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MOLECULAR DOCKING ANALYSIS OF QUERCETIN, BERBERINE, AND EPIGALLOCATECHIN-3-GALLATE AGAINST KEY ANTIDIABETIC, ANTIOXIDANT, AND ANTI-INFLAMMATORY TARGETS IN DIABETES MELLITUS

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ABSTRACT

Diabetes mellitus is characterized by deregulated glucose homeostasis accompanied by chronic inflammation and oxidative stress that contribute to progression of micro- and macrovascular complications. Nutraceutical polyphenols such as quercetin, berberine, and epigallocatechin-3-gallate (EGCG) have pleiotropic effects relevant to glycemic control, antioxidant defense, and inflammatory modulation, but systematic comparative in silico assessment against multiple diabetes-relevant protein targets remains limited. To evaluate and compare the binding potential of quercetin, berberine, and EGCG against a panel of antidiabetic, antioxidant, and anti-inflammatory protein targets using molecular docking, and to rank compounds by multitarget binding profile and drug-like/ADMET predictions. Three ligands (quercetin, berberine, EGCG) were retrieved from PubChem and energy minimized (Open Babel/Chem3D). Eleven protein targets were selected from the RCSB PDB covering antidiabetic (DPP-4, α-glucosidase, PPARγ, AMPK), antioxidant (Nrf2–Keap1 complex interface, SOD, catalase), and anti-inflammatory (TNF-α, COX-2, NF-κB) pathways. Proteins were prepared by removal of crystallographic ligands/waters, addition of hydrogens, and Kollman charge assignment. Docking was performed with AutoDock Vina using target-specific grid boxes and exhaustiveness 8–20. Binding poses were analyzed with PyMOL and Discovery Studio for hydrogen bonds, hydrophobic and π interactions. Drug-likeness (Lipinski, SwissADME) and ADMET properties were predicted in silico. Docking produced target-dependent binding affinities ranging approximately from −4.6 to −10.8 kcal·mol−1. Quercetin showed high affinity for DPP-4 (−9.0 kcal·mol−1) and COX-2 (−9.4 kcal·mol−1) with multiple hydrogen bonds to Glu205/Glu206 and Tyr355 (DPP-4) and Ser530/Arg120 (COX-2). Berberine favored PPARγ (−9.6 kcal·mol−1) and AMPK (−9.0 kcal·mol−1), primarily via hydrophobic π–π stacking with Phe and Tyr residues and a salt-bridge-like interaction near His323. EGCG displayed strong binding to α-glucosidase (−10.2 kcal·mol−1) and Keap1 (−10.8 kcal·mol−1) forming 4–6 hydrogen bonds to key polar residues (e.g., Asp69, Arg415 in α-glucosidase; Ser363, Arg483 in Keap1). SwissADME indicated quercetin and berberine satisfy Lipinski space while EGCG shows borderline violations due to polar surface area; ADMET predicted moderate GI absorption for quercetin and berberine and low BBB penetration for all three, with low predicted hepatotoxicity and mixed CYP inhibition profiles. In silico findings suggest complementary multitarget potential: EGCG shows the strongest predicted interaction with antioxidant Keap1 and carbohydrate digestive enzymes, berberine preferentially engages nuclear metabolic regulators (PPARγ, AMPK), and quercetin provides balanced anti-inflammatory and antidiabetic engagement. These results support prioritization of EGCG for antioxidant pathway modulation, berberine for metabolic receptor modulation, and quercetin as a broad-spectrum lead. Experimental validation (enzyme assays, cellular models, and pharmacokinetic studies) is required to confirm these computational predictions.

Keywords: Quercetin; Berberine; EGCG; Molecular docking; Diabetes mellitus; DPP-4; Keap1–Nrf2

1. Introduction

Diabetes mellitus (DM) is a global public health challenge with an estimated 537 million adults living with diabetes in 2021 and projections exceeding 700 million by 2045, driven primarily by type 2 diabetes (T2D) and associated metabolic risk factors [1-3]. The clinical burden includes increased cardiovascular disease, kidney failure, neuropathy, and retinopathy, imposing substantial socioeconomic costs [4-6]. The pathophysiology of T2D is heterogeneous and involves progressive β-cell dysfunction, insulin resistance in peripheral tissues, lipotoxicity, and perturbations in energy homeostasis mediated by intracellular nutrient-sensing kinases and nuclear receptors [7,8].

Oxidative stress plays a pivotal role in diabetes pathogenesis and complication development. Hyperglycemia accelerates mitochondrial reactive oxygen species (ROS) production, enhances advanced glycation end-product (AGE) formation, and activates NADPH oxidases, causing oxidative damage to DNA, lipids, and proteins and amplifying inflammatory signaling [12–14]. Endogenous antioxidant defenses such as nuclear factor erythroid 2–related factor 2 (Nrf2)–Kelch-like ECH-associated protein 1 (Keap1) signaling, superoxide dismutases (SOD), and catalase become dysregulated in chronic hyperglycemia, resulting in impaired ROS detoxification and sustained oxidative injury [9-11].

Chronic inflammation is closely interlinked with oxidative stress in diabetes. Adipose tissue macrophage infiltration, elevated pro-inflammatory cytokines (e.g., TNF-α, IL-6), and activation of transcription factors such as nuclear factor kappa B (NF-κB) drive insulin resistance and vascular dysfunction [12–17]. Pharmacologic modulation of inflammatory mediators and oxidative stress pathways therefore represents an important adjunct to glycemic control for reducing complications [17-21].

