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PANCREATIC ENZYME MODULATION AND METABOLIC PATHWAY REGULATION BY BAUHINIA VARIEGATA FLAVONOIDS: AN INTEGRATED IN SILICO, BIOCHEMICAL AND KEGG PATHWAY INVESTIGATIONS

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Abstract
Bauhinia variegata is a rich source of bioactive flavonoids, like Apigenin 7 -O -glucoside, Kaempferol-3-glucoside, and Quercetin, which have received a lot of attention as far as their therapeutic potential in glucose and lipid metabolism is concerned. The enzyme modulatory properties of these flavonoids have been investigated in silico through docking, in vitro enzyme assays and KEGG pathway enrichment. Docking of Apigenin 7-O-glucoside and Kaempferol-3-glucoside in-silico revealed good binding affinity with enzymes like alpha-amylase and lipase which are involved in the breakdown of starch and fats. The inhibitory activity of Bauhinia variegata extracts against alpha-amylase and lipase was also validated on rats with hyperglycemia in-vitro and it would be beneficial in the regulation of glucose release from the postprandial period and lipid absorption. The role of these flavonoids in important metabolic pathways was identified by KEGG pathway analysis; they are involved in starch and sucrose metabolism, cholesterol metabolism and other pathways, such as biosynthesis of bile acids and metabolism of lipids and steroids, etc. Toxicity forecast indicated that Apigenin 7-O-glucoside, Kaempferol-3-glucoside were less toxic and thus safer to use therapeutically and Quercetin had moderate toxicity, particularly neuro- and hepatotoxicity. The obtained results suggest that Bauhinia variegata flavonoids is an interesting class of natural enzyme inhibitors with two beneficial effects in diabetes and obesity control. Further research is needed to evaluate the long-term clinical efficacy, bioavailability, and potential synergistic effects with other diabetic medications, which could aid in the formulation of comprehensive treatment plans.

Keywords: Bauhinia variegate; Flavonoids; Pancreatic enzyme modulation; Diabetes Mellitus; Metabolic regulation; Toxicity profiling.

INTRODUCTION

The Bauhinia variegata is also referred to as the orchid tree and has been used in traditional medicine for centuries due to its many therapeutic properties. As a native source of tropical Asia, this medicinal plant has become a promising source of numerous bioactive compounds, such as flavonoids, alkaloids, glycosides, and tannins, with promising effects on glucose regulation and diabetes control (Sharma et al., 2025). The flavonoids also possess interesting and specific properties, including antioxidant, anti-inflammatory, and enzyme-modulating. In particular, Apigenin 7-O-glucoside, Kaempferol-3-glucoside and Quercetin can be potentially important players for altering enzymes involved in carbohydrate and fat digestion, which play a role in glucose metabolism regulation (Shahana et al., 2017).

Amylase and lipase are two enzymes which help break down the food material that has carbohydrates or fats, respectively. It is known that amylase converts starch to glucose, whereas lipase helps in the digestion of fat, the breakdown of triglycerides into free fatty acid and glycerol (Mukherjee, 2003; Whitcomb and Lowe, 2007). The dysregulation of these enzymes can easily result in metabolism disruptions, which are the basis of many diseases, such

as obesity and type 2 diabetes, with rapid glucose absorption and inefficient metabolism of fat that can lead to high blood sugar levels and insulin resistance (Harper, 2018). Regulation of amylase and lipase activity is considered an important strategy for maintaining glucose and lipid homeostasis in metabolic disorders (Tucci et al., 2010; Singh et al., 2019). Herbal medicines have additionally been shown to inhibit some of the digestive enzymes of carbohydrates and lipids that may prove to be a good alternative in the management of such conditions (Sompong et al., 2016).
Interestingly, Bauhinia variegata has demonstrated potential to control these enzymes, although the exact compounds that cause it are under investigation. In this paper, we are concentrating on three key flavonoid-based phytochemicals, namely Apigenin 7-O-glucoside, Kaempferol-3-glucoside, and Quercetin, which were found in a large pool of 104 phytochemicals that were retrieved in the Indian Medicinal Plants, Phytochemistry and Therapeutics Database (Singh et al., 2026). The reasons behind selecting these include their reported enzyme-modulatory, antioxidant and antidiabetic activities. In silico docking studies will be conducted in order to determine their ability to bind with these enzymes and to examine their inhibitory properties. This study aims to offer more insight into the mechanism by which the Bauhinia variegata compounds might contribute to glucose regulation and act as future therapeutic agents to treat diabetes by getting to understand the molecular interactions of these flavonoids with the enzymes (Upadhyay et al., 2026).
The research not only seeks to justify the therapeutic value of these flavonoids but also highlights the incorporation of the current computational methods in the investigation of the traditional plant-based remedies. The findings may lead to new and evidence-based methods of treating type 2 diabetes, and the final aim is to have better management of glycemic levels and lessen the impact of the chronic disease.

MATERIALS AND METHODS

Plant collection and extraction

Collection of plant material
Leaves of Bauhinia variegata were freshly collected at the campus of Hamdard University, Karachi, Pakistan. The leaves were carefully selected to make sure that they were healthy and no damage had occurred, so that quality samples could be used in the study.

Plant authentication and voucher specimen
The plant specimen was identified by the botanical experts from Hamdard University (Botanical Department) Karachi. One voucher specimen type of the sample is deposited in the University herbarium for future identification with the reference number: **A00185.**

Preparation of extract
The dried leaves of Bauhinia variegata were pulverized and subjected to a double maceration process to obtain the hexane extract. Specifically, 1 kg of the powdered leaves was immersed in 5 litres of ethanol and hexane and left for a period of 15 days, following the method described by Younus et al. (2020). The mixture was then filtered with Whatman Grade-1 filter paper, and then the filtrate was concentrated with a rotary evaporator under reduced pressure (-760 mm Hg) and controlled temperature. The resulting extract was a viscous, semi-solid residue of brownish-black color, as reported by Sachan et al. (2020).

