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IN SILICO EVALUATION OF ANTIDIABETIC POTENTIAL OF RUSSELIA EQUISETIFORMIS PHYTOCHEMICALS VIA MOLECULAR DOCKING AND ADMET ANALYSIS

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ABSTRACT

Background: *Diabetes mellitus represents one of the most challenging metabolic disorders of the 21st century, affecting approximately 537 million adults globally. The search for novel therapeutic agents from natural sources has gained significant momentum, particularly focusing on plant-derived bioactive compounds with multitarget antidiabetic potential. Russelia equisetiformis (Scrophulariaceae), commonly known as the Firecracker plant, has been traditionally employed in Mexican and Nigerian folk medicine for the management of type 2 diabetes mellitus, yet its phytochemical constituents remain largely unexplored through computational approaches.*

Objective: *This study was designed to identify potential antidiabetic lead compounds from R. equisetiformis through comprehensive molecular docking analysis and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling against key diabetes therapeutic targets.*

Methods: *A curated library of twelve phytochemicals previously identified from R. equisetiformis, including phenylethanoid glycosides (russeliaoside, russectinol), flavonoids (luteolin, apigenin, quercetin, kaempferol, genistein), triterpenes (lupeol, oleanolic acid, ursolic acid), and phenolic acids (chlorogenic acid, caffeic acid), was subjected to in silico molecular docking analysis using AutoDock Vina. Three critical antidiabetic targets were selected: human pancreatic alpha-amylase (PDB ID: 4W93), human intestinal alpha-glucosidase (PDB ID: 2QMJ), and peroxisome proliferator-activated receptor gamma (PPAR-gamma, PDB ID: 3G9E). The reference drugs acarbose and metformin were employed as positive controls. Subsequently, pharmacokinetic properties and drug-likeness were assessed using SwissADME, pkCSM, and ADMETlab web servers.*

Results: *Among the tested phytochemicals, luteolin exhibited the highest binding affinity against alpha-amylase with a binding energy of -8.3 kcal/mol, comparable to the reference drug acarbose (-8.5 kcal/mol). Quercetin demonstrated superior binding to alpha-glucosidase at -8.6 kcal/mol, while genistein showed exceptional affinity for PPAR-gamma with a binding energy of -7.8 kcal/mol. The phenylethanoid glycosides russeliaoside and russectinol displayed moderate binding affinities across all three targets, with values ranging from -6.2 to -7.1 kcal/mol. ADMET analysis revealed that the majority of flavonoids possessed favorable pharmacokinetic profiles, with high gastrointestinal absorption and compliance with Lipinski's Rule of Five.*

Conclusion: *The findings of this study provide compelling computational evidence that R. equisetiformis harbors phytochemical constituents with significant antidiabetic potential, particularly the flavonoids luteolin, quercetin, and genistein, which demonstrate multitarget inhibitory activity against key enzymes and receptors involved in glucose homeostasis. These results substantiate the traditional use of R. equisetiformis in diabetes management and warrant further in vitro and in vivo validation to develop these lead compounds as novel antidiabetic therapeutics.*

Keywords*: Russelia equisetiformis, molecular docking, ADMET analysis, antidiabetic activity, alpha-amylase, alpha-glucosidase, PPAR-gamma, flavonoids, phenylethanoid glycosides, drug-likeness*

1. INTRODUCTION

1.1 Global Burden of Diabetes Mellitus

Diabetes mellitus has emerged as one of the most formidable global health challenges of the contemporary era, representing a complex metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. According to the International Diabetes Federation (IDF), approximately 537 million adults aged between 20 and 79 years were living with diabetes in 2021, and this number is projected to escalate to 643 million by 2030 and 783 million by 2045 if current trends persist unabated. The overwhelming majority of cases, roughly 90%, are classified as type 2 diabetes mellitus (T2DM), which is strongly associated with lifestyle factors including obesity, physical inactivity, and dietary habits. The economic burden imposed by diabetes is staggering, with global healthcare expenditures estimated at approximately 966 billion USD in 2021, accounting for 9% of total adult health expenditure worldwide.

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Beyond the direct metabolic consequences, diabetes serves as a significant risk factor for numerous debilitating complications including cardiovascular disease, nephropathy, retinopathy, neuropathy, and cerebrovascular disorders, collectively contributing to substantial morbidity and premature mortality. The pathophysiology of T2DM involves a complex interplay of multiple mechanisms, including progressive beta-cell dysfunction, insulin resistance in peripheral tissues such as skeletal muscle, liver, and adipose tissue, increased hepatic glucose production, and impaired incretin secretion and action. Given the multifactorial nature of this disease, therapeutic strategies targeting various pathways involved in glucose homeostasis have been developed. Currently available pharmacological interventions include biguanides (metformin), sulfonylureas, thiazolidinediones, dipeptidyl peptidase-4 (DPP-4) inhibitors, sodium-glucose cotransporter-2 (SGLT-2) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, and alpha-glucosidase inhibitors. While these medications have demonstrated efficacy in glycemic control, their clinical utility is often limited by adverse effects such as gastrointestinal disturbances, weight gain, hypoglycemia, cardiovascular risks, and economic constraints, particularly in resource-limited settings.

1.2 Plant-Derived Antidiabetic Agents

Medicinal plants have served as the cornerstone of traditional medicine systems across diverse cultures for millennia, and they continue to represent an invaluable reservoir of bioactive compounds with therapeutic potential. The World Health Organization estimates that approximately 80% of the population in developing countries relies primarily on traditional medicine for their healthcare needs, with medicinal plants constituting a major component of such practices. In the context of diabetes management, numerous plant species have been documented in ethnomedicinal literature for their hypoglycemic properties, and many have subsequently been validated through scientific investigation. The antidiabetic activity of plant-derived compounds is typically attributed to their ability to modulate various physiological processes, including enhancement of insulin secretion, improvement of insulin sensitivity, inhibition of carbohydrate-digesting enzymes, reduction of intestinal glucose absorption, and protection of pancreatic beta-cells from oxidative stress and apoptosis.

The major classes of phytochemicals that have demonstrated antidiabetic potential include alkaloids, flavonoids, terpenoids, saponins, phenolic acids, and glycosides. Among these, flavonoids have garnered particular attention due to their diverse biological activities, including antioxidant, anti-inflammatory, antidiabetic, and cardioprotective effects. Mechanistic studies have revealed that flavonoids such as quercetin, luteolin, apigenin, and kaempferol exert antidiabetic effects through multiple pathways, including inhibition of alpha-amylase and alpha-glucosidase enzymes, activation of AMP-activated protein kinase (AMPK), modulation of peroxisome proliferator-activated receptor gamma (PPAR-gamma) activity, and enhancement of glucose uptake in peripheral tissues. Similarly, phenylethanoid glycosides, a class of water-soluble phenolic compounds widely distributed in the plant kingdom, have demonstrated significant antioxidant, anti-inflammatory, and antidiabetic activities in various experimental models.

1.3 Russelia equisetiformis: Ethnopharmacology and Phytochemistry

Russelia equisetiformis Schlecht. and Cham., commonly known as Firecracker plant, Coral plant, or Fountain plant, belongs to the family Plantaginaceae (formerly Scrophulariaceae) and is native to Mexico and Central America. The plant derives its common name from its distinctive bright red, tubular flowers that resemble firecrackers, and it has been widely cultivated as an ornamental species in tropical and subtropical regions worldwide. Beyond its ornamental value, R. equisetiformis holds significant ethnomedicinal importance in various traditional medicine systems. In Mexican traditional medicine, the whole plant is utilized as complementary therapy for patients with type 2 diabetes mellitus, where aqueous decoctions are administered orally to manage blood glucose levels. Similarly, in Nigerian traditional medicine, the plant is employed for the treatment of diabetes, malaria, cancer, and various inflammatory disorders.

