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In-silico Estimation of Flavonoids through Molecular Docking and Assessment of ADMET properties as DPP-IV inhibitor in the treatment of Diabetes Mellitus

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Abstract

Flavonoids are polyphenolic compounds identified in greater quantities in plants, fruits, and vegetables with varying proportions. Their subgroups comprise six main classes, including flavanols, flavones, flavanones, and isoflavones. The focus of the work was to identify their receptor-drug interactions against dipeptidyl peptidase IV (DPP-IV) enzymes through *in-silico* drug design. Their chemical structure was drawn using ACD labs Chemsketch and energy minimized using Avogadro energy minimization software. To test the binding interaction and mode of flavonoids with the DPP-IV receptor, molecular docking simulations were executed using AutoDock Tools 4.2v, and ADMET predictions were performed. The overall quality of the protein chosen for performing docking analysis was assessed using ERRAT and the score of the model was around 98.386%. Among the 19 flavonoids used in this experiment for *in-silico* studies, their binding energy was calculated using Autodock. Molecular docking analysis revealed quercetin and kaempferol as the most promising candidates based on their low binding energy of -9.3 and -9.2 kcal/mol, respectively. These compounds demonstrated strong molecular interactions through H-bonding and other hydrophobic interactions with key amino acid residues (Pro475, Gly476, Met509, Pro510, Ser511, Lys512, Gln527, Ile529, Asp545, Asp556, Val558, Phe559, Arg560, Asn562, Ala564 and Thr565). Lipinski's rule of five identified the drug-like behavior of the molecules and all the compounds were screened using the SWISS ADME software tool. Both quercetin and kaempferol exhibited optimal drug-like characteristics, and showed complete compliance with Lipinski's rule, supporting their potential for therapeutic development.

Keywords: Flavonoids, polyphenols, *in-silico* docking, SWISS ADME, quercetin

Introduction

Flavonoids are naturally occurring phenolic compounds with lower molecular weight as one of their unique properties(1). Its chemical structure is a 15-carbon nucleus with a fusion of two benzene ring systems, A and B. The backbone of flavonoids consists of a skeletal arrangement (C6-C3-C6), C6 belongs to the aromatic C ring and C3 is a heterocyclic moiety Figure.1 (2). Their ubiquitous presence in many plant components is of greater importance in contributing to multiple health benefits such as anti-inflammatory, anti-viral, neuroprotective, cardioprotective, and cancer preventive. It occupies a larger position in the daily human

diet(3). Some fruits apples, cherries, berries, and citrus are active sources of flavonoids. The percentage of flavonoids content are found to be high in onions (quercetin), herbs (flavonols and flavones), parsley (apigenin), thyme, beets, cabbage, cauliflower (luteolin), red potatoes and red onions (anthocyanidins), red grapes and berries (anthocyanins), cocoa (flavan-3-ol) (4). Of these, the highest concentration of flavonoid of quercetin is found in onion. Their bioavailability and medicinal benefits depend on the type of flavonoid and its mechanism of action. Due to these reasons, they are considered an attractive target in drug discovery and development (5).

