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In silico molecular docking analysis of major plant flavanols and proteins associated with glucose homeostasis

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ABSTRACT

Diabetes mellitus remains to be a significant global metabolic disorder, affecting both human and animal populations. Plant-derived medications have gained focus for diabetes management due to their perceived safety compared to conventional drugs. Recent research has emphasized the potential of polyphenols, particularly flavonols, in modulating genes related to insulin secretion and signalings pathways, indicating their potential role in preventing type II diabetes. This study investigates the interactions of three plant flavonols—quercetin, kaempferol, and myricetin—with two critical proteins involved in glucose homeostasis: PPAR-γ and GLUT-4 through *in-silico* docking analysis performed using Biovia Discovery Studio (version 2020). Our docking results revealed that kaempferol showed significant binding affinity for PPAR-γ, with a LibDock score of 95.462, interacting notably with residues THR269 and ASP271. Quercetin demonstrated moderate binding affinity (LibDock score of 46.657), while myricetin exhibited no interactions with PPAR-γ. For GLUT-4, myricetin had the highest docking score of 98.347, indicating strong binding affinity, with favorable interactions at residues SER412, LEU410, SER480, and LYS266. Quercetin also interacted with GLUT-4 (LibDock score: 95.317), binding to residues SER412, LYS266, and LEU410. Kaempferol with a dock score of 92.997 interacted with HIS484 and LYS266. The common interaction of amino acid residue LYS266 with all flavonols underscores its role in mediating their effects. The findings thus highlight the potential of flavonols in influencing glucose homeostasis through interactions with key proteins. Further pharmacokinetic studies and *in vitro* and *in vivo* validations are necessary to establish their efficacy and safety as anti-diabetic agents.

Keywords: *Diabetes mellitus*, PPAR-γ, GLUT-4, Quercetin, Kaempferol, Myricetin.

INTRODUCTION

Diabetes mellitus remains to be one of the most important metabolic disorders affecting a wide range of human and animal population. In recent years, plant derived medications have found immense use in the management of diabetes mellitus as they are considered as a safer alternative to allopathic medication. Wide array of plant derived active principles exhibiting different type of biological activity have been demonstrated to possess anti-diabetic activity. Polyphenols are a large and heterogeneous group of phytochemical compounds of plant origin and are divided into flavonoids, phenolic acids, stilbenes and lignans. Among all polyphenols, flavonoids are the most abundant and are found to have a potential role in the prevention of type II diabetes [1,2].

More than 5000 different plant-based flavonoids have been isolated and identified. These compounds are widely distributed in all photosynthesizing plant cells. Structurally, flavonoids consist of a 15-carbon skeleton (C6–C3–C6) formed by two benzene rings connected by a linear three-carbon chain. Flavonoids are divided into six major subgroups: flavonols (e.g., quercetin, kaempferol, and myricetin), flavanones (e.g., eriodictyol, hesperetin, and naringenin), isoflavonoids (e.g., daidzein, genistein, and glycitein), flavones (e.g., apigenin and luteolin), flavans-3-ol (e.g., catechin), and anthocyanins (e.g., cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin) [3].

Amongst the flavonols, quercetin and kaempferol are widely distributed in fruit and vegetables. Several studies have reported the anti-diabetic activity of plants such as *Garcinia pedunculata*, *Allium cepa*, *Glycine max L. Merrill*, *Macrotyloma uniflorum* which are rich source of quercetin, kaempferol and myricetin [4,5]. Such dietary polyphenols and phenolic compounds have been shown to regulate the expression of genes involved in insulin secretion (e.g., Sirtuin1-Sirt1 and glucose transporter 2- Glut2), in β-cells [6] and insulin signalling mechanisms (e.g., glucose transporter 4 - Glut4 and peroxisome proliferator-activated receptor gamma, PPAR-γ) in adipocytes [7]. Pharmacological studies in mouse models have shown that PPAR-γ activation enhances the expression of insulin-dependent glucose transporters (GLUT-4 and GLUT-1) leading to increased glucose uptake in the liver and skeletal muscles, thereby reducing plasma glucose levels [8].

Visualization of such plant based polyphenolic compounds and their interaction with the effector protein at an atomic level using molecular docking tools would enhance our understanding and aid in selection of specific compound for *in-vivo* efficacy studies. As per the literature, there are limited docking studies on quercetin, kaempferol and myricetin to detect their relative interactions with protein associated with insulin signalling. Hence, the present work was designed to study the interactions between the three plant flavonols viz., quercetin, kaempferol & myricetin with the two proteins associated with glucose homeostasis *viz*. GLUT-4 and PPAR-γ by *in-silico* docking analysis.

