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INSILICO DOCKING ANALYSIS OF KAEMPHEROL AND LUTEOLIN AGAINST DIABETIC DRUG TARGET PEPCK1

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ABSTRACT

The fast and electronic gadgetic, lifestyle have led mankind to a complicated mesh leading to many diseases that passively spreads into the population producing an unhealthy society. Diabetes is one such disease that is gaining ground due to eating habits and sedentary lifesyte. Even though there are many pharmaceutical products available many patients prefer to revert back to the slow healthy approach integrated with natural therapeutics. In recent years, the demands on drug discovery process have increased dramatically, partly because of the necessity to recognize novel target that are both pertinent to disease and chemically tractable. The emergence of bioinformatics gives room to investigate diseases at the molecular level using computational techniques. In this study, some of the flavonoids present in the tamarind seed coat is used in the *insilico* docking analysis against phosphoenolpyruvate carboxykinase (PEPCK1). The phytochemical investigation of the methanolic extract of the tamarind seed coat was positive for Kaempherol and luteolin. These two flavonoids were selected for the *insilico* docking analysis against the target protein PEPCK1. The aim of the present study is to describe antidiabetic role of kaempherol and lately against PEPCK1.

KEYWORDS: phosphoenolpyruvate carboxykinase; luteolin; kaempherol; diabetes; flavonoids; *insilico* docking analysis.

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1.INTRODUCTION

Diabetes mellitus, one of the most common endocrine, metabolic disorders has caused significant morbidity and mortality due to microvascular (retinopathy,neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications[1]. From the past four decades the incidence of type 2 diabetes mellitus (T2DM) has been raised intensely. Type 2 diabetes mellitus has been described as:the resistance of insulin, hyperinsulinaemia and hyperglycaemia. Eminent hepatic gluconeogenesis producing surplus of glucose through aberrantly way is the vital indicator of hyperglycaemia in type 2 diabetes mellitus.[2,3] There is clinical correlation to the insulin resistance causing the increase of glucose level .This results due to the lack of inhibition of two enzymes involving in gluconeogenic process, phosphoenol pyruvatecarboxykinase (PEPCK1) and G6Pase (glucose-6-phosphatase) subunit. Several enzymes catalyzed the gluconeogenesis pathway, i.e., PEPCK. Insulin is considered the most significant hormone that play role in the inhibition of gluconeogenesis. Primarily, it acts by suppressing the expression PEPCK and G6Pase genes for the gluconeogenic enzymes. In the first rate limiting steps of gluconeogenesis, PEPCK catalyzes the reaction of oxaloacetic acid to phosphoenolpyruvate, and then in the final step of gluconeogenesis, G6Pase catalyzes the glucose 6-phosphate into glucose. Glucagon and glucocorticoids induced the expression of genes for PEPCK and G6Pase during fasting and periods of stress, respectively.[4–6] Plant materials have been used traditionally as medicine for treating ailments and maintaining health Tamarind or *Tamarindus indica* L. of the Fabaceae, subfamily Caesalpinoideae, is an important food in the tropics. It is a multipurpose tree of which almost every part finds atleast some use either nutritional or medicinal.[7] *Tamarindus indica* L. is one of the reported ancient herbal medicine plants.[8] The healing power of tamarind is first mentioned in the traditional Sanskrit literatures. In Europe, the medical properties of tamarind were well known after it has been introduced by the Arab traders. *Tamarindus indica* L. fruit is useful as an agent of antihelmintic, antidiarrheal, and antiemetic.[9] Water extract of tamarind seed was found to reduce blood sugar level in Streptozotocin-induced diabetic male rats.[10] In addition, tamarind seeds known to have high inhibitory activity against human neutrophil elastase.[11] Human neutrophil elastase is release by neutrophil during inflammation but excessive production will lead to emphysema. Tamarind seed polysaccharide (TSP) has been shown to improve dry eye syndrome, to assist release of drug in the human body and intraocular penetration of Rufloxacin.[12,13] Herbal medicines are naturally occurring; plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices. For a long time, herbal medicines or their extracts have been used to cure various diseases,[14–16] because plant products are frequently considered to be less toxic and more free from side effects than synthetic ones.[17] Luteolin and kaempherol are plant-derived flavonoids, have a variety of biological activities including well-known anti-inflammatory,[18] antimutagenic, and antitumorigenic[19] properties. Moreover, it possesses direct antioxidant activity,[20] which may be useful in treatment of many chronic diseases associated with oxidative stress, such as cardiovascular diseases,[21] liver diseases,[22,23] diabetes,[24] and aging.[25] Glucose uptake and utilisation by peripheral tissues, such as liver, muscle, and fat, is crucial for maintaining normal blood glucose level. Many recent studies reveal that antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models[26,27] as well as reducing the severity of diabetic complications.[28] Flavonoids are abundant plant phenolic compounds. More than 6000 have been identified to date, and some have been shown to possess hypoglycemic and antidiabetic activities.[29]