Biochemical enzyme assay
Blood samples were collected and centrifuged systematically on the 28 th day of the treatment regimen to isolate the plasma. The resulting supernatant plasma was then assayed to determine the level of pancreatic enzymes, namely Amylase and Lipase, using commercially available diagnostic kits. The activity of amylase was measured by the use of the α-amylase kit assay (Cat. No. 5.17160.0001) and analyzed with the help of the Selectra Pro M automatic analyzer and the levels of lipase were measured by the use of the BIO-BAS Lipase Kit Assay (Cod. 38297) and analyzed with the help of the same analyzer.

INSILICO EXPERIMENTS

Retrieval and selection of Bauhinia variegata phytochemicals
A phytochemical profile of Bauhinia variegata was systemically retrieved in IMPPAT 2.0 (Indian Medicinal Plants, Phytochemistry and Therapeutics), which is a manually curated database that combines chemical annotation and therapeutic metadata of Indian medicinal plants (Mohanaraj et al., 2018; Vivek-Ananth et al., 2023; Sil et al., 2025). An extensive search of B. variegata provided 104 phytochemical components, which were described in various plant tissues, their molecular frameworks, chemical identifiers, and canonical SMILES.

Based on this primary data, three prominent phytoconstituent flavonoid compounds, which include Quercetin, Apigenin 7-O-glucoside and Kaempferol-3-glucoside, were selected as key compounds to be studied in silico. Repeated phytochemical reports in B. variegata leaves, structural completeness adequate for computational modeling, and reported enzyme-modulatory and anti-diabetic activity in published literature. The structural data of the chosen ligands were cross-validated, and they were retrieved in the SDF format of PubChem (Kim et al., 2019). Compounds with no complete structural data or with stereochemical inconsistencies were filtered out, leaving a final ligand library to be analyzed with computation.

Ligand preparation

The chosen ligands were subjected to docking analysis by transforming SDF files into 3D structures with the assistance of Open Babel, with 3D coordinate generation where mandatory (O'Boyle et al., 2011).

Insilico toxicity prediction

ProTox-II was used to carry out toxicological profiling (Banerjee et al., 2018). The parameters analyzed were predicted LD50, toxicity, hepatotoxicity, mutagenicity, carcinogenicity, and immunotoxicity. Only compounds that fall under the lower toxicity risk groups and which are predicted to be non-mutagenic and non-hepatotoxic were selected for further molecular docking analysis.

Target protein retrieval and preparation

The three-dimensional crystal structures of Alpha-amylase and Pancreatic lipase were retrieved from the RCSB Protein Data Bank (Berman et al., 2000). To define active sites, structures were selected on the basis of high resolution (< 2.5 Å) and presence of co-crystallized inhibitors. Protein preparation involved removal of water molecules and heteroatoms, addition of polar hydrogens, and assignment of Kollman charges using AutoDock Tools. The catalytic residues of α-amylase (Asp -Glu-Asp) and the catalytic triad of pancreatic lipase (Ser-His-Asp) were verified based on literature and structural information to make sure of proper docking grid placement.

Molecular docking analysis using MOE

Molecular docking of the identified flavonoids Apigenin 7-O-glucoside, Kaempferol-3-glucoside, and Quercetin with Alpha-amylase and Pancreatic lipase as proteins was undertaken using the Molecular Operating Environment (MOE) 2023.08 suite, which is commonly used to perform protein-ligand docking and scoring (Jain, 2006). The crystal structures of the target proteins in three dimensions were obtained at the RCSB Protein Data Bank, where high-resolution structures (< 2.5 Å) were obtained, and the co-crystallized inhibitors were available to define the active sites accurately.

Protein preparation in MOE involved the correction of missing residues, addition of hydrogen atoms, assignment of AMBER99 charges, and energy minimization using the default MOE force field to relieve steric clashes. The active site of the respective enzymes was determined as per the coordinates of the co-crystallized ligand, and catalytic residues established by literature (Asp-Glu-Asp triad of 8-amylase and Ser-His-Asp of pancreatic lipase).

The ligands were imported into MOE in 3D format, and the protonation was changed to pH 7.4. Conformational flexibility of ligands was considered using the “Conformation Import” protocol, and energy-minimized structures were used for docking. Docking was performed using the Triangle Matcher placement algorithm, and the London dG scoring function was used to first evaluate the pose through docking. The top 5based on each ligand, was further refined via the Induced Fit protocol to include flexibility of the side-chain and more natural ligand-protein interactions. The binding energies (ΔG) and docking scores were determined, and the ligand protein interactions, including hydrogen bonding, π-π stacking and hydrophobic interactions, were observed using the LigX module of MOE.

To compare these, the Acarbose and Orlistat standard inhibitors were also docked under the same conditions to confirm the predictive power of the docking protocol (Sanner, 1999).

KEGG pathway analysis

KEGG pathway enrichment was performed to comprehend the molecular interactions of the chosen flavonoids and their possible role in metabolic regulation. KEGG Pathway Analysis for Apigenin 7-O-glucoside, Kaempferol-3-glucoside, and Quercetin revealed their involvement in carbohydrate and lipid metabolism pathways, including starch and sucrose metabolism and cholesterol metabolism (Kanehisa et al., 2008). These pathways are essential in understanding the regulation of α-amylase and lipase activities during glucose and lipid metabolism.

RESULTS

Invitro enzyme assay

In vitro enzyme assays were done to determine the presence of pancreatic enzymes, namely Amylase and Lipase, in the plasma of treated animals.

Amylase inhibition assay

The ethanolic and Hexane extracts of Bauhinia variegata were assessed for their effect on amylase activity in streptozotocin-induced hyperglycemic rats. The mean level of amylase in the control group was 939 ± 8.88 U/L. A significant reduction in amylase activity was observed in the diabetic control group (610 ± 45.74 U/L, $P < 0.01$ compared to the control group). Glibenclamide (standard drug) treatment recovered amylase activity to 775.66 ± 10.17 U/L ($P < 0.01$ compared to diabetic control), whereas sitagliptin had a value of 754.33 ± 4.05 U/L ($P < 0.05$).
Ethanolic extracts at concentrations of 50mg/kg and 100mg/kg reduced the amylase levels significantly (712.66 ± 8.98U/L and 717.66 ± 43.87U/L, respectively, $P < 0.01$ compared to diabetic control). However, the Hexane extract at both doses (50 mg/kg and 100 mg/kg) had higher levels (866 ± 16.50 U/L and 951.66 ± 15.10 U/L, respectively), but still showed significant inhibition ($P < 0.01$ compared to control) as shown in Table 1.

