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In Silico Molecular Docking of Phytochemicals for Type 2 Diabetes Mellitus Therapy: A Network Pharmacology Approach

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ABSTRACT

Identification of potential lead molecules in herbal medicines is crucial not only for validation but also for drug discovery. This study was focused on identifying the therapeutic mechanisms of 10 common herbs used to treat type 2 diabetes mellitus (T2DM) using network pharmacology and docking studies. Details pertaining to medicinal plants and their phytoconstituents were obtained from Indian Medicinal Plants, Phytochemistry, and Therapeutics and Dr. Duke's database, respectively. MolSoft was used to assess their drug likeness. Prediction of protein targets for the screened phytochemicals and the list of target genes involved in T2DM were obtained using Swiss TargetPrediction and GeneCards respectively. STRING; Cytoscape; Database for Annotation, Visualization, and Integrated Discovery; and PyRx were used for network construction, network analysis, gene ontology analysis, and molecular docking, respectively. The protein targets MAPK1, AKT1, PI3K, and EGFR were identified to play a crucial role in the progression of T2DM. Furthermore, molecular docking indicated that nimbaflavone exhibited high binding affinities for MAPK1 (−8.7 kcal/mole) and PI3K (−9.6 kcal/mole), whereas rutin and 10-hydroxyaloin-B showed high binding affinities for AKT1 (−7.4 kcal/mole) and EGFR (−8.1 kcal/mole), respectively. The findings from this study suggest that flavonoids are the major phytoconstituents that display antidiabetic activity by interacting with key protein molecules related to the MAPK and PI3K-AKT signaling pathways, thereby aiding in the treatment of T2DM. The activation of these pathways alters Ras-GTPase activity and enhances the expression of GLUT4, a glucose transporter, resulting in the uptake of glucose from the bloodstream.

Keywords: Type 2 diabetes mellitus, network pharmacology, gene ontology, molecular docking

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Introduction

Diabetes mellitus is an endocrine disorder. Type 2 diabetes mellitus is defined as a defect in the metabolism of carbohydrates, lipids, and proteins owing to decreased insulin production or increased insulin resistance or a combination of both (1). In 2022, according to the International Diabetes Federation (IDF) (https://idf.org/), 537 million and 90 million people had diabetes globally and in Southeast Asia, respectively. Of the 90 million individuals, 77.4 million are Indians, which is expected to exceed 134 million by 2045. According to the IDF, the percentage of diabetes occurrence in the Indian population is 8.9. As per World Health Organization (WHO) data, 2% of all deaths in India are due to diabetes and its complex clinical implications, such as retinopathy, neuropathy, nephropathy, cardiovascular disease, and skin disorders (https://www.who.int/). T2DM has a complicated pathophysiological process that involves the concerted action of various factors, which results in disease development (2). Hence, it is important to target multiple proteins in the T2DM pathway. Highly interactive proteins in different pathways of the disease must be identified for a multitarget approach (3).

Network pharmacology (NP) reveals the complex interactions of a pharmacological molecule in a living cell using computational power. The method is useful for identifying the underlying intricate interactions among proteins in the entire body and is an unbiased method of studying new potential target proteins (4). Target selection is based on the use of NP analysis to screen out highly interacting proteins. NP is a computational approach for identifying the hub of proteins that possibly interact with multiple ligands. Using this approach, the complex association between phytoconstituents and specific disease targets can be predicted (5).

In Indian Medicine, most practitioners create and formulate their own medicines. The WHO has gathered a list of 21,000 medicinal plants that are in use across the world. Currently, 2500 species of medicinal plants are available in India, of which 150 are in commercial use. India, the world's botanical paradise, is the largest producer of medicinal herbs. Of late, the use of traditional plant remedies for diabetes has gained significant attention. However, as many as 400 such remedies lack scientific and medical testing to ascertain their efficacy and safety, which limits the integration of herbal medicine into modern medical practices. Therefore, clinical investigations using *in vitro* assays and toxicity and safety testing are essential to determine the viability of herbal medications (6). The conventional drug discovery method of "one target–one drug" may not be efficient in treating diseases. Hence, multiple targets involved in the pathways associated with a disease must be targeted while developing a potential drug. NP is a computational approach for identifying the hub of proteins that possibly interact with multiple ligands. Using the NP approach, the complex association between phytoconstituents and specific disease targets can be predicted (5). Antidiabetic medications, increase insulin production by beta cells and glucose reabsorption in the kidney and lower bad cholesterol, and oral medications, such as thiazolidinediones, biguanides, and meglitinides, work by decreasing the blood glucose level. All these drugs, besides interacting with their target, also interact with and disrupt other metabolic interactions, thereby causing several side effects (7). The abnormality in a gene not only contributes to one disease but also leads to several associated diseases. To overcome this issue, NP is expected to aid in further drug development by providing a platform for systematic exploration, allowing diseases, genes, and pathways to be identified (5).

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Materials and methods

Selection of Herbal Plants

Indian Medicinal Plants, Phytochemistry, and Therapeutics (IMPPAT) (https://cb. imsc.res. in/imppat/home) is an extensive online database on Indian medicinal plants that assists in computer-aided natural product-based drug discovery (8). The web server provides a graphical output that depicts how closely the plant species alleviates the disease. In this study, the plants were screened by searching "diabetes mellitus" in therapeutic use. Based on the results obtained from IMPPAT and literature survey, 10 plants were selected for the docking studies, and the list of phytochemicals present in them was downloaded from Dr. Duke's website (https://phytochem.nal.usda.gov/phytochem/search).

Ligand Screening

The drug likeness characteristics of the selected compounds were assessed using MolSoft (https://molsoft.com/mprop/), with their canonical SMILES as the input (9). When the drug likeness score (DLS) of a ligand is >0.8, it exhibits good activity. Hence, phytochemicals with a DLS of 0.8–1.12 were selected for further studies (10).

Swiss TargetPrediction

Understanding the molecular mechanisms behind bioactivity and anticipating potential side effects or cross-reactivity require mapping the targets of phytochemicals. In Swiss TargetPrediction (http://www.swisstargetprediction.ch/), based on the idea that two similar bioactive molecules probably share their protein targets, the structure of the query ligand was compared with existing drugs to predict protein targets (11). From the screened phytochemicals, the target corresponding to a specific drug was identified using Swiss TargetPrediction. The canonical SMILES for the phytochemicals were obtained from PubChem, and target prediction was performed for the 13 screened phytochemicals. The result included a list of potential target genes along with their probability score.

