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Integrative Network Pharmacology, Molecular Docking, and *In-Vitro* Analysis of the Antidiabetic Potential of Diosmin

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ABSTRACT:

Background: Diosmin, a citrus-derived flavonoid, exhibits antioxidant, anti-inflammatory and metabolic regulatory properties; however, its molecular mechanisms and multitarget interactions in diabetes mellitus remain poorly understood and not fully clear. **Methods:** An integrative strategy combining network pharmacology, molecular docking and *in vitro* enzyme inhibition assays were employed in this study. Diosmin-associated targets were retrieved from the Comparative Toxicogenomics Database (CTD; accessed January 2025) and intersected with diabetes mellitus–related genes obtained from GeneCards (version 5.18; relevance score ≥10). Protein–protein interaction (PPI) networks was constructed using STITCH (confidence score ≥0.08) and analyzed by Cytoscape (version 3.9.1). Gene Ontology (GO) and KEGG pathway enrichment analysis were performed using g: Profiler. Molecular docking was conducted using AutoDock Vina against key insulin-signaling proteins including insulin receptor (IR), insulin receptor substrate-1 (IRS-1), PI3K, AKT1 and GLUT4. *In vitro* α-amylase and α-glucosidase inhibition assays were performed, using acarbose as the reference drug. **Results:** Network pharmacology analysis identified 39 common targets, which are primarily involved in insulin signaling, AMPK signaling and glucose metabolism pathways although some overlap existed. The ADME profile supports diosmin as a safe and pharmacologically relevant nutraceutical or lead compound rather than a conventional orally bioavailable small molecule drug. Docking analysis suggested favorable binding propensities of diosmin toward selected hub proteins, ranging from −9.3 to −11.3 kcal/mol; with GLUT4 and PI3K showing the highest affinities among the targets. *In vitro* assays demonstrated dose-dependent inhibition of α-amylase and α-glucosidase by diosmin, although it was less potent when compared than acarbose. **Conclusion:** Diosmin may have potential antidiabetic property through multitarget modulation of insulin signaling and carbohydrate-hydrolyzing enzymes, suggesting a systems-level mechanism. A limitation of this study is the absence of cellular and in vivo validation which may affect interpretation, warranting further experimental confirmation in future studies.

KEYWORDS: ADMET, Diabetes Mellitus, Diosmin, Enzyme Inhibition, Enzyme Inhibition, Insulin Signaling, Insulin Signaling, Molecular Docking, Network Pharmacology, Systems Biology

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1. INTRODUCTION

Diabetes mellitus (DM) is complex chronic metabolic disorder, characterized by persistently high blood glucose levels due to defect in insulin secretion insulin action or both [1] It is one of fastest growing global health concern. Recent epidemiological data indicate substantial increase diabetes prevalence over past decades affecting more than 500 million people worldwide, with type 2 diabetes mellitus accounts for approximately 90 to95 % of cases. [2] This is closely associated with sedentary lifestyle, obesity, aging populations, and genetic factors that make DM the leading cause of morbidity and mortality due to complications such as diabetic neuropathy, nephropathy, retinopathy, cardiovascular disease, and stroke. [3]

The present treatment options of diabetes involve the use of oral antidiabetic drugs, insulin, and behavior modification. Although these methods are effective in managing diabetes, they are often related to adverse events like gastrointestinal irritation, episodes of hypoglycemia, weight gain, poor patient compliance, and a relatively high cost of treatment [4]. The long-term use of artificial antidiabetic drugs can cause a loss of efficacy and adverse effects. This highlights the need to develop alternative therapies that are safe, effective, and affordable. In this context natural products particularly plant derived bioactive compounds, have gained significant attention due to broad pharmacological properties minimal side effects, and multiple mechanism of action [5,6]

Flavonoids, a large class of polyphenolic compounds abundant in fruits vegetables and medicinal plants, are well known for antioxidant, anti-inflammatory, and metabolic regulatory activities [7,8] Diosmin an aglycone flavonoid glycoside derived from citrus fruits, has emerged as promising bioactive molecule with wide range therapeutic potentials [9] Chemically diosmin is 3’,5,7-trihydroxy-4’-methoxyflavone 7-rhamnoglucoside, and exhibit strong antioxidant, anti-inflammatory, vasoprotective and antihyperlipidemic properties (figure 1) [10]. These effects make it potentially beneficial for managing metabolic disorder such as diabetes. Clinically diosmin is widely used for conditions like chronic venous insufficiency and hemorrhoidal disease, demonstrating excellent safety profile in human [11]

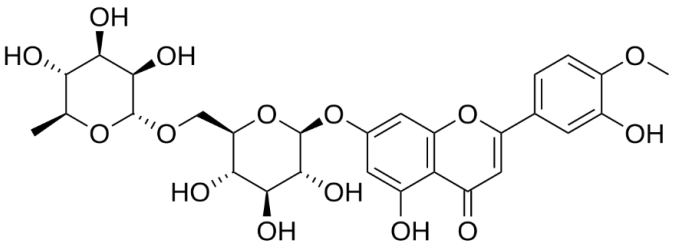


Figure 1: Structure of Diosmin

Oxidative stress and chronic low -grade inflammation are key contributors to developement and progression of diabetes mellitus. Elevated blood glucose generates reactive oxygen species ROS, impairing pancreatic β-cell function reducing insulin secretion and inducing insulin resistance in peripheral tissues. Diosmin has the potential to counteract oxidative stress damages scavenging free radicals, which inturn promotes antioxidant defense mechanisms like SOD, CAT, and GPx [12, 13]. Apart from this, Diosmin can also modulate inflammation primarily through inhibition of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and NF-κB activation, which plays a significant role in restoring metabolic homeostasis [14, 15].