MATERIALS AND METHODS

Molecular Docking studies

The docking studies were performed by using Biovia Discovery studio (Version 2020) at the Department of Bioinformatics and ARIS cell, Madras Veterinary College, TANUVAS, Chennai (https://www.tanuvas.ac.in/mvc_bioinformatics.php).

Ligand Preparation

Chemical structure of the ligands *viz.* quercetin, kaempferol & myricetin wereretrieved from Pub chemcompound database (https://pubchem.ncbi.nlm.nih. gov/) in SDF format (Table 1). The 3-Dimensional structure of the ligands were visualized and their structural analysis (viz. molecular weight, atom composition, presence of sulphur atom) were carried out using BIOVIA Discovery Studio Visualizer (version 2020). Ligands were prepared for docking by making them flexible using the "Prepare Ligand" module to enable accurate simulations by adopting various conformations.

Protein selection and preparation

The crystallographic three-dimensional structures of the selected target protein PPAR-γ was retrieved from Protein Data Bank database (PDB ID:6FZP). As 3-D structure of GLUT-4 was not available in PDB database, it was modelled using I-Tasser server (https://zhanglab.ccmb. med.umich.edu/I-TASSER/) with the amino acid sequence retrieved from the UniProt (Accession no: P14672) (Figure 1). The amino acid sequences, number of binding sites, molecular weight, etc were analysed and the protein molecule was made flexible using the 'prepare protein' module in BIOVIA Discovery Studio. After preparation, hydrogen was from the receptor cavities of the protein to facilitate binding with the ligand.

Docking analysis

The ligands were in a flexible condition when interacting with macromolecules under rigid conditions. Individual ligands were subjected to docking with the receptor, and the resulting complexes were analyzed for their binding modes using three-dimensional receptor-ligand poses. To investigate specific interactions such as hydrogen bonding and hydrophobic interactions, two-dimensional diagrams of the receptor-ligand complexes were employed.

Among the various conformations generated for each ligand, the ligand pose with the highest LibDock score and the least binding energy was considered for predicting the optimal ligand binding conformation. Hydrogen bonds and various hydrophobic interactions play a pivotal role in mediating protein-ligand interactions. To assess the stability of the best-docked pose for these compounds, the hydrogen bonding interactions between the protein and compounds were examined, revealing the critical amino acids involved in hydrogen bond formation. In addition to hydrogen bonding interactions, other non-bonded interactions such as hydrophobic bonding were also investigated.

RESULT AND DISCUSSION

Docking analysis revealed that the selected flavonols viz., quercetin, kaempferol & myricetin had a good binding affinity against the two proteins associated with glucose homeostasis *viz*. PPAR-γ and GLUT-4 (Table 2).

Among the flavonols, kaempferol exhibited a high binding affinity with PPAR-γ. The structural model of the active site of PPAR-γ and the binding pattern of kaempferol are shown in Figure 1. Computational studies showed that kaempferol interacts with residues THR 269 and ASP 271 of PPAR-γ through hydrogen bonds (Fig. 2). Specifically, THR269 served as a hydrogen bond donor, while ASP271 acted as a hydrogen bond acceptor. This interaction is significant as PPAR-γ agonists are known to enhance insulin sensitivity by increasing the transcription of genes involved in glucose and lipid metabolism [9]. More recent studies have further supported this, demonstrating that PPAR-γ agonists continue to play a vital role in managing insulin resistance through similar mechanisms [10]. The high binding affinity of kaempferol supports its potential to influence PPAR-γ activity and suggests its use as a potential therapeutic agent for managing type II diabetes.

Quercetin displayed only a moderate binding affinity, which was lower than kaempferol but still notable. In contrast, myricetin showed least interaction with PPAR-γ, indicating that its role in modulating PPAR-γ activity may be limited suggesting alternate mechanisms of glucose homeostasis. Some studies have suggested that myricetin administration might be beneficial for increasing insulin sensitivity and inhibiting islet β-cell apoptosis by acting as Glucagon-like peptide-1 (GLP-1) agonist [11].

Amongst the various proteins associated with glucose homeostasis, GLUT-4 plays a vital role in glucose-sensing as it facilitates absorption of 15% of the blood glucose by adipose tissue and the remaining 85% by muscle in healthy individuals [12]. GLUT-4 continuously recycles between intracellular stores (vesicles) and the plasma membrane. Insulin shifts GLUT4 translocation towards the plasma membrane while glucagon shifts GLUT4 translocation towards the intracellular stores [13].