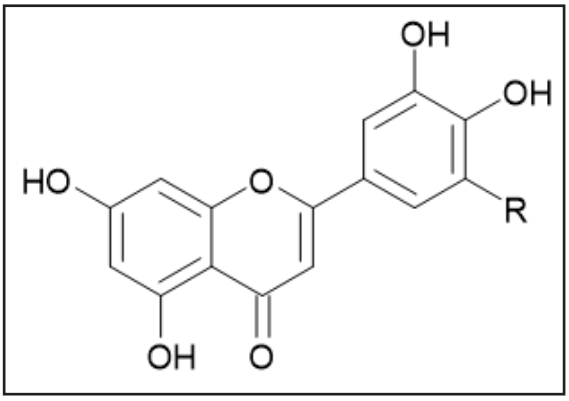


Figure.1 Chemical Structure of a flavonoid

Flavanoids are sub-classified and are categorized into six different forms, ketone form being flavones, flavanones and isoflavones, flavanol, and flavon-3-ol with its alcoholic functional group, and anthocyanins(6). About 18 categorizations of flavonoid structures including quercetin, hesperidin, silymarin, apigenin, luteolin, etc., differ in the chemical structures of the functional groups present in C ring, generic structure, and the position of attachment of B ring to C ring Fig.1. In a recent study conducted in USA about 2,00,000 men and women were found to lower diabetic risk to consumption of fruits such as apple blueberry and pears. The hypothesis states that their bioactivity is due to the presence of hydroxyl and ketonic groups. The structural activity relationship of flavonoids further supports their anti-diabetic efficacy. In the A ring, the presence of hydroxyl groups at 5,6, and 7 and in the B ring the double bond at C2-C3 and C ring with hydroxyl groups at C3` and C4` are the most important characteristics responsible for causing anti-diabetic activity. The hydroxyl group donates electrons causing resonance stabilization and is responsible for biological activity (7). Modifications that increase the activity are positioned in the C ring system and belong to the functional groups, methoxy, rhamnose, chlorine atom, and galloyl group. Deterioration in diabetic activity is seen in glycosylation at the C7 position (8). Supportive in-vitro tests suggest that they act as effective supplements of flavonoids used in the management and prevention of diabetes mellitus (9). They have the potential to combat diabetes and inflammation by protecting the body against free radical generation and other mechanisms such as maintaining blood glucose levels and regulating glucose uptake and insulin secretion (10).

T2DM necessitates the need to regulate insulin by increasing its secretion and activity. Delayed gastric emptying and carbohydrate absorption are ways to maintain glucose levels. Incretin hormones namely, Glucagon Like Peptide-1 (GLP-1) and Gastric Inhibitory Peptide (GIP) increase insulin secretion whereas Dipeptidyl peptidase IV (DPP-IV) enzymes inactivate the available incretins in circulation. Although numerous synthetic DPP-IV inhibitors, including gliptins, have been developed, pharmacokinetic limitations and adverse effects associated with these DPP-4 inhibitors remain a major challenge. Notable adverse effects encompass cardiovascular risks such as heart failure, polyarthritis, and allergies. Therefore, identifying safer and more effective inhibitors is crucial. Consequently, inhibition of DPP-IV utilizing natural compounds represents a promising therapeutic approach. Natural products, particularly flavonoids, have the capability in producing glu-

cose by inhibition of α-amylase and α-glucosidase and also have the capability to retard the absorption of glucose by GLUT4 transporters. They also play a role in insulin resistance (11).

A most recent study by Lu and colleagues (2024) employed computational screening of 1,668 flavonoid derivatives derived from natural product databases, utilizing quantitative estimate of drug-likeness (QED) scores integrated with quantitative structure-activity relationship (QSAR) modeling. The result identified three flavonoid derivatives (5,7,3',5'-tetrahydroxyflavone, 3,7-dihydroxy-5,3',4'-trimethoxyflavone, and 5,7,2',5'-tetrahydroxyflavone) exhibiting potent anti-DPP-IV activity (12). Despite extensive reports on the antidiabetic properties of flavonoids, comprehensive evaluations of their drug-likeness, pharmacokinetic behavior, and molecular interactions with DPP-IV are limited. In this study, 18 different flavonoid structures were analyzed for drug likeliness using Lipinski rule of five. The pharmacokinetic parameters to understand (absorption, distribution, metabolism, and toxicity) were estimated using pkCSM. The molecules that satisfy drug-like character were subjected to molecular modeling using auto dock tools to identify the interaction between the flavonoids and proteins. The objective of this present study was to explore the capability of flavonoids to interact with DPP-IV (1X70) as an inhibitor approach (13).

Materials and Methods

Protein quality determination and validation

Dipeptidyl peptidase -IV inhibitor with RCSB PDB Code: 1X70 was downloaded from the Protein Data Bank to be used for the study. The overall quality factor of the protein was assessed using ERRAT and a higher value denotes that the protein is of better quality. The acceptable quality factor is higher than 50 for structures of high resolution and greater than 95% for low-resolution structures (14).

Binding pocket prediction

The human dipeptidyl peptidase IV in complex with beta amino acid inhibitor was downloaded in .pdb protein Data Bank. The active site of the region to perform docking was predicted using CASTp (15) (Computed Atlas of Surface Topology of proteins) server from the link CASTp 3.0: Computed Atlas of Surface Topography of proteins (uic.edu) (16).