Docking is a process by which one can predict the significant orientation of one molecule to a second when bound to each other to form a stable complex[30,31]Docking is mostly used for finding the binding between the ligand and the receptor. Hence, in drug designing docking plays a vital role.[32] Between the two molecules, the binding affinities strength is predicted using the preferred orientation. For docking we require 3D structure of the protein and ligands as the input, for which the bound conformation of the ligand with that of the protein active site is predicted.[33,34] We need a search algorithm which evolves new low energy conformations of the macromolecule with that of the micro among all the possible orientations. Genetic algorithm is one such algorithm. Large amount of ligands are there in the data bank, because of the exponential time complexity wet lab is not preferred first. Computer assisted drug design is preferred, and then go for wet lab only if there is good interaction between the ligands and the receptor. The docking job is done in dry lab using several commercial docking programs.[35] AutoDock is a suite of programmed docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure.[36]

2.MATERIALS AND METHODS

Dry healthy tamarind seeds were collected, cleaned and sun dried. The seed coat and cotyledons were then separated using mortar and pestle. The seedcoat was then finely powdered and the methanolic extract was prepared by using the the soxhlet apparatus.The dried extract was then subjected to HPLC analysis. the results were positive for luteolin and kaemperol.

2.1. Pubchem

PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information NCBI, a component of the National Library of Medicine, which is part of the United States National Institutes of Health NIH. PubChem can be accessed for free through a web user interface. Millions of compound structures and descriptive datasets can be freely downloaded via FTP. PubChem contains substance descriptions and small molecules with fewer than 1000 atoms and 1000 bonds. The American Chemical Society tried to get the U.S. Congress to restrict the operation of PubChem, because they claim it competes with their Chemical Abstracts Service.[37]http://pubchem.ncbi.nlm.nih.gov/

2.2. Uniprot

UniProt is a comprehensive, high-quality and freely accessible database of protein sequence and functional information, many entries being derived from genome sequencing projects. It contains a large amount of information about the biological function of proteins derived from the research literature. The UniProt/Swissprot Knowledgebase UniProtKB is the central access point for extensive curated protein information, including function, classification, and cross-reference. It consists of two sections: UniProtKB/Swiss-Prot which is manually annotated and is reviewed and UniProtKB/TrEMBL which is automatically annotated and is not reviewed. The UniProt Reference Clusters UniRef databases provide clustered sets of sequences from the UniProtKB and selected UniProt Archive records to obtain complete coverage of sequence space at several resolutions while hiding redundant sequences.[38] http://www.uniprot.org/.

2.3. PDB

The Protein Data Bank PDB is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the websites of its member organizations. The PDB is a key resource in areas of structural biology, such as structural genomics. Most major scientific journals, and some funding agencies, such as the NIH in the USA, now require scientists to submit their structure data to the PDB. If the contents of the PDB are thought of as primary data, then there are hundreds of derived i.e., secondary databases that categorize the data differently. For example, both SCOP and CATH categorize structures according to type of structure and assumed evolutionary relations;

GO categorize structures based on genes.[39] http://www.rcsb.org/pdb/home/home.do.

2.4. PFam

The Pfam database contains information about protein domains and families. Pfam-A is the manually curated portion of the database that contains over 10,000 entries. For each entry a protein sequence alignment and a hidden Markov model is stored. These hidden Markov models can be used to search sequence databases with the HMMER package written by Sean Eddy. Because the entries in Pfam-A do not cover all known proteins, an automatically generated supplement is provided called Pfam-B. Pfam-B contains a large number of small families derived from clusters produced by an algorithm called ADDA.[40]http://pfam.sanger.ac.uk

2.5. Autodock

Auto Dock is a suite of automated docking tools. The software is used for modelling flexible small molecule such as drug molecule binding to receptor proteins of known three dimensional structure. It uses Genetic Algorithms for the conformational search and is a suitable method for the docking studies. The technique combines simulated annealing for conformation searching with a rapid grid based method of energy evaluation. Auto Dock tools are used to prepare, run and analyze the docking simulations, in addition to modeling studies. Auto Dock is the most cited docking software because it is very fast, it provides high quality predictions of ligand conformations and good correlations between inhibition constants and experimental ones.[35] http://autodock.scripps.edu/resources/tools