Table 1. Effect of Ethanolic and Hexane extracts of Bauhinia variegate on Pancreatic Enzymes in streptozotocin-induced hyperglycemic rats.

Pancreatic Enzyme	**Experimental Groups**	**Amylase (U/L)**
Control Group		939 ± 8.88
Diabetic Control Group	Induction	610 ± 45.74**
Glibenclamide Standard		775.66 ± 10.17**^^
Sitagliptin Standard		754.33 ± 14.05**^
Bauhinia Ethanolic (50 mg/kg)	Treated Group	712.66 ± 8.98**
Bauhinia Ethanolic (100 mg/kg)	Treated Group	717.66 ± 43.87**
Bauhinia Hexane (50 mg/kg)	Treated Group	866 ± 16.50^^
Bauhinia Hexane (100 mg/kg)	Treated Group	951.66 ± 15.10^^

The findings are presented as the mean ± standard error of mean (SEM) =12. Statistical significance is indicated by **$P < 0.01$, when compared to the control group and ^$P < 0.05$, ^^$P < 0.01$, when compared to the diabetic control group. The study examined the effects of standard, ethanolic, and Hexane extracts of Bauhinia variegata.

Lipase inhibition assay

In the lipase inhibition assay, the control group had a lipase level of 75.20 ± 4.45 U/L. The diabetic control group showed a significantly reduced lipase activity (39.80 ± 1.77 U/L, $P < 0.01$ compared to the control group). Treatment with glibenclamide restored lipase activity to 66.40 ± 1.77 U/L ($P < 0.01$ compared to diabetic control), and sitagliptin showed 71.60 ± 1.07 U/L.
For the Bauhinia variegata extracts, the ethanolic extract at 50 mg/kg and 100 mg/kg showed lipase levels of 72.60 ± 2.74 U/L and 79.20 ± 1.85 U/L, respectively. The hexane extract at 50 mg/kg and 100 mg/kg resulted in higher lipase activity, 90.20 ± 1.98 U/L and 90.40 ± 2.89 U/L, respectively ($P < 0.01$ compared to the control group), as shown in Table 2.

Table 2. Effect of Ethanolic and Hexane extracts of Bauhinia variegate on Pancreatic Enzymes in streptozotocin-induced hyperglycemic rats.

Pancreatic Enzyme	**Experimental Groups**	**Lipase (U/L)**
Control Group		75.20 ± 4.45
Diabetic Control Group	Induction	39.80 ± 1.77**
Glibenclamide Standard		66.40 ± 1.77^^
Sitagliptin Standard		71.60 ± 1.07^^
Bauhinia Ethanolic (50 mg/kg)	Treated Group	72.60 ± 2.74^^
Bauhinia Ethanolic (100 mg/kg)	Treated Group	79.20 ± 1.85^^
Bauhinia Hexane (50 mg/kg)	Treated Group	90.20 ± 1.98**^^
Bauhinia Hexane (100 mg/kg)	Treated Group	90.40 ± 2.89**^^

The results are reported as the mean ± standard error of the mean (SEM) for n=12. Statistical significance is indicated by **$P < 0.01$, when compared to the control group and ^$P < 0.05$, ^^$P < 0.01$, when compared to the diabetic control group. The study examined the effects of standard, ethanolic, and Hexane extracts of Bauhinia variegata.

INSILICO ANALYSIS

Retrieval and selection of Bauhinia variegata phytochemicals

Among the 104 phytochemicals obtained from the IMPPAT 2.0 database, three flavonoid compounds, Apigenin 7-O-glucoside, Kaempferol-3-glucoside, and Quercetin, were chosen for further in silico investigation. The compounds were selected based on the common occurrence of these compounds in B. variegata leaves, structural completeness to be used in computational modeling, and known therapeutic effects associated with the compounds on enzyme modulation and anti-diabetic effects. The resulting ligand library was verified and downloaded in SDF format to PubChem, eliminating compounds without complete structure data or stereochemical inconsistencies. The structures of the selected phytochemicals are shown in Figure 1.

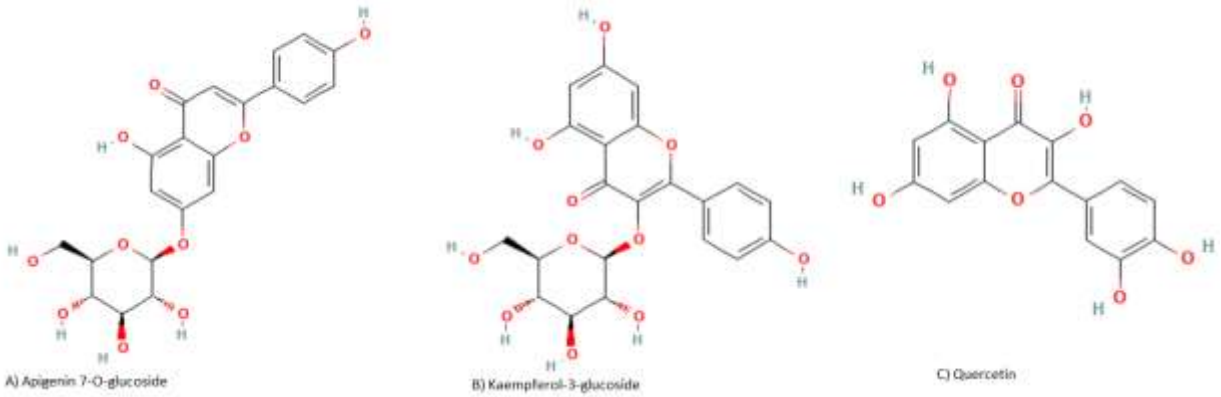

Figure 1. Structural representations of the selected flavonoid-based phytochemicals from Bauhinia variegata for insilico analysis. (a) Apigenin 7-O-glucoside, (b) Kaempferol-3-glucoside, and (c) Quercetin.