Selection of Target Genes

To precisely identify relevant disease targets, information on T2DM-associated target genes was retrieved from GeneCards (https://www.genecards.org/) (12). From the obtained result, the top 500 disease-associated genes were screened based on their relevance score.

Protein–Protein Interaction (PPI)

The list of genes obtained from GeneCards and Swiss TargetPrediction was given as input for Venny2.1 (https://bioinfogp.cnb.csic.es/tools/venny/), which provides overlapping target genes between them, indicating higher disease associated with the genes. Ninety-eight genes were identified to be overlapping targets. These genes were fed as multiple protein input to the STRING database (https://string-db.org/), which provides functional associations among proteins using sources, including co-occurrence, neighborhood, text mining, experiments, coexpression, and database search (13). The obtained PPI network was given as input to Cytoscape version 3.8.2.

Network Construction

Cytoscape was used to integrate, visualize, and analyze biological networks. Cytoscape may be expanded via plugins, allowing a large community of scientists to add helpful features. This growth has occurred naturally as a result of the separate work of various writers, producing a potent and diversified set

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of tools. Cytoscape 3.8.2 was used to analyze the network and nodes representing active chemicals, target genes, and pathways. The edges show how phytochemicals interact with the targets. The degree, which represents the relevance of a component/target/pathway in the network, was calculated using the analyze tool available in Cytoscape (14). The different types of networks, namely, Target–Pathway, Target–Phytochemicals, and Target–Phytochemical–Pathway, were constructed (15).

Gene Ontology (GO) Analysis

GO enrichment analyses are commonly used to perform biological interpretation of the gene (16). This analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using DAVID 6.8 (https://david.ncifcrf.gov/). The overlapped gene targets were given as input for DAVID GO analysis. The obtained result included three different analysis reports, namely, biological process, molecular function, and cellular component. The top 10 pathways were chosen based on their significance using the p-value. Analyzing the results indicated that genes obtained from the network analysis contributed to most of the pathways in T2DM. GO visualization was performed using R 4.1.2 (17).

Molecular Docking

The 3D structures of the selected ligands were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in the SDF format (18). The crystal structures of the hub target proteins were downloaded

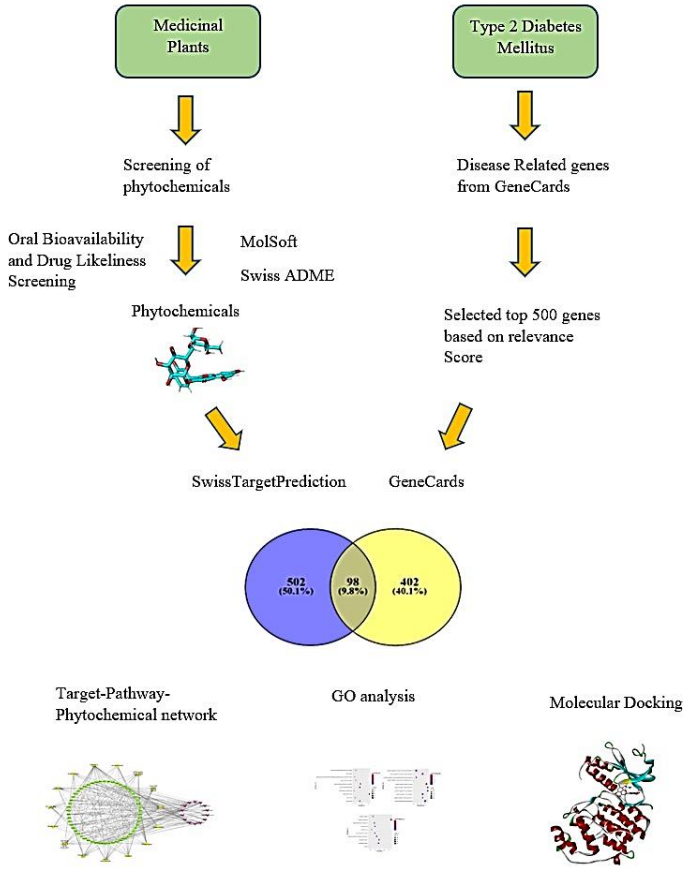

Fig. 1. Framework based on the Network Pharmacology Strategy

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from RCSB PDB (https://www.rcsb.org/) in the PDB format. The protein was prepared using PyMol 2.5.2 by removing water molecules, adding polar hydrogens, and removing additional molecules. Protein and ligand were imported into PyRx 0.8 and converted into PDBQT format. Molecular docking was used for the prediction and design of new probable drugs via possible docking modes, and the binding affinity between the PDB structure of the target protein and the ligand molecule was analyzed (19). The interactions of the docked complex were visualized using BIOVIA Discovery Studio 2021. The overall framework of the present study is depicted in Figure 1.

Results

Mining of Target Proteins

The overlapping targets of phytochemicals and disease database is shown in Figure 2. The PPI network of 98 overlapped targets obtained from the STRING database (Figure 3) was given as input to Cytoscape 3.8.2. Degree, one of the significant topological characters, was used to retrieve the hub targets. The combined analysis of the PPI network and GO provided the lead to identify the effective drug to control T2DM.

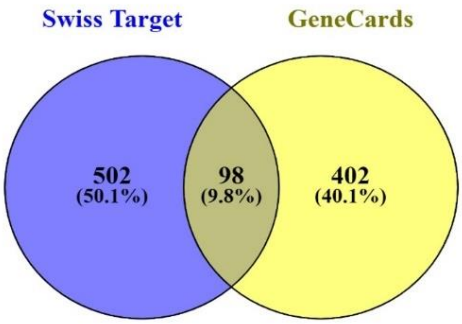

Fig. 2. Venn diagram of targets obtained from Swiss target prediction and GeneCards.

DLS and Phytochemicals

The canonical SMILES for the 724 phytochemicals and their structures were obtained from PubChem. The canonical SMILES for each of the compounds were fed as input to MolSoft. The DLS for all the 724 phytochemicals ranged from −1.98 to −1.5. Phytochemicals exhibiting the properties of drugs should have DLS ≥ 0.8. In this study, the selected phytochemicals had DLS > 0.8. Table 1 lists the phytochemicals that were screened, and Table 2 lists their DLS.