2.6. Discovery Studio Visualizer

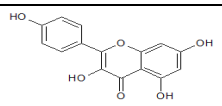
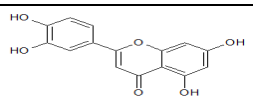
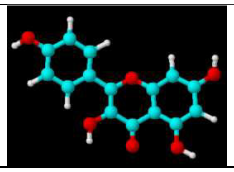
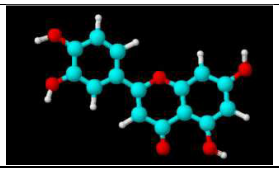
Molecular visualization is a key aspect of the analysis and communication of modeling studies. High Performance Publication Quality Graphics: andle very large macromolecule systems (E.g., Ribosomes); Support a range of stereo graphics options (E.g., split screen, hardware stereo); Hardware graphics acceleration support for a range of AMD(ATI) and nVidia cards; and Depth cueing, blur and shading capabilities.[41]

3. RESULTS AND DISCUSSION

3.1. Preparation of Ligands

HPLC analysis of tamarind seed coat shows positive results for flavonoids. Hence, for further docking analysis two flavanoids namely kaempherol and luteolin was selected. The two-dimensional structures of the ligands (kaempherol and luteolin) were generated using the ACD/ChemSketch tool. This software contains tools for 2D cleaning, 3D optimization, and viewing. These data are saved as a molecular format file (MDL MOL format). The molecular format converter tool (Open Babel) is used to convert this file into the PDB format and is used during docking analysis. The structure and molecular formula of inhibitors was shown in Table 1.

Table1
Structure of Inhibitors

	Kaempherol	**Luteolin**
Molecular Formula	**$C_{15}H_{10}O_6$**	**$C_{15}H_{10}O_6$**
2D Structure		
3D Structure		

3.2. Drug likeliness and Bioavailability

Lipinski rule states, that most "drug-like" molecules have logP <= 5, molecular weight <= 500, number of hydrogen bond acceptors <= 10, and number of hydrogen bond donors <= 5. Molecules violating more than one of these rules may have problems with bioavailability.[42,43] Lipinski's rule is called "Rule of 5," because the border values are 5, 500, 2*5, and 5. Drug likeliness and bioavailability analysis are shown in Table 2.

Table 2
Lipinski's rule of Kaempherol and Luteolin

..	Kaempherol	Luteolin
LIPINSKI'S RULE		
Molecular Weight (g/mol)	286.236	286.2363
XLogP3	1.9	1.4
H-Bond Donor	4	4
H-Bond Acceptor	6	6

3.3. Retrieval of Target Protein Sequence

The protein sequence for the PEPCK1 was obtained from the protein sequence data base of Uniprot (http://www.uniprot.org/uniprot/P35558). The source organism is *Homo sapiens*.

3.4. Domain Analysis

The functional analysis of PEPCK1 was predicted using the Pfam data base (http://www.pfam.sanger.ac.uk/). The predicted domain region is PEPCK (29–622) and shown in Figure 1.