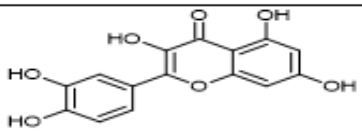
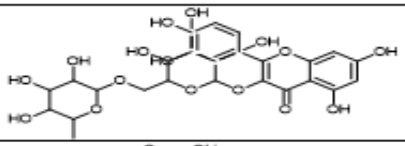
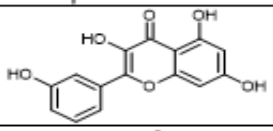
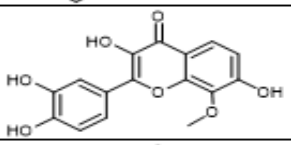
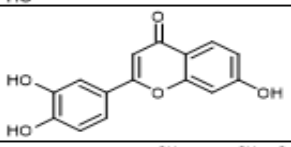
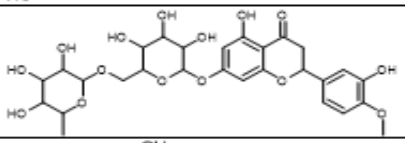
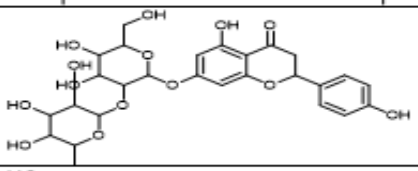
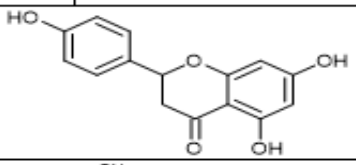
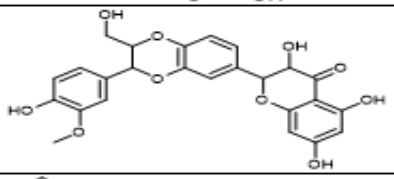
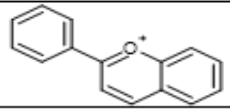
Identification of ligands and their optimization

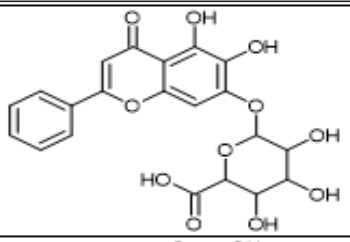
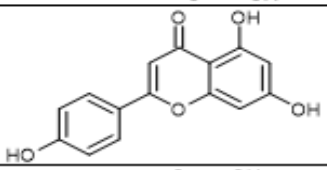
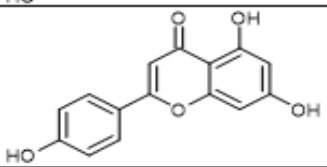
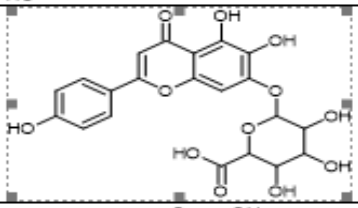
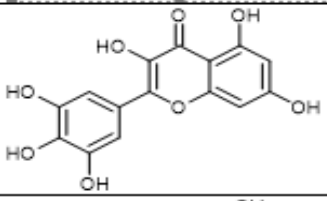
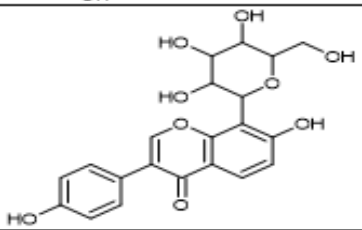
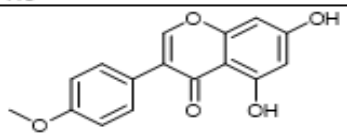
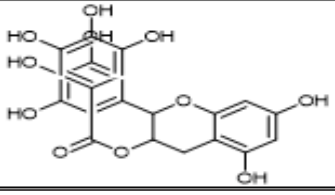
A database of flavonoids consisting of 18 chemical structures (Table 1) was sketched using ACD Labs Chemsketch (17), an online software used for drawing chemical structures. It further underwent optimization using energy minimization software such as Avogadro and his molecules were saved in the .pdb format.

Molecular docking

It is a virtual screening technique that calculates the interaction energies between the receptor protein and ligand. The binding energy exhibited as a result of the docking procedure explains the affinity of the protein-ligand binding within the active site (18). The cavity space of the protein was found using CB-Dock, a freely available software that gives the center and size of the volume occupied. Furthermore, docking could be performed in the weblink, CB-Dock2: An accurate protein-ligand blind docking tool (lab share.cn) by uploading optimizing protein and ligand and carrying out an auto blind docking (19). The protein preparation was carried out with the help of Autodock 4.2 by deleting waters, adding polar hydrogens, and saving in .pdb format and the ligands were prepared in a similar fashion by detecting the roots, setting the number of torsions and saved in .pdb format (20). The grid size measurements identified included the center to be at x=58, y=44, and z=27 occupying a cavity volume of 1403 A°.