Antidiabetic properties of diosmin have been established in a few preclinical studies. Evidence suggests diosmin's ability to lower glucose tolerance, improve insulin sensitivity, modulate the enzyme responsible for carbohydrate metabolism, and lower insulin resistance [16]. It also works by decreasing LDL cholesterol, triglycerides, but increasing HDL cholesterol, offering cardioprotective effects in diabetic conditions [17]. In addition, diosmin modulates glucose-related hepatic enzymes glucokinase, glucose-6-phosphatase, and Fructose-1,6-bisphosphatase, establishing diosmin's role in glucose regulation in the liver [18,

Despite these promising findings, a clear understanding of specific cell and molecular mechanisms underlying antidiabetic activity of diosmin has remained poorly clarified. Most work has primarily focused on in vivo and biochemistry studies, without any concerted effort to investigate mechanistically by molecular docking, target prediction, and pathway analysis. Computational simulations are important tools to analyze ligand-receptor interaction, identification of molecular targets, as well as predictions on drug action mechanisms in vivo, complementing *in vitro* studies. However, molecular interaction of diosmin with critical glucose controlling enzyme receptors, such as α-amylase, α-glucosidase, DPP-IV, PPARγ, and insulin receptor substrates, has not been fully explored.

Moreover, while the antioxidant activity of diosmin has been confirmed by *in vitro* experiments, very little research has investigated the effect of diosmin on glucose uptake or other cellular processes related to diabetes [20]. As a result, the use of both prediction and experimentation may result in more concrete proofs for the enhancement of the role of diosmin.

Network pharmacology was chosen as the suitable framework, given the polyfactorial nature of diabetes mellitus which is a complicated condition, along with the multitarget pharmacological property of flavonoids in general. Network pharmacology can be useful in the identification of interactions between a molecule, a target and a pathway although not always direct.

The explicit hypothesis of this study is that diosmin exerts antidiabetic effects by modulating multiple targets involved in insulin signaling, and glucose metabolism including insulin receptor related pathways and carbohydrate hydrolyzing enzymes. To test this we employed an integrated approach combining network pharmacology molecular docking, and in vitro assays to elucidate its mechanism of action molecular interactions, and efficacy. This work aim to address existing knowledge gaps, and support the development of natural flavonoid based therapies for diabetes management.

2. MATERIALS AND METHODS

Data Availability and FAIR Compliance

The datasets used in this study are all extracted from publicly available and carefully maintained databases. The datasets are FAIR (Findability, Accessibility, Interoperability, and Reusability) compliant using persistent URLs gene identifiers, and clear inclusion criteria. Nevertheless because of the natural differences in the database organization and annotation schemes, the partial limitations in interoperability were overcame by normalization and cross validation. The target genes associated with Diosmin were extracted from the Comparative Toxicogenomics Database (CTD; https://ctdbase.org/ ; last accessed January 2025, CTD; CAS 520-27-4) using the keyword “Diosmin”. Genes associated with diabetes mellitus was obtained from GeneCards (version 5.18; https://www.genecards.org/) with a relevance score cutoff of ≥10 , to filter out irrelevant information.

Protein protein interactions (PPI) and chemical protein interactions were extracted from the STITCH database (https://stitch.embl.de/) using a minimum interaction confidence score of 0.08 . All genes and proteins were annotated using HGNC approved gene symbols and UniProt identifiers to facilitate multi-platform analysis. The network files gene sets, interaction scores and analysis parameters are available upon reasonable request to ensure transparency and reproducibility, in accordance with accepted network pharmacology reporting practices. Here, NPRS refers solely to data transparency expectations in network pharmacology studies and not to nonribosomal peptide synthetase sequence deposition.

Target identification, DEG integration and PPI network construction

Diabetes mellitus associated genes were retrieved from GeneCards (version 5.18) with a relevance score filter ≥10.

Diosmin target genes was gathered from CTD, followed by validation of chemical gene interactions using STITCH with a confidence level of 0.08. Overlapping genes between diabetes mellitus associated genes and diosmin target genes was determined to enhance robustness, and biological plausibility. Overlapping genes were visualized using FunRich v3.1.3 to determine common molecular features connecting diabetes mellitus and diosmin pharmacological action. These common genes was then employed to build protein protein interaction and chemical protein interaction networks using Cytoscape (version 3.9.1)

Hub Gene Identification and Network Topology Analysis

To enhance methodological rigor and respond to reviewer comments, hub gene identification was conducted using multiple topological measures rather than degree centrality alone. Topological measures of network topology such as degree centrality betweenness centrality, and closeness centrality were calculated using the CytoHubba plugin in Cytoscape (version 3.9.1)

Hub genes were identified using consensus ranking based on multiple topological measures, choosing genes that ranked in the top nodes in at least two different centrality measures. This multi measure strategy help to eliminate selection bias inherent in single measure approaches and is consistent with best practices in network pharmacology and NPRS methodological guidelines

Highly interconnected subgraphs were obtained using the MCODE plugin with the following parameters degree cutoff = 2, node score cutoff = 0.2, K core = 2, and maximum depth = 100. The identified modules were regarded as biologically significant clusters that may be involved in diosmin induced modulation of diabetes related pathways

Functional Enrichment Analysis

Functional enrichment analysis was conducted using the g:Profiler platform to detect Gene Ontology (GO) terms and KEGG pathways including biological processes molecular functions, and cellular components. The Benjamini Hochberg false discovery rate (FDR) correction was used and p values ≤ 0.05 was regarded as statistically significant.

Molecular Docking

Molecular docking analysis was performed to predict the binding affinity of diosmin with selected hub proteins including insulin receptor (IR) insulin receptor substrate-1 (IRS-1), AKT1, phosphatidylinositol 3-kinase (PI3K) and glucose transporter-4 (GLUT4), using AutoDock Vina version 1.2.0 to evaluate the interaction patterns.