Pathway Enrichment and Network Analysis

In the constructed network of phytochemicals, targets, and pathways that contained 127 nodes and 1096 edges, MAPK1, PI3K, AKT1, EGFR HRAS, MMP9, TP53, RAF1, MTOR, and MMP2 were the top 10 proteins targeted by the phytochemicals. Figure 4 shows the network of targets and phytochemicals. Similarly, pathways in cancer, lipid and atherosclerosis, proteoglycans in cancer, AGE-RAGE signaling pathway in diabetic complications, hepatitis B, endocrine resistance, prostate cancer, HIF-1 signaling

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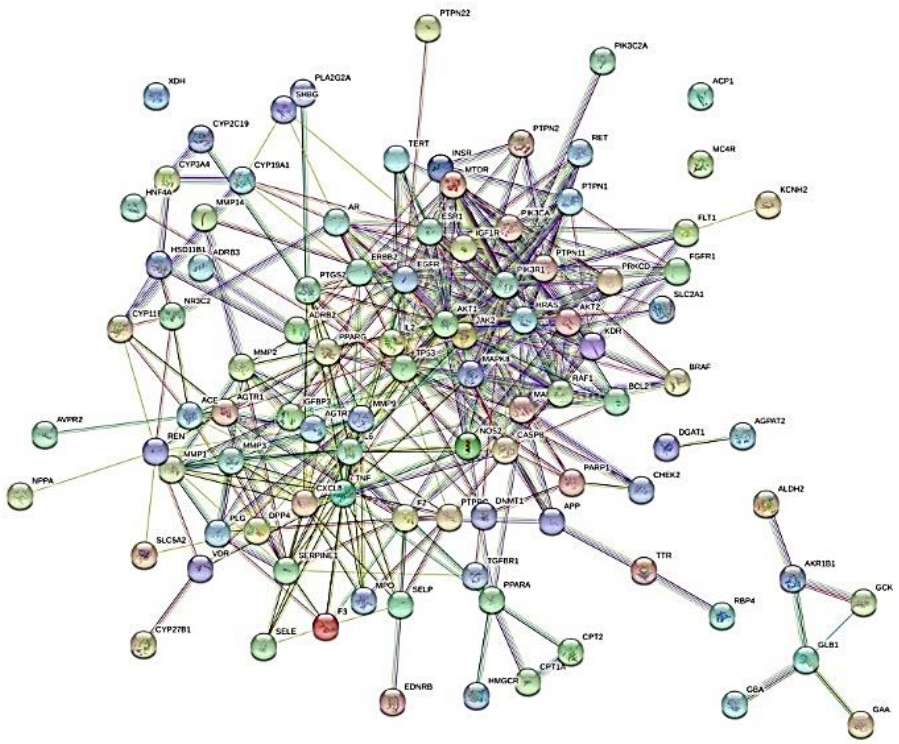

Fig. 3. Protein-Protein interaction network.

Table 1. List of screened phytochemicals.

1.	Aloe vera	10-Hydroxyaloin-A	14889736	$C_{21}H_{22}O_{10}$	434.4
2.	Aloe vera	10-Hydroxyaloin-B	14889737	$C_{21}H_{22}O_{10}$	434.4
3.	Aloe vera	Isorabaichromone	10370832	$C_{29}H_{32}O_{12}$	572.6
4.	Aloe vera	Folacin	135398658	$C_{19}H_{19}N_7O_6$	441.4
5.	Aloe vera Cannabis sativa	Pteroylglutamic-acid	137217037	$C_{20}H_{22}N_8O_6$	470.4
6.	Aloe vera Cannabis sativa Carica papaya Gymnema-sylvestre Momordica charantia Murraya koenigii	Thiamin	1130	$C_{12}H_{17}N_4OS^+$	265.36
7.	Azadirachta indica	Rutin	5280805	$C_{27}H_{30}O_{16}$	610.5
8.	Azadirachta indica	Quercitrin	5280459	$C_{21}H_{20}O_{11}$	448.4
9.	Azadirachta indica	Nimbaflavone	14492795	$C_{26}H_{30}O_5$	422.5
10.	Carica papaya Momordica charantia	Gamma-carotene	5280791	$C_{40}H_{56}$	536.9
11.	Coccinia grandis	Betulin	72326	$C_{30}H_{50}O_2$	442.7
12.	Momordica charantia	Map30	451600	$C_{22}H_{29}FO_5$	392.5
13.	Momordica charantia	Vicine	135413566	$C_{10}H_{16}N_4O_7$	304.26

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Table 2. Drug Likeness Score of the Screened Phytochemicals.

S.No.	Phytochemicals	NHBA	NHBD	MolLogP	DLS	Oral Bioavailability
1.	Quercitrin	11	7	0.32	0.82	0.17
2.	Nimbaflavone	5	2	6.61	0.84	0.55
3.	Vicine	8	9	-3.83	0.84	0.17
4.	Gamma-carotene	0	0	14.77	0.86	0.17
5.	10-Hydroxyaloin-A	10	8	-0.75	0.86	0.55
6.	10-Hydroxyaloin-B	10	8	-0.75	0.86	0.55
7.	Thiamin	4	3	0.29	0.87	0.55
8.	Isorabaichromone	12	6	1.71	0.881	0.17
9.	Rutin	16	10	-1.55	0.914	0.17
10.	Map30	4	1	4	0.91	0.85
11.	Betulin	5	3	9.06	1.08	0.17
12.	Folacin	9	7	-1.85	1.09	0.11
13.	Pteroylglutamic-acid	10	8	-1.55	1.5	0.11