Ligands for docking, toxicity, and ADMET studies

Apigenin 7-O-glucoside, Kaempferol-3-glucoside, and Quercetin were the ligands that were used in this study, and each of them has been reported to have potential biological activities. The Apigenin 7-O-glucoside ($C_{21}H_{20}O_{10}$, MW: 432.4 g/mol) is a flavonoid glycoside compound with the functional groups of hydroxyl and glucose forms, which increases its receptor-binding specificity. The structure can be denoted as the SMILES code:
C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O[C@H]4C@@HO)O).
Likewise, Kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$, MW: 448.4 g/mol) is a flavonoid glycoside containing hydroxyl groups and a glucose moiety, which is involved in receptor interaction. The SMILES code of this compound is C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O[C@H]4C@@HO)O.
Quercetin ($C_{15}H_{10}O_7$, MW: 302.23 g/mol) is a common flavonoid with several hydroxyl groups, which increases its affinity to the receptors. Its structure is represented using the SMILES notation:
C1=CC(=C(C=C1C)N(C3=NC(=O)N(C3=NC(=O)C3=N2)CC(=O)O.
These flavonoids were chosen because they possess the ability of binding to receptor as a result of hydrogen bonding and π-π interaction that are fundamental to their biological functionality. The functional groups and their structural complexity make them a potential enzyme inhibitor and hence good candidates to be studied in future pharmacological research.

Toxicity Analysis

Oral toxicity prediction of Apigenin 7-O-glucoside, Kaempferol-3-glucoside and Quercetin showed that they had a significant variation in their toxicity profile. Both Apigenin 7-O-glucoside and Kaempferol-3-glucoside belonged to Toxicity Class 5 and are predicted to have a low toxicity LD50 5000 mg/kg, as shown in Figure 2a and 2b. Also, the compounds were found to have low logP values (0.05 Apigenin 7-O-glucoside and -0.24 Kaempferol-3-glucoside), which implied that they were not as lipophilic and therefore could be absorbed and distributed throughout the body, not accumulating to excessive levels in fat tissues.
Conversely, Quercetin had a much lower LD50 of 159 mg/kg, which is classified in Toxicity Class 3, which is correlated with moderate toxicity (Figure 2c). Quercetin also had a higher value of logP of 1.99, which denotes high lipophilicity. This increased lipophilicity implies that Quercetin can be more readily stored in fatty tissues, so it could have more toxicological dangers.

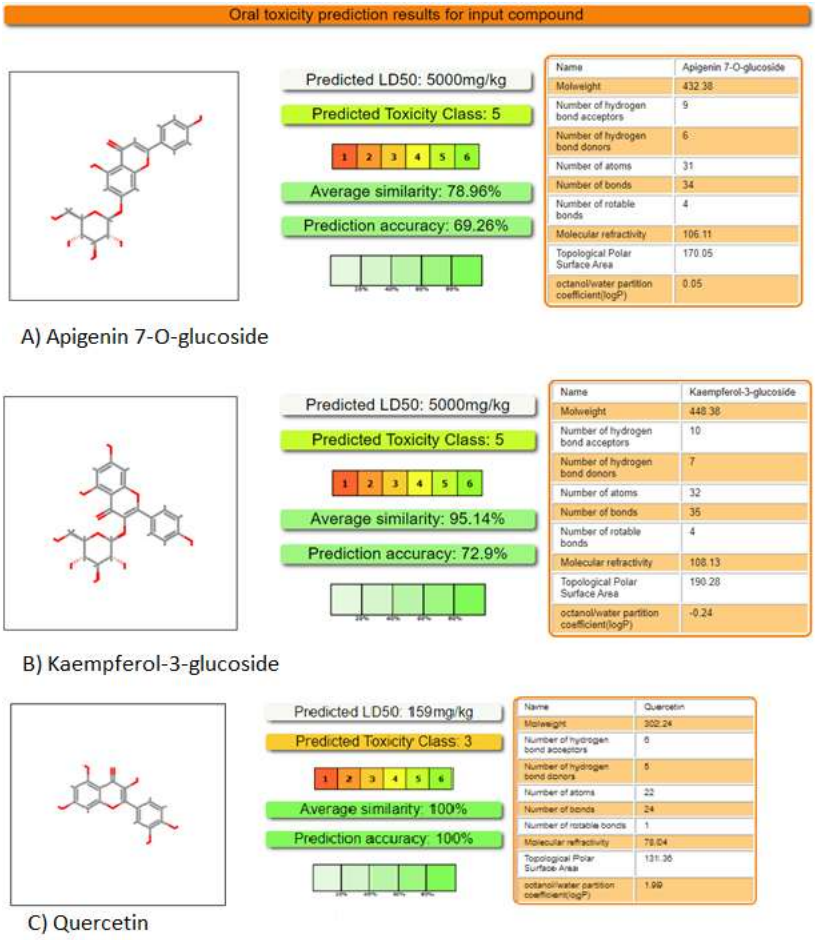

Figure 2. Comparative Oral Toxicity Prediction of (A) Apigenin 7-O-glucoside , (B) Kaempferol-3-glucoside , and (C) Quercetin.

In terms of toxicity-related bioactivity, Quercetin displayed the highest level of interaction with toxicity pathways, particularly Neurotoxicity (100%) and Hepatotoxicity (83%) (Figure 3c). This was significantly more than Apigenin 7-O-glucoside and Kaempferol-3-glucoside, which only had moderate activity in these places (Figure 3a and 3b). The radar plots as well as the network graphs also suggested that the reactions of Quercetin were mostly linked to the toxicological pathways such as Neurotoxicity, Carcinogenicity and Mutagenicity, which made it a more toxic compound.