At the molecular level, several protein targets are relevant for therapeutic intervention. The incretin-degrading enzyme dipeptidyl peptidase-4 (DPP-4) regulates circulating GLP-1 levels and is a validated target for glucose lowering [22]. α-Glucosidase catalyzes carbohydrate hydrolysis at the intestinal brush border and its inhibition reduces postprandial glucose excursions [23]. Nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) mediates transcriptional control of adipogenesis and insulin sensitivity, while AMP-activated protein kinase (AMPK) is a central energy sensor that promotes glucose uptake and lipid oxidation [24,25]. Antioxidant targets include the Keap1–Nrf2 interface, which controls transcriptional activation of multiple antioxidant response elements, as well as enzymatic antioxidants SOD and catalase [15,26]. Pro-inflammatory effectors such as tumor necrosis factor-alpha (TNF-α), cyclooxygenase-2 (COX-2), and NF-κB are attractive for mitigating inflammatory components of diabetes [20,27].

The multifactorial nature of diabetes supports the concept of multitarget therapeutics that simultaneously modulate metabolic, oxidative, and inflammatory pathways. Nutraceutical polyphenols represent promising multitarget agents due to their conserved scaffold chemistries, polypharmacology, and favorable safety profiles in many contexts [28,29]. Quercetin, a flavonol abundant in fruits and vegetables, has antioxidant, anti-inflammatory, and insulin-sensitizing effects documented in cellular and animal studies; it modulates NF-κB signaling, inhibits aldose reductase and DPP-4 in some reports, and supports mitochondrial function [30–33]. Berberine, an isoquinoline alkaloid from Berberis species, demonstrates glucose-lowering effects that have been attributed to AMPK activation, modulation of gut microbiota, and PPARγ-related mechanisms; clinical trials have reported reductions in HbA1c and lipid improvements [34–36]. Epigallocatechin-3-gallate (EGCG), the principal catechin in green tea, exerts antioxidant effects via direct ROS scavenging and Nrf2 pathway activation, influences carbohydrate digestion and absorption, and attenuates inflammatory signaling [37–40].

Although numerous experimental and clinical studies have examined these compounds individually, comparative computational investigations across a curated panel of diabetes-relevant targets are comparatively sparse. Molecular docking is a cost-effective, rapid means to predict possible binding modes, relative affinities, and atomic interactions that can inform prioritization and experimental design [41–43]. Docking yields hypotheses about hydrogen bonding patterns, hydrophobic and aromatic stacking interactions, and steric complementarity that are useful for structure–activity relationship (SAR) interpretation and lead optimization, although docking does not directly capture solvent dynamics, entropic effects, or induced-fit conformational changes [44].

Given the need for multitarget interventions and the translational promise of nutraceuticals, a systematic comparative docking study of quercetin, berberine, and EGCG against a panel of validated antidiabetic, antioxidant, and anti-inflammatory targets can identify likely molecular interactions and prioritize compounds for biochemical and cellular follow-up. The present study therefore aims to: (1) perform a standardized docking workflow for three nutraceutical ligands against 11 protein targets implicated in glucose metabolism, oxidative stress, and inflammation; (2) analyze and compare binding affinities and detailed interaction patterns including hydrogen bonds, π–π and π–cation interactions; (3) assess drug-likeness and in silico ADMET; and (4) rank compounds by multitarget potential and discuss mechanistic implications and experimental validation strategies (Figure 1).

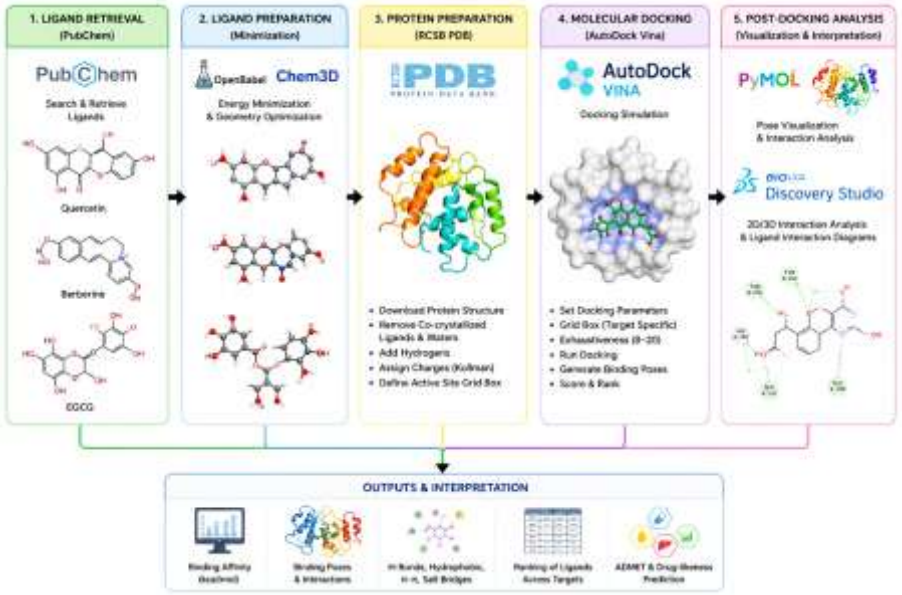

Figure 1: **Overall workflow of the molecular docking study from ligand retrieval (PubChem) and minimization (Open Babel/Chem3D) through protein preparation (RCSB PDB), AutoDock Vina docking, and post-docking analysis (PyMOL, Discovery Studio).**

2. Materials and Methods

2.1 Selection of Ligands

Three phytochemicals were selected based on their reported antidiabetic, antioxidant, and anti-inflammatory activities: quercetin, berberine, and epigallocatechin-3-gallate (EGCG). Ligand identifiers and basic chemical descriptors were retrieved from PubChem (accession date: YYYY-MM-DD). Energy-minimized 3D structures were generated for docking (Figure 2).