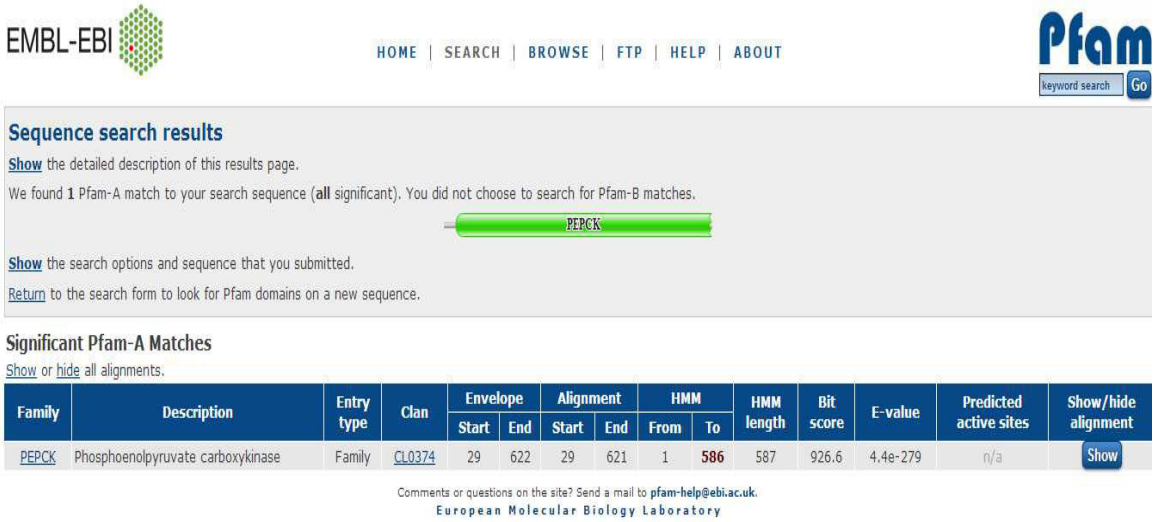


Figure 1
PFam Domain anaysis of PEPCK1 showing PEPCK domain

3.5. Structure Retrieval

The three-dimensional structure of PEPCK1 was available in the PDB database. The PDB id is 1KHB (A-chain). The 3D structure was visualized using the Rasmol Tool (shown in Fig. 2).

Figure 2
Three dimensional structure visualization of PEPCK1 using RASMOL

3.6. Docking of Luteolin and Kaempherol with PEPCK1

The inhibitors Flavonoids are one of the biologically active chemical constituents of plants. These natural products are readily available to man in the form of vegetables and fruits.[44] These flavonoids namely Luteolin and Kaempherol docked with PEPCK1 receptor using Autodock software (Version 4.2) . The Graphical User Interface program "Auto-Dock Tools" was used to prepare, run, and analyze the docking simulations. Kollman united atom charges, solvation parameters and polar hydrogens were added into the receptor PDB file for the preparation of protein in docking simulation. AutoDock[45–47] requires precalculated grid maps, one for each atom type present in the flexible molecules being docked and its stores the potential energy arising from the interaction with rigid macromolecules. This grid must surround the region of interest in the rigid macromolecule. The grid box size was set at 70, 70 and 70 A° (*x, y*, and *z*) to include all the amino acid residues that present in rigid macromolecules. AutoGrid 4.2 Program, supplied with AutoDock 4.2 was used to produce grid maps. The spacing between grid points was 0.375 angstroms. The Lamarckian Genetic Algorithm (LGA)[48] was chosen search for the best conformers. During the docking process, a maximum of 10 conformers was considered. The population size was set to 150 and the individuals were initialized randomly. Maximum number of energy evaluation was set to 500000, maximum number of generations 1000, maximum number of top individual that automatically survived set to 1, mutation rate of 0.02, crossover rate of 0.8, step sizes were 0.2 Å for translations, 5.0° for quaternions and 5.0° for torsions. Cluster tolerance 0.5Aº, external grid energy 1000.0, max initial energy 0.0, max number of retries 10000 and 10 LGA runs were performed. All the AutoDock docking runs were performed in Intel(R) Xeon(R) CPU 5150 @ 2.66GHz, 2GB RAM in Apple system. AutoDock was compiled and run under Windows XP operating system. Autodock results were analyzed to study the interactions and the binding energy of the docked structure. The best ligand-receptor structure from the docked structures was chosen based on the lowest energy and minimal solvent accessibility of the ligand. The docking results were visualized using the Acceryls Visualizer discovery studio tool. A bond is formed between two atoms by overlapping the atomic orbitals. This overlap of atomic orbitals to form molecular orbitals occurs only at certain distances between the atom. When the amino acid residues of the active site is closer, then the interactions is much higher than the other sites. On the basis of the available evidence, when a hydrogen atom lies between two atoms having high negativity, it shows a unique property of forming a bond or bridge between them, holding one of the atoms by a covalent bond and other by purely electrostatic forces.

Hydrogen bonding is very important when the electronegative atoms are O, F, N. These three elements are negative enough for the necessary attraction to exist. The effectiveness is due to their high electronegativity and their small size. Many reports indicate that flavonoids can access intracellular locations, because of their benzyl structures, justifying their ability to attenuate oxidative stress induced by diverse stimuli. The chemical structure of rutin may contribute to its direct antioxidant properties.[49] Binding energy is correlated with the probability of affinity and stable bound between ligand and its receptor.[50] Binding energy values may also predict the bioactivity value for a ligand to the corresponding receptor.

