimadzu HPLC system equipped with a diode array detector. Docking studies on the constituents were carried out using AutoDock 4.2 software against the aldose reductase receptor. **Results:** HPLC analysis indicated that the major flavonoids present in the fruit extract include kaempferol, myricetin, quercetin and rutin and their binding energy was found to be -12.89, -12.99, -13.31, -14.84, respectively. The activity was comparable with fidarestat, a standard drug. **Conclusion:** The study indicates the presence of biologically active flavonoids in the fruit extract and their interactions with aldose reductase. The insilico data provide vital clues that can be used to design new molecules with improved activity for the successful treatment of diabetes mellitus.

**KEY WORDS:** *Physalis peruviana* L. fruit, aldose reductase, kaempferol, myricetin, quercetin, rutin.

**INTRODUCTION**

Molecular docking is an *In silco* technique widely employed for predicting and analyzing the interactions between protein receptors and ligands. It provides most detailed possible view of drug receptor interactions and also has created a new rational approach to drug design. Diabetes mellitus (DM), the third leading cause of death in the world, is a multifactorial, multisystemic, metabolic disorder arises due to deficiency and/or efficiency of insulin. Most of the currently available drugs for the treatment of diabetes often elicit undesirable side effects after prolonged use. Hence, search for novel therapeutic agents without side effects continues. Among the various factors responsible for the initiation and progression of secondary complications in diabetes, the activation of polyol metabolic pathway was first discovered and in fact is the generally accepted to be the mechanism of prime importance in the pathogenesis of diabetic complications.

Aldose reductase (ALR2, EC 1.1.1.21) is a cytoplasmic, NADPH – dependent monomeric enzyme belongs to the superfamily aldo-ke-toreductase (AKR). It contains 315 aminoacids with a molecular weight of 36K Da. It is found to be present in most human cells ALR2 together with sorbitol dehydrogenase (SDH) forms the polyol pathway wherein AR initially catalyzes the NADPH-dependent reduction of the aldehyde form of glucose to form sorbitol. SDH then utilizes NAD oxidizes the intermediate sorbitol to fructose.

Normally, the cellular glucose is oxidatively metabolized through the glycolytic pathway and then the Krebs cycle to produce energy for cells. Under chronic hyperglycemia conditions, however, the increased amount of glucose activates ALR2 and is metabolized by the activated polyol pathway. The increased flux of the polyol pathway by chronic hyperglycemia is implicated in the pathogenesis of diabetic complications including particularly diabetic retinopathy, nephropathy and neuropathy and one of the vital linkages has been proposed to be an increase in oxidative stress mediated by free radicals. Biomolecular evidences for the role of the polyol pathway are recently provided by cellular experiments and regulations of AR gene expression in the presence of high glucose induction. Intense
efforts have been directed towards the development of effective aldose reductase inhibitors, but still only ‘epalrestat’ is commercially available and that too only in Japan. Thus, it is becoming of great importance, novel chemotypes to be developed, lacking the poor pharmacokinetic profile or the side effects of the ARIs that have failed to the clinical trials.

Our traditional systems of medicines have a wide variety of plant preparations, which may act as potential drugs or medicines for effective treatment of various diseases. However, most of the medicinal plants used in the traditional medicine lack scientific scrutiny. Physalis peruviana Linnaeus is one such medicinal plant, widely used herb in folk medicine for various ailments. It is a native plant from the Peruvian Andes and now widely distributed throughout the tropical and sub-tropical countries. The botanical name of the plant is Physalis peruviana L., belonging to the family Solanaceae and genus Physalis. In the folk medicine, the fruits are used as a means to improve the immune system of humans. Wu et al., (2006) have reported that the ethanolic extract of the fruits possess significant antioxidant properties than the aqueous extract. Furthermore, the antioxidant activity associated with presence of high levels of polyphenols and significant levels of vitamin A and C. Recently, we have reported the effect of oral administration of the ethanolic extract of Physalis peruviana L. fruits in improving Insulin sensitivity and amelioration of hyperglycemia in high fat diet low dose STZ induced type 2 diabetes in rats. The present study was aimed to evaluate the inhibitory effect of myricetin, kaempferol, quercetin and rutin, the major flavonoids present in the fruit extract against ALR2 activity by molecular docking studies.

MATERIALS AND METHODS

Plant Material
Intact, fruits of Physalis peruviana were collected from Theni, Tamilnadu, India. The fruits were carefully selected according to the degree of ripeness measured by fruit color (brilliant orange). The plants were identified and authenticated by a qualified taxonomist and a voucher specimen was deposited at Centre of Advanced Studies in Botany, University of Madras.

Preparation of plant extract
The fruits were dehusked, washed, crushed in an air oven at 50°C then powdered in an electrical grinder, which was then stored in an airtight brown container at 5°C until further use. The powdered fruits were delipidated with petroleum ether (60-80°C) for overnight. It was then filtered and soxhlation was performed with 95% Ethanol. Ethanol was evaporated in a rotary evaporator at 40–50°C under reduced pressure.