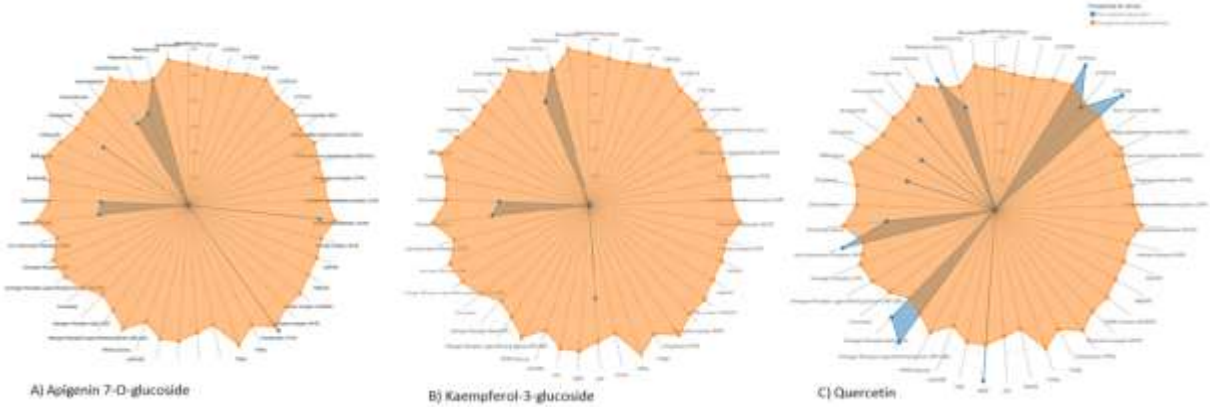

Figure 3. Comparative Analysis of Toxicity and Bioactivity Profiles for (A) Apigenin 7-O-glucoside, (B) Kaempferol-3-glucoside, and (C) Quercetin Based on Radar Plot Data.

Further analysis in Figure 4 compares the receptor binding and activity profiles of these flavonoids, which can provide more insights into the interactions of these flavonoids in their toxicity and the bioactivity in different receptors. Apigenin 7-O-glucoside and Kaempferol-3-glucoside had much weaker affinities to toxicologically important receptors, which confirms their relatively lower toxicological risks than Quercetin, which had strong affinities to toxic receptors, especially those associated with neurotoxicity and carcinogenicity. These results of Figure 4 also give more evidence that Quercetin (Figure 4c) is the strongest regarding toxicity and receptor binding interactions, and the other relatively safer compounds are Apigenin 7 -O -glucoside and Kaempferol-3 -glucoside (Figure 4a and b), which may be more appropriate in the treatment of metabolic diseases.

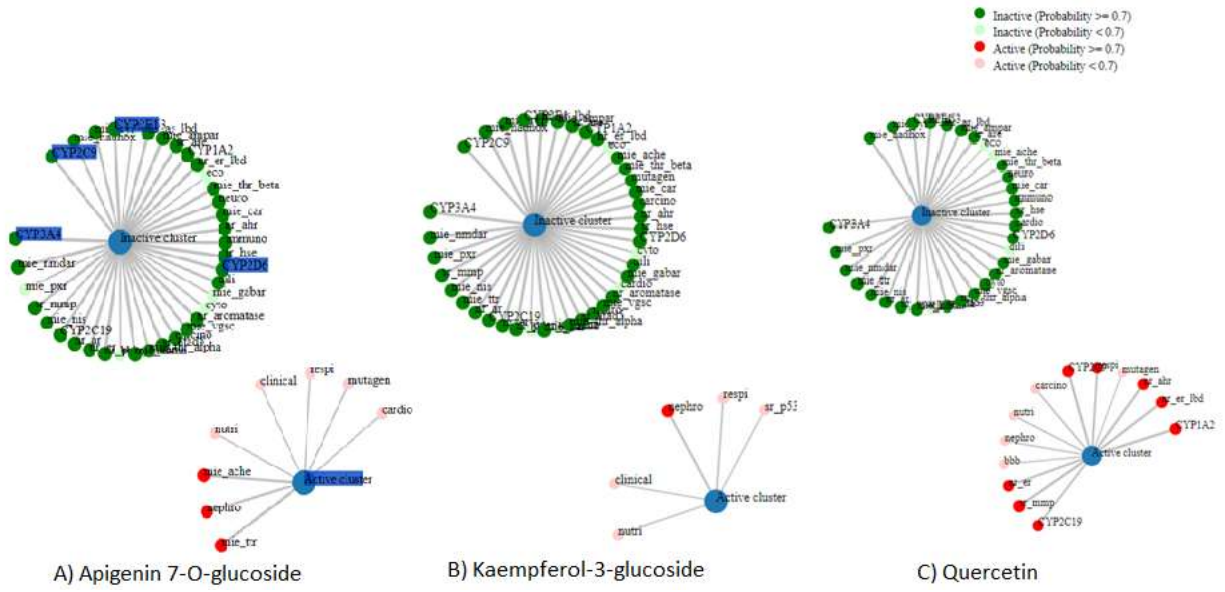

Figure 4. Comparative Analysis of Receptor Binding and Activity Profiles for Apigenin 7-O-glucoside.

Therefore, Quercetin is the most toxic and receptor-binding interaction, apigenin 7-O-glucoside and Kaempferol-3-glucoside are safer, and their toxicity profiles belong to Class 5. These results imply that Quercetin is more biologically active but also has more adverse effects that can be researched to make its use safe.

Protein to ligand interaction analysis

The docking protein to ligand interaction studies that were carried out to determine the inhibitory potential of three flavonoids, namely Apigenin 7-O-glucoside, Kaempferol-3-glucoside, and Quercetin, against Alpha-amylase and Pancreatic lipase provided a lot of insight into their enzyme-modulating effect. These flavonoids were chosen because of their established biological activity and potential in managing glucose and lipid metabolism, especially regarding type 2 diabetes.

Alpha-amylase (1OSE) docking results

The docking of Alpha-amylase (1OSE) showed that the interactions between the chosen flavonoids and the active location residues of the enzyme were strong and may result in an inhibitory effect, which can help in the management of diabetes. Apigenin 7-O-glucoside was shown to form hydrogen bonds with GLU 233 (H-donor) and TYR 151 (H-acceptor) and had binding energies of -0.6 kcal/mol and -0.6 kcal/mol, respectively. Additionally, it formed a hydrogen bond with HIS 201 (H-acceptor) at a binding energy of -2.2 kcal/mol (Figure 5a). Kaempferol-3-glucoside showed even stronger binding with GLU 233 (H-donor) at 2.73 Å and 3.00 Å, with binding energies of -4.0 kcal/mol and -2.9 kcal/mol, respectively. The compound also exhibited significant π-H stacking interactions with VAL 163 (Figure 5b). Quercetin exhibited a lower binding affinity, but still depicted a significant hydrogen bonding with GLU 233 (H-donor) at 2.91Å with a binding energy of -1.4 kcal/mol (Figure 5c).