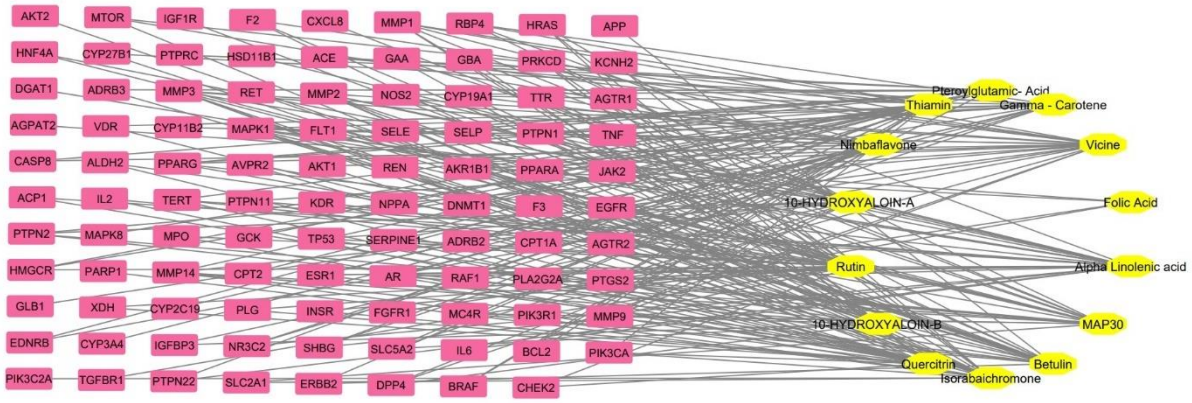

Fig.4. Network interaction of phytochemicals with their plausible target genes. The yellow colored hexagon represents the bioactives and the pink rectangle shows the target genes.

pathway, EGFR tyrosine kinase inhibitor resistance, and insulin resistance pathways had a significant number of genes. The targets-pathways network is depicted in Figure 5. In addition, Figure 6 depicts the interactions of various phytochemicals with their respective proteins and pathways. Of the 527 biological processes identified via GO analysis, positive regulation of MAPK cascade (GO: 0043410) had the lowest false discovery rate (FDR). Furthermore, of the 104 molecular functions, insulin receptor (IR) substrate binding (GO: 0043560) displayed the lowest FDR. In addition, 47 cellular components were identified in GO analysis, of which the receptor complex (GO: 0043235) exhibited the lowest FDR. Bubble plot on top 10 significant biological process, cellular component and molecular function with respect to gene ratio is shown in Figure 7.

Table 3 represents the top 10 pathways enriched in KEGG analysis. Moreover, in the network analysis, the phytochemicals rutin, thiamin, vicine, quercitrin, nimbaflavone, betulin, 10-hydroxyaloin A, and 10-

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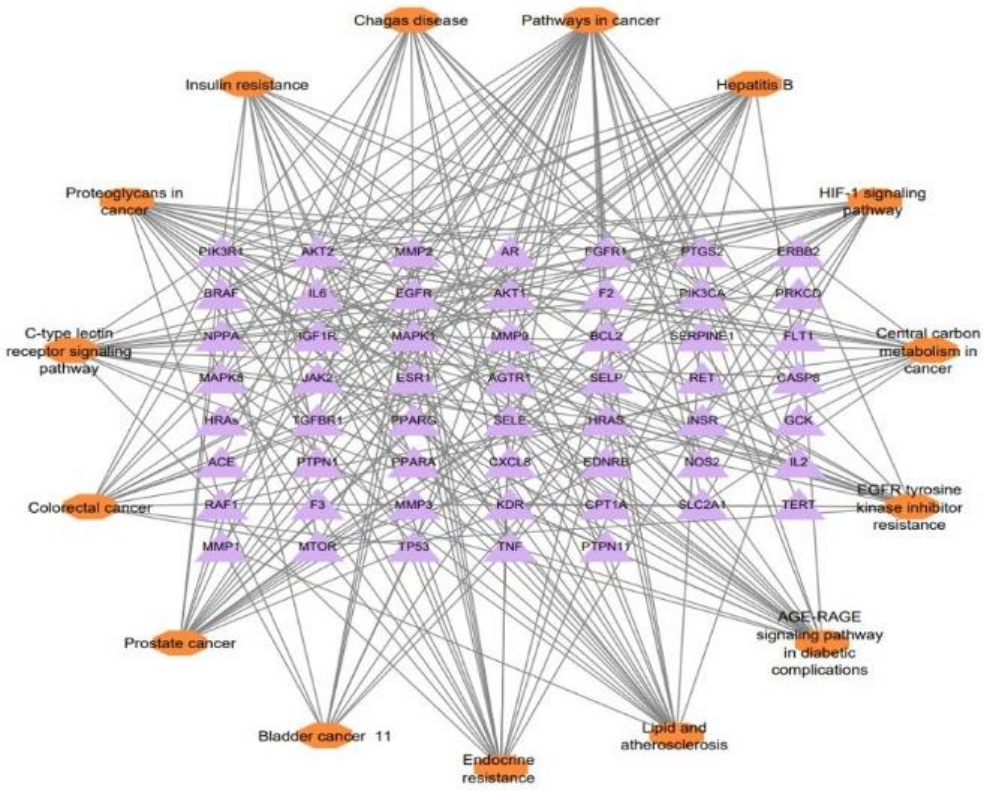

Fig. 5. Targets-pathways network. The lavender triangle nodes represent the targets of T2DM and the brick red hexagon represent the related pathways.

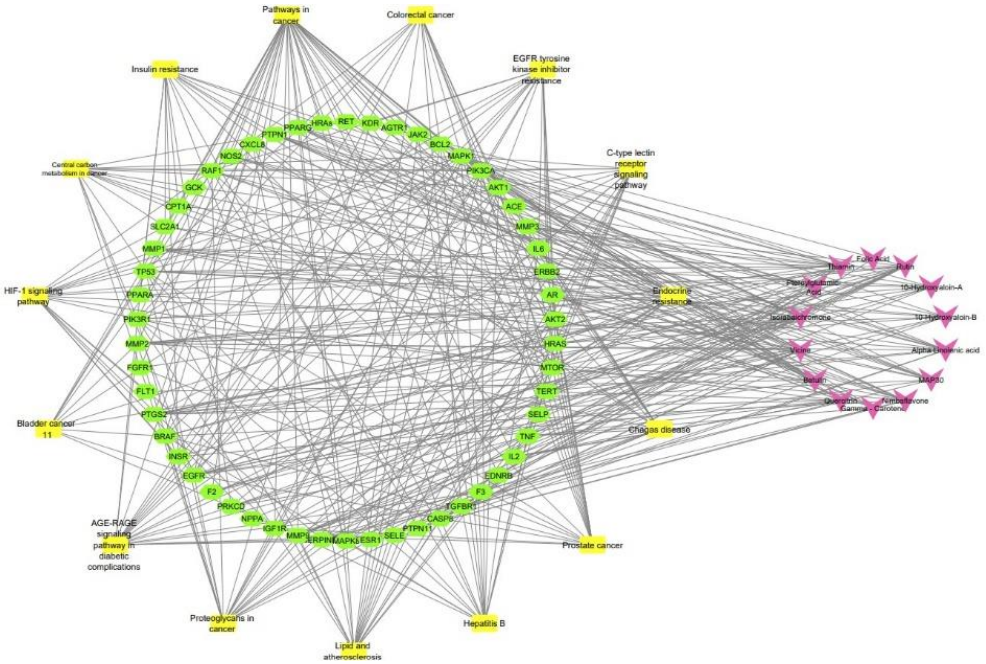

Fig. 6. Network diagram of phytochemicals/target genes/pathways. The purple arrow nodes represent the bioactives, the green ellipse nodes and yellow rectangle nodes represent the target genes and pathways respectively.