Table 1. Seven major flavonoids from plant sources along with their subclassifications and chemical structures.

S.No	TYPE	FLAVANOIDS	CHEMICAL STRUCTURE
1.	Flavanols	Quercetin	
		Rutin	
		Kaempferol	
		Isorhamnetin	
		Fisetin	
2.	Flavanones	Hesperidin	
		Naringin	
		Naringenin	
3.	Flavanonols	Silymarin	
4.	Anthocyanins	Anthocyanin	

5.	Flavones	Baicalin	
		Apigenin	
		Luteolin	
		Scutellarin	
		Myricetin	
6.	Isoflavones	Puerarin	
		Biochanin A	
7.	Flavan-3-ol	(−)-Epigallocatechin-3-gallate (EGCG)	

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Drug-Likeliness screening

A chemical compound is considered to be a drug-like molecule when it is subjected to follow Lipinski's rule of five. This rule states that the molecular weight must be less than 500 daltons, and should have a number of hydrogen bond donors and hydrogen bond acceptors be less than 5 and 10 respectively (21). Log P <5 and the number of rotatable bonds to be <10. Based on the above rule, all the compounds were screened using the SWISS ADME software tool.

Prediction of ADMET properties

The pharmacokinetic parameters identify the Absorption, distribution, metabolism, excretion, and toxicity of the chemicals using pkCSM, a freeware pkCSM (uq.edu.au). It is used to predict small-molecule properties using a graph-based technique. The chemical structures are converted into SMILES format uploaded in the string format and run for calculation that estimates the properties (22).

Results and Discussion

Protein Preparation

An ERRAT analysis predicted the protein (1X70) with a scoring model of 98.386% (Figure.2) and is proven to be of good quality. It is a human dipeptidyl peptidase IV complex with a beta amino acid inhibitor and the analysis further confirms it to be an attractive target for drug development (23). The arrangement of atoms and non-bonded interactions contributed to a higher score resulting in a higher quality of the model.

Binding Site Prediction

The binding pockets are identified using an online server CASTp (http://sts.bioe.uic.edu/castp/) that uses alpha shape and pocket algorithm in the development of computational geometry in a molecular structure. It ensures all the available residues are involved in the designing process (24). The active site identified using the CASTp server were as follows: A630, A708, A740, B630, B708, B740, and the total surface area of the protein was 5345.71A^2

Molecular docking

Docking is an *in-silico* technique capable of estimating the ligand-receptor interactions and further its binding energy in Kcal/mol. Flavonoids are abundantly present in natural dietary sources, but the quantity of their content is still unknown (25). They are widely explored for their biological activities and their efficiency in the prevention of diabetes insighted to explore the interaction efficiency between flavonoid and dipeptidyl peptidase IV with RCSB PDB code: 1X70.Figure.3 shows the binding cavity of the ligand within the protein 1X70 and X-ray crystallographic structure of DPP-IV protein downloaded from the data bank 1X70. The affinity of the drug to bind and elicit different types of

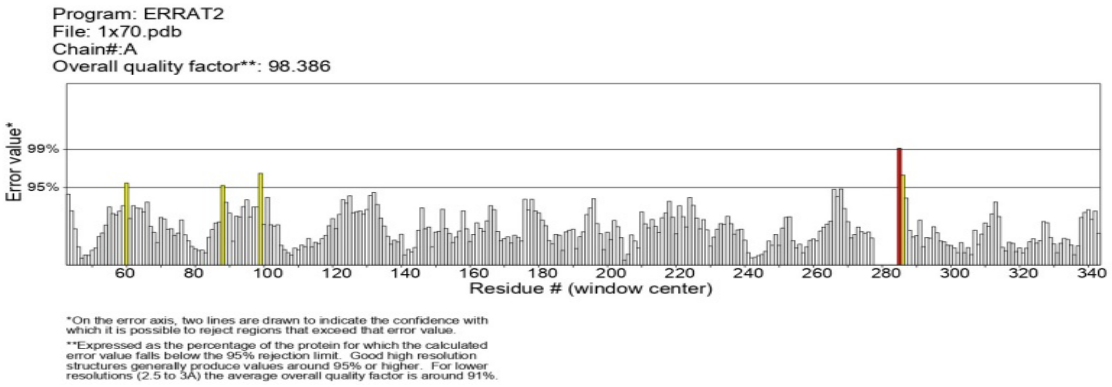

Figure.2 ERRAT Analysis of the Protein Structure PDB ID: 1X70.

interactions such as hydrophobic, hydrophilic, and electrostatic, Vanderwaal's helps in understanding the binding affinity of molecular interaction (26).