Metformin, a cornerstone in the management of type 2 diabetes, also promotes GLUT4 translocation, facilitating glucose uptake in cells [14]. Although generally well-tolerated, it can rarely cause lactic acidosis, a serious condition with symptoms such as dizziness, severe drowsiness, muscle pain, fatigue, chills, cyanosis, rapid or difficult breathing, irregular heartbeat, abdominal pain with diarrhoea, nausea, and vomiting [15]. Thus, plant-based therapeutics have gained an increased demand due to their easy availability, affordability and minimal side effects.

Numerous studies have suggested the role of flavonoids and phenolic compound in enhancement of GLUT-4 expression and glucose uptake. Quercetin and procyanidins have been reported to possess antidiabetic properties by up-regulation of mRNA level of GLUT-4 and its translocation to the cell membrane in adipocytes and skeletal muscle cells [12]. Kaempferol has been reported to improve diabetes mellitus through the promotion of glucose metabolism in skeletal muscle, and the suppression of hepatic gluconeogenesis [16,17].

The present i*n silico* study revealed that all three flavonols had favorable interaction with GLUT-4 protein (Fig.3). Molecular interactions of GLUT-4 and myricetin was found to be the most favorable with the highest libdock score and strong binding affinity suggesting that myricetin might be a potent enhancer of GLUT-4 activity. Myricetin formed favorable hydrogen bonds with active site residues SER412, LEU410, SER480, and LYS266 (Table 2). Quercetin also showed significant bindingand interacting with SER412, LYS266, and LEU410 of GLUT-4 while Kaempferol exhibited interactions with HIS484 and LYS266.

The *in-silico* analysis also highlighted the interaction of all three flavonols with LYS266 residue of GLUT-4 which was particularly noteworthy suggesting its possible role in mediating their effects on GLUT-4. Presence of lysine residue in the active site of GLUT-4 may play crucial role in the function and regulation of these transporters. Emerging evidence indicates that methylation of lysine residues of key enzymes and proteins is an important epigenetic modification which can significantly impact glucose and lipid metabolism. Studies suggest that lysine methylation affects glucose uptake efficiency in carbohydrate metabolism and can directly influences GLUT activity [18]. Also, ubiquitination of GLUT4 at its lysine residue helps in translocation to the cell surface in response to insulin [19].

Table 1: Details of the flavonols used in the study

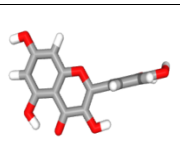
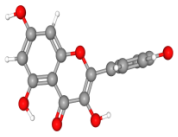
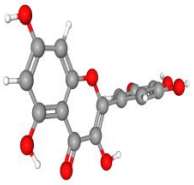
S. No	Name of the Compound	Compound ID	Molecular formula	Smile	Chemical Structure
1.	Quercetin	PubChem CID 5280343	C15H10O7	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C = C(C=C3O2)O)O)O)O)O	
2.	Kaempferol	PubChem CID 5280863	C15H10O6	C1=CC(=CC=C1C2=C(C(=O)C3=C(C= C(C=C3O2)O)O)O)O	
3	Myricetin	PubChem CID 5281672	C15H10O8	C1=C(C=C(C(=C1O)O)O)C2=C(C(=O) C3=C(C=C (C=C3O2) O)O)O	

Table 1: LibDock scores, interaction data of the docked flavonols with the binding site of PPAR-γ and GLUT-4

Protein	Flavonol	LibDock score	No. of Hydrogen bonds	Interacting atoms	Bond Distance
PPAR-γ	Kaempferol	95.462	2	Thr 269	2.512
				Asp 271	2.514
GLUT-4	Kaempferol	92.9976	2	His 484	3.053/2.354
				Lys 266	1.679
	Quercetin	95.317	3	Ser 412	2.454
				Lys 266	2.896
				Leu 410	2.641
	Myricetin	98.347	4	Ser 412	1.821
				Lys 266	2.97/2.352
				Leu 410	2.786
				Ser 480	2.495