3.6.1. Luteolin and PEPCK1

Docking of PEPCK1 against luteolin produced seven clusters of conformers using RMSD-tolerance of 2.0 Å out of 10 docking runs. Cluster Rank 1 with binding energy -7.58 kcal/mol at eighth run has formed eight hydrogen bond with active binding sites of PEPCK1 namely three bonds with THR 339 (OG1, OG1, N); one bond with PRO 337 (O); one bond with VAL 335 (N); one bond with PHE 333 (O); one bond with LYS 290 (NZ); one bond with ALA 287 (N). Docking conformation between the Luteolin and the PEPCK1 is shown in Figure 3(a), docking score is shown in Figure 3(b). The interactions between atoms of Luteolin and atoms of aminoacids of PEPCK1 is shown in Figure 3(c) and interaction along with distance is shown in Figure 3(d). Hydrogen bond distance between the donor and acceptor atoms was shown in Table 3.

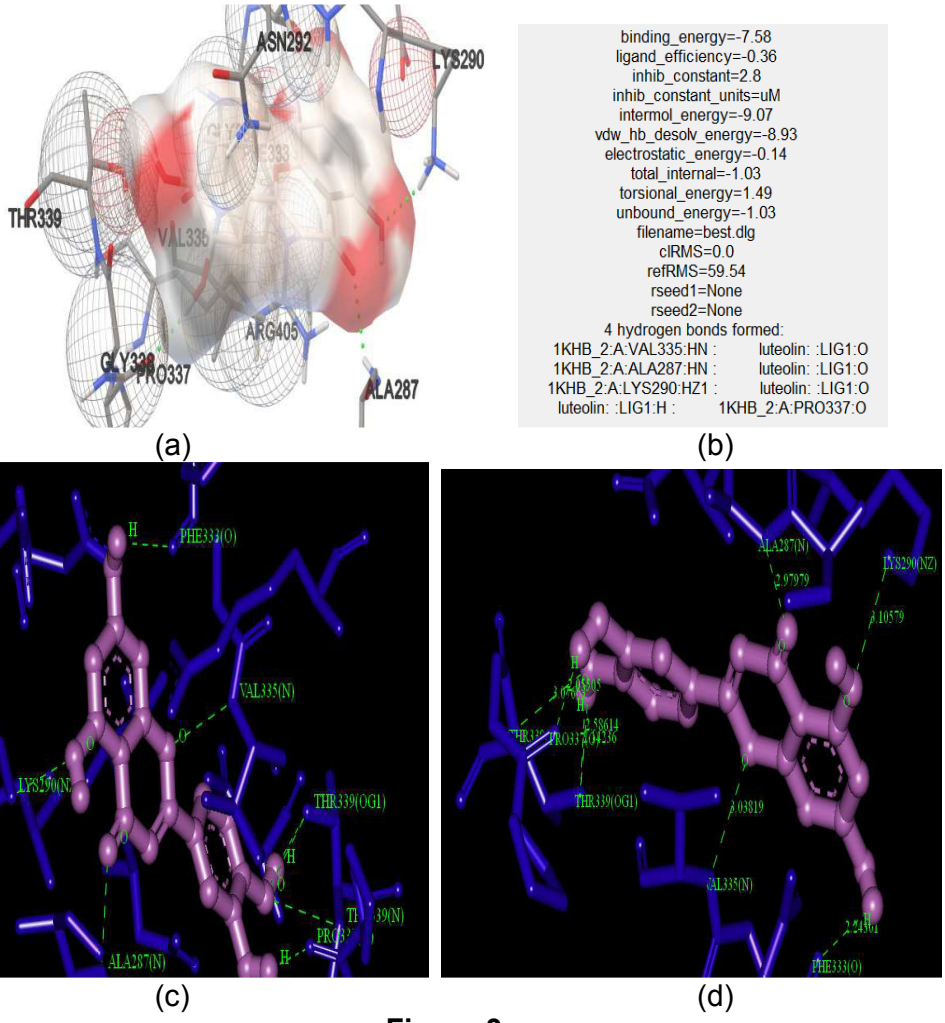


Figure 3

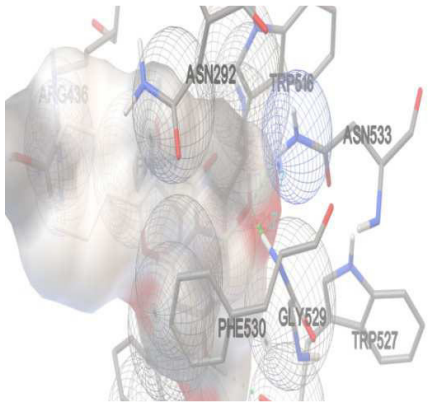
(a) Conformation between the Luteolin and the PEPCK1; (b) docking score; (c) the interactions between donor and acceptor atoms of Luteolin and PEPCK1; (d) interaction along with distance between donor and acceptor atoms.