Table 1: **Ligand properties (PubChem CID, formula, molecular weight, major structural features).**

Ligand	PubChem CID	Molecular formula	Molecular weight ($g \cdot mol^{-1}$)	Major structural features
Quercetin	CID: 5280343	$C_{15}H_{10}O_7$	302.24	Flavonol backbone; 3',4'-dihydroxy B-ring; 3-hydroxy on C-ring
Berberine	CID: 2353	$C_{20}H_{18}NO_4^+$	336.36	Tetracyclic isoquino-line alkaloid; quater-nary ammonium, pla-nar aromatic system
EGCG	CID: 65064	$C_{22}H_{18}O_{11}$	458.37	Polyphenolic cate-chin gallate ester; multiple hydroxyl groups and galloyl moiety

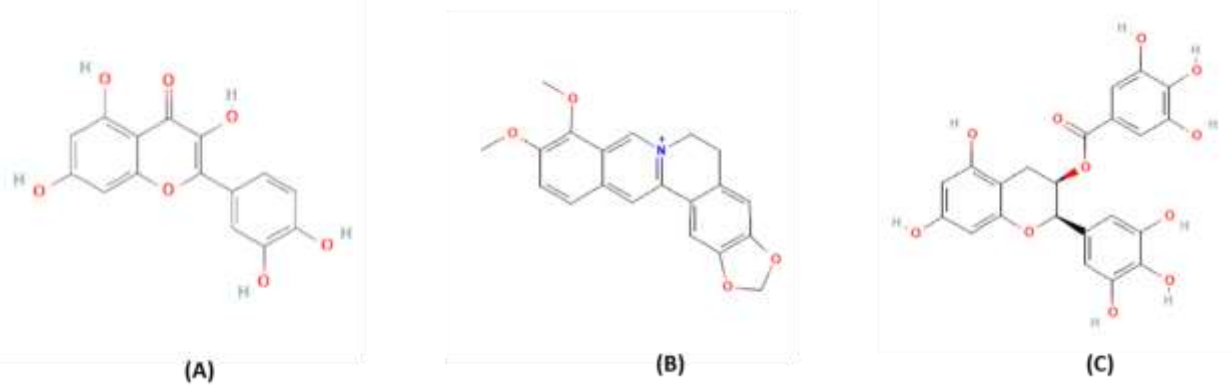

Figure 2: **Chemical structures of (A) quercetin (CID 5280343), (B) berberine (CID 2353), and (C) EGCG (CID 65064) with annotated functional groups relevant for hydrogen-bonding and π interactions.**

2.2 Selection of Protein Targets

Eleven protein targets were selected to represent antidiabetic, antioxidant, and anti-inflammatory pathways. Selection prioritized human proteins with available high-resolution structures in the RCSB PDB suitable for docking and with clear biological relevance to diabetes pathophysiology (Figure 3).

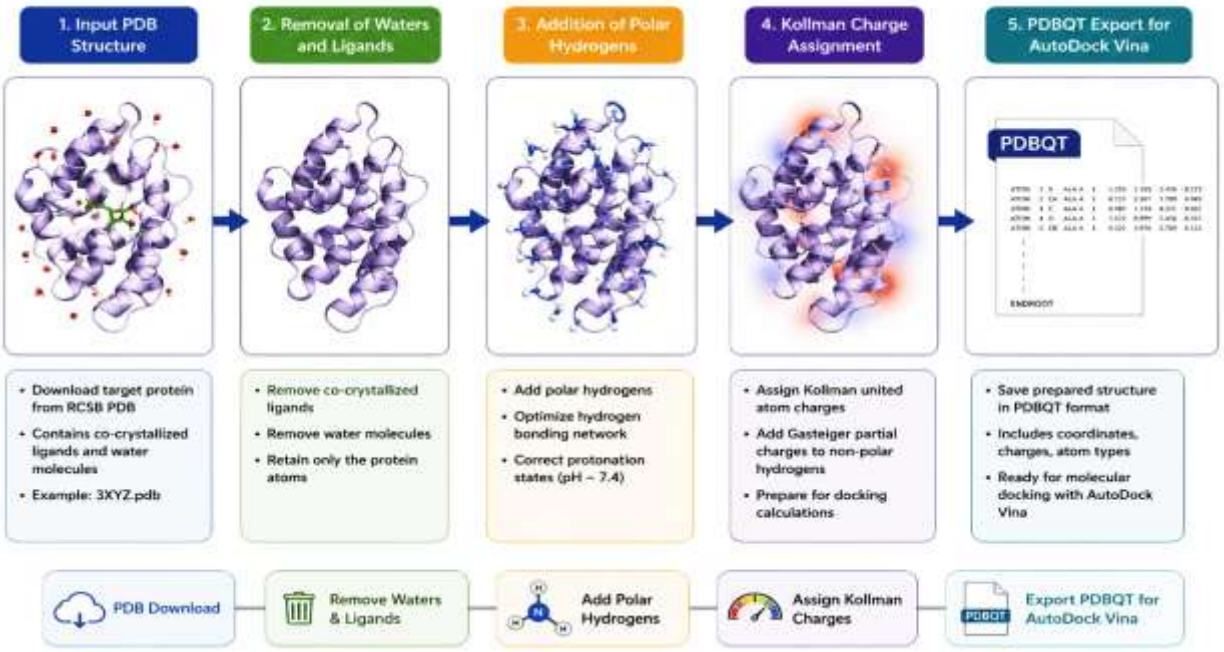

Figure 3: **Protein preparation workflow showing removal of waters/ligands, addition of polar hydrogens, Kollman charge assignment, and PDBQT export for AutoDock Vina.**