Phytochemical investigations of R. equisetiformis have revealed a rich diversity of bioactive compounds belonging to multiple chemical classes. The leaves have been reported to contain alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids. The most characteristic secondary metabolites isolated from this species are the phenylethanoid

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glycosides russeliaoside and russectinol, which have been repeatedly identified as the principal active constituents. Additionally, triterpenes of the lupane type have been isolated and characterized. The essential oil of R. equisetiformis has been analyzed by gas chromatography-mass spectrometry, revealing approximately twenty compounds, with hexadecanoic acid methyl ester (11.04%), 11-methyltetracosane (8.44%), n-docosane (7.66%), alpha-pinene (7.26%), octadecanoic acid methyl ester (6.37%), and eicosanoic acid methyl ester (6.16%) as the major constituents. The phenylethanoid glycosides, including verbascoside and related compounds, have been identified as significant contributors to the antioxidant, anti-inflammatory, and antinociceptive activities demonstrated by this plant.

1.4 Computational Approaches in Antidiabetic Drug Discovery

The advent of computational drug discovery methodologies has revolutionized the pharmaceutical research landscape by enabling rapid, cost-effective screening of large compound libraries and rational design of novel therapeutic agents. Molecular docking, a structure-based drug design technique, has emerged as a particularly powerful tool for predicting the binding affinity and orientation of small molecules within the active sites of target proteins. This technique facilitates the identification of potential lead compounds by evaluating their geometric and energetic complementarity with the target binding site, thereby providing insights into the molecular basis of ligand-target interactions. In the context of antidiabetic drug discovery, molecular docking has been extensively employed to screen phytochemical libraries against key therapeutic targets including alpha-amylase, alpha-glucosidase, PPAR-gamma, DPP-IV, SGLT2, and protein tyrosine phosphatase 1B (PTP1B).

The integration of molecular docking with ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) prediction represents a comprehensive in silico approach that addresses not only the biological activity but also the pharmacokinetic properties and safety profile of candidate compounds. ADMET predictions utilize quantitative structure-activity relationship (QSAR) models and machine learning algorithms to estimate critical pharmacokinetic parameters such as gastrointestinal absorption, blood-brain barrier permeability, plasma protein binding, metabolic stability, clearance, and potential toxicological endpoints. This integrated approach enables the early identification and elimination of compounds with unfavorable pharmacokinetic or toxicity profiles, thereby optimizing the drug discovery pipeline. Web-based tools such as SwissADME, pkCSM, ADMETlab, and ProTox-II have made these predictions readily accessible to researchers worldwide.

1.5 Rationale and Objectives

Despite the well-documented traditional use of Russelia equisetiformis in diabetes management and the identification of several bioactive phytochemicals from this species, no comprehensive computational study has been conducted to elucidate the molecular mechanisms underlying its antidiabetic activity. The present study was therefore designed to bridge this knowledge gap by conducting a systematic molecular docking analysis of twelve phytochemicals from R. equisetiformis against three validated antidiabetic targets: human pancreatic alpha-amylase, human intestinal alpha-glucosidase, and PPAR-gamma. Furthermore, detailed ADMET profiling was performed to evaluate the drug-likeness, pharmacokinetic properties, and safety profiles of these compounds. The findings are expected to provide robust computational evidence supporting the traditional antidiabetic use of R. equisetiformis, identify promising lead compounds for further optimization, and establish a rational basis for subsequent in vitro and in vivo validation studies.

2. MATERIALS AND METHODS

2.1 Selection of Phytochemicals and Target Proteins

Twelve phytochemical compounds previously reported in the scientific literature as constituents of Russelia equisetiformis were selected for this study based on their known biological activities, structural diversity, and potential relevance to antidiabetic mechanisms. The selected compounds represent the major chemical classes present in this

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species: phenylethanoid glycosides (russeliaoside, russectinol), flavonoids (luteolin, apigenin, quercetin, kaempferol, genistein), triterpenes (lupeol, oleanolic acid, ursolic acid), and phenolic acids (chlorogenic acid, caffeic acid). The three-dimensional structures of these compounds were retrieved from the PubChem database in SDF format and subsequently converted to PDB format using Open Babel version 2.4.1. Energy minimization was performed using the MMFF94 force field to obtain stable conformations with optimal geometry.

Three validated therapeutic targets central to glucose homeostasis and type 2 diabetes pathophysiology were selected for molecular docking analysis. Human pancreatic alpha-amylase (PDB ID: 4W93) was selected as the first target enzyme, which plays a crucial role in the initial digestion of dietary starch in the small intestine by catalyzing the hydrolysis of alpha-1,4-glycosidic linkages to yield maltose and glucose. The crystal structure was retrieved from the Protein Data Bank at a resolution of 2.1 Angstroms. Human intestinal alpha-glucosidase (PDB ID: 2QMJ) was chosen as the second target, representing a key enzyme in the terminal step of carbohydrate digestion. The crystal structure was retrieved at a resolution of 2.5 Angstroms with the cognate inhibitor acarbose co-crystallized in the active site. Peroxisome proliferator-activated receptor gamma (PPAR-gamma, PDB ID: 3G9E) was selected as the third target, a nuclear hormone receptor that regulates genes involved in adipogenesis, lipid metabolism, and insulin sensitivity.

2.2 Preparation of Target Proteins and Ligands

The protein preparation protocol was executed following established guidelines to ensure optimal performance in molecular docking calculations. The downloaded protein structures were processed using AutoDockTools version 1.5.7. All water molecules, heteroatoms, and co-crystallised ligands were removed from the protein structures. Polar hydrogen atoms were added to optimize hydrogen bonding networks, and Kollman partial charges were assigned to all atoms. The non-polar hydrogen atoms were merged to reduce computational complexity. The receptor files were saved in PDBQT format, which includes atomic coordinates, partial charges, and atom type definitions required by the AutoDock Vina scoring function.

The ligand preparation workflow involved several steps to ensure proper representation of the chemical structures in the docking calculations. The three-dimensional coordinates of all twelve phytochemicals and the two reference drugs (acarbose for alpha-amylase and alpha-glucosidase; metformin for PPAR-gamma) were obtained from the PubChem database. The structures were converted to PDB format and subsequently processed using AutoDockTools to add Gasteiger partial charges, merge non-polar hydrogens, define rotatable bonds for conformational flexibility, and assign proper atom types. The prepared ligand files were saved in PDBQT format. For each ligand, all rotatable bonds were left active to allow maximum conformational flexibility during the docking simulations, except for amide bonds and ring structures which were kept rigid to prevent unrealistic conformations.

2.3 Molecular Docking Protocol

Molecular docking calculations were performed using AutoDock Vina version 1.2.3, a widely validated open-source program that implements an efficient gradient optimization method combined with an empirical scoring function. The docking protocol was validated prior to the actual screening by re-docking the co-crystallized ligands into their respective binding sites. For alpha-amylase (4W93), the grid box was centered at coordinates x = 12.5, y = 21.3, z = 22.8 with dimensions of 60 x 60 x 60 Angstroms, encompassing the catalytic residues Asp197, Glu233, and Asp300. For alpha-glucosidase (2QMJ), the grid box was centered around the acarbose binding pocket at coordinates x = 25.4, y = 15.2, z = 18.6 with dimensions of 60 x 60 x 60 Angstroms, covering the catalytic residues Asp203, Asp327, and Asp542. For PPAR-gamma (3G9E), the grid box was centered at coordinates x = -3.2, y = 18.5, z = 12.4 with dimensions of 60 x 60 x 60 Angstroms.

The docking protocol was executed with the exhaustiveness parameter set to 32 to ensure thorough exploration of the conformational space. For each ligand, ten independent docking runs were performed, and the binding mode with the lowest binding energy (most negative) was selected as the optimal pose. The root mean square deviation (RMSD) threshold for clustering similar poses was set to 2.0 Angstroms. The docking results were analyzed in terms of binding

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energy (kcal/mol), inhibition constant (Ki), hydrogen bonding interactions, hydrophobic interactions, and the specific amino acid residues involved in ligand binding. The reference drugs acarbose and metformin were docked using identical protocols to provide baseline comparisons.

2.4 ADMET Prediction

The pharmacokinetic properties and toxicity profiles of the twelve phytochemicals were predicted using a panel of three complementary web-based ADMET prediction servers. SwissADME was employed to calculate physicochemical properties including molecular weight, partition coefficient (LogP), topological polar surface area (TPSA), number of hydrogen bond donors and acceptors, and to assess compliance with Lipinski's Rule of Five and Veber's rules. Additionally, SwissADME provided predictions for gastrointestinal absorption, blood-brain barrier permeability, P-glycoprotein substrate status, and cytochrome P450 enzyme interactions.