The first step involved the preparation of protein by downloading it and removing water molecules. The hydrogens were added during the process and energy was optimized through the addition of Kollman's charges and stabilizing protein for molecular docking. The chemical structures of flavonoids were drawn using an open-source software Chemsketch and energy minimised using Avogadro's energy minimisation technique. The ligands for binding were saved in .mol format. The interaction efficiency was assessed using online software for binding CB-dock, wherein the prepared protein and ligand were uploaded (27). Similarly, the process was also performed with a standard DPP-IV inhibitor, Sitagliptin. Table.5 shows results of the binding energy in kcal/mol. Molecular Interactions of Flavonoids: Residue Binding and Hydrogen Bonding Analysis Using AutoDock are reported in Table.6.

Quercetin, a subclassification of flavanol was reported with the highest docking score of about -9.3kcal/mol, myricetin belonging to flavones showed a moderate binding score of -9.0 kcal/mol, and anthocyanins with the lowest score of -8.1kcal/mol (Figure.4). Sitagliptin was used as the standard throughout the molecular docking simulations with a score of -9.5 kcal/mol. Among the flavonoids used in the study, quercetin was found to show highest efficacy and the interacting amino acids Pro475, Gly476, Met509, Pro510, Ser511, Lys512, Gln527, Ile529, Asp545, Asp556, Val558, Phe559, Arg560, Asn562, Ala564, Thr565.

Drug like behavior

Based on the binding energy calculations, flavonoids showed increased binding interactions with DPP-IV, and the drug likeliness score was calculated for all the categories of flavonoids. Among these, rutin, hesperidin, naringenin, baicalin, Scutellarin, (−)-Epigallocatechin-3-gallate (EGCG) showed either one or two violations (Table 2) (28). The resultant 12 flavonoids have drug-like behavior predicted using SWISS ADME.

ADMET analysis

Estimating the pharmacokinetic properties of flavonoids is the most important step in understanding drug-like behavior. Absorption, distribution, metabolism, elimination, and toxicity give a clear picture of the applicability of the flavonoids to be converted into phytopharmaceuticals (29). The human intestinal absorption of quercetin (94.987), plasma protein binding (-1.56), the volume of distribution (Nil), clearance (2.045), and hepatotoxicity (0.558). The highest proportion of intestinal absorption was for Anthocyanins (96%), the plasma protein binding and volume of distribution must be low in order for the drug to be available in greater quantities in the circulation. Table.3 and Table.4 reports the ADMET data obtained using pkCSM for all the flavonoids used in the study (30).

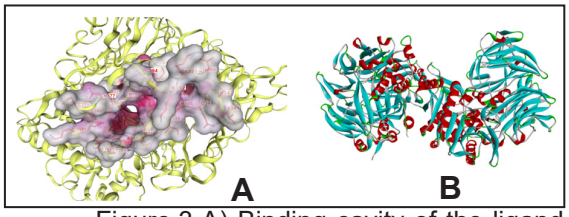

Figure.3 A) Binding cavity of the ligand within the protein 1X70 B) X-ray crystallographic structure of DPP-IV protein downloaded from the data bank 1X70

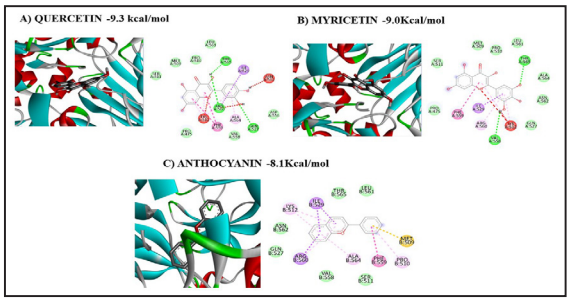

Figure.4 Flavonoid compounds identified with best binding scores A) Quercetin B) Myricetin and moderate binding score C) Anthocyanins with its 2D ligand interaction diagram