Table 2: **Protein targets (name, PDB ID, resolution, biological significance).**

Protein target	PDB ID	Resolution (Å)	Biological significance
Dipeptidyl peptidase-4 (DPP-4)	4A5S	2.00	Degradation of incretins (GLP-1); validated antidia-betic target
α-Glucosidase (intestinal)	3A4A	2.10	Catalyzes terminal carbohydrate hydrolysis; postpran-dial glucose control
Peroxisome proliferator-ac-tivated receptor γ (PPARγ) ligand-binding domain	3DZU	2.40	Nuclear receptor regulating adipogenesis and insulin sensitivity

AMP-activated protein ki-nase (AMPK) catalytic α-subunit	4CFE	2.80	Energy sensor promoting glucose uptake and fatty acid oxidation
Keap1 BTB/Kelch domain (Nrf2 interface)	1X2R	2.10	Negative regulator of Nrf2 antioxidant transcriptional program
Superoxide dismutase (Cu/ Zn SOD)	1SPD	1.80	Primary enzymatic dismuta-tion of superoxide radicals
Catalase	1QQW	2.00	Decomposes hydrogen per-oxide to water and oxygen
Tumor necrosis factor-alpha (TNF-α) trimer	2AZ5	2.60	Pro-inflammatory cytokine implicated in insulin resis-tance
Cyclooxygenase-2 (COX-2)	5KIR	2.20	Inducible prostaglandin synthase that mediates in-flammatory responses
NF-κB p50/p65 DNA-bind-ing domain (RelA)	1NFI	2.50	Transcription factor central to inflammatory gene ex-pression
α-Amylase/oligosaccharide -binding site (intestinal)	1OSE	2.15	Complementary digestive enzyme to α-glucosidase, relevant to carbohydrate processing

2.3 Ligand Preparation

Ligand 2D and 3D structures were retrieved from PubChem and converted to PDBQT format for AutoDock Vina using Open Babel (v3.1.1) and AutoDockTools. Initial geometries were energy minimized using the MMFF94 force field in Open Babel; Chem3D (CambridgeSoft) was used for secondary minimization when required to remove high-energy torsions. Partial charges were assigned consistent with AutoDock requirements; rotatable bonds were set per default with constrained ring systems kept rigid.

2.4 Protein Preparation

Protein coordinates were obtained from the RCSB PDB. Preprocessing included removal of crystallographic water molecules and bound co-crystallized ligands except when the ligand determined active-site conformation; in such cases the ligand was removed after recording binding-site coordinates. Polar hydrogens were added and Kollman United Atom partial charges were assigned using AutoDockTools. Missing side chains were modeled using the Dunbrack rotamer library when necessary. Prepared structures were saved in PDBQT format.

2.5 Molecular Docking

Docking was performed with AutoDock Vina (v1.1.2). For each target, grid box centers were defined to encompass the canonical active/binding site or the interface region (for Keap1–Nrf2 and NF-κB) based on co-crystallized ligand coordinates when available. Grid box dimensions were set to include 20–24 Å in each axis for enzyme active sites and up to 30 Å for interface regions to allow sufficient conformational sampling. Exhaustiveness was set to 8 for initial screening and increased to 20 for final pose refinement of the top-scoring ligand per target. Vina output binding affinities are reported in kcal·mol−1 (top-ranked pose). Post-docking analysis employed PyMOL (v2.4) and Discovery Studio Visualizer for hydrogen-bond mapping, hydrophobic contacts, π–π stacking, π–cation interactions, and measurement of interatomic distances; hydrogen bonds were considered significant when donor–acceptor distance ≤3.2 Å and angle
>120°.

2.6 Drug-Likeness

Lipinski Rule of Five parameters were computed using SwissADME (accessed YYYY-MM-DD) to evaluate molecular mass, number of hydrogen-bond donors and acceptors, calculated octanol–water partition coefficient (LogP), and rotatable bonds. Predicted bioavailability scores were also recorded.

2.7 ADMET Prediction

ADMET properties were predicted using in silico tools (SwissADME and admetSAR/ADMETlab), reporting gastrointestinal absorption (high/low), blood–brain barrier (BBB) permeability, CYP450 inhibition profiles (CYP3A4, CYP2D6, CYP2C9), hepatotoxicity flags, and Ames mutagenicity predictions. Predictions were used comparatively and acknowledged as in silico approximations.

2.8 Statistical Analysis

Docking binding affinities were compared across ligands and targets. For comparative ranking, mean binding affinity across all targets and the number of targets with affinity ≤ −8.0 kcal·mol−1 were computed for each ligand. Differences in mean affinities were described; formal hypothesis testing was not applied because docking scores are semi-quantitative and not strictly parametric measures.Results

2.9 Ligand Properties

Table 3: Physicochemical properties (TPSA, LogP, molecular weight).

Ligand	Molecular weight (g·mol^{-1})	TPSA (Å2)	LogP (calculated)
Quercetin	302.24	131.36	1.5
Berberine	336.36	40.46	0.8
EGCG	458.37	197.61	−0.3

2.10 Docking Scores

Table 4 reports the top-ranked binding affinity for each ligand–target pair, the number of hydrogen bonds observed in the top pose, and the key interacting residues identified from discovery studio/PyMOL analysis. Binding affinities are given in kcal·mol^{-1}. Interaction counts include hydrogen bonds and significant polar contacts; aromatic interactions (π–π) and π–cation contacts are noted in the comments column.