Protein and Ligand Preparation

Protein structures (IR, IRS, Akt, PI3K, Glut4) was retrieved from Protein Data Bank (PDB). Structures were selected based on the availability of high-resolution X-ray crystallographic data (≤ 3.0 Å resolution) and relevance to the study focus, ensuring proper selection criteria were applied. Water molecules and hetero atoms were removed manually. Proteins were then subjected to addition of Kollman charges and polar hydrogens. Likewise, the 3D structure of diosmin compound was downloaded from PubChem and Open Babel software was applied for energy minimization using MMFF94 force field.

Docking analysis

Docking studies were performed using AutoDockTools version 1.5.7. Grid parameters were defined based on previously reported active-site residues and co-crystallized ligand binding positions of each target protein, as documented in the Protein Data Bank and were given in the Table 1. Grid spacing was fixed at 1.0 Å and grid box size was set as 40 × 40 × 40 Å. Grid centers were adjusted to active site coordinates for every protein. Docking parameters such as exhaustiveness of eight, energy range of three kcal/mol and ten binding modes were applied. Docked conformations were clustered using an RMSD tolerance of 2.0 Å, with respect to the lowest-energy pose and the most populated cluster was considered, for interaction analysis.

Table 1: Grid box parameters

Targets	Center	Dimensions (Å)
AKT	39.0837x31.7623x118.4972	50.4162x52.3851x60.5364
GLUT4	101.469x102.689x107.7769	76.5229x43.8450x71.5492
IR	-24.002x37.9724x11.0066	64.8622x48.3872x60.5675
IRS1	9.9158x45.0756x14.0197	50.0091x40.2694x109.7820
PI3K	14.9328x35.6191x33.7772	78.6669x103.4176x92.9384

Visualization of docked molecules

To validate the docking protocol, re-docking of co-crystallized ligands was performed for selected protein targets and the resulting binding poses were compared with experimentally resolved conformations as reported in the Protein Data Bank, for proper assessment. Visualization of docked molecules was analyzed using PyMOL and Discovery Studio Visualizer softwares. From docking output, the top ranked poses showing lowest binding free energy values (ΔG, kcal/mol) were selected. Additionally, hydrogen bonds, hydrophobic interactions and π–π interactions were manually examined.

ADMET analysis

Using the SwissADME online program (http://www.swissadme.ch), the absorption, distribution metabolism, excretion and Toxicity (ADMET) characteristics of diosmin were predicted. Diosmin's canonical SMILES was obtained from the PubChem database, and submitted to the SwissADME service for examination. ESOL Ali, and SILICOS-IT are validated predictive models for solubility and Lipinski, Veber Egan, and Muegge filters for drug likeness. The platform also assesses physicochemical descriptors lipophilicity, water solubility, pharmacokinetic behavior drug likeness, and medicinal chemistry friendliness. The oral bioavailability and pharmacokinetic feasibility of diosmin were evaluated using the expected ADME properties which were created using default settings.

***In vitro* analysis:**

Chemicals

All the chemicals used in this study are from analytical standards purchased from Sisco Research Laboratories Pvt. Ltd India, except the study compound diosmin (CAS No.: 520-27-4; Molecular Weight: 608.54). It is procured from Sigma Aldrich Chemicals Pvt. Ltd. India. Additionally, α-amylase (Product Code 28588 CAS No 9000-90-2) and α-glucosidase (Product Code 75551 CAS No 9001-42-7) were also purchased from Sisco Research Laboratories Pvt. Ltd, India.

α-amylase enzyme inhibitory activity

The α-amylase inhibitory activity of diosmin was examined by adding 50 µL phosphate buffer (pH 6.8), 10 µL of α-amylase enzyme, and diosmin at different concentrations (10–50 µg/mL) in a 96-well plate. Then 20 µL of starch solution (1%) prepared in phosphate buffer was added as substrate. The reaction mixture was pre incubated at 37°C for 30 minutes. After incubation, 100 µL of color reagent 3,5-dinitrosalicylic acid (DNS) was added followed by boiling for 10 minutes to stop the reaction. Formation of reddish-brown colored complex was measured at 540 nm using ELISA Plate reader (Model: Spark Make: TECAN). Acarbose (10–50 µg/mL) was used as standard and all tests were done in triplicate for analysis. IC_{50} values were determined from dose–responses curves by interpolation of percentage inhibition versus concentration. These values are reported as approximate value derived by linear interpolation and rounded to the nearest whole number.

α-glucosidase enzyme inhibitory activity

The inhibitory effect of diosmin on α-glucosidase was evaluated using the method described by Sancheti et al. [8]. Briefly, different volumes of diosmin solution (10, 20, 30, 40 and 50 µL) were mixed with 25 µL of 4-nitrophenyl α-D-glucopyranoside, 50 µL phosphate buffer (pH 7.0) and 25 µL freshly prepared α-glucosidase enzyme solution to make total volume of 110 µL. The reaction mixture was incubated at 37°C for 30 minutes, then 100 µL of 0.2 M sodium carbonate was added to terminate the reaction. The liberated p-nitrophenol

was measured at 410 nm along with blank, acarbose (standard) and negative control Using ELISA PLATE reader (Model: Spark Make: TECAN). All reactions were performed in triplicate. IC_{50} value were determined from dose–responses curves by interpolation of percentage inhibitions versus concentration. These values are reported as approximate value derived by linear interpolation and rounded to the nearest whole number.

Statistical analysis

This study used freely accessible bioinformatics platforms for computational analysis; therefore, formal statistical validation was not applicable. For *in vitro* assays, results were expressed as mean ± SEM and analyzed using two-way ANOVA followed by Bonferroni's post-hoc test using GraphPad Prism version 8.0. Values with $p < 0.05$ was considered statistically significant.