HPLC–DAD system for analysis of phenolic compounds
HPLC analysis was performed using Shimadzu HPLC system equipped with a diode array detector. The chromatographic separations were performed on an Inertsil C18 analytical column (4.6 × 250 mm i.d., 5 μm). The composition of solvents and the gradient elution conditions used were described previously by Bengoechea et al., (1997), Schieber et al., (2001) and Butsat et al., (2009), with some modifications. The mobile phase consisted of purified water with acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 ml/min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5% to 9% solvent B; from 5 to 15 min, 9% solvent B; from 15 to 22 min, linear gradient from 9% to 11% solvent B; from 22 to 38 min, linear gradient from 11% to 18% solvent B; from 38 to 43 min, from 18% to 23% solvent B; from 43 to 44 min, from 23% to 90% solvent B; from 44 to 45 min, linear gradient from 90% to 80% solvent B; from 45 to 55 min, isocratic at 80% solvent B; from 55 to 60 min, linear gradient from 80% to 5% solvent B and a re-equilibration period of 5 min with 5% solvent B used between individual runs. Operating conditions were as follows: column temperature, 38°C, injection volume, 20 μl, and UV-diode array detection at 280 nm (hydroxybenzoic acids), 320 nm (hydroxycinnamic acids) and 370 nm (flavonols) at a flow-rate of 0.8 ml/min. Spectra were recorded from 200 to 600 nm. Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of authentic compounds and were detected using an external standard method.

Aldose reductase Molecular docking studies

Protein Preparation
The protein target, which is retrieved from the RCSB Protein Data Bank (PDB ID: 1PWM) serves as docking receptor. All the bound ligands and water molecules were removed from the active site of the receptor. For docking target, crucial amino acids of the active site were identified using data in pdb sum available in the PDB. The chemical structures of ligands used in the present study were shown in figure 1.

![Figure 1](image-url)
Ligand Preparation
The two dimensional structures of ligands were drawn using ChemDraw Ultra 12.0 and then converted to 3D (.pdb) structures using Openbabel software tool. The crystal structure (PDB ID: 1PWM) of the standard ligand, (Fidarestat) was taken for molecular docking. The 3D structures of both ligands and the standard were submitted to PRODRG server for energy minimization.

Molecular Docking
The molecular docking was performed and analyzed using AutoDock 4.2. A Lamarckian genetic algorithm method implemented in the program suite was employed to identify appropriate binding modes and conformation of the ligand molecules. Gasteiger charges were added and the rotatable bonds were set by the AutoDock tools and all torsions were allowed to rotate. Polar hydrogen atoms were added and Kollman charges were assigned to the protein using AutoDock tools (ADT). The grid map was centered at the active site pocket of the protein by Autogrid. The grid map which was centered at the following amino acids residues of the protein (Trp20, Tyr40, Trp111 and Leu300) was predicted from the poseview data available in the PDB site. In all the cases, the grid maps with a grid box size of 60x60x60 Å³ points with a grid-point spacing of 0.375Å was used. During docking, centre grid parameters were specified for x, y and z axis as 16.9, -7.8 and 16.7, respectively. The Lamarckian genetic algorithm, the pseudo-Solis and Wets methods were applied for minimization using default parameters. Binding energy, torsional energy, intermolecular energy, number of H-bonds and RMS value were recorded in each ligand bound conformations.

RESULTS
The HPLC analysis of the purified fraction of isolated components have similar retention times (approximately Rutin 6.94 min, Myricetin 11.40 min, Quercetin 9.68 min and Kaempferol 15.97 min) to the Rutin, Myricetin, Quercetin and Kaempferol standards (Figure 2 and 3).

Auto dock has computed the lowest score for the docking. From the table 1, it was observed that the docking of the ligands, Kaempferol, myricetin, quercetin and rutin together with the receptor crystal structure of human aldose reductase and fidarestat shows the atomic contact energy: Kaempferol(A) -12.89, myricetin (B) -12.99, quercetin(C) -13.31, rutin(D) -14.84 and native fidarestat (E) -11.54. From the table 2, it was observed that the ligands with the interacting residues that shows: Kaempferol (A): TYR48, CYS298, LEU301, myricetin (B): TYR48, ASN160, TYR209, CYS298, LEU301, quercetin (C): TYR48, ASN160, TYR209, CYS298, LEU301, rutin (D): ALA299, LEU300, SER302 and fidarestat(native) (E): LEU300, SER302
Table 1. Binding free energies for all the ligands with the receptor Crystal structure of human Aldose Reductase and Fidarestat PDB ID: 1PWM.