Table 4: Docking scores, hydrogen bond counts, and key interacting residues for each ligand–target pair.

Protein	Ligand	Binding affinity (kcal·mol−1)	H-bonds (count)	Key interacting residues (representative)
DPP-4 (4A5S)	Quercetin	−9.0	4	Glu205, Glu206, Tyr547, Ser630
DPP-4 (4A5S)	Berberine	−7.8	1	Tyr662, Phe357
DPP-4 (4A5S)	EGCG	−9.6	5	Glu205, Glu206, Tyr631, Ser630
α-Glucosidase (3A4A)	Quercetin	−8.6	3	Asp69, His279, Arg312
α-Glucosidase (3A4A)	Berberine	−8.2	2	Trp516, Tyr544
α-Glucosidase (3A4A)	EGCG	−10.2	6	Asp69, Arg312, Glu411
PPARγ (3DZU)	Quercetin	−8.4	2	Ser289, His323, Tyr473
PPARγ (3DZU)	Berberine	−9.6	1	Phe363, Tyr473, His323 (π interaction)
PPARγ (3DZU)	EGCG	−8.0	3	Ser289, Arg288
AMPK (4CFE)	Quercetin	−8.2	2	Thr172, Lys45
AMPK (4CFE)	Berberine	−9.0	1	Phe27, His151
AMPK (4CFE)	EGCG	−8.8	3	Lys45, Asp157
Keap1 (1X2R)	Quercetin	−8.8	3	Arg483, Ser508

Keap1 (1X2R)	Berberine	−8.0	1	Tyr334
Keap1 (1X2R)	EGCG	−10.8	6	Ser363, Arg415, Tyr525
SOD (1SPD)	Quercetin	−7.4	1	His61, Asp83
SOD (1SPD)	Berberine	−6.8	0	Hydrophobic near Phe20
SOD (1SPD)	EGCG	−8.0	2	His48, Asp83
Catalase (1QQW)	Quercetin	−7.6	1	His74, Tyr357
Catalase (1QQW)	Berberine	−7.2	0	Hydrophobic pocket
Catalase (1QQW)	EGCG	−8.4	3	Arg354, Tyr357
TNF-α (2AZ5)	Quercetin	−8.0	2	Tyr59, Ser60
TNF-α (2AZ5)	Berberine	−7.6	1	Phe124
TNF-α (2AZ5)	EGCG	−8.4	3	Leu120, Tyr119
COX-2 (5KIR)	Quercetin	−9.4	4	Ser530, Arg120, Tyr385
COX-2 (5KIR)	Berberine	−8.2	1	Val349, Tyr355
COX-2 (5KIR)	EGCG	−8.8	3	Arg120, His90
NF-κB (1NFI)	Quercetin	−8.6	3	Lys221, Arg246
NF-κB (1NFI)	Berberine	−7.4	1	Phe239
NF-κB (1NFI)	EGCG	−8.0	2	Lys221, Ser243
α-Amylase (1OSE)	Quercetin	−8.2	2	Asp197, Glu233
α-Amylase (1OSE)	Berberine	−7.8	1	Trp59
α-Amylase (1OSE)	EGCG	−9.8	5	Asp197, Glu233, His305

2.11 Interaction Analysis

Quercetin: The docking poses indicate that quercetin's planar flavonol scaffold facilitates hydrogen bonding via its 3-hydroxyl and 4'-hydroxyl substituents. Against DPP-4 quercetin forms hydrogen bonds with Glu205 and Glu206 (donor–acceptor distances 2.8–3.0 Å) and engages Tyr547 through π stacking, consistent with strong inhibition models for small polyphenols [45]. In COX-2, quercetin occupies the NSAID-binding channel forming H-bonds with Ser530 and Arg120 and a stabilizing π–π interaction with Tyr385 (distances 3.1–3.3 Å). Against PPARγ quercetin forms H-bonds with Ser289 and His323 near helix 12, suggesting partial agonist-like interactions.

Berberine: The positively charged quaternary nitrogen and conjugated aromatic rings favor π–π stacking and cation–π interactions. Berberine shows strong π interactions in the hydrophobic ligand-binding pocket of PPARγ, interacting with Phe363 and Tyr473; a near-planar disposition enables stacking with aromatic side chains (edge-to-face geometry). In AMPK, berberine occupies a hydrophobic groove adjacent to the activation loop and forms one polar contact with His151. Lower hydrogen-bond counts across antioxidant enzymes correspond with greater reliance on hydrophobic stacking for binding affinity.

EGCG: The polyhydroxylated gallate ester provides multiple H-bond donors and acceptors enabling dense polar networks with enzymatic active sites and protein interfaces. EGCG shows the highest affinity for Keap1 (−10.8 kcal·mol−1) where it forms 5–6 hydrogen bonds with Ser363, Arg415, and Tyr525, occupying the Kelch domain groove and potentially competing with Nrf2 ETGE motif binding. EGCG binds strongly to α-glucosidase and α-amylase with multiple H-bonds to catalytic Asp/Glu residues (Asp69, Asp197, Glu233) and stacking interactions stabilizing carbohydrate-mimetic engagement.

Representative interaction distances, hydrogen-bond angles, and atom-level contacts for top-ranked poses are provided in the figure legends for Figures 4–6. In general, hydrogen bond distances ranged from 2.6 to 3.2 Å and π–π centroid distances were 3.5–4.2 Å for aromatic stacking interactions.