Table 2. Evaluation of flavonoids Based on Lipinski's Rule of five

S.No	Flavonoids	Molecular Weight (<500 Daltons) g/mol	Log P (< 5)	Hydrogen Bond Acceptor (<10)	Hydrogen Bond Donor (<5)	Number of Rotatable Bonds (< 10)
1.	Quercetin	302.24	1.23	7	5	1
2.	Rutin	610.52	-1.29	16	10	6
3.	Kaempferol	286.24	1.58	6	4	1
4.	Isorhamnetin	316.26	1.65	7	4	2
5.	Fisetin	286.24	1.55	6	4	1
6.	Hesperidin	610.56	-0.72	15	8	7
7.	Naringin	580.53	-0.79	14	8	6
8.	Naringenin	272.25	1.84	5	3	1
9.	Silymarin	482.44	1.59	10	5	4
10.	Anthocyanin	207.25	2.64	1	0	1
11.	Baicalin	446.36	0.22	11	6	4
12.	Apigenin	270.24	2.11	5	3	1
13.	Luteolin	286.24	1.73	6	4	1
14.	Scutellarin	462.36	-0.20	12	7	4
15.	Myricetin	318.24	0.79	8	6	1
16.	Puerarin	416.38	0.27	9	6	3
17.	Biochanin A	284.26	2.44	5	2	2
18.	(−)-Epigallocatechin-3-gallate (EGCG)	458.38	1.01	11	8	4

Table.3 ADMET Pharmacokinetic Parameters of 18 Flavonoids: Human Intestinal Absorption, Plasma Protein Binding, and Volume of Distribution (L/kg)

S.No	Compounds	Human Intestinal Absorption (≥30%: HIA+)	Plasma Protein Binding (%)	Volume of Distribution (L/kg)
1.	Quercetin	94.987	0.206	-1.56
2.	Rutin	23.446	0.381	1.663
3.	Kaempferol	74.29	0.178	1.274
4.	Isorhamnetin	76.014	0.091	1.123
5.	Fisetin	83.752	0.166	0.718
6.	Hesperidin	0	0.101	0.011
7.	Naringin	25.797	0.159	0.619
8.	Naringenin	91.31	0.064	-0.015
9.	Silymarin	61.861	0	0.369
10.	Anthocyanin	96.182	0.147	0.24
11.	Baicalin	26.224	0.294	0.267
12.	Apigenin	93.25	0.147	0.822

13.	Luteolin	81.13	0.168	1.153
14.	Scutellarin	13.836	0.295	0.904
15.	Myricetin	65.93	0.238	1.317
16.	Puerarin	67.446	0.187	0.377
17.	Biochanin A	93.028	0.03	-0.341
18.	Epigallocatechin-3-gallate(EGCG)	47.395	0.215	0.806

Table.4 Cytochrome P450 3A4 (CYP3A4) Metabolism, Elimination Clearance Rate, and Hepatotoxicity of 18 Flavonoids.

S.No	Compounds	CYP3A4 Metabolism	Elimination Clearance Rate mL/min/kg	Toxicity Hepatotoxicity
1.	Quercetin	No	2.045	0.558
2.	Rutin	No	0.369	0.438
3.	Kaempferol	No	0.477	0.531
4.	Isorhamnetin	No	0.508	0.576
5.	Fisetin	No	0.421	0.579
6.	Hesperidin	No	-41.808	0.525
7.	Naringin	No	0.318	0.43
8.	Naringenin	No	0.06	-0.176
9.	Silymarin	No	-0.103	0.65
10.	Anthocyanin	No	0.716	0.453
11.	Baicalin	No	0.04	0.652
12.	Apigenin	No	0.566	0.328
13.	Luteolin	No	0.495	0.499
14.	Scutellarin	No	0.481	0.538
15.	Myricetin	No	0.422	0.51
16.	Puerarin	No	-0.007	0.642
17.	Biochanin A	No	0.247	0.4
18.	Epigallocatechin-3-gallate(EGCG)	No	0.292	0.441

Table.5. Binding Energy in Kcal/mol of flavonoids with DPP-IV inhibitory activity

S.No	Flavonoids	Binding Energy (Kcal/Mol)
1.	Quercetin	-9.3
2.	Kaempferol	-9.2
3.	Isorhamnetin	-8.7
4.	Fisetin	-8.9
5.	Naringenin	-8.6
6.	Silymarin	-9.0
7.	Anthocyanin	-8.1
8.	Apigenin	-8.6
9.	Luteolin	-8.6
10	Myricetin	-9.0
11	Puerarin	-9.1
12	Biochanin A	-9.4
13	Rutin	-9.4
14	Sitagliptin	-9.5