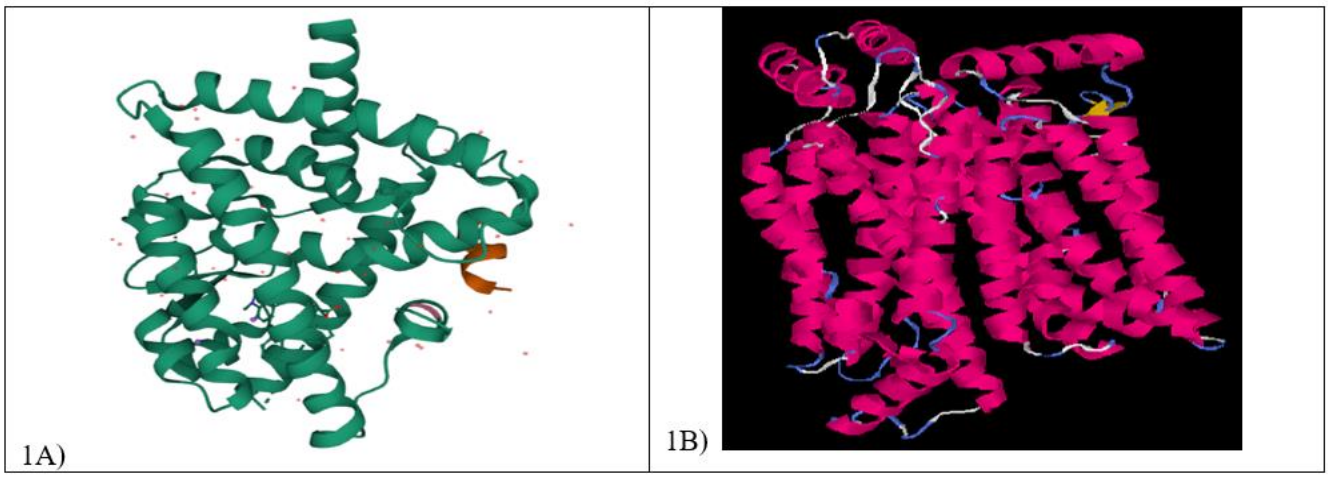


Figure 1: Three-dimensional structure of target protein taken for the study. 1A. Structure of PPAR- γ and 1B. Structure of GLUT-4 modelled using I-Tasser server

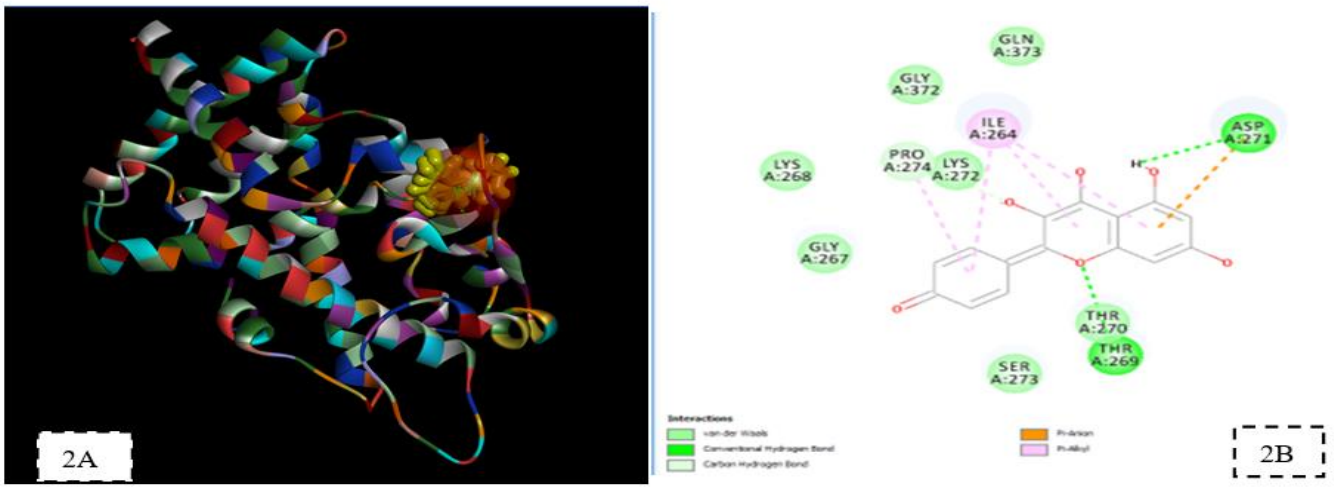


Figure 2: Molecular structure represents the interaction of kaempferol with PPAR-γ, 2A. The 3D interaction of Kaempferol showing residue interaction, 2B. The 2D interaction plot of kaempferol showing the interaction types