Comparatively, Acarbose, which is an established Alpha-amylase inhibitor, exhibited more significant interactions, especially with GLU 233 and TYR 151, with a binding energy of -4.5 kcal/mol (Figure 5d). Nevertheless, the flavonoids, despite displaying weaker binding affinities, may still be able to inhibit Alpha-amylase, which is one of the main enzymes involved in carbohydrate digestion, moderately. This implies that these flavonoids could have a role in the management of diabetes through decreasing the conversion of starches into glucose and the postprandial

elevation of blood sugar. The moderate inhibitory action of the flavonoids on Alpha-amylase suggests their usefulness as natural substitutes or supplements in the management of diabetes, though not as potent as Acarbose. Inclusion in dietary supplements can offer an adjunctive approach to glucose metabolism regulation and long-term control of diabetes.

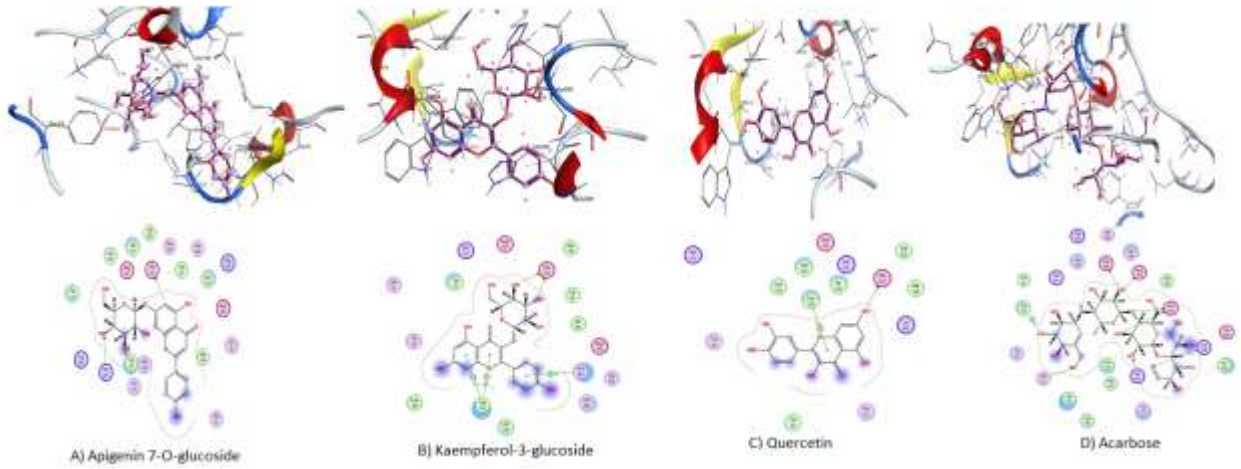

Figure 5. Docking Interaction of (A) Apigenin 7-O-glucoside, (B) Kaempferol-3-glucoside, (C) Quercetin, and (D) Acarbose with Alpha-Amylase (1OSE).

Pancreatic lipase (1HPL) results

The docking results of Pancreatic Lipase (1HPL) showed that there were strong interactions between the active site of the enzyme and the selected flavonoids. Apigenin 7-O-glucoside had a high hydrogen bonding capacity with SER 108 (H-donor) at 2.92 A, GLU 440 (H-donor) at 2.98 Å, and LYS 107 (H-acceptor) at 2.70 Å (binding energy ranged between -0.9 kcal/mol and -5.7 kcal/mol) (Figure 6a). Kaempferol-3-glucoside was hydrogen-bonded to LYS 107 (H-acceptor) at 3.13 Å and ASN 409 (H-acceptor) at 3.03 A with a binding energy value of -2.1 kcal/mol and -2.7 kcal/mol, respectively (Figure 6b). Quercetin was found to show strong interaction with PRO 413 at 2.91 Å (H-donor) with a binding energy of -2.4 kcal/mol, and π-H stacking interactions with ASN 409 at 4.67 Å (Figure 6c).

Comparatively, Orlistat, a well-known Pancreatic Lipase inhibitor, showed stronger associations with major active site residues and, in particular, with SER 108, GLU 440, and LYS 107 with a binding energy of -7.8 kcal/mol (Figure 6d). Although Orlistat has high binding affinity, the flavonoids showed a good competitive binding, indicating their good potential as pancreatic lipase inhibitors. This evidence suggests that the flavonoids might have a role in the management of Type 2 Diabetes by inhibiting fat digestion that eventually may lead to the regulation of glucose. Their capacity to control postprandial blood sugar levels makes them promising natural compounds to be investigated in the future in the context of Type 2 diabetes management.

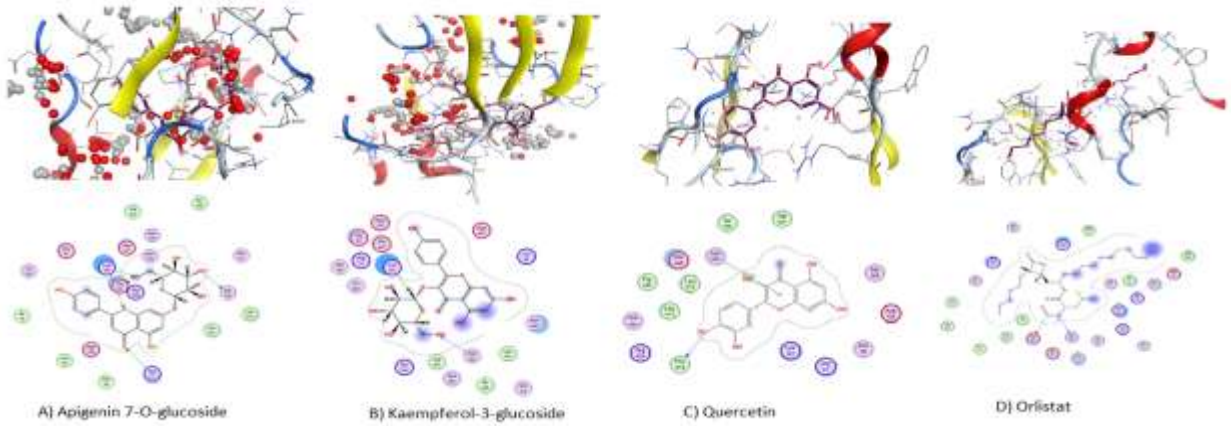

Figure 6. Docking Interaction of (A) Apigenin 7-O-glucoside, (B) Kaempferol-3-glucoside, (C) Quercetin, and (D) Acarbose with Pancreatic Lipase (1HPL).