Table.6 Molecular Interactions of Flavonoids: Residue Binding and Hydrogen Bonding Analysis Using AutoDock

S.No	Flavonoids	Interactions Residue	Hydrogen Interactions
1.	Quercetin	Pro475 Gly476 Met509 Pro510 Ser511 Lys512 Gln527 Ile529 Asp545 Asp556 Val558 Phe559 Arg560 Asn562 Ala564 Thr565	Lys512 Gln527 Ile529 Phe559 Arg560 Asn562 Thr565
2.	Kaempferol	Pro475 Gly476 Met509 Pro510 Ser511 Lys512 Gln527 Ile529 Lys554 Asp556 Val558 Phe559 Arg560 Leu561 Asn562 Ala564 Thr565	Pro475 Pro510 Lys512 Gln527 Ile529 Phe559 Arg560 Ala564
3.	Isorhamnetin	Pro475 Gly476 Met509 Pro510 Ser511 Lys512 Gln527 Ile529 Asp556 Thr557 Val558 Phe559 Arg560 Leu561 Asn562 Ala564 Thr565	Pro475 Lys512 Gln527 Ile529 Phe559 Arg560 Asn562 Thr565
4.	Fisetin	Pro475 Gly476 Met509 Pro510 Ser511 Lys512 Gln527 Ile529 Asp545 Asp556 Val558 Phe559 Arg560 Asn562 Ala564 Thr565	Pro475 Lys512 Gln527 Ile529 Phe559 Arg560 Ala564
5.	Naringenin	Pro475 Gly476 Pro510 Ser511 Lys512 Gln527 Ile529 Asp556 Val558 Phe559 Arg560 Leu561 Asn562 Ala564 Thr565	Lys512 Ile529 Val558 Phe559 Thr565
6.	Silymarin	Ile346 Glu347 Met348 Ser349 Thr350 Thr351 Gly352 Trp353 Val354 Gly355 Ile375 Ser376 Asn377 Glu378 Arg382 Cys385 Phe387 Lys392 Cys394 Phe396 Asp588 His592	Glu347 Met348 Ser349 Thr350 Thr351 Cys394
7.	Anthocyanin	Pro475 Met509 Pro510 Ser511 Lys512 Gln527 Ile529 Val558 Phe559 Arg560 Leu561 Asn562 Ala564 Thr565	Met509 Pro510 Lys512 Ile529 Phe559 Arg560 Ala564
8.	Apigenin	Pro475 Gly476 Met509 Pro510 Ser511 Lys512 Gln527 Ile529 Asp556 Val558 Phe559 Arg560 Asn562 Ala564 Thr565	Lys512 Ile529 Val558 Phe559 Asn562 Thr565
9.	Luteolin	Pro475 Gly476 Met509 Pro510 Ser511 Lys512 Gln527 Ile529 Asp556 Val558 Phe559 Arg560 Leu561 Asn562 Ala564 Thr565	Pro510 Lys512 Ile529 Phe559 Arg560 Asn562 Thr565
10	Myricetin	Pro475 Gly476 Met509 Pro510 Ser511 Lys512 Gln527 Ile529 Asp556 Val558 Phe559 Arg560 Leu561 Asn562 Ala564 Thr565	Lys512 Ile529 Val558 Phe559 Thr565
11	Puerarin	Ile346 Glu347 Met348 Ser349 Thr350 Thr351 Gly352 Trp353 Val354 Gly355 Ile375 Ser376 Asn377 Glu378 Glu379 Gly380 Arg382 Phe387 Lys392 Asp393 Cys394 Asp588 His592	Ser349 Thr350 Thr351 Ile375 Ser376 Glu378 Cys394
12	Biochanin A	Pro475 Leu504 Met509 Pro510 Ser511 Lys512 Gln527 Ile529 Lys554 Asp556 Val558 Phe559 Arg560 Leu561 Asn562 Ala564 Thr565	Leu504 Met509 Pro510 Lys512 Gln527 Ile529 Val558 Phe559 Arg560 Ala564 Thr565
13	Rutin	Gln344 His345 Ile346 Glu347 Met348 Ser349 Thr350 Thr351 Gly352 Trp353 Val354 Gly355 Arg356 Ile374 Ile375 Ser376 Asn377 Glu378 Glu379 Gly380 Tyr381 Arg382 Cys385 Phe387 Lys391 Lys392 Asp393 Cys394 Thr395 Phe396 Asp588 Met591 His592	Glu347 Thr350 Thr351 Trp353 Val354 Gly355 Ser376 Glu378 Arg382
14	Sitagliptin	Arg125 Glu205 Glu206 Val207 Phe208 Ser209 Phe357 Arg358 Tyr547 Ser630Tyr631 Val656 Trp659 Tyr662 Asp663 Tyr666 Asn710 Val711 His740	Arg125 Glu205 Glu206 Val207 Ser209 Phe357 Arg358 Tyr662 Tyr666 Asn710