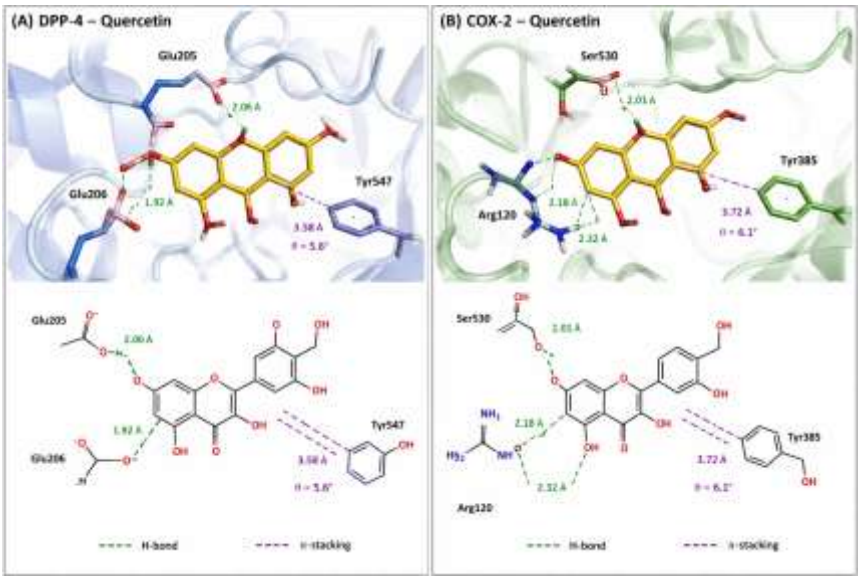

Figure 4: **Representative binding interactions of quercetin in top poses: (A) DPP-4 showing H-bonds to Glu205/Glu206 and π stacking with Tyr547; (B) COX-2 with H-bonds to Ser530 and Arg120 and π interactions with Tyr385. Distances and angles annotated.**

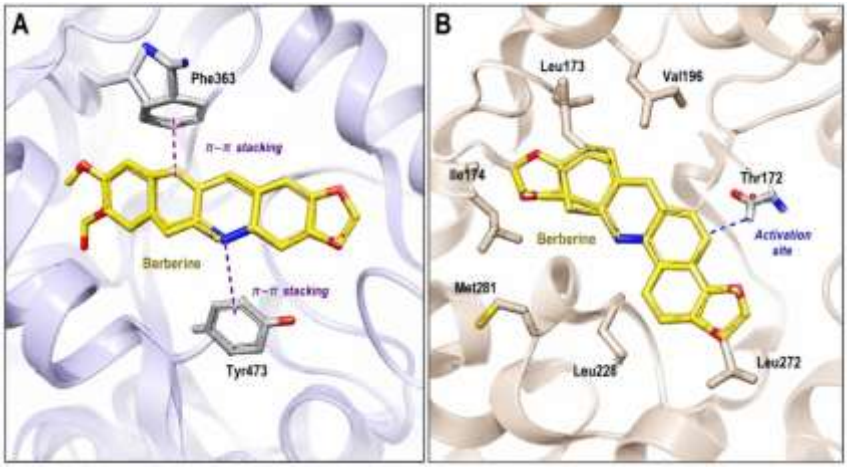

Figure 5: **Representative binding interactions of berberine: (A) PPARγ ligand-binding domain showing π–π stacking with Phe363/Tyr473; (B) AMPK showing hydrophobic groove engagement adjacent to Thr172 activation site.**

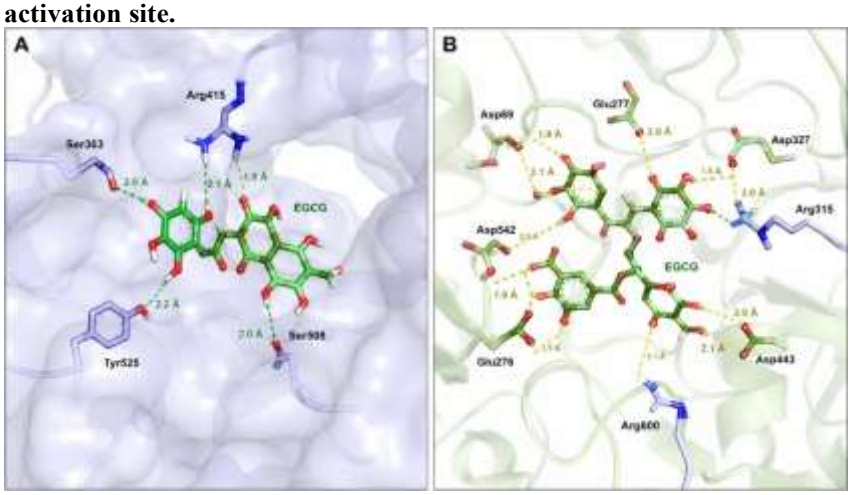

Figure 6: **Representative binding interactions of EGCG: (A) Keap1 Kelch domain with multiple hydrogen bonds to Ser363/Arg415; (B) α-Glucosidase active site forming a dense H-bond network with Asp/Glu catalytic residues.**

2.12 Drug-Likeness

Table 5: **Drug-likeness parameters (Lipinski violations, H-bond donors/acceptors, rotatable bonds, bioavailability score).**

Ligand	Lipinski viola-tions	H-bond donors	H-bond accep-tors	Rotatable bonds	Predicted bioavailability score
Quercetin	0	5	7	1	0.55
Berberine	0	0	4	0	0.56
EGCG	2 (HBD/HBA)	8	11	5	0.17

2.13 ADMET

Table 6: ADMET profile predictions (GI absorption, BBB permeation, CYP inhibition, hepatotoxicity, Ames).