Based on the binding energy values and the interactions with critical residues**,** Kaempferol-3-glucoside emerged as the most potent compound for Alpha-amylase inhibition, while Apigenin 7-O-glucoside was the most potent for Pancreatic lipase inhibition (Table 3). These compounds exhibited the highest binding affinities and interactions on the active sites of their respective enzymes, arguing that they could be used to enhance the regulation of glucose and help in controlling diabetes.

Table 3. Docking Results of Selected Ligands with Alpha-Amylase and Pancreatic Lipase.

Ligand	**Target**	**Interaction**	**Distance (Å)**	**Binding Energy (kcal/mol)**
Apigenin 7-O-glucoside	Alpha-amylase	GLU 233 (H-donor)	3.44	-0.6
Kaempferol-3-glucoside	Alpha-amylase	GLU 233 (H-donor)	2.73	-4.0
Quercetin	Alpha-amylase	GLU 233 (H-donor)	2.91	-1.4
Acarbose	Alpha-amylase	ASP 300 (H-donor)	2.51	-2.9
Apigenin 7-O-glucoside	Pancreatic lipase	SER 108 (H-donor)	2.92	-0.9
Kaempferol-3-glucoside	Pancreatic lipase	LYS 107 (H-acceptor)	3.13	-2.1
Quercetin	Pancreatic lipase	PRO 413 (H-donor)	2.91	-2.4
Orlistat	Pancreatic lipase	ASN 404 (H-donor)	2.89	-1.8

KEGG pathway analysis

Pathway enrichment analysis showed that the flavonoids among the selected flavonoids, including Apigenin 7-O-glucoside, Kaempferol-3-glucoside, and Quercetin, are important in the critical metabolic pathways, such as starch and sucrose metabolism, cholesterol metabolism, and other related pathways that are essential in regulating the digestion of glucose and lipids (Figure 7). These pathways play a significant role in explaining the effects of these flavonoids on carbohydrate and lipid metabolism, which are crucial in the management of diabetes and obesity.

The application of these flavonoids to the main digestive enzymes, including α -amylase (digesting starch to glucose) and pancreatic lipase (digesting triglycerides to fatty acids), is an essential factor in the regulation of the postprandial glucose release and the absorption of lipids. These flavonoids may influence carbohydrate metabolism through interaction with amylase-associated pathways, and thus they prevent postprandial hyperglycemia. Likewise, their interaction with lipid metabolic pathways may contribute to improved lipid utilization and metabolic balance.

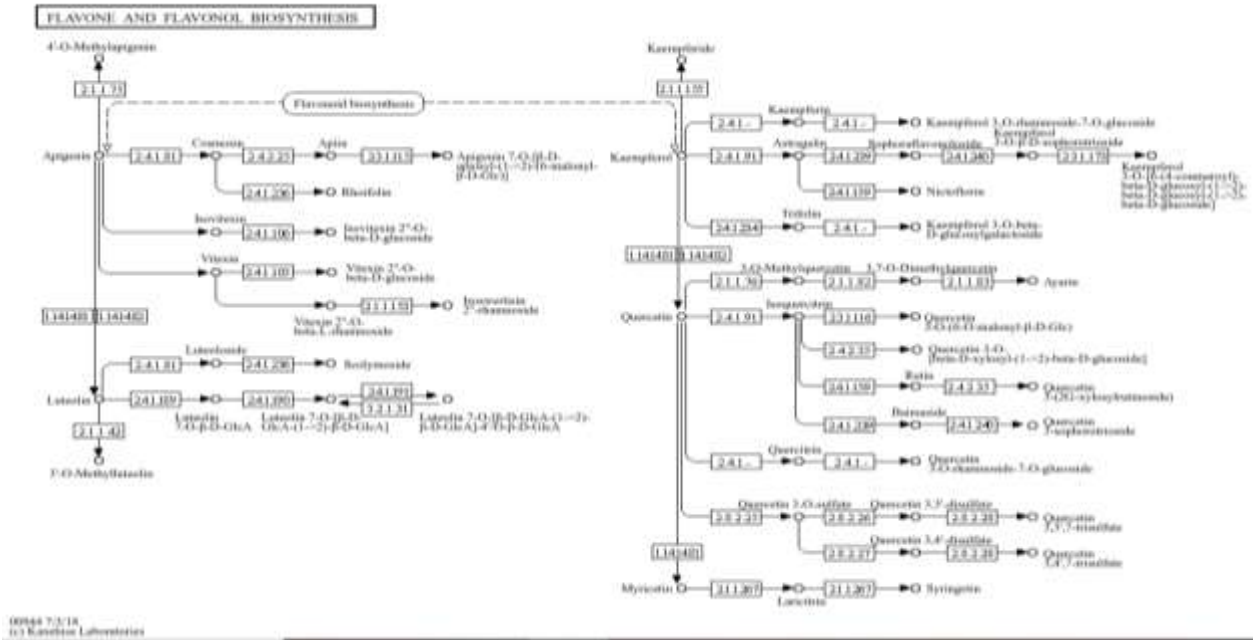

Figure 7. Interaction of flavonoid synthesis with glucose and lipid metabolism.

The pathway of KEGG Flavone and Flavonol Biosynthesis shows the synthesis of flavonoid glycosides, such as Apigenin 7 O-glucoside, Kaempferol 3-glucoside, and Quercetin. The derivatives of these upstream intermediates, Apigenin, Kaempferol and Quercetin aglycones, are involved in carbohydrate and lipid digestion regulation.
This dual mechanism of activity connects the plant flavonoid biosynthesis directly with the glucose and lipid metabolic control, indicating the therapeutic value of Bauhinia variegata flavonoids in the treatment of metabolic disorders. The results of the pathway enrichment analysis, therefore highlights how the natural compounds may be used as effective natural inhibitors in the regulation of the metabolism processes to offer an integrated method of controlling the metabolism of both fat and sugar in the case of diabetes and obesity (Sompong et al., 2016; Kanehisa et al., 2008).