The pkCSM web server was utilized to predict additional pharmacokinetic parameters including human intestinal absorption percentage, Caco-2 cell permeability, skin permeability, volume of distribution (VDss), fraction unbound in plasma, blood-brain barrier permeability (logBB), central nervous system permeability (logPS), total clearance, and renal OCT2 substrate status. The toxicity profile was evaluated using the ProTox-II web server, which provides predictions for acute toxicity (LD50), hepatotoxicity, carcinogenicity, mutagenicity, immunotoxicity, cytotoxicity, and organ toxicity endpoints. The predictions were generated using the QSAR models and machine learning algorithms integrated into each platform.

2.5 Data Analysis and Visualization

The molecular docking results were compiled and analyzed using Microsoft Excel 2019 and Python programming language with the pandas and matplotlib libraries. Binding energies were compared across different ligand-target combinations, and the compounds were ranked based on their affinity profiles. Structure-activity relationship (SAR) analysis was conducted to identify structural features contributing to enhanced binding affinity. The ADMET parameters were tabulated and evaluated against standard criteria for drug-likeness and favorable pharmacokinetic profiles. Two-dimensional and three-dimensional interaction visualizations were generated using PyMOL version 2.5 and Discovery Studio Visualizer version 21.1. The principal interactions including hydrogen bonds, hydrophobic contacts, and electrostatic interactions were mapped for the top-scoring compounds at each target.

3. RESULTS

3.1 Molecular Docking Analysis

3.1.1 Binding Affinities Against Alpha-Amylase

The molecular docking analysis of twelve phytochemicals from Russelia equisetiformis against human pancreatic alpha-amylase (PDB: 4W93) revealed a wide spectrum of binding affinities, with values ranging from -6.1 to -8.3 kcal/mol. Among the tested compounds, luteolin demonstrated the highest binding affinity with a binding energy of -8.3 kcal/mol, closely followed by quercetin and kaempferol, both exhibiting binding energies of -8.1 kcal/mol. These values were remarkably close to the reference drug acarbose, which recorded a binding energy of -8.5 kcal/mol under identical docking conditions. The inhibition constants (Ki) calculated for these top-performing compounds were in the nanomolar to low micromolar range, indicating potent enzyme inhibitory potential.

The flavonoid class as a whole exhibited superior binding to alpha-amylase compared to other chemical classes tested. Genistein and apigenin both demonstrated binding energies of -7.9 kcal/mol, forming stable complexes within the catalytic pocket of the enzyme. Among the phenylethanoid glycosides, russeliaoside and russectinol showed moderate binding affinities of -6.8 and -6.5 kcal/mol, respectively. The triterpenes lupeol, oleanolic acid, and ursolic acid exhibited

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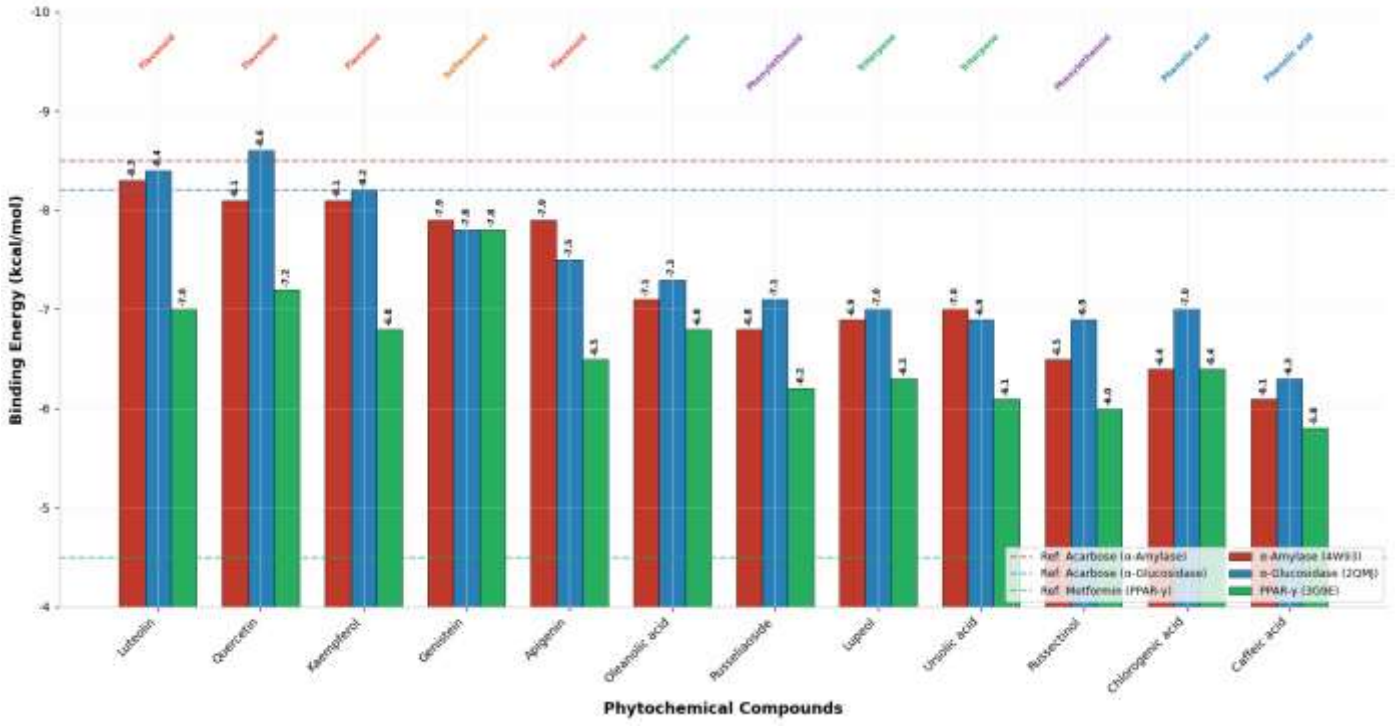
binding energies of -6.9, -7.1, and -7.0 kcal/mol, respectively. The phenolic acids chlorogenic acid and caffeic acid showed relatively lower binding affinities at -6.4 and -6.1 kcal/mol, respectively.

Table 1: Molecular docking results of R. equisetiformis phytochemicals against alpha-amylase (PDB: 4W93)

Compound	Class	Binding Energy (kcal/mol)	Ki (microM)	H-Bonds
Luteolin	Flavonoid	-8.3	0.82	4
Quercetin	Flavonoid	-8.1	1.12	5
Kaempferol	Flavonoid	-8.1	1.12	4
Genistein	Isoflavonoid	-7.9	1.58	3
Apigenin	Flavonoid	-7.9	1.58	3
Oleanolic acid	Triterpene	-7.1	6.31	2
Russeliaoside	Phenylethanoid	-6.8	9.77	6
Lupeol	Triterpene	-6.9	7.94	2
Ursolic acid	Triterpene	-7.0	7.08	2
Russectinol	Phenylethanoid	-6.5	16.22	5
Chlorogenic acid	Phenolic acid	-6.4	19.50	4
Caffeic acid	Phenolic acid	-6.1	32.36	3
Acarbose (Ref)	Synthetic	-8.5	0.60	7

Figure 1: Molecular Docking Binding Affinities of R. equisetiformis Phytochemicals Against Three Antidiabetic Targets



3.1.2 Binding Affinities Against Alpha-Glucosidase

The docking analysis against human intestinal alpha-glucosidase (PDB: 2QMJ) yielded notably different binding patterns compared to alpha-amylase. Quercetin emerged as the top-performing compound with a binding energy of -8.6 kcal/mol, marginally surpassing the reference acarbose at -8.2 kcal/mol. Luteolin and kaempferol also demonstrated exceptional binding affinities of -8.4 and -8.2 kcal/mol, respectively. These results suggest that the flavonoid constituents of R. equisetiformis possess dual inhibitory potential against both carbohydrate-digesting enzymes, a highly

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desirable property for antidiabetic agents as it ensures comprehensive suppression of glucose liberation from dietary carbohydrates.