3. RESULTS

Network pharmacology was employed to systematically investigate the potential mechanisms of diosmin in the treatment of diabetes. The workflow includes active compound screening, target prediction, diabetes-related target identification, network construction, protein–protein interaction analysis, and functional enrichment analysis. The complete network pharmacology pipeline is summarized in a schematic workflow figure-2 to clearly present the analytical strategy of this study**.**

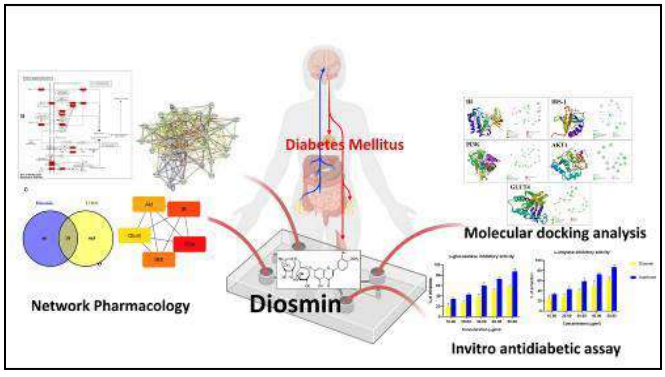


Figure 2: Schematic workflow of the network pharmacology analysis of diosmin against diabetes.

Data Collection and Identification of Common Targets

The Venn diagram show the diosmin associated genes (66), T2DM related genes (465) and a total of 39 common target genes. The pie chart describes the targets and their distribution among major protein classes, such as enzymes, kinases, receptors, oxidoreductases, transcription factors and transporters derived from the 39 shared genes. Using CTD and GeneCards online data bases, the genes linked with diosmin (66 genes) and type 2 diabetes mellitus (465 genes) was collected. These genes were analyzed by FunRich software, which revealed 39 common genes and are represented in Fig. 2. Further analysis of the common genes showed that secreted proteins (27%), enzymes (13%), oxidoreductases (12%), and family A/G protein coupled receptors (7%) were the dominant molecular classes, along with other minor categories, as shown in Fig.3.

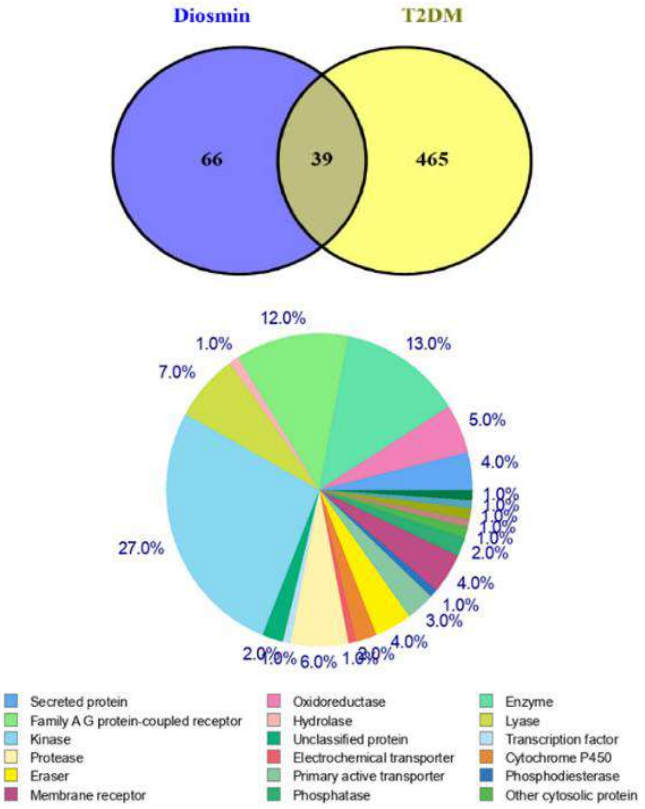
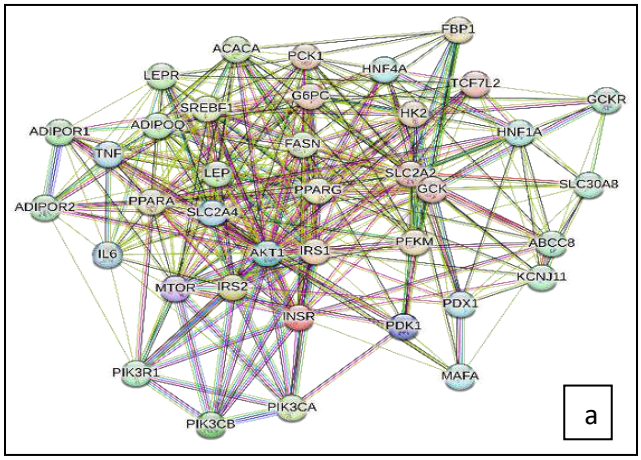


Figure 3: Identification and functional classification of diosmin–T2DM common targets.

Protein–Protein Interaction (PPI) Network Analysis

The PPI network results and hub genes associated with diosmin and DM is illustrated, where the right-side panel highlights the major hub genes IR, IRS, PI3K, AKT and GLUT4 identified by CytoHubba analysis. These hub genes represent important interactions within the insulin signaling pathway, as shown in Fig. 4. Circular nodes represent diabetes-associated target genes with larger nodes indicating hub genes based on network topology, Edges denote interaction between targets. Edge colors correspond pathway associations: green- insulin signaling, yellow- AMPK signaling pink- adipocytokine signaling, and blue- glucose metabolism. As revealed from the STITCH database, the 39 common genes were linked to both diosmin and DM and also demonstrated strong inter connectivity among key metabolic and signaling related proteins. Proteins such as INSR, IRS1, AKT1, PI3K sub units, GLUT4, PPARG, FASN, GCK and others could be visualized in this interaction network. Additionally, top hub genes including IR (INSR), IRS1, PI3K, AKT1 and GLUT4 were filtered based on degree centrality, each forming strong regulatory connections within the insulin signaling axis as shown in Fig.4. According to MCODE cluster analysis, these signaling components dominated a highly connected cluster, suggesting that diosmin mainly targets core elements of the IR–IRS–PI3K–AKT–GLUT4 pathway, which plays a vital role in insulin sensitivity and glucose uptake.