DISCUSSION

Therapeutic discovery has traditionally been based on natural products, especially in the context of metabolic disorders. Of these, flavonoids have been widely examined in terms of their antioxidant, anti-inflammatory and enzyme-modulating properties. Bauhinia variegata is a well-known plant used in a wide variety of medicinal ways, and it contains bioactive compounds, including Apigenin 7-O-glucoside, Kaempferol-3-glucoside, and Quercetin. These flavonoids have been demonstrated to have an effect on glucose metabolism and lipid metabolism, which are essential in the management of type 2 diabetes and obesity (Shahana et al., 2017; Sharma et al., 2025). According to recent research, it has been reported that the phytochemicals of Bauhinia variegata could regulate amylase and lipase, which are enzymes that play a vital role in breaking down carbohydrates and fats. These flavonoids have the potential to support glucose and lipid homeostasis through modulation of digestive enzymes and associated metabolic pathways (Mukherjee, 2003; Whitcomb and Lowe, 2007).
In-silico docking was used to examine the molecular interactions of these flavonoids with α-amylase and pancreatic lipase. Kaempferol-3-glucoside had the highest binding affinity with α-amylase, and Apigenin 7-O-glucoside had strong interactions with lipase. The docking outcomes showed that flavonoids formed a hydrogen bond and π- π stacking complex with the active site residues that were important, indicating their ability to interact with catalytic residues and potentially modulate enzyme function. This becomes especially important in the management of diabetes, where inhibiting alpha-amylase may slow the process of breaking down starch to glucose, and inhibiting lipase may slow down the breakdown of dietary fats, which ultimately causes a decrease in blood glucose and fat absorption (Sanner, 1999; Jain, 2006). This is consistent with the previous research that has emphasized the use of flavonoid-based compounds to regulate digestive enzymes (Tucci et al., 2010; Singh et al., 2019).
Interestingly, the biochemical findings did not demonstrate suppression of amylase or lipase activities. Instead, treatment with Bauhinia variegata extracts restored enzyme activities toward normal physiological levels in diabetic animals. This observation suggests a possible pancreatic protective or functional restorative effect rather than direct enzymatic inhibition. The apparent discrepancy between docking predictions and biochemical findings may arise because molecular docking evaluates direct ligand–enzyme interactions under static conditions, whereas in vivo biochemical measurements reflect integrated physiological responses involving pancreatic tissue integrity, endocrine regulation and metabolic adaptation (Sompong et al., 2016).
Moreover, in order to explain a greater mechanistic position of these flavonoids, KEGG pathway analysis was conducted. The results of the analysis identified Apigenin 7-O-glucoside, Kaempferol-3-glucoside, and Quercetin as compounds taking part in important metabolic processes, such as starch metabolism, sucrose metabolism and cholesterol metabolism. These pathways play a critical role in the regulation of glucose and lipid digestion, which is crucial to regulating postprandial glucose and fat absorption. These pathways were inhibited by α-amylase and lipase, which substantiated the hypothesis that these flavonoids may play a role in better glycemic regulation and lipid metabolism (Kanehisa et al., 2008). The flavonoids would inhibit glucose absorption and fat digestion by affecting these important enzymes, and this corresponds to the results of other studies concerning the pharmacological role of flavonoids in the regulation of metabolic processes (Rao et al., 2004).
This is in addition to the encouraging data on the biochemical assays, it is important to test the toxicity profiles of the flavonoids before they can be utilized clinically. ProTox-II analysis identified Apigenin 7-O-glucoside and Kaempferol-3-glucoside to have a low toxicity that fits the Toxicity Class 5, which has a predicted LD50 value of above 5000 mg/kg, indicating a good safety profile. However, Quercetin fell in the Toxicity Class 3 group with a predicted LD50 of 159 mg/kg, indicating moderate toxicity. This increased toxicity was also linked to neurotoxicity and hepatotoxicity, as anticipated by the software, indicating the possibility that Quercetin exhibits increased biological activity but can be associated with increased risk of adverse effects, in contrast to the other two flavonoids. These results confirm the significance of toxicity tests in the process of drug development, as well as the promising qualities of Apigenin 7 -O -glucoside and Kaempferol -O -glucoside because of their low toxicity, although additional

research and potential dose adjustment will be required to allow safe therapeutic application of Quercetin (Banerjee et al., 2018).

The biochemical assays, in-silico docking, and KEGG pathway analysis findings presented were very strong evidence of the enzyme-modulating effect of Bauhinia variegata flavonoids. The findings may indicate that the compounds may be useful as natural inhibitors of α-amylase and lipase, which are key enzymes in the management of diabetes. Nevertheless, more studies have to be conducted to completely justify such compounds to be used in a clinical setting. Future research ought to be concerned with the use of long-term in-vivo experiments to determine the safety and effectiveness of these flavonoids in animal models and clinical trials on humans. Also, their bioavailability and pharmacokinetics will be investigated, which will make it possible to establish the most suitable dose and formulation to be used in a clinical setting. The scientific potential in terms of understanding how these flavonoids interact synergistically with other diabetic agents also allows the combination therapies to be developed that will provide a more integrated approach to the management of the metabolic disorders. In addition, proteomics and metabolomics may be utilized to study the global molecular processes by which these compounds control metabolic pathways, providing information on their multi-target therapeutic value in diabetes and obesity (Sharma et al., 2025).

CONCLUSION

This paper offers great information on the enzyme-modulating capability of Bauhinia variegata flavonoids (Apigenin 7-O-glucoside, Kaempferol-3-glucoside and Quercetin). The findings of the in-silico docking experiments, in-vitro enzyme analysis, and KEGG pathway show that these flavonoids possess significant potential to interact with and modulate α-amylase and lipase while contributing to metabolic regulation. The flavonoids may support normalization of digestive enzyme function and metabolic homeostasis. Moreover, the toxicity profile showed that Apigenin 7-O-glucoside and Kaempferol-3-glucoside had low toxicity; they are safer in therapeutic uses than Quercetin, with moderate toxicity. Although the results are encouraging, it requires more long-term research in vivo and clinical trials to have an in-depth understanding of the safety, efficacy, and bioavailability. Combining modern computational methods with natural herbal remedies might result in the emergence of an effective, natural treatment of metabolic disorders and a way forward in future research on combination therapy and multi-target interventions in diabetes treatment.

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