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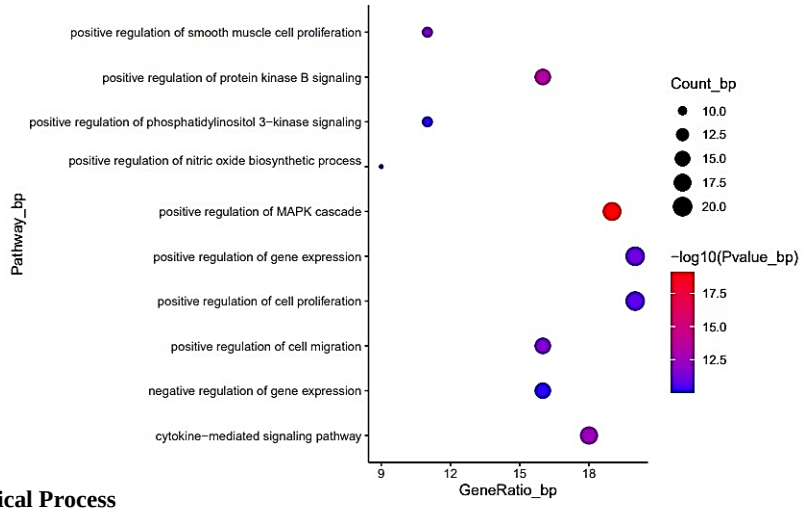
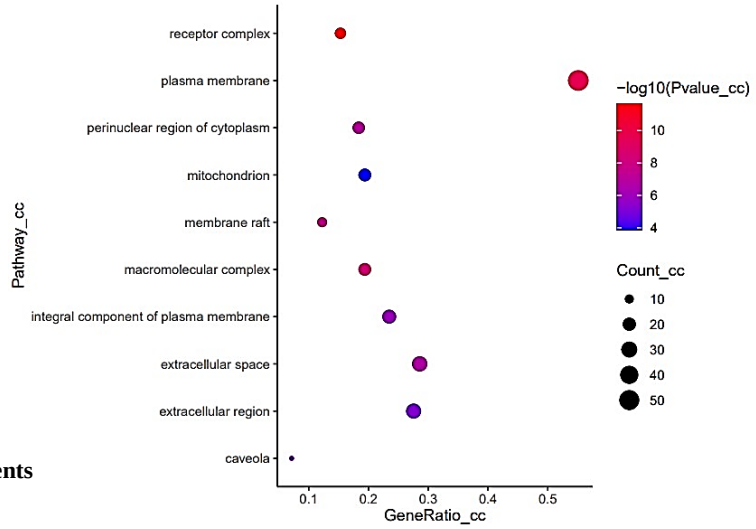
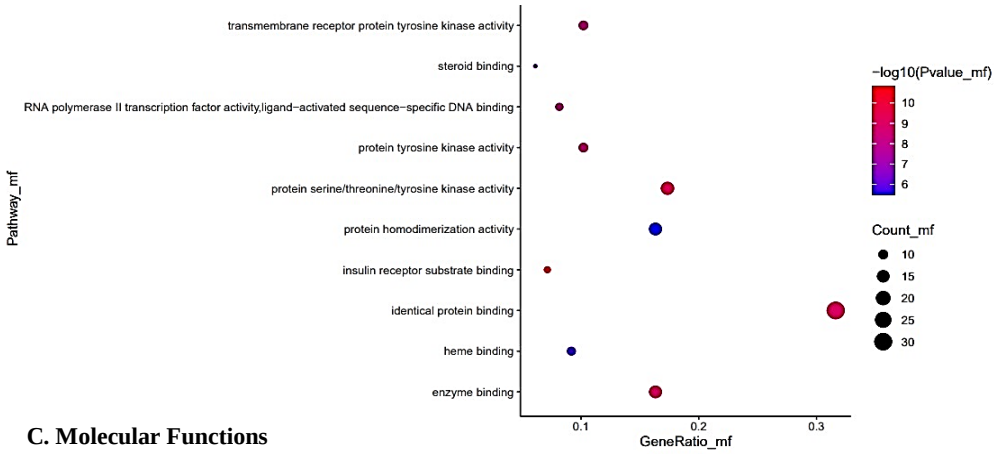


A. Biological Process



B. Cellular Components



C. Molecular Functions

Fig. 7. GO analysis for the targets in treating T2DM. The X-axis depicts the Gene ratio. The Y-axis shows enriched pathways in Biological processes (A), Cellular components (B) and Molecular functions (C). The higher the gene ratio, the higher level of enrichment. The size of the dot indicates the number of target genes in the pathway and the colour of dot shows the different $-\log_{10}$ (P value) range.

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Table 3. Number of genes involved in respective KEGG pathways.