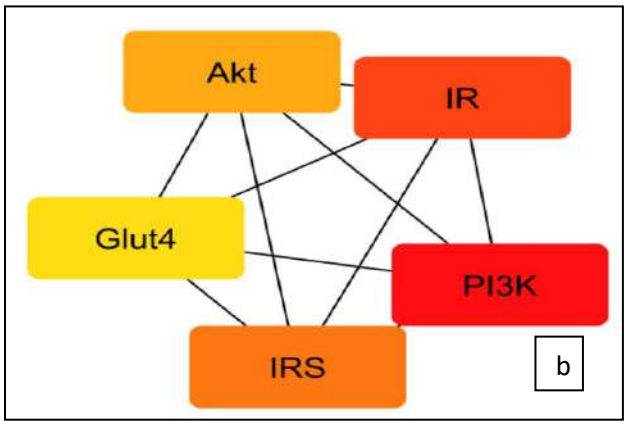


Figure 4. Protein – Protein interaction analysis (a) PPI network (b) hub-gene identification

Circular nodes: diabetes-associated target genes, larger nodes: hub genes, Edge colors Green: insulin signaling, Yellow:AMPK signaling, Pink: adipocytokine signaling, and Blue: glucose metabolism.

Enrichment analysis and pathway mapping of diosmin–T2DM common targets

The enrichment analysis and pathway mapping of common targets between diosmin and T2DM are illustrated, where the image represents KEGG pathway enrichment showing several significant pathways linked with the intersected genes. These include insulin signaling, AMPK signaling, adipocytokine signaling and T2DM related pathways. The MCODE derived cluster represent the most densely connected protein module inside the PPI network, as shown in Fig. 5. Type II diabetes mellitus, adipocytokine signaling, AMPK signaling and insulin resistance were identified as highly enhanced metabolic regulatory pathways involved by the intersected genes (39 genes) analyzed using GO enrichment. Furthermore, it also showed the influence of other pathways like glycolysis / gluconeogenesis, carbohydrate metabolism and HIF-1 signaling, which indicate a strong association with glucose homeostasis. These targets, particularly within the IRS1–PI3K–Akt signaling cascade and adipocytokine mediated regulation, were also highlighted in KEGG pathway mapping (Fig.5). As a

results, this result suggests that the intersected genes may have regulatory role in insulin metabolism.

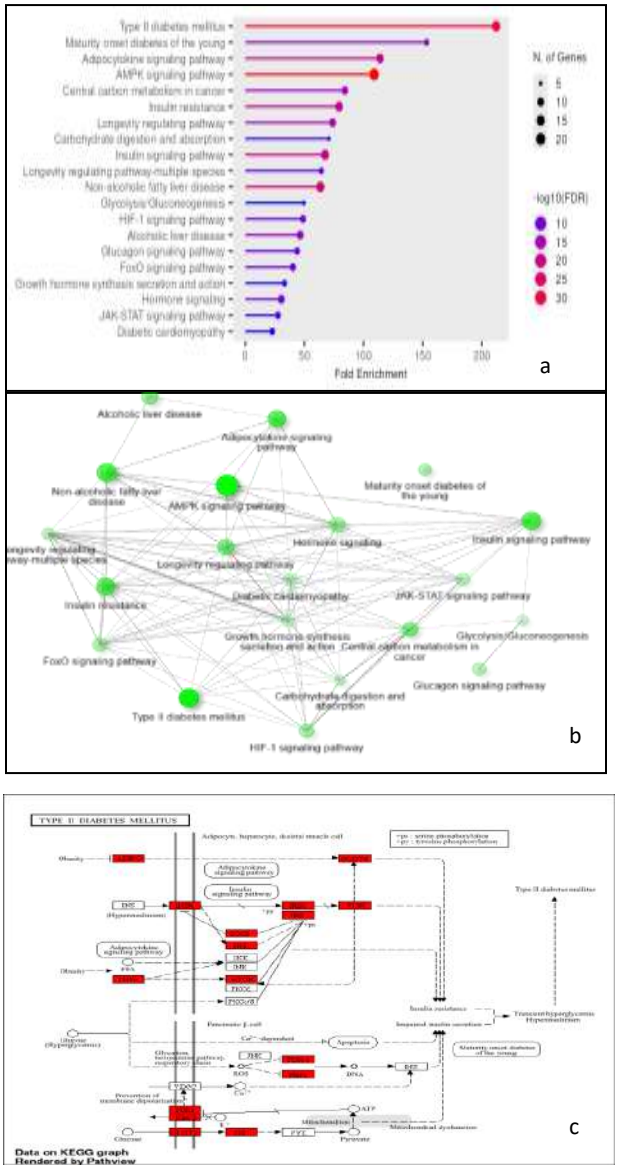


Figure 5: a) Enrichment Analysis of diosmin b) Gene Ontology of diosmin and c) KEGG pathway

Molecular Docking Results

Molecular docking analysis reveals the strong binding affinities of diosmin with hub genes (IR, IRS-1, PI3K, AKT1, and GLUT4) with the binding energy ranging from –9.3 to –11.3 kcal/mol as mentioned in table 2. Docking results of GLUT4 (–11.3 kcal/mol) were the highest followed by PI3K (–10.0 kcal/mol), IR (–9.3 kcal/mol), AKT1 (–10.1 kcal/mol), and IRS-1 (–9.4 kcal/mol). Subsequently, amino acids interactions were also studied, in which ASP1132, THR1154, and HIS1130 in IR; ASP241 and ASP242 in IRS-1; ASN677, HIS676, and LYS678 in PI3K; HIS207 in AKT1; and ASN411, GLN283, and SER80 in GLUT4 were involved using hydrogen bonds (Fig. 6). All selected protein–ligand complexes exhibited stable binding conformations within the accepted RMSD clustering threshold (≤ 2.0 Å), indicating a consistency of the predicted binding poses, and showing that the poses was reliable. The RMSD values corresponding to IR, IRS-1, PI3K, AKT1, and GLUT4 were provided in Supplementary Tables S1–S5. The docking results were consistent across repeated runs, confirming the consistency of the predicted binding positions.