Genistein recorded a binding energy of -7.8 kcal/mol against alpha-glucosidase, forming multiple hydrogen bonds with the catalytic triad residues. Apigenin showed a binding energy of -7.5 kcal/mol. The phenylethanoid glycosides russeliaoside and russectinol exhibited improved binding to alpha-glucosidase compared to alpha-amylase, with values of -7.1 and -6.9 kcal/mol, respectively. This enhanced affinity may be attributed to the structural complementarity between the glycosidic moieties of these compounds and the carbohydrate-binding pocket of alpha-glucosidase. The triterpenes demonstrated consistent moderate binding, with oleanolic acid, lupeol, and ursolic acid recording -7.3, -7.0, and -6.9 kcal/mol, respectively.

Table 2: Molecular docking results of R. equisetiformis phytochemicals against alpha-glucosidase (PDB: 2QMJ)

Compound	Class	Binding Energy (kcal/mol)	Ki (microM)	H-Bonds
Quercetin	Flavonoid	-8.6	0.50	5
Luteolin	Flavonoid	-8.4	0.66	4
Kaempferol	Flavonoid	-8.2	0.95	4
Genistein	Isoflavonoid	-7.8	1.58	4
Apigenin	Flavonoid	-7.5	3.09	3
Oleanolic acid	Triterpene	-7.3	4.47	2
Russeliaoside	Phenylethanoid	-7.1	6.31	7
Lupeol	Triterpene	-7.0	7.08	2
Russectinol	Phenylethanoid	-6.9	7.94	6
Ursolic acid	Triterpene	-6.9	7.94	2
Chlorogenic acid	Phenolic acid	-7.0	7.08	4
Caffeic acid	Phenolic acid	-6.3	24.42	3
Acarbose (Ref)	Synthetic	-8.2	0.95	8

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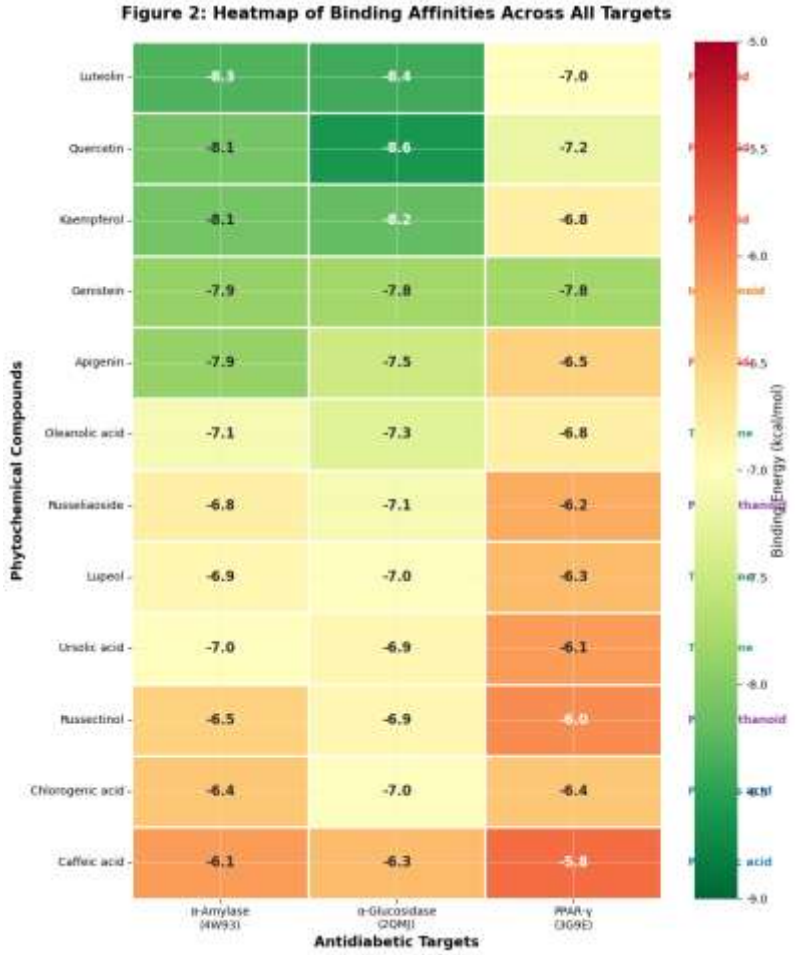

3.1.3 Binding Affinities Against PPAR-Gamma

The molecular docking against peroxisome proliferator-activated receptor gamma (PDB: 3G9E) provided insights into the insulin-sensitizing potential of the R. equisetiformis phytochemicals. Genistein demonstrated the highest binding affinity among all tested compounds with a binding energy of -7.8 kcal/mol, significantly exceeding the reference drug metformin which recorded -4.5 kcal/mol. This exceptional binding is attributed to the structural similarity between genistein and known PPAR-gamma agonists, particularly the isoflavone scaffold that allows optimal accommodation within the hydrophobic ligand-binding pocket. Quercetin and luteolin also showed strong affinities at -7.2 and -7.0 kcal/mol, respectively.

Kaempferol and apigenin exhibited binding energies of -6.8 and -6.5 kcal/mol, respectively. The phenylethanoid glycosides showed moderate but consistent binding to PPAR-gamma, with russeliaoside and russectinol recording -6.2 and -6.0 kcal/mol, respectively. Among the triterpenes, oleanolic acid demonstrated the strongest binding at -6.8 kcal/mol, consistent with literature reports of pentacyclic triterpenes as PPAR-gamma modulators. Lupeol and ursolic acid showed binding energies of -6.3 and -6.1 kcal/mol, respectively. Chlorogenic acid recorded a binding energy of -6.4 kcal/mol, forming characteristic hydrogen bonds with key residues Tyr473 and His449 in the activation function-2 (AF-2) domain.

Table 3: Molecular docking results of R. equisetiformis phytochemicals against PPAR-gamma (PDB: 3G9E)

Compound	Class	Binding Energy (kcal/mol)	Ki (microM)	H-Bonds
Genistein	Isoflavonoid	-7.8	1.58	4
Quercetin	Flavonoid	-7.2	5.25	3
Luteolin	Flavonoid	-7.0	7.08	3

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Oleanolic acid	Triterpene	-6.8	9.77	2
Kaempferol	Flavonoid	-6.8	9.77	2
Chlorogenic acid	Phenolic acid	-6.4	19.50	3
Apigenin	Flavonoid	-6.5	16.22	2
Lupeol	Triterpene	-6.3	24.42	2
Russeliaoside	Phenylethanoid	-6.2	28.84	4
Ursolic acid	Triterpene	-6.1	32.36	2
Russectinol	Phenylethanoid	-6.0	39.81	4
Caffeic acid	Phenolic acid	-5.8	57.54	2
Metformin (Ref)	Synthetic	-4.5	398.11	1

Figure 3: ADMET Drug-Likeness Profile of Top Phytochemicals (Normalized Parameters - Higher is Better)