Pathway description	Gene Count	-$\log_{10}$ (FDR)	Targets
Pathways in cancer	36	591	RET, CXCL8, SLC2A1, PIK3R1, PTGS2, EGFR, IGF1R, MAPK8, CASP8, EDNRB, TERT, AKT2, ERBB2, AKT1, MAPK1, JAK2, HRAS, NOS2, MMP1, MMP2, BRAF, F2, MMP9, ESR1, TGFBR1, IL2, MTOR, AR, IL6, PIK3CA, BCL2, AGTR1, PPARG, RAF1, TP53, FGFR1
Proteoglycans in cancer	20	8.51	MMP2, BRAF, PTPN11, PIK3R1, ESR1, TNF, MMP9, EGFR, MTOR, IGF1R, PIK3CA, AKT2, ERBB2, KDR, AKT1, MAPK1, RAF1, HRAS, TP53, FGFR1
Lipid and atherosclerosis	20	8.11	CXCL8, MMP1, MMP3, PIK3R1, SELE, TNF, MMP9, SELP, IL6, MAPK8, CASP8, PIK3CA, AKT2, BCL2, AKT1, MAPK1, PPARG, JAK2, HRAS, TP53
PI3K-Akt signalling pathway	20	4.93	FLT1, INSR, PIK3R1, EGFR, IL2, MTOR, IGF1R, IL6, PIK3CA, AKT2, ERBB2, KDR, BCL2, AKT1, MAPK1, JAK2, RAF1, HRAS, TP53, FGFR1
AGE-RAGE signalling pathways in diabetics complications	18	16.57	CXCL8, MMP2, PRKCD, SERPINE1, PIK3R1, SELE, F3, TNF, TGFBR1, IL6, MAPK8, PIK3CA, AKT2, BCL2, AGTR1, AKT1, MAPK1, JAK2, HRAS
Prostate cancer	18	16.19	MMP3, BRAF, PIK3R1, MMP9, EGFR, MTOR, IGF1R, AR, PIK3CA, AKT2, ERBB2, BCL2, AKT1, MAPK1, RAF1, HRAS, TP53, FGFR1
Endocrine resistance	**18**	**16.02**	**MMP2, BRAF, PIK3R1, ESR1, MMP9, EGFR, MTOR, IGF1R, MAPK8, PIK3CA, AKT2, ERBB2, BCL2, AKT1, MAPK1, RAF1, HRAS, TP53**
Hepatitis B	18	9.69	CXCL8, BRAF, PIK3R1, TNF, MMP9, TGFBR1, IL6, MAPK8, CASP8, PIK3CA, AKT2, BCL2, AKT1, MAPK1, JAK2, RAF1, HRAS, TP53
Diabetic cardiomyopathy	18	7.73	ACE, PARP1, MMP2, INSR, PRKCD, SLC2A1, PIK3R1, MMP9, TGFBR1, MTOR, MAPK8, CPT2, PIK3CA, AKT2, AGTR1, AKT1, REN, PPARA
Chemical carcinogenesis-receptor activation	18	7.41	VDR, PIK3R1, ADRB2, CYP3A4, ESR1, EGFR, MTOR, AR, PIK3CA, ADRB3, AKT2, BCL2, AKT1, MAPK1, JAK2, RAF1, PPARA, HRAS

hydroxyaloin B were identified to interact with the highest number of proteins; MAPK1, PI3K, AKT1, and EGFR were the primarily targeted proteins, with the MAPK1 signaling pathway being the targeted signaling pathway. The details of hub genes and topology characteristics obtained from network analysis are shown in Table 4.

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Table 4. Hub genes with PDB ID and Degree obtained from network analysis.

Gene Symbol	MAPK1	PI3K	AKT1	EGFR
PDB ID	4ZZN	1E7U	1UNQ	EGFR
Degree	22	17	16	15
Average Shortest Path Length	1.841463415	1.963414634	1.963414634	2.109756098
Betweenness Centrality	0.06624349	0.03676533	0.02805188	0.02486974
Closeness Centrality	0.543046	0.515723	0.509317	0.473988
Degree	221	17	16	15
Eccentricity	4	3	3	4
Neighborhood Connectivity	15.31818182	17.47058824	17.75	15.46666667
Number Of Undirected Edges	22	17	16	15
Radiality	0.97663	0.97392	0.97324	0.96917
Stress	15224	11098	8908	6338
Topological Coefficient	0.27534965	0.31076582	0.31603774	0.30138889

KEGG Pathway Enrichment

GO analysis identified 98 genes involved in 158 pathways. Furthermore, the pathways associated with T2DM were studied using KEGG pathway enrichment analysis. The top 10 pathways selected based on the FDR were mainly modulated by the following targets: EGFR, IGF1R, MAPK8, CASP8, EDNRB, TERT, AKT2, ERBB2, AKT1, MAPK1, JAK2, HRAS, NOS2, MMP1, MMP2, BRAF, F2, MMP9, ESR1, TGFBR1, IL2, MTOR, AR, IL6, PIK3CA, BCL2, AGTR1, PPARG, RAF1, TP53, and FGFR1. KEGG pathway enrichment on top 10 key pathways is shown in Figure 8.

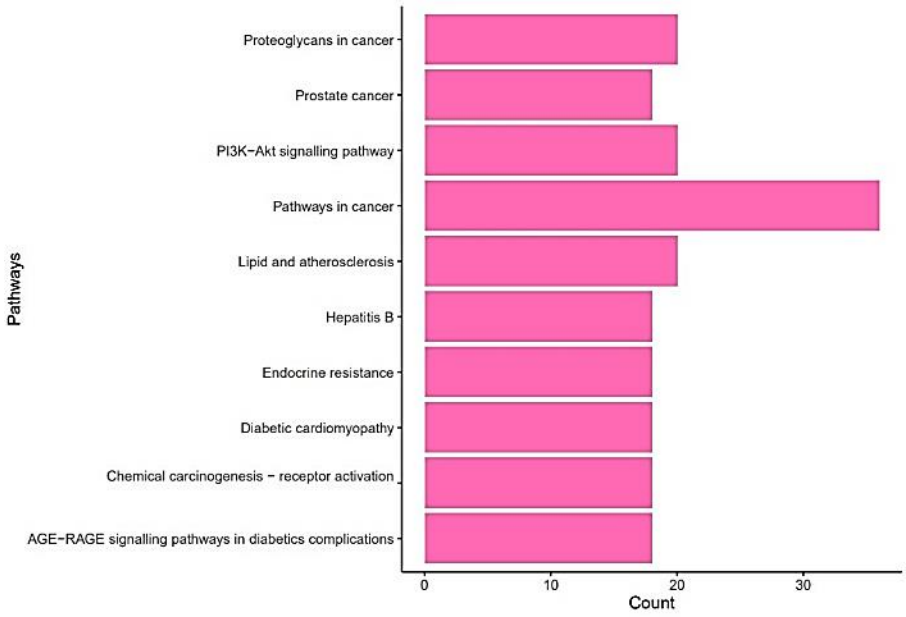

Figure 8. KEGG pathway enrichment.