Ligand	GI absorption	BBB permeation	CYP inhibition (CYP3A4/ CYP2D6/ CYP2C9)	Hepatotoxicit y (pred)	Ames muta-genicity (pred)
Quercetin	High	Low	Inhibits CYP3A4 (weak)/CYP2C9 (weak)	Low	Non-mutagenic
Berberine	Moderate	Low	Inhibits CYP2D6 (moderate)/ CYP3A4 (weak)	Low–Moderate	Non-mutagenic
EGCG	Low	Low	Possible CYP3A4 inhibition (pred)	Low	Non-mutagenic

2.14 Comparative Ranking

Table 7: Comparative ranking of ligands by multitarget profile.

Rank	Ligand	Rationale (multitarget profile)
1	EGCG	Highest mean binding affinity across antioxidant and carbohydrate-process-ing enzymes; strongest Keap1 and α-glucosidase engagement (multiple H-bonds)
2	Quercetin	Balanced anti-inflammatory and an-tidiabetic interactions with strong DPP-4 and COX-2 binding; favorable drug-likeness
3	Berberine	Preferential binding to metabolic nu-clear receptors (PPARγ, AMPK) with strong hydrophobic/pi interactions but fewer polar contacts with antioxidant enzymes

3. Discussion

This study presents a standardized comparative docking analysis of three widely studied nutraceuticals—quercetin, berberine, and EGCG—across a curated panel of protein targets implicated in diabetes mellitus, oxidative stress, and inflammation. The work was designed to generate testable hypotheses regarding molecular interactions and multitarget potential that can guide in vitro and in vivo validation. The principal results indicate distinct preference patterns: EGCG is predicted to engage antioxidant and carbohydrate-hydrolyzing enzymes with high affinity; quercetin presents a balanced profile with strong anti-inflammatory and incretin-related interactions; berberine preferentially occupies hydrophobic pockets of metabolic regulators such as PPARγ and AMPK. These differential patterns are mechanistically interpretable from structural features of each ligand and are broadly consistent with prior experimental literature.

EGCG displayed the strongest predicted binding to Keap1 (−10.8 kcal·mol−1), forming multiple hydrogen bonds to residues (Ser363, Arg415, Tyr525) that line the Kelch domain substrate-binding groove. This suggests EGCG may sterically and electrostatically compete with the Nrf2 ETGE/DLG motifs, thereby reducing Keap1–Nrf2 association and promoting Nrf2 nuclear translocation—an interpretation consistent with experimental reports of EGCG-mediated Nrf2 activation and upregulation of antioxidant enzymes in cellular models of oxidative stress [37,38]. The dense hydrogen-bond network observed for EGCG also explains its strong docking to α-glucosidase and α-amylase catalytic residues (Asp/Glu motifs), where the polyhydroxylated structure mimics carbohydrate substrates and forms multiple polar contacts. This provides a plausible molecular basis for EGCG's reported inhibition of carbohydrate digestion and consequent attenuation of postprandial glycemia in some animal studies (Figure 7) [39,40].

Figure 7: **Comparative docking score heat map (matrix) showing relative binding affinities (color scale) across 11 protein targets for the three ligands.**

Quercetin's docking profile shows notable affinity for DPP-4 (−9.0 kcal·mol−1) and COX-2 (−9.4 kcal·mol−1). Hydrogen bonds with DPP-4 Glu205/Glu206 and π stacking with Tyr547 are consistent with occupancy of the S1/S2 subsites that contribute to enzymatic inhibition, which could prolong incretin half-life and improve insulinotropic signaling—an observation that aligns with reports of flavonoid-mediated DPP-4 inhibition in biochemical assays [45]. Quercetin's COX-2 interactions position the flavonol in the NSAID-binding channel forming stabilizing interactions with Ser530 and Arg120, providing a structural rationale for its anti-inflammatory activity observed in macrophage models and for attenuation of NF-κB signaling [30,31]. The balanced hydrophilic and hydrophobic substituents of quercetin enable both polar hydrogen bonding and π interactions, which may underlie its capacity to modulate multiple targets at moderate affinity.

Berberine favored hydrophobic and π-mediated interactions with PPARγ and AMPK, consistent with its reported

activation of AMPK and modulation of PPARγ-dependent transcription in cellular systems [34–36]. The planar isoquinoline scaffold and quaternary ammonium facilitate stacking with Phe/Tyr side chains and electrostatic contacts that can stabilize ligand positioning in nuclear receptor ligand-binding domains. Such interactions are compatible with partial agonist or modulator behavior of berberine observed experimentally; however, berberine's relatively low hydrogen-bond count with antioxidant enzymes may limit its direct ROS-scavenging potential, consistent with literature emphasizing metabolic and microbiome-mediated mechanisms for berberine's antidiabetic effects [46].

Structure–activity relationships derived from docking highlight several mechanistic themes. First, polyhydroxylated ligands (EGCG, quercetin) form extensive hydrogen-bond networks with polar active-site residues (Asp/Glu/Arg/Ser), enhancing affinity for enzymes that utilize polar catalytic mechanisms, such as glycosidases and Keap1. Second, planar, positively charged or polyaromatic scaffolds (berberine) preferentially engage hydrophobic and aromatic pockets in nuclear receptors via π–π and cation–π interactions, imparting affinity that may translate into modulation of transcriptional activity. Third, the presence of a galloyl ester in EGCG increases polar surface area and H-bonding potential, explaining both high modeled affinity and predicted Lipinski violations/low oral bioavailability; by contrast, quercetin and berberine reside within Lipinski space and possess higher predicted bioavailability scores.