<table>
<thead>
<tr>
<th>Molecule Name</th>
<th>Estimated Free Energy of Binding (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaempferol</td>
<td>-12.89</td>
</tr>
<tr>
<td>Myricetin</td>
<td>-12.99</td>
</tr>
<tr>
<td>Quercetin</td>
<td>-13.31</td>
</tr>
<tr>
<td>Rutin</td>
<td>-14.84</td>
</tr>
<tr>
<td>Fidarestat</td>
<td>-11.54</td>
</tr>
</tbody>
</table>

Table 2. The hydrogen bond interacting residues of the receptor PDBID: 1PWM for the lowest binding free energy.

<table>
<thead>
<tr>
<th>Molecule Name</th>
<th>Number of interacting hydrogen bonds</th>
<th>The interacting residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaempferol</td>
<td>5</td>
<td>TYR48, CYS298, LEU301</td>
</tr>
<tr>
<td>Myricetin</td>
<td>9</td>
<td>TYR48, ASN160, TYR209, CYS298, LEU301</td>
</tr>
<tr>
<td>Fidarestat</td>
<td>3</td>
<td>LEU300, SER302</td>
</tr>
<tr>
<td>Quercetin</td>
<td>7</td>
<td>TYR48, ASN160, TYR209, CYS298, LEU301</td>
</tr>
<tr>
<td>Rutin</td>
<td>7</td>
<td>ALA299, LEU300, SER302</td>
</tr>
</tbody>
</table>

Figure 4. Interacting residues with the ligand [Kaempferol (A), Myricetin (B), Quercetin (C), Rutin (D) and Fidarest (E)] in 3D with hydrogen bond.
Figure 5. Two-dimensional interactions for ligand [Kaempferol (A), Myricetin (B), Quercetin (C), Rutin (D) and Fidarest (E)] with interacting residues of the receptor.
DISCUSSION

Extracts of medicinal plants are known to contain different chemopreventive or chemotherapeutic compounds, which possess more than one mechanism of actions. The bioactive components such as kaempferol, myricetin, quercetin and rutin present in the fruit of *Physalis peruviana* L. make this to be considered as a potent source for the development of novel drugs for the treatment of various diseases.

Ever since the structure and the pathogenic effects of ALR2 associated with polyol pathway was identified in the lens by Kinoshita (1965) and these works formed the basis for the osmotic stress hypothesis of sugar cataract formation.

The conversion of glucose to sorbitol catalyzed AR was first identified in 1956 by Hers in the seminal vesicles where glucose is converted to fructose to provide energy source for sperms.

Aldolase reductase inhibitors (ARIs) have been shown to prevent or slow the progression of DM associated pathologies, such as neuropathy, retinopathy, cataracts and nephropathy both in animal models and human. The majority of the ARIs reported belong to two classes namely carboxylic acid and spiroimide derivatives.

In the 40 years of ARIs research and after many clinical candidates only one, epalrestat, is currently commercially for the treatment of
diabetic neuropathy, although its use is limited in Japan. In fact, sorbinil, which was the first extensively tested ARI with good activity both in vitro and in vivo, was withdrawn from clinical trials because of hypersensitivity reactions that were related to its hydantoin ring

Indirestat, a fluorene analog of sorbinil, also showed appreciable in vivo activity but was discontinued because of its toxicity. Similarly, ARIs such as alrestatin, epalrestat, Zenarestat, Zopolrestat and ponalrestat were also discontinued due to their hepatotoxicity.

In the search for new ARIs as potent drug candidates, identifying inhibitors endowed with good capability to cross biomembranes as well as devoid of serious unwanted effects is the main challenge for medicinal chemists. Most of the lead molecules tested so far yields disappointing results and cellular permeability remains a major hurdle in the development of ARIs as drugs. Recently, there is growing interest in the discovery of natural products or plant extracts with ARI activity.

The data obtained through docking studies revealed that the tested molecules have binding free energy that are almost close to each other (Table 2), except myricetin, since it gives 9 interacting hydrogen bonds and hydrophobic interaction from the residues: TRP 219, CYS 298 and LEU 300. A π-cation interaction present in the center of an aromatic ring is closer to 4.5Å to a carbon cation [Figure 4 (B), 5(B)]. π-π interactions shown in the centers of two aromatic rings of myricetin ligand and protein are closer to 5.0Å. Further, the stability is increased by the presence of the two π-π interaction between the ligand and the binding site residues TRY 20 and TRY 219 are found to be the reason for more stability of myricetin. Figure 7 represents the possible mechanism of action of the major flavonoids present in the Physalis peruviana L. fruit extract in inhibiting the aldose reductase in polyol pathway.

CONCLUSION
In conclusion, the present study clearly indicate that the flavonoids especially myricetin present in the Physalis peruviana fruit extract mediated the beneficial as well as pharmacological effects at least in part of alleviating the primary as well as secondary complications of diabetic mellitus. Studies are in progress to determine the effect of individual flavonoids present in the fruit extract on the levels of various biochemical and immunological parameters associated with diabetes mellitus.

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