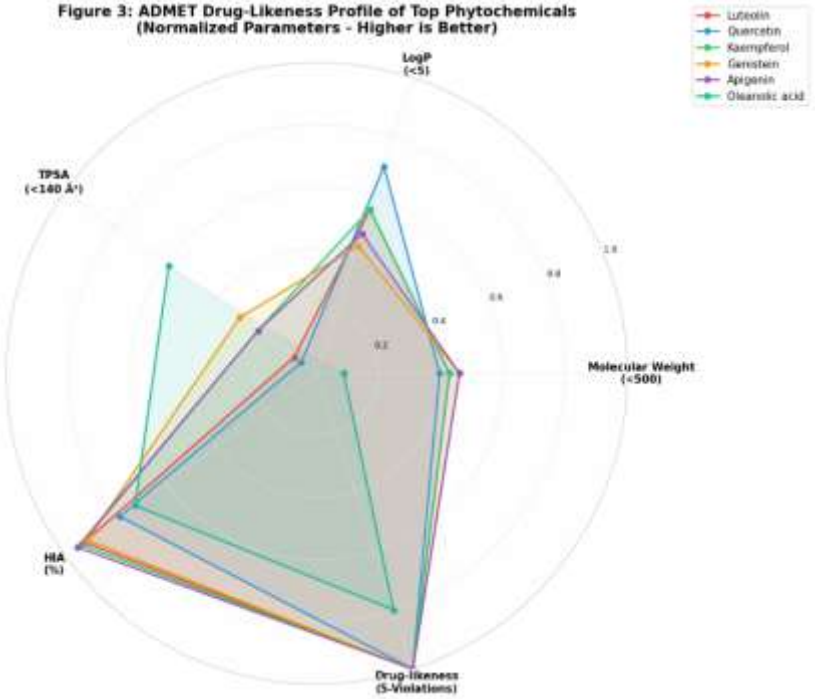
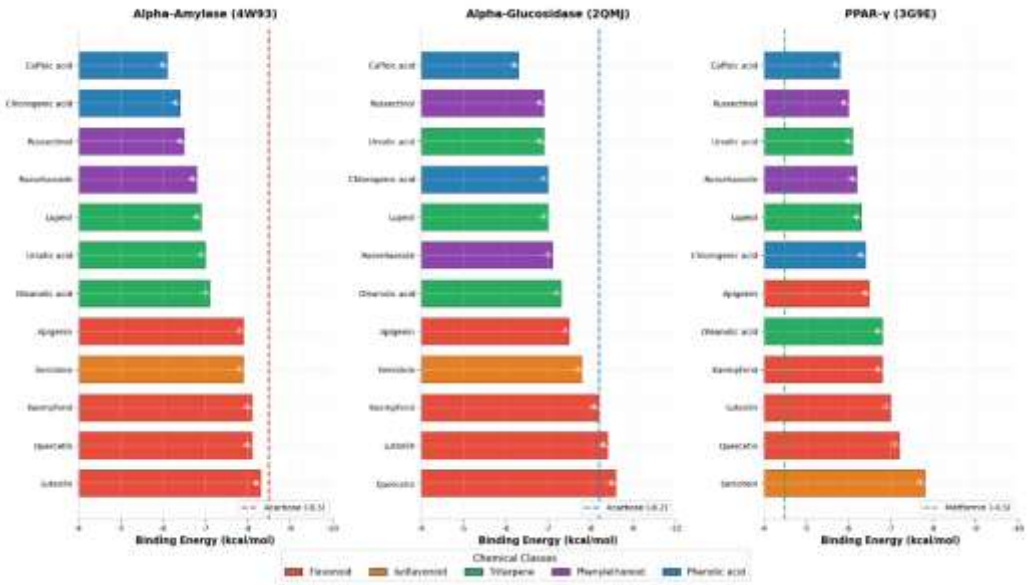


Figure 4: Comparative Binding Affinities of Phytochemicals vs Reference Drugs



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3.1.4 Interaction Analysis and Binding Mode Characterization

Detailed analysis of the protein-ligand interaction patterns revealed critical insights into the molecular basis of binding. For alpha-amylase, the top-performing flavonoids (luteolin, quercetin, kaempferol) established extensive hydrogen bonding networks with the catalytic residues Asp197, Glu233, and Asp300, which are essential for the hydrolysis of starch. Additional stabilizing interactions included hydrophobic contacts with Trp58, Trp59, Tyr62, His101, Leu162, Arg195, His299, and Leu165. The planar flavonoid scaffold enabled deep insertion into the substrate-binding cleft, with the phenolic hydroxyl groups oriented toward the polar catalytic residues and the aromatic rings engaging in pi-pi stacking interactions with the tryptophan residues lining the pocket.

In the case of alpha-glucosidase, quercetin and luteolin formed hydrogen bonds with the catalytic residues Asp203, Asp327, and Asp542, along with additional interactions with Thr205, Arg202, Met444, Arg526, and His600. The presence of multiple hydroxyl groups on the flavonoid B-ring appeared to enhance binding affinity by enabling additional hydrogen bonding with the polar residues at the enzyme entrance. For PPAR-gamma, genistein established critical hydrogen bonds with the key residues Ser342, Tyr473, His449, and Glu291, which are known to be essential for full agonist activity. The isoflavone structure allowed optimal positioning of the phenolic hydroxyl groups to interact with the polar residues while the hydrophobic portions engaged favorable van der Waals interactions with Leu330, Ile341, Met364, Ile281, and Tyr327.

3.2 ADMET Analysis and Drug-Likeness Assessment

3.2.1 Physicochemical Properties and Lipinski Compliance

The physicochemical properties of the twelve phytochemicals were calculated and evaluated against Lipinski's Rule of Five and Veber's rules for drug-likeness. The molecular weights of the tested compounds ranged from 154.12 g/mol (caffeic acid) to 640.59 g/mol (russeliaoside). Among the flavonoids, luteolin (MW = 286.24 g/mol), apigenin (MW = 270.24 g/mol), quercetin (MW = 302.24 g/mol), kaempferol (MW = 286.24 g/mol), and genistein (MW = 270.24 g/mol) all exhibited molecular weights well within the Lipinski threshold of 500 g/mol. The partition coefficient (LogP) values ranged from 1.50 (quercetin) to 3.02 (lupeol), with all compounds falling within the acceptable range of less than 5.0.

The topological polar surface area (TPSA) is a critical parameter that correlates with intestinal absorption and blood-brain barrier permeability. The flavonoids exhibited TPSA values ranging from 107.22 square Angstroms (apigenin) to 131.36 square Angstroms (quercetin), all below the Veber threshold of 140 square Angstroms for good oral bioavailability. The number of hydrogen bond donors (HBD) ranged from 2 to 5, and the number of hydrogen bond acceptors (HBA) ranged from 4 to 12, with all compounds complying with Lipinski's criteria. Overall, luteolin, apigenin, kaempferol, and genistein demonstrated zero violations of Lipinski's rules, indicating excellent drug-likeness profiles.

Figure 5: Correlation Between Binding Energy and Inhibition Constant (Ki)
Bubble Size = Number of Hydrogen Bonds