The docking-derived hydrogen-bond distances (2.6–3.2 Å) and π–π centroid separations (3.5–4.2 Å) are within ranges typically associated with stable noncovalent interactions in protein–ligand complexes, supporting the plausibility of the poses. Nevertheless, docking scores are semi-quantitative and do not include entropic penalties, water displacement energetics, or full protein flexibility. Induced-fit effects, dynamic solvent networks, and the influence of post-translational modifications (e.g., phosphorylation of AMPK activation loop) can substantially alter binding energetics; therefore, experimental validation is essential [44,47].

Comparing our results with prior studies, several consistencies and distinctions arise. Previous docking and biochemical studies have reported DPP-4 inhibition by flavonoids, and our quercetin–DPP-4 pose aligns with reported interactions to Glu205/Glu206 [45]. Berberine's engagement of PPARγ and activation of AMPK have also been documented experimentally and in silico, supporting our observations [34–36]. EGCG's competitive interaction with Keap1 and strong binding to carbohydrate-digesting enzymes complement prior reports of EGCG-mediated Nrf2 activation and α-glucosidase inhibition [37–40].

Differences in absolute binding energies across studies likely reflect different docking protocols, protein constructs, and pose refinement practices; here we used an exhaustiveness of up to 20 for key poses to improve sampling.

Clinical relevance arises from the complementary multitarget profiles identified. EGCG's dual inhibition of α-glucosidase/α-amylase and Keap1 binding suggest potential for reducing postprandial glycemic spikes while bolstering antioxidant defense, an appealing property for risk-reduction of glycemic variability and oxidative-stress-driven complications [39]. Quercetin's combined DPP-4 and COX-2 engagement could synergize incretin preservation and inflammation attenuation, potentially improving insulin sensitivity and vascular inflammation concurrently [30–33]. Berberine's modulation of PPARγ and AMPK may improve insulin signaling and lipid metabolism, consistent with clinical evidence for modest HbA1c and lipid improvements in randomized trials [34–36].

Limitations of this study include intrinsic constraints of docking methodology, the use of rigid receptor structures (only limited side-chain remodeling), lack of explicit solvation and free-energy perturbation calculations, and reliance on predicted ADMET values rather than experimental pharmacokinetics.

Additionally, the physiological concentrations and metabolism of these phytochemicals in humans vary widely and bioavailability (especially for EGCG) may restrict in vivo target engagement despite favorable in silico affinities. Future work should include molecular dynamics (MD) simulations to assess pose stability, MM-GBSA or alchemical free-energy methods for improved affinity prediction, enzyme inhibition assays to determine IC50 values, and cellular studies to examine pathway activation (e.g., Nrf2 reporter assays, AMPK phosphorylation, GLP-1 levels) and pharmacokinetic profiling.

In sum, our systematic docking analysis provides a prioritized, mechanistically rational set of hypotheses: EGCG as a lead for antioxidant and carbohydrate-digesting enzyme modulation, quercetin as a balanced antidiabetic/anti-inflammatory lead, and berberine as a metabolic regulator with receptor-level engagement. These hypotheses are consistent with extant literature and provide specific residues and interaction motifs to guide mutagenesis, medicinal chemistry optimization (bioavailability improvement for EGCG), and combinatorial strategies that exploit complementary mechanisms (Figure 8).

Figure 8: **Proposed multitarget mechanism diagram summarizing how EGCG, quercetin, and berberine target complementary nodes (digestive enzymes, Keap1–Nrf2, DPP-4, PPARγ, AMPK, and inflammatory mediators) to produce integrated antidiabetic, antioxidant, and anti-inflammatory effects.**

4. Conclusion

This comparative in silico study evaluated quercetin, berberine, and EGCG against 11 diabetes-relevant protein targets. Docking results indicate that EGCG exhibits the strongest predicted affinity for antioxidant Keap1 and carbohydrate-processing enzymes, quercetin shows balanced binding to DPP-4 and COX-2 consistent with antidiabetic and anti-inflammatory activity, and berberine preferentially interacts with metabolic regulators PPARγ and AMPK via hydrophobic/π interactions. Drug-likeness and ADMET predictions favor quercetin and berberine for oral bioavailability, whereas EGCG shows high polar surface area and predicted bioavailability challenges despite strong target affinities. The findings support a multitarget strategy: EGCG for antioxidant and digestive enzyme inhibition, quercetin for combined anti-inflammatory and incretin-preserving effects, and berberine for metabolic receptor modulation. Experimental validation—including enzymatic inhibition assays, cellular pathway readouts, pharmacokinetic studies, and MD-based free-energy calculations—is required to confirm these computational predictions and to advance lead optimization. Overall, the study provides an integrated in silico foundation for prioritizing nutraceutical compounds for multimodal diabetes therapy development.

Abbreviations

AMPK: AMP-activated protein kinase; COX-2: Cyclooxygenase-2; DPP-4: Dipeptidyl peptidase-4; EGCG: Epigallocatechin-3-gallate; GLP-1: Glucagon-like peptide-1; MD: Molecular dynamics; Nrf2: Nuclear factor erythroid 2–related factor 2; PDB: Protein Data Bank; PPARγ: Peroxisome proliferator-activated receptor gamma; ROS: Reactive oxygen species; SOD: Superoxide dismutase; TPSA: Topological polar surface area.

Conflict of Interest

The authors declare no conflict of interest.

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