

Available online on 15.04.2023 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

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Research Article

Unraveling the Multi-Target Pharmacological Mechanism of *Brassica rapa* in Diabetes Treatment: Integration of Network Pharmacology and Molecular Docking Approaches

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Article Info:

Abstract



Article History:

Received 10 Feb 2023
Reviewed 14 March 2023
Accepted 22 March 2023
Published 15 April 2023

Cite this article as:

Suresh Kumar G, Manivannan R, Nivetha B, Kamalakannan D, Bhuvaneshwari K, Ammu R, Aravinthan A, Anitha K, Dinesh Kumar V, Unraveling the Multi-Target Pharmacological Mechanism of *Brassica rapa* in Diabetes Treatment: Integration of Network Pharmacology and Molecular Docking Approaches, Journal of Drug Delivery and Therapeutics. 2023; 13(4):13-27

DOI: <http://dx.doi.org/10.22270/jddt.v13i4.5783>

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Brassica rapa has been widely reported as an anti-diabetic plant and is widely used in traditional medicine for the treatment of various disorders. However, the molecular mechanism underlying the plant's anti-diabetic activity has not been elucidated. Therefore, the present study aimed to investigate the possible molecular mechanism of *B.rapa* for managing diabetes mellitus through network pharmacology and molecular docking studies. The active ingredients and associated target proteins were obtained from a literature review and the Swiss Target Prediction platform and validated using the PubChem database. The disease-associated genes were retrieved from the Genecard database. The *B. rapa*-DM target network was analyzed using the STRING database, and the results were integrated and visualized using Cytoscape software. The