Table 3
Molecular Interaction between Luteolin and PEPCK1

PEPCK1		Luteolin	Distance (Å)
Residue	Atom		
THR339	OG1	H	2.14
THR339	OG1	O	2.58
THR339	N	O	3.07
PRO337	O	H	2.05
VAL335	N	O	3.03
PHE333	O	H	2.24
LYS290	NZ	O	3.10
ALA287	N	O	2.97

3.6.2. Kaempherol and PEPCK1

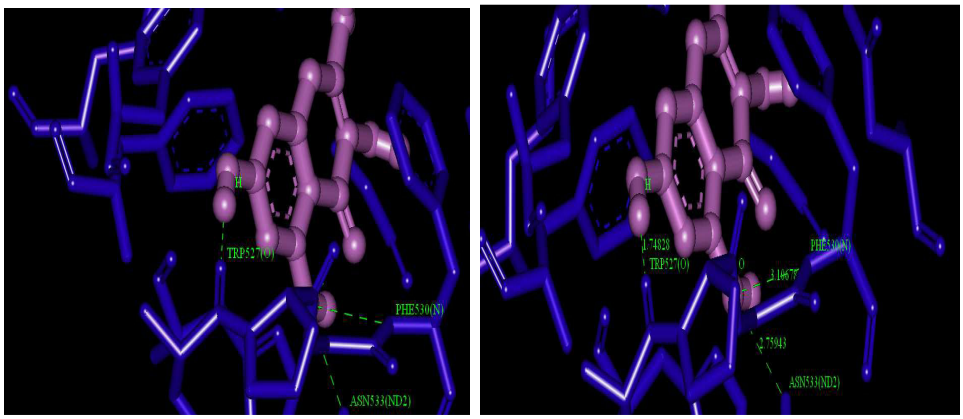
Docking of PEPCK1 against kaempherol produced eight clusters of conformers using RMSD-tolerance of 2.0 Å out of 10 docking runs. Cluster Rank 1 with binding energy -6.44 kcal/mol at sixth run has formed three hydrogen bond with active binding sites of PEPCK1 namely three hydrogen bonds with TRP 257 (O); ASN 533 (ND2); PHE 530 (N), respectively. Docking conformation between the kaempherol and the PEPCK1 is shown in Figure 4(a), docking score is shown in Figure 4(b). The interactions between atoms of kaempherol and PEPCK1 were shown in Figure 4(c) and interaction along with distance is shown in Figure 4(d). Hydrogen bond distance between the donor and acceptor atoms was shown in Table 4.



(a)

binding_energy=-6.44
ligand_efficiency=-0.31
inhib_constant=18.99
inhib_constant_units=uM
intermol_energy=-7.93
vdw_hb_desolv_energy=-7.79
electrostatic_energy=-0.14
total_internal=-0.99
torsional_energy=1.49
unbound_energy=-0.99
filename=best.dlg
cIRMS=0.0
refRMS=63.22
rseed1=None
rseed2=None
2 hydrogen bonds formed:
1KHB_2:A:PHE530:HN : kaempherol: :LIG1:O
1KHB_2:A:ASN533:HD21 : kaempherol: :LIG1:O

(b)



(c) (d)

Figure 4
(a) ***Conformation between the kaempherol and the PEPCK1; (b) docking score; (c) the interactions between donor and acceptor atoms of kaempherol and PEPCK1; (d) interaction along with distance between donor and acceptor atoms.***

Table 4
Molecular Interaction between Kaempherol and PEPCK1

PEPCK1		Kaempherol	Distance (Å)
Residue	Atom		
TRP257	O	H	1.74
ASN533	ND2	O	2.75
PHE530	N	O	3.10

CONCLUSION

The results of the present study show that luteolin and Kaempherol has good interactions eight and three hydrogen bonds with the diabetes drug target PEPCK1, respectively, with the docking score of –7.58 and –6.44 Kcal/Mol. Hence, luteolin and Kaempherol bioflavonoid which can be obtained from commonly available tamarind seed coat that exhibits antioxidant activity when extracted with ethyl acetate and ethanol[51] can be used as an antidiabetic agent for treatment of diabetes mellitus. Therefore, these results may offer therapeutic advantages in the treatment and prevention of Diabetes.

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