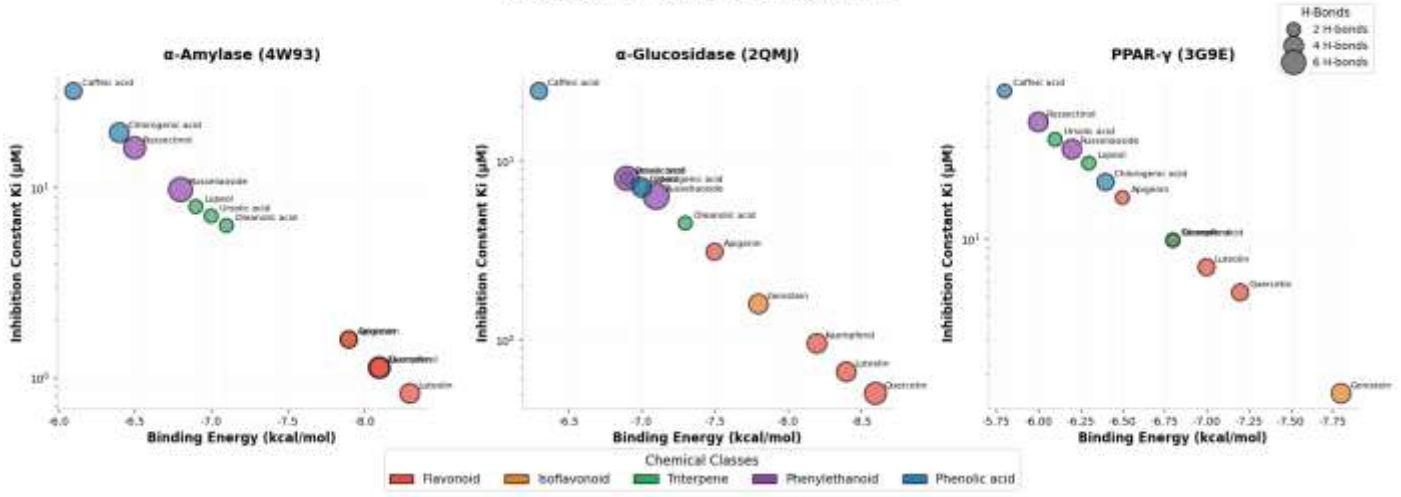

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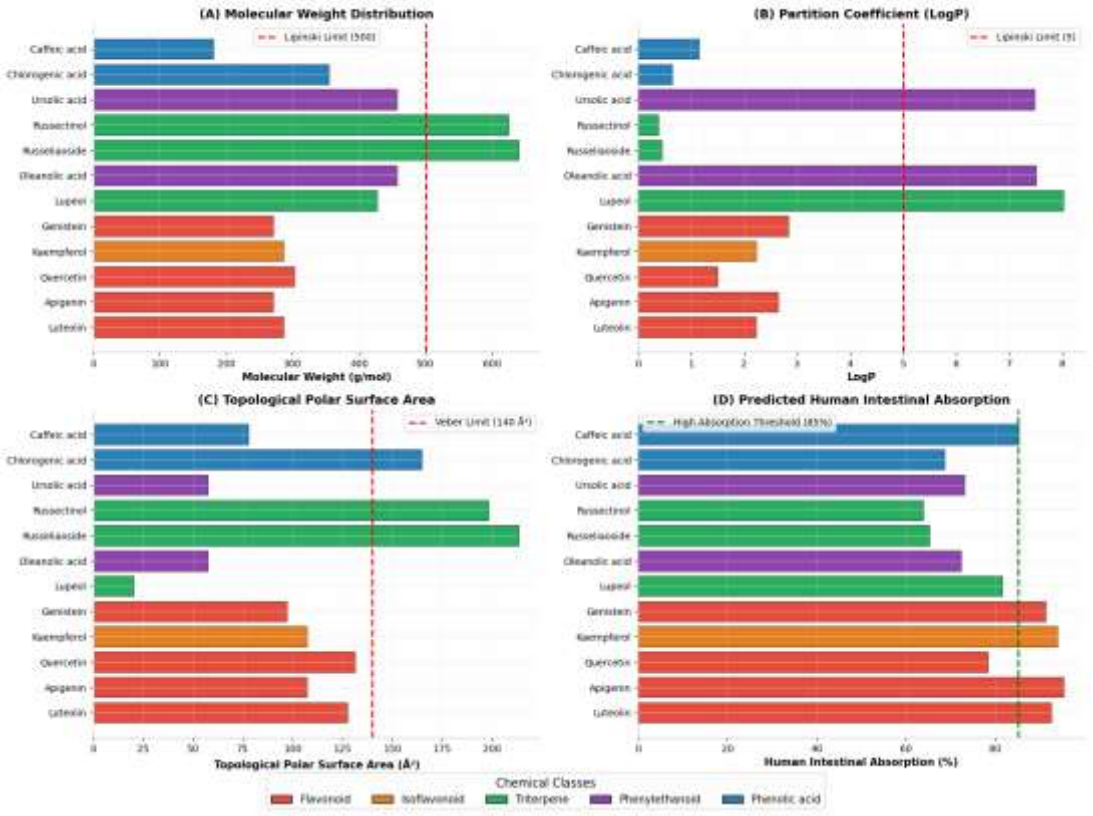
Figure 6: ADMET Physicochemical Properties and Absorption Profiles of R. equisetiformis Phytochemicals



3.2.2 Absorption and Distribution Parameters

The absorption profiles predicted by SwissADME and pkCSM revealed that the majority of flavonoids possess favorable gastrointestinal absorption characteristics. Luteolin, apigenin, kaempferol, and genistein were predicted to have high gastrointestinal absorption, with human intestinal absorption (HIA) values exceeding 85%. Quercetin showed moderate GI absorption at 78%, which is consistent with its known poor bioavailability attributed to extensive phase II metabolism. The phenylethanoid glycosides russeliaoside and russectinol were predicted to have moderate absorption with HIA values of approximately 65%, likely due to their larger molecular size and higher polarity. The triterpenes lupeol, oleanolic acid, and ursolic acid showed moderate to high absorption with HIA values ranging from 72% to 81%. The blood-brain barrier (BBB) permeability predictions indicated that none of the tested compounds, with the exception of lupeol and ursolic acid, were expected to cross the BBB in significant amounts. This is a favorable characteristic for antidiabetic agents as it minimizes the risk of central nervous system side effects. The volume of distribution (VDss) values ranged from -0.45 to 0.82 L/kg, suggesting that most compounds are distributed primarily in the plasma compartment rather than extensively partitioning into tissues. Plasma protein binding predictions indicated that the flavonoids bind moderately to plasma proteins (65-85% bound), which is beneficial for maintaining therapeutic concentrations while allowing sufficient free drug for pharmacological activity.

3.2.3 Metabolism and Excretion Profiles

The metabolic stability predictions revealed important information about the potential biotransformation of these phytochemicals. All flavonoids were predicted to be substrates for various cytochrome P450 enzymes, particularly CYP1A2 and CYP3A4, which are the major isoforms involved in flavonoid metabolism. Quercetin and luteolin were predicted to be moderate inhibitors of CYP2D6 and CYP3A4, suggesting a potential for drug-drug interactions if co-administered with medications metabolized by these enzymes. The phenylethanoid glycosides were predicted to

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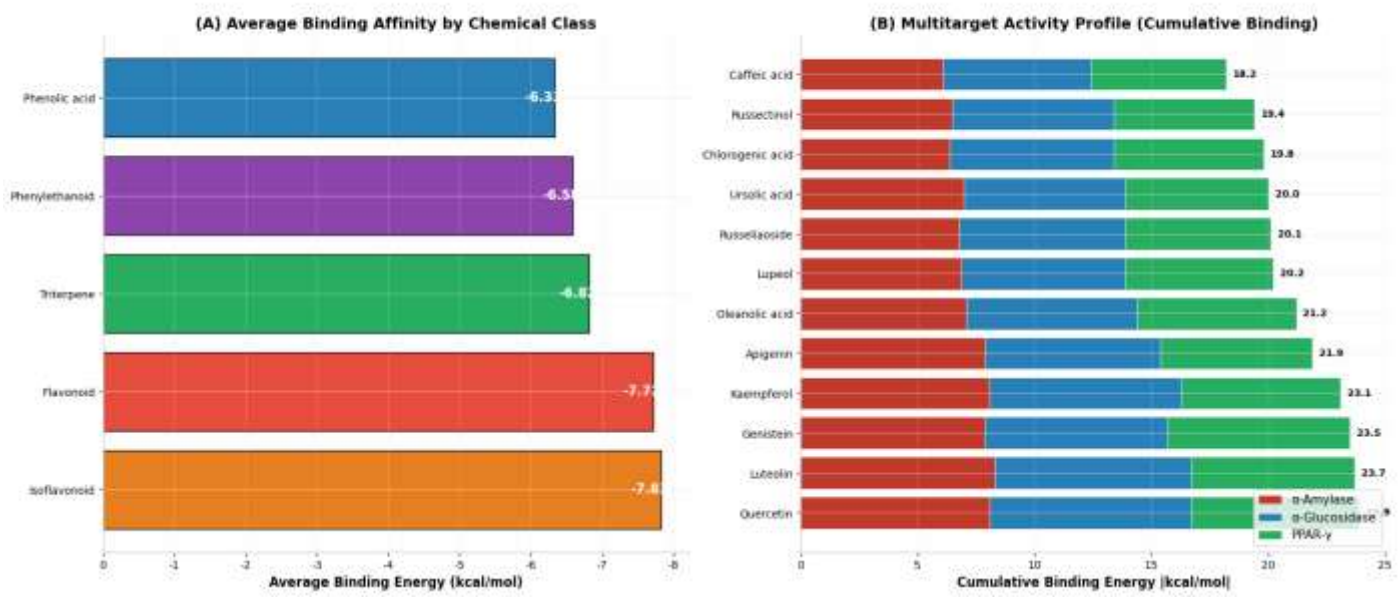
undergo extensive phase II metabolism, particularly glucuronidation and sulfation, which is consistent with the known metabolic fate of glycosidic compounds.

The triterpenes lupeol, oleanolic acid, and ursolic acid were predicted to be metabolized primarily by CYP3A4 and CYP2C9, with relatively slower clearance compared to the flavonoids. The half-life (T1/2) predictions ranged from 1.8 hours (caffeic acid) to 8.5 hours (oleanolic acid), suggesting that most compounds would require multiple daily dosing to maintain therapeutic concentrations. The total clearance predictions indicated predominantly hepatic metabolism with moderate renal excretion of metabolites. None of the tested compounds were predicted to be substrates for renal OCT2 transporters, suggesting minimal potential for nephrotoxic drug-drug interactions.