ADMET analysis

SwissADME prediction on diosmin was tabulated in the table3. The prediction shows that it possesses high molecular weight (608.54 g/mol) and high polarity (TPSA = 238.20 Å²), which is associated with limited membrane permeability and low gastrointestinal absorption. The compound exhibited hydrophilic character (consensus LogP = −0.52) with soluble to moderately soluble aqueous behavior. Diosmin was predicted to be a P glycoprotein substrate with no blood brain barrier permeation and showed no inhibition of major CYP450 enzymes, suggesting a low risk of drug drug interactions

Although diosmin did not comply with Lipinski's rule of five and showed a low predicted bioavailability score (0.17), no PAINS or Brenk alerts was detected. Overall, the ADME profile support diosmin as a safe pharmacologically relevant nutraceutical or lead compound, rather than a conventional orally bioavailable small molecule drug.

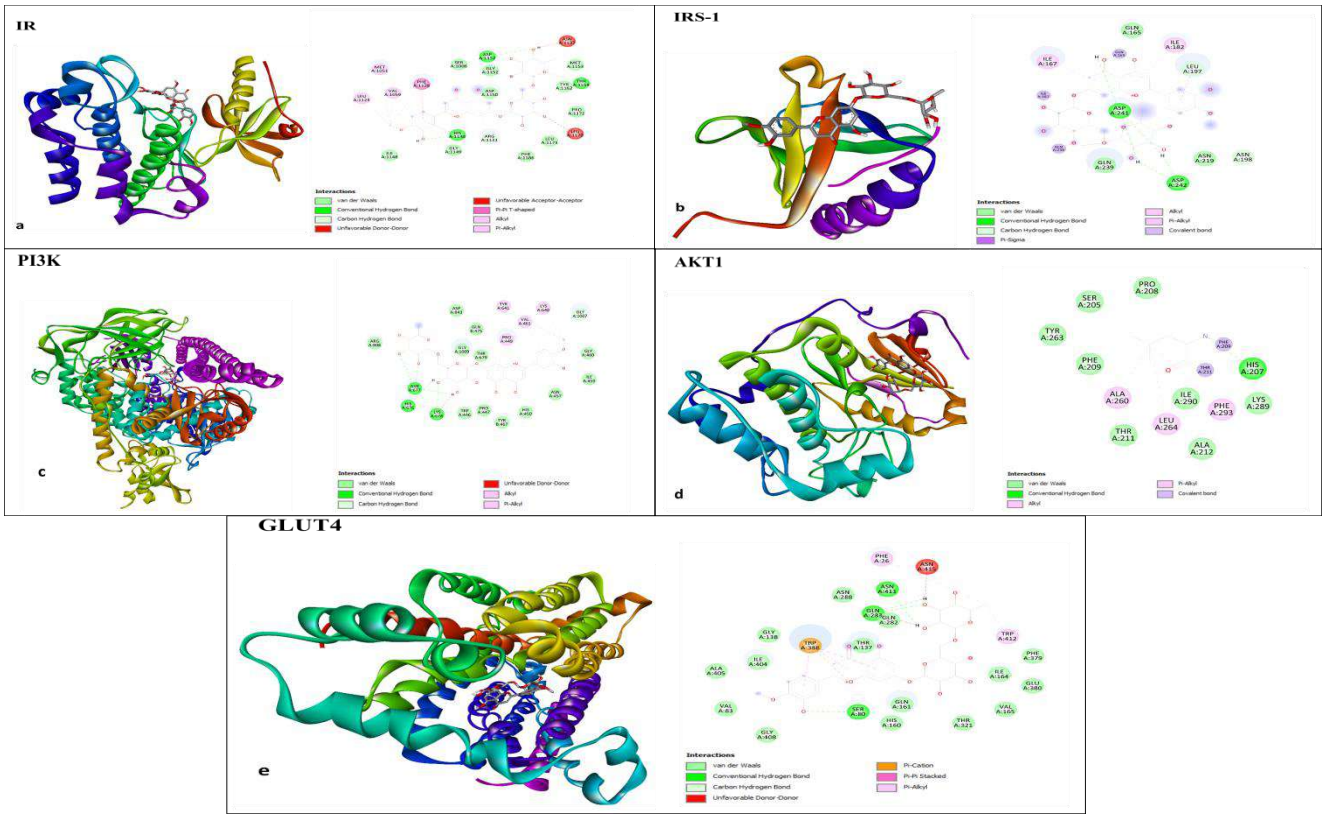


Figure 6: Molecular docking and 2D and 3D interaction maps of diosmin with key insulin-signaling proteins a) IR, b) IRS-1, c) PI3K, d) AKT1, e) GLUT4.

Table 2: Molecular docking results of diosmin with key insulin-signaling proteins.