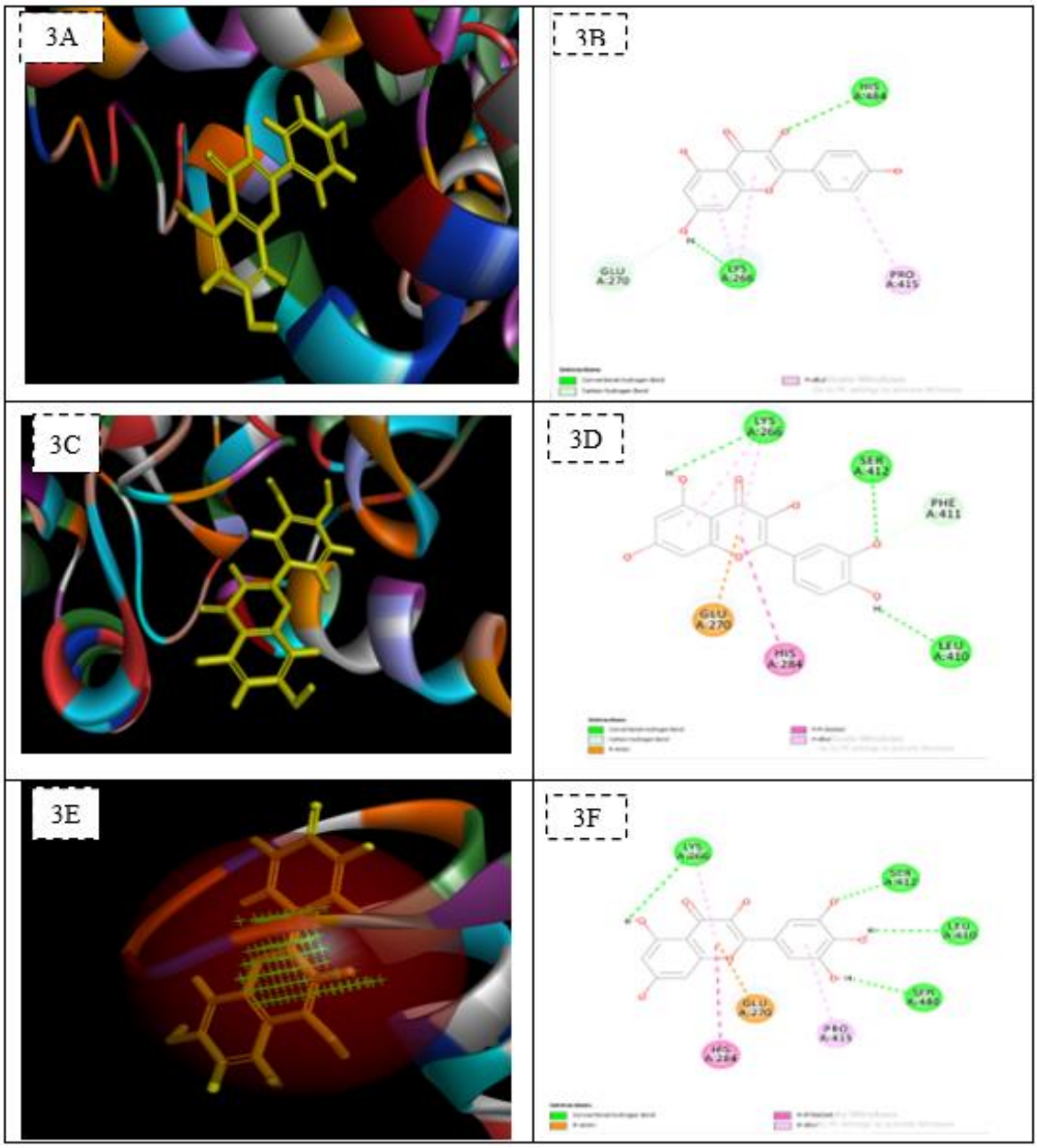


Figure 3: Molecular structure represents the interaction of flavonols with GLUT-4, 3A. The 3D interaction of Kaempferol showing residue interaction, 3B. The 2D interaction plot of kaempferol showing the interaction types, 3C. 3D interaction of quercetin showing residue interaction, 3D. The 2D interaction plot of quercetin showing the interaction types, 3E. 3D interaction of myricetin showing residue interaction, Fig. 3F. The 2D interaction plot of myricetin showing the interaction types

CONCLUSION

The present molecular docking study thus revealed a favourable interaction of all three flavonols *viz*. quercetin, kaempferol and myricetin with GLUT-4 than PPAR-γ suggesting that the anti-diabetic potential of plants rich in these flavonols might be associated with alteration in the expression levels of GLUT-4. The significance of the LYS266 residue of GLUT-4 in the interactions with the flavonols demands further investigation. Studies on the pharmacokinetics properties of these flavonols followed by *in vitro* and *in vivo* studies may prove the potential of these compounds to be developed into an effective anti-diabetic therapeutic agent.

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Conflict of interest

The authors declared no conflict of interest.

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