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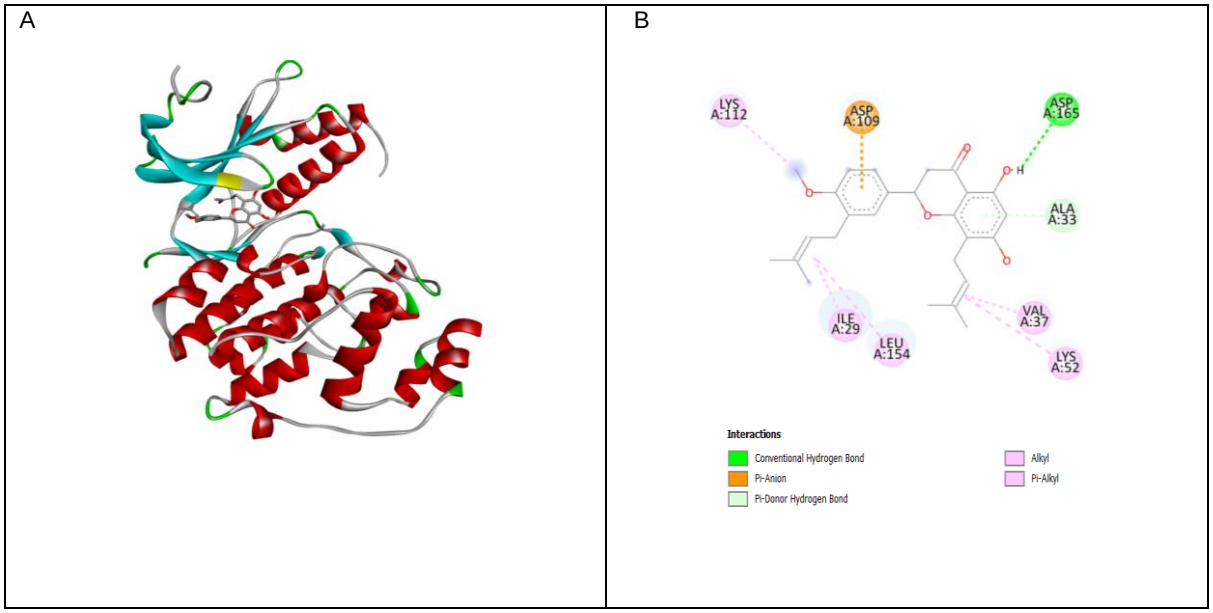
Molecular Docking Studies

The result obtained from GO and network analysis showed that PI3K/AKT signaling pathway, AGE-RAGE signaling pathway, and endocrine resistance pathway may be highly related to T2DM. The PPI network analysis suggested that MAPK1, PI3K, AKT1, and EGFR were the key targets of the abovementioned pathways. Hence, these targets were selected as hub genes. The crystal structures of the target proteins obtained from RCSB PDB were MAPK1 (4ZZN), PI3K (1E7U), AKT1 (1UNQ), and EGFR (1IVO). Molecular docking analysis of the 13 selected phytochemicals and the target proteins was performed using PyRx 0.8. Table 5 shows the docking scores and binding residues of compounds with the highest binding affinity. The binding energies of nimbaflavone with MAPK1 and PI3K were −8.7 kcal/mol and −9.6 kcal/mol, respectively. Similarly, the binding energy of EGFR with 10-hydroxyaloin B was −8.1 kcal/mol and that of rutin with AKT1 was −7.4 kcal/mol. These compounds demonstrated effective binding with the target proteins and may interfere with their molecular mechanism. In this study, nimbaflavone, rutin, and 10-hydroxyaloin B were found to act as lead molecules in the treatment of T2DM. The docking sites and type of interactions of phytochemicals with proteins were visualized using BIOVIA Discovery Studio 21.1.0, as shown in Figure 9. The interactions of these compounds with their respective protein targets based on molecular docking studies have not been reported so far.

Table 5. Docking analysis

S.No.	Target Protein	Phytochemical	Docking Score (kcal/mole)	Binding Residues
1.	MAPK1	Nimbaflavone	-8.7	Ile29, Ala33, Val37, Lys52, Asp109, Lys112, Leu154, Asp165
2.	PI3K	Nimbaflavone	-9.6	Leu657, His 658, Asp788, Arg849, Leu865
3.	AKT1	Rutin	-7.4	Arg15, Gly16, Glu17, Tyr18, Lys20, Ile74, Val83, Ile84, Glu85, Arg86, Thr87
4.	EGFR	10-Hydroxyaloin B	-8.1	Gln8, Gly9, Phe380, Lys407