Protein	Binding energy (kcal/mol)	Number of H-bonding	H-bonding
IR (1IRK)	-9.3	3	ASP A:1132, THR A:1154, HIS A:1130
IRS-1 (1IRS)	-9.4	2	ASP A:241, ASP A:242
PI3K (4JPS)	-10.0	3	ASN A:677, HIS A:676, LYS A:678
AKT1 (6CCY)	-10.1	1	HIS A:207
GLUT4 (4PYP)	-11.3	3	ASN A:411, GLN A:283, SER A:80

Table:3 Summary of Biological Activity and ADME Evaluation of Diosmin as an Antidiabetic Lead

Category	Parameter	Result	Interpretation / Relevance
Physicochemical properties	Molecular weight	608.54 g/mol	High MW limits permeability
	TPSA	238.20 Å²	Indicates high polarity
	H-bond donors / acceptors	8 / 15	Explains low oral absorption
	Rotatable bonds	7	Moderate molecular flexibility
Lipophilicity	Consensus Log P	−0.52	Hydrophilic nature
Water solubility	Solubility class	Soluble–moderately soluble	Favors formulation flexibility
Pharmacokinetics	GI absorption	Low	Limits oral bioavailability
	BBB permeation	No	Reduces CNS side effects
	P-gp substrate	Yes	Efflux reduces absorption
	CYP inhibition	None	Low drug–drug interaction risk

Drug-likeness	Lipinski rule	Not compliant	Typical for flavonoid glycosides
	Bioavailability score	0.17	Predicts poor oral exposure
Medicinal chemistry	PAINS alerts	0	No assay interference
	Brenk alerts	0	Favorable safety profile
	Synthetic accessibility	6.48	Moderate synthetic complexity
Overall assessment	Therapeutic potential	Promising	Suitable as nutraceutical / lead compound

α-Amylase Inhibition Assay

Diosmin showed a clear concentration dependent inhibition of α-amylase activity across the tested range (10–50 µg/mL), with a gradual reduction in absorbance at 540 nm when compared with control group (Fig. 7). The highest concentration of diosmin (50 µg/mL) showed a significant inhibitory effect ($p < 0.05$). Although diosmin was effective in suppressing enzyme activity, acarbose consistently showed stronger inhibition at all comparable concentration, and was used as positive reference standard. Dose– response analysis reveals that diosmin exhibited an IC_{50} values of approximately 40 µg/mL against α-amylases, whereas the reference drug acarbose shows a lower IC_{50} value of approximately 22 µg/mL, indicating higher inhibitory potency.

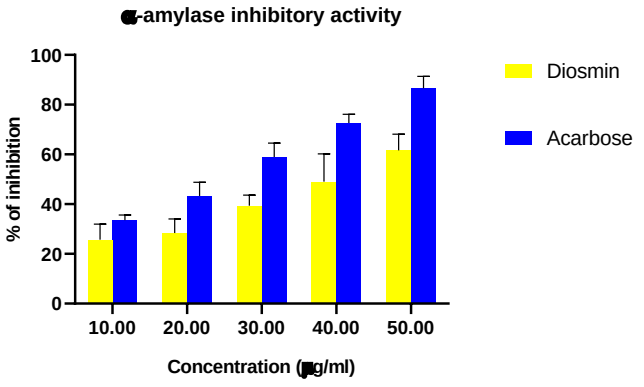


Figure 7: Concentration-dependent inhibition of α-amylase activity by diosmin compared with acarbose.

α-Glucosidase Inhibition Assay

Diosmin inhibited the α-glucosidase activity in a dose dependent manner. Diosmin gradually reduce the enzyme activity, while acarbose showed much stronger inhibition at all the tested concentrations (fig 8). The level of p-nitrophenol released was decrease with increasing diosmin concentration (10–50 µg/mL). Significant inhibition was mainly observed at the higher diosmin concentration ($p < 0.05$). Acarbose display the highest inhibitory effect among all the tested groups. Blank and negative control did not show any inhibition. The IC_{50} values of diosmin against α-glucosidases was estimated to be approximately 38 µg/mL, while acarbose demonstrated a stronger inhibitory effect with an IC_{50} of approximately 20 µg/mL.

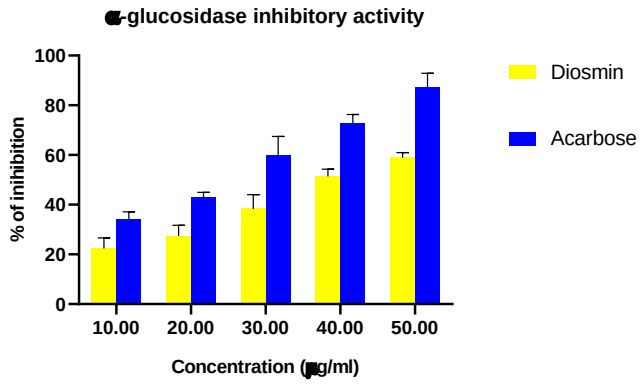


Figure 8: Concentration-dependent inhibition of α-glucosidase by diosmin compared with acarbose.

4. DISCUSSION

The antidiabetic potential of diosmin, a natural bioflavonoid compound was analytically explored in the present work by using an integrated approach including network pharmacology, molecular docking and *in vitro* enzyme inhibition studies. The in-silico approach was applied to analysis the network pharmacology of diosmin using various online software tools followed by molecular docking studies to predict its interaction with insulin signaling related proteins such as IR, IRS-1, PI3K, AKT1 and GLUT4. Similarly, the *in vitro*

analysis like α-amylase and α-glucosidase inhibition was also carried out to evaluate the diosmin antidiabetic potential. Overall, diosmin appears to be a strong natural therapy candidate for DM improvement through regulation of glucose metabolism.

The identification of genes linked with diosmin and diabetes mellitus is considered as the initial step in network pharmacology analysis. Hence, the genes associated with diosmin and DM were analysed using online tools and total 39 intersected genes were identified and grouped. This analysis indicated the regulatory influence of diosmin on glucose metabolism pathways, insulin signaling pathways as well as inflammatory pathways. Diosmin, being a flavone glycoside, may influence these pathway mechanisms which leads to the regulation of hub genes associated with DM [22]. However, it should be noted that network pharmacology prediction is based on database-driven associations and do not confirm direct biological effects [23].