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Discussion

This study evaluates DPP-4 as a promising therapeutic target for diabetes treatment. Several clinical guidelines position DPP-4 inhibitors as a relatively safer alternative to sulfonylureas for diabetes treatment (31). However, as described above, the risk of hospitalization due to heart failure was observed with the use of DPP-4 inhibitors (32). Natural flavonoids offer promising leads for developing novel DPP-4 inhibitors.

The present research work evaluated eighteen naturally derived flavonoids, classified according to their structural subgroups, as potential DPP-4 inhibitors using computational study. Critical parameters including physicochemical properties, pharmacokinetic characteristics, and drug-likeness are fundamental factors to determine a potential candidate for drug development. Based on drug-likeness analysis, rutin, hesperidin, naringenin, baicalin, scutellarin, and EGCG violated Lipinski's rule of five and were excluded as viable candidates.

Previously reported SAR analysis by Pan et al. (2022) confirms that hydroxylation at positions 3' and 4' of ring B and position 6 of ring A enhanced DPP-4 inhibition, while hydroxylation at position 3 of ring C reduced inhibitory effectiveness (33). These findings align with observations by Sarian et al. (2017), who compared chrysin (lacking B-ring substitution), which showed no activity at the highest tested concentration, with quercetin and kaempferol demonstrated IC_{50} values of 21.75 ± 5.81 mM and 45.93 ± 8.61 mM, respectively (34). Our results support these experimental findings, as both quercetin and kaempferol exhibited strong binding scores (-9.3 and -9.2 kcal/mol) and optimal drug-likeness properties. Similarly, methylation of hydroxyl groups at positions C3', C4', and C7 of the flavonoid structure, such that in the case of biochanin A lowers DPP-4 inhibitory activity. Despite biochanin A's favorable binding score (-9.4 kcal/mol) and Lipinski compliance, we eliminate it as a viable candidate based on the reported SAR analysis.

Experimental evidence from Fan et al. (2013) demonstrated that while various flavonoids including kaempferol and quercetin showed effective DPP-4 inhibition, rutin failed to inhibit the enzyme (35). Morikawa et al. (2015), also reported that rutin showed no DPP-4 inhibitory activity at 100 μM, the maximum tested concentration (36). Despite showing the strongest binding affinity *in-silico* (−9.4 kcal/mol), rutin fails to meet Lipinski's rule of five and lacks experimental evidence of DPP-4 inhibition.

In our computational study, myricetin demonstrated a higher binding affinity of −9.0 kcal/mol, which is notably stronger than the −8.03 kcal/mol reported by Pan et al. (2022). However, conflicting data exist regarding the DPP-4 inhibitory potency of kaempferol. While Fan et al. reported a strong IC_{50} of 0.49 μM, Zhao et al. (2016) observed a much weaker IC_{50} of 45.96 μM (approx. 100-fold higher), suggesting possible discrepancies due to experimental conditions or compound variability (37). This discrepancy between computational binding scores and experimental results emphasizes the critical need for experimental validation in drug discovery.

Conclusion

Dipeptidyl peptidase-4 (DPP-4) inhibitors emerged as a promising class of antidiabetic agents in recent years. In this study, an in-silico approach was employed to screen and identify potential flavonoid-based DPP-4 inhibitors. Among the eighteen flavonoids evaluated, quercetin and kaempferol emerged as the most promising candidates, exhibiting strong binding affinities and favorable drug-likeness profiles. The structural quality of the DPP-4 protein model (PDB ID: 1X70) was validated using ERRAT analysis, confirming its reliability for molecular docking studies. These findings suggest that flavonoids hold significant potential as scaffolds for the development of novel antidiabetic compounds. Modifying their structures to enhance drug-likeness could pave the way for more ef-

fective DPP-4 inhibitors in the future. However, to substantiate these findings and advance the development of effective lead compounds, comprehensive in vitro and in vivo studies are essential.

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Conflict of Interest

The Authors declare that there is no conflict of interest.

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