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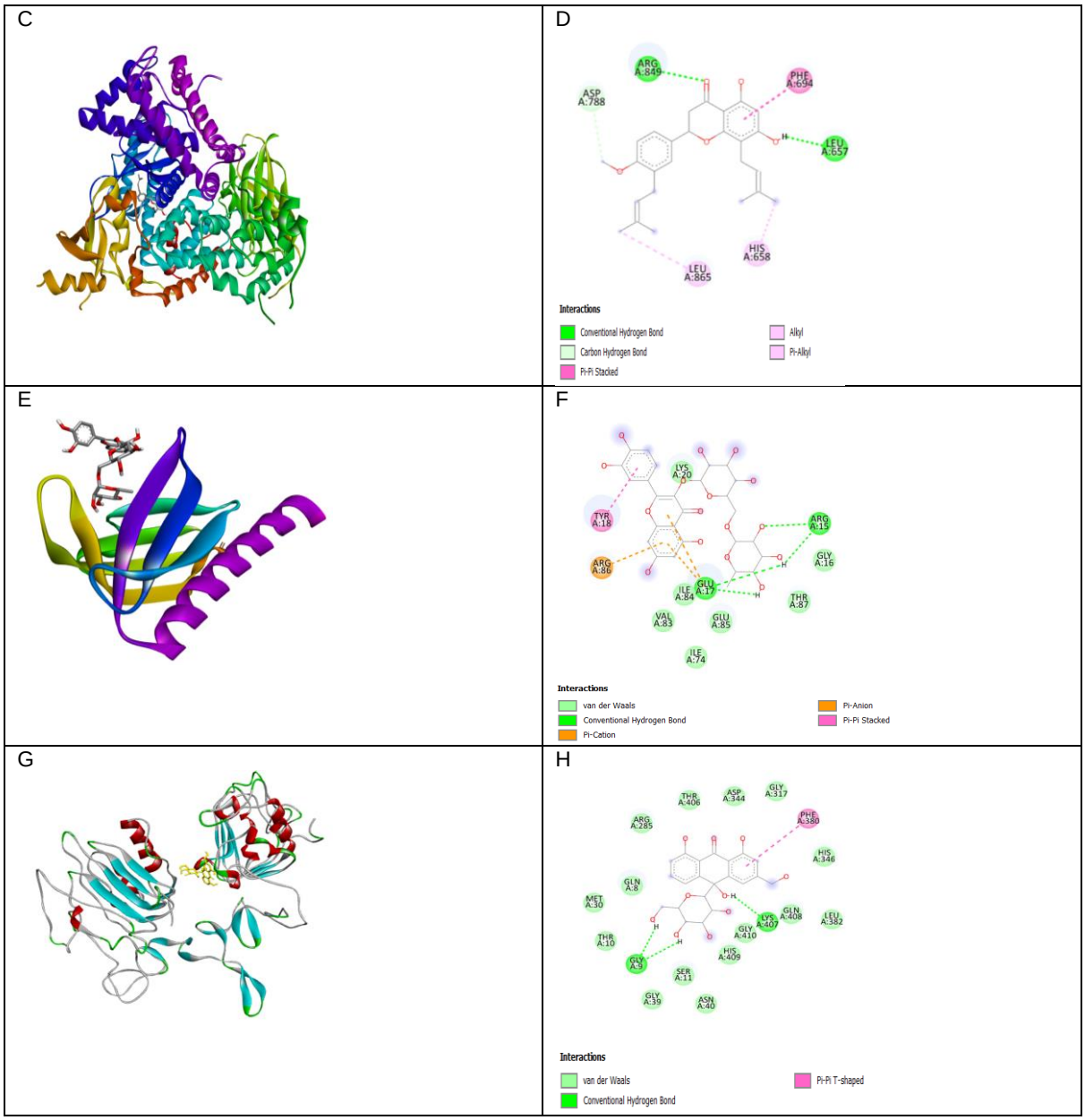

Fig. 9. 2-D interaction of the target protein and ligand: MAPK1-Nimbaflavone Interaction (A-B), PI3K-Nimbaflavone interaction (C-D), AKT1-Rutin interaction (E-F) and EGFR- 10-Hydroxyaloin B interaction (G-H).

Discussion

The potential of phytochemicals in treating T2DM was investigated using NP. This study identified that nimbaflavone, rutin, and 10-hydroxyaloin B were 3 of the 13 phytochemicals that exhibited potential efficacy in the treatment of T2DM. PPI networks were constructed for the associated pathways in the enrichment analysis. The PI3K/AKT pathway plays a key role in cellular physiology owing to its involvement in growth factor signal mediation during cell growth and cellular process. Furthermore,

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this pathway aids in maintaining blood glucose homeostasis, protein synthesis, and cell survival and proliferation (20).

Insulin is responsible for glucose transport in mammalian skeletal muscles via a mechanism that involves several intracellular proteins (21). The phosphorylation of Insulin Receptor Substrates (IRS1 and IRS2) is caused by the binding of insulin with insulin receptor, which results in the activation of phosphatidylinositol 3-kinase (PI3K). P13K activation is also mediated by the activation of the Ras protein. Consequently, PI3K phosphorylates phosphatidylinositol 4, 5-biphosphate (PIP_2) to phosphatidylinositol 3,4,5-triphosphate (PIP_3). PIP_3, upon activation, phosphorylates AKT1. AKT1 phosphorylation is catalyzed by 3-phosphoinositide-dependent kinase 1 (PDK1) and mTORC2 at Thr-308 and Ser-473, respectively (22). Both PDK1 and mTORC2 are activated via the PI3K pathway. AKT1 in the skeletal muscle is responsible for glycogen synthesis. AKT1 phosphorylates AS160, which increases Ras-GTPase activity that regulates GLUT4 translocation (23).

The expression of MAPK1 is required for the complete activation of GLUT4. Oxidant stress directly induces insulin resistance in the skeletal muscle via the p38-MAPK pathway (24). This stress-associated activation of the MAPK pathway decreases insulin function. Two mechanisms that play major roles in the development of insulin resistance are mitochondrial hydrogen peroxide production and NADPH oxidase activation (25). Hyperglycemia stimulates the overproduction of superoxide in the mitochondria and increases the levels of inflammatory cytokines. Tumor necrosis factor-alpha (TNF-α) causes insulin resistance via the MAPK pathway by increasing IRS-1 serine phosphorylation and decreasing insulin-stimulated IRS-1 tyrosine phosphorylation, Akt phosphorylation, and endothelial nitric oxide synthase activity (26).

EGFR activates the RAS-RAF-MEK-MAPK pathway, which directs gene transcription, cell cycle progression, cell proliferation, and PI3K-AKT pathway. Activation of the epidermal growth factor receptor (EGFR) occurs via the binding of ligands, such as epidermal growth factor and TNF-α, to the extracellular domain of EGFR. This binding event triggers dimerization via the phosphorylation of specific tyrosine residues in the intracellular domain of EGFR. The phosphorylated tyrosine residues serve as binding sites for signaling molecules, such as Ras, which in turn phosphorylate downstream molecules. The binding of growth-factor-receptor bound protein 2 and Src-homology-2-containing domain to the phosphorylated EGFR is required for the activation of MAPK cascades upon binding with Ras. However, the PI3K/AKT pathway cannot be directly activated by EGFR and is associated with the RAS-MAPK and RAS-PI3K-AKT pathways (27)

In this study, the molecular mechanism of phytoconstituents present in medicinal plants for T2DM treatment was predicted using the NP approach. GO and KEGG analyses of the target genes helped in the identification of key targets related to T2DM. The screened phytochemicals were observed to interact with MAPK1, PI3K, AKT1, and EGFR associated with the MAPK and PI3K-AKT signaling pathways and aid in the treatment of T2DM. The activation of these pathways enabled the positive regulation of the MAPK cascade, thereby increasing Ras-GTPase activity, and facilitated the expression of GLUT4, which is responsible for the transport of glucose from the bloodstream. Overall, this study has provided valuable

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insights into the therapeutic action of the screened phytochemicals based on computational tools, which should be confirmed via *in vitro* and *in vivo* studies.

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