The interaction of genes in relation to different pathways was analyzed using CytoHubba analysis, which revealed highly interactive nodes associated with insulin signaling, glucose metabolism and inflammatory pathways, along with diosmin potential to regulate these pathways [24, 25]. Additionally, this PPI network analysis also minimized the number of genes for further analysis by identifying hub genes such as IR, IRS-1, PI3K, AKT1 and GLUT4 and showing their interactions. These results which support the role of diosmin in regulating multiple metabolic pathways were further strengthened by the detection of dense clusters using MCODE analysis [26, 27]. Nevertheless, similar hub genes have also been reported in other phytochemicals-based network studies, suggesting that these targets may represent common metabolic regulators rather than diosmin-specific effects [28].

With the hub genes obtained from PPI network, GO and KEGG functional enrichment analysis was further proceeded to understand the drug–disease relationship. This analysis highlighted that diosmin has significant impact on insulin signaling, glucose metabolism, oxidative stress response and inflammatory pathways by altering key members of these pathways, which in turn influence DM progression [29]. Similarly, previous studies also reported that this flavone compound has the potential to alter metabolic processes as observed through GO and KEGG enrichment patterns [30, 31]. Conversely, some studies have reported limited bioavailability and inconsistent in *vivo* efficacy of flavonoid glycosides, which may restrict their translational relevance despite promising *in silico* enrichment results [32]. Collectively, this research supported that diosmin may reduce insulin resistance by regulating these hub genes, which was further examined using molecular docking approach.

Molecular docking was becoming an important tool to analysis the interaction between bioactive compounds and target proteins. In this regard, we analyzed the molecular docking of diosmin with the selected hub genes. Molecular docking suggested energetically favorable binding pose of diosmin with selected hub proteins IR, IRS, PI3K, Akt and GLUT4. Among the selected protein, lower docking scores were observed for GLUT4 and PI3K; however, these values should be interpreted cautiously, as docking does not confirm functional modulation, particularly for transporter protein such as GLUT4. Likewise, few earlier studies have reported that diosmin shows higher affinity for binding with metabolic enzymes and insulin signaling molecules responsible for DM pathogenesis [33, 34, 35]. These findings indicate that diosmin may have the potential to interact with proteins involved in glucose metabolism, warranting further experimental validation hence to confirm these in silico results, in vitro analysis was further designed. Although molecular docking provides valuable insights into potential ligand protein interactions, it does not confirm biological activity target engagement, or downstream functional effects. Docking scores represent predicted binding energies under

static conditions and may not reflect physiological binding dynamics protein conformational flexibility, or intracellular accessibility. Furthermore, proteins such as IRS-1 and GLUT4 are not classical druggable targets and docking results for such proteins should be interpreted as exploratory rather than confirmatory. Therefore, the docking results in this study are intended to support hypothesis generation rather than mechanistic validation.

In the *in vitro* studies, the α-amylase inhibition assay was performed to explore the natural compound potential in controlling blood sugar levels. Therefore, we investigated the antidiabetic activity of diosmin using α-amylase inhibition assay, where acarbose was used as standard drug. The results demonstrated that diosmin exhibited concentration dependent α-amylase inhibitory effect [36]. This antidiabetic activity of diosmin was comparable to other citrus flavonoids reported earlier, which delay starch breakdown and showed similar inhibitory patterns [37, 38]. Despite these findings, the inhibitory potency of diosmin was moderate, indicating that it may function as a supportive rather than primary antidiabetic agent.

Another important glucose metabolic enzyme, α-glucosidase, which plays key role in reducing intestinal glucose absorption was also evaluated in the present study [39]. In the α-glucosidase inhibitory assay, diosmin showed significant inhibition at higher doses compared to the standard acarbose, which is in agreement with earlier studies reporting citrus flavonoids inhibitory effects on carbohydrate hydrolyzing enzymes [40, 41]. This effect may be due to its ability to inhibit disaccharide breakdown, thereby preventing glucose release. Although diosmin demonstrated measurable inhibitory activity against α-amylase and α-glucosidase, its IC_{50} value were higher than acarbose, indicating moderate potency and supporting its potential role as an adjunct antidiabetic, or nutraceutical compounds rather than a primary enzymes inhibitor.

Strengths and Limitations:

The present study benefits from an integrated strategy combining network pharmacology, molecular docking and in vitro enzyme inhibition assays to explore the antidiabetic potential of diosmin at a systems level however. The network pharmacology analysis is limited by database bias as gene target associations are prediction based and may not represent diosmin specific effects clearly. Molecular docking provides only theoretical binding estimates under static conditions and does not confirm functional modulation, particularly for non-classical drug targets such as IRS-1 and GLUT4. Additionally, the lack of in vivo and clinical validation limits translational relevance, as bioavailability, pharmacokinetics and therapeutic efficacy in humans remain to be established. Hence forth, Diosmin may represents a promising adjunct or lead compounds for further investigation in diabetes management, though further studies using animal models are required.

6. CONCLUSION

The present study described the antidiabetic potential of diosmin, a natural citrus flavone, using an integrated approach of *in silico* and *in vitro* studies. Network pharmacology and molecular docking analysis identified a set of genes responsible for DM pathogenesis, which were also influenced by diosmin compound. Based on these findings, *in vitro* assays were performed to support the computational data. Diosmin are predicted to exhibit antidiabetic potentials by inhibiting key enzymes involved in glucose uptake and breakdown, thereby modulating glucose metabolisms and insulin signaling pathways. However, this study has few limitations, as further validation using animal models and cell line studies are needed, which may strengthen the current findings and support diosmin as a potential therapeutic agent for DM.

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