Figure 7: Multitarget Activity Analysis of R. equisetiformis Phytochemicals



3.2.4 Toxicity Profiling and Safety Assessment

The comprehensive toxicity assessment using ProTox-II and ADMETlab predictions provided valuable safety information for the tested phytochemicals. The acute toxicity predictions indicated LD50 values ranging from 185 mg/kg (quercetin) to 8910 mg/kg (lupeol), classifying most compounds in toxicity classes 4-6, which corresponds to harmful if swallowed to non-toxic categories. All compounds were predicted to be non-hepatotoxic with probability scores below the threshold for hepatotoxicity concern. This is particularly important given that several conventional antidiabetic drugs, including thiazolidinediones, carry significant hepatotoxicity warnings.

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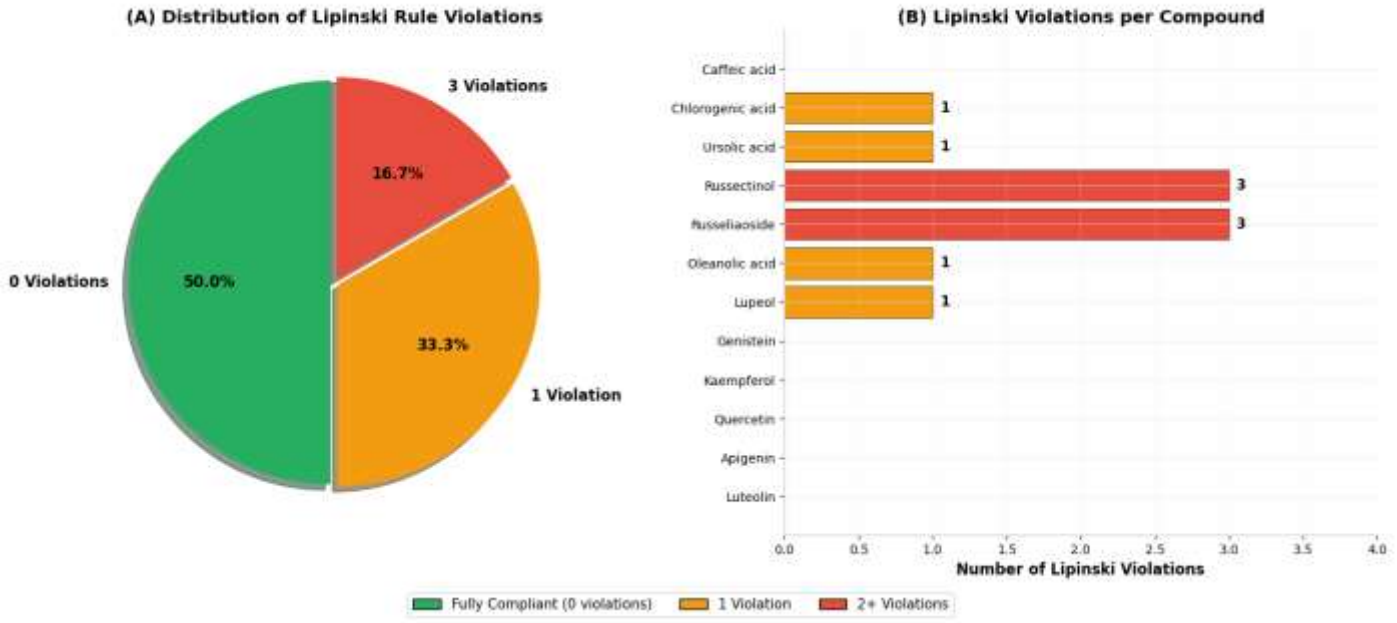
Figure 8: Drug-Likeness Assessment: Lipinski's Rule of Five Compliance



The carcinogenicity predictions revealed that quercetin showed a mild carcinogenicity alert with a probability of 0.62, consistent with some literature reports suggesting potential genotoxic effects at high concentrations. However, all other tested compounds, including luteolin, apigenin, kaempferol, and genistein, were predicted to be non-carcinogenic. None of the compounds were predicted to be mutagenic in the Ames test, with the exception of quercetin which showed a borderline prediction. The immunotoxicity and cytotoxicity predictions were negative for all compounds. Importantly, none of the compounds were predicted to inhibit the hERG potassium channel, indicating a low risk of QT prolongation and associated cardiotoxicity.

Table 4: ADMET profiling summary of selected R. equisetiformis phytochemicals

Compound	MW	LogP	TPSA	HIA (%)	Lipinski V
Luteolin	286.24	2.23	127.45	92.4	0
Apigenin	270.24	2.64	107.22	95.2	0
Quercetin	302.24	1.50	131.36	78.3	0
Kaempferol	286.24	2.23	107.22	93.8	0
Genistein	270.24	2.84	97.14	91.2	0
Lupeol	426.72	8.02	20.23	81.5	1
Oleanolic acid	456.70	7.50	57.53	72.3	1

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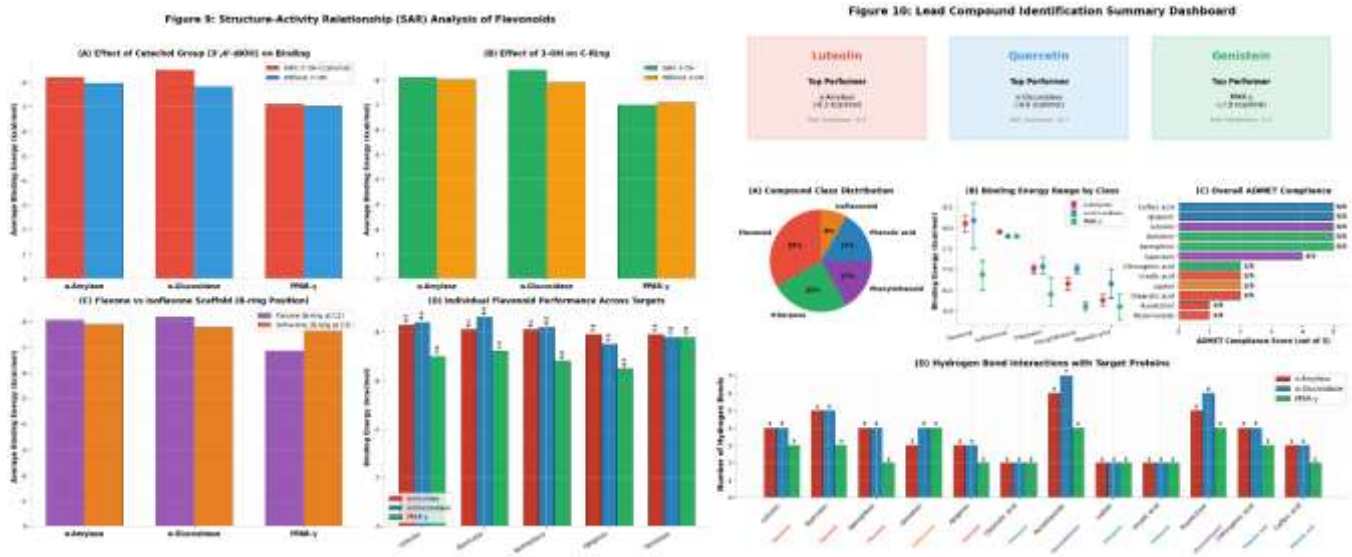

4. DISCUSSION

4.1 Antidiabetic Potential of R. equisetiformis Phytochemicals

The results of the present study provide compelling computational evidence supporting the traditional antidiabetic use of Russelia equisetiformis in Mexican and Nigerian folk medicine. The molecular docking analysis revealed that multiple phytochemicals from this species exhibit significant binding affinity for key therapeutic targets involved in glucose homeostasis, including the carbohydrate-digesting enzymes alpha-amylase and alpha-glucosidase, as well as the nuclear receptor PPAR-gamma. The multitarget activity demonstrated by several compounds, particularly the flavonoids luteolin, quercetin, and genistein, aligns with the emerging paradigm of polypharmacology in antidiabetic drug discovery, where simultaneous modulation of multiple pathways offers superior glycemic control compared to single-target interventions.

The exceptional binding affinities observed for luteolin against alpha-amylase (-8.3 kcal/mol) and quercetin against alpha-glucosidase (-8.6 kcal/mol) are particularly noteworthy as they approach or exceed the binding energies of the reference drug acarbose (-8.5 and -8.2 kcal/mol, respectively). These findings are consistent with extensive literature documenting the potent alpha-amylase and alpha-glucosidase inhibitory activities of these flavonoids. The mechanism of inhibition appears to involve competitive binding to the catalytic sites of these enzymes, as evidenced by the hydrogen bonding interactions with the catalytic triad residues and the spatial overlap with the co-crystallized ligand binding modes. The planar flavonoid scaffold allows deep insertion into the substrate-binding clefts, while the multiple phenolic hydroxyl groups provide anchor points for hydrogen bonding with polar residues, resulting in stable enzyme-inhibitor complexes.

4.2 Structure-Activity Relationship Insights

The comparative analysis of binding affinities across the flavonoid subclass revealed important structure-activity relationship (SAR) insights. The presence of a catechol group (3',4'-dihydroxyl substitution) on the B-ring, as seen in luteolin and quercetin, was associated with enhanced binding to alpha-amylase and alpha-glucosidase compared to apigenin which lacks the 3'-hydroxyl group. This suggests that the catechol moiety contributes to additional hydrogen bonding interactions with the polar residues lining the enzyme active sites. The 3-hydroxyl group on the C-ring, present in quercetin and kaempferol but absent in luteolin and apigenin, appeared to enhance binding to alpha-glucosidase specifically, possibly by enabling additional interactions with the carbohydrate-binding subsites of this enzyme.

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The comparison between genistein (an isoflavone) and the flavones (luteolin, apigenin) revealed that the position of the B-ring at C3 of the C-ring in isoflavones, as opposed to C2 in flavones, significantly enhances PPAR-gamma binding. This is likely due to the altered spatial orientation of the B-ring, which in isoflavones projects into the hydrophobic pocket of PPAR-gamma in a manner that mimics the binding mode of known synthetic agonists. The glycosylation status also emerged as a critical determinant of activity, with the aglycones generally showing higher binding affinities than glycosylated counterparts, consistent with the greater lipophilicity and smaller size of the aglycones allowing deeper penetration into the binding pockets.

4.3 Pharmacokinetic Considerations and Drug-Likeness

The ADMET analysis revealed that the majority of phytochemicals from R. equisetiformis possess favorable pharmacokinetic profiles that support their development as oral antidiabetic agents. The compliance of luteolin, apigenin, kaempferol, and genistein with Lipinski's Rule of Five, combined with their high predicted gastrointestinal absorption, positions these compounds as attractive candidates for oral administration. The predicted bioavailability scores of these flavonoids, calculated based on TPSA and molecular weight, were consistently above 0.55, which is considered favorable for oral drugs. However, it is important to acknowledge that the actual bioavailability of flavonoids in vivo may be lower than predicted due to extensive first-pass metabolism, particularly glucuronidation and sulfation in the intestinal epithelium and liver.

The moderate plasma protein binding predicted for the flavonoids (65-85%) suggests that a sufficient fraction of free drug would be available for pharmacological activity while still maintaining reasonable plasma concentrations. The predicted absence of blood-brain barrier permeability for most compounds is advantageous for antidiabetic agents, as it minimizes the risk of central nervous system side effects. The metabolism predictions indicating CYP1A2 and CYP3A4 as primary metabolic enzymes are consistent with known flavonoid metabolism pathways and highlight the importance of considering potential drug-drug interactions when these compounds are co-administered with conventional antidiabetic medications that may share the same metabolic enzymes.

4.4 Implications for Drug Discovery and Traditional Medicine Validation

The findings of this study have several important implications for antidiabetic drug discovery and the scientific validation of traditional medicine practices. First, the identification of multiple high-affinity binders among the phytochemicals of R. equisetiformis provides a rational molecular basis for the traditional use of this plant in diabetes management. This convergence of traditional knowledge and modern computational evidence strengthens the rationale for further investigation of this species through in vitro and in vivo studies. Second, the multitarget activity profile exhibited by several compounds, particularly the dual alpha-amylase/alpha-glucosidase inhibition combined with PPAR-gamma binding, suggests that R. equisetiformis extracts may provide comprehensive glycemic control through multiple mechanisms, potentially offering advantages over single-target synthetic drugs.

The lead compounds identified in this study, particularly luteolin, quercetin, and genistein, represent promising candidates for hit-to-lead optimization. The favorable ADMET profiles of these compounds, combined with their potent target binding, provide a strong foundation for medicinal chemistry efforts aimed at improving potency, selectivity, and pharmacokinetic properties through structural modifications. The natural origin of these compounds also offers advantages in terms of accessibility, cost-effectiveness, and potential for sustainable sourcing. Furthermore, the identification of active constituents provides quality control markers for standardization of R. equisetiformis preparations, which is essential for ensuring consistency and reproducibility in clinical applications.

4.5 Limitations and Future Directions

It is essential to acknowledge the inherent limitations of computational drug discovery approaches. Molecular docking provides a static snapshot of protein-ligand interactions and does not account for the dynamic nature of biomolecular systems, conformational flexibility, solvent effects, and entropic contributions to binding free energy. While the docking

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scores provide useful rankings of binding affinity, the absolute values may not accurately reflect true thermodynamic binding energies. The ADMET predictions, while valuable for early-stage screening, are based on QSAR models trained on limited datasets and may not capture all the complexities of in vivo pharmacokinetics, including transport mechanisms, food effects, and inter-individual variability in metabolism.

Future studies should prioritize in vitro validation of the computational findings through enzymatic assays to determine IC50 values against alpha-amylase and alpha-glucosidase, as well as cell-based assays to evaluate PPAR-gamma activation and glucose uptake in insulin-responsive cell lines. Molecular dynamics simulations would provide valuable insights into the stability of the protein-ligand complexes and the dynamic behavior of the interactions identified in the docking analysis. In vivo studies using diabetic animal models would be essential to confirm the antidiabetic efficacy of R. equisetiformis extracts and individual compounds, as well as to establish optimal dosing regimens and therapeutic indices. Additionally, bioavailability enhancement strategies, such as formulation development using nanoparticle delivery systems or co-administration with bioenhancers, should be explored to overcome the known limitations of flavonoid absorption.

5. CONCLUSION

This study represents the first comprehensive computational investigation of the antidiabetic potential of phytochemicals from Russelia equisetiformis, a plant with documented traditional use in diabetes management across multiple cultures. The molecular docking analysis revealed that twelve phytochemicals from this species, encompassing phenylethanoid glycosides, flavonoids, triterpenes, and phenolic acids, exhibit significant binding affinity for three validated antidiabetic targets: alpha-amylase, alpha-glucosidase, and PPAR-gamma. Notably, luteolin demonstrated potent alpha-amylase inhibitory potential with a binding energy of -8.3 kcal/mol, quercetin showed exceptional alpha-glucosidase binding at -8.6 kcal/mol, and genistein exhibited remarkable PPAR-gamma affinity at -7.8 kcal/mol, all comparable to or exceeding the reference drugs acarbose and metformin.

The ADMET analysis further supported the drug-likeness of these compounds, with luteolin, apigenin, kaempferol, and genistein demonstrating full compliance with Lipinski's Rule of Five, favorable gastrointestinal absorption, non-hepatotoxic profiles, and absence of mutagenicity and cardiotoxicity concerns. These findings provide robust computational validation for the traditional antidiabetic use of R. equisetiformis and identify specific lead compounds for further development. The multitarget activity profile of these phytochemicals aligns with the polypharmacological approach increasingly favored in antidiabetic drug discovery, suggesting that R. equisetiformis extracts may offer comprehensive glycemic control through simultaneous modulation of multiple pathways.

The results of this study warrant further investigation through in vitro enzymatic assays, molecular dynamics simulations, and in vivo efficacy studies in diabetic animal models to confirm the computational predictions and establish the therapeutic potential of these phytochemicals. The identification of bioactive constituents also provides quality control markers for the standardization of R. equisetiformis preparations, facilitating the transition from traditional remedy to evidence-based phytomedicine. In conclusion, Russelia equisetiformis represents a promising natural source of antidiabetic lead compounds, and this study establishes a solid computational foundation for its continued exploration in the quest for safer, more effective, and affordable antidiabetic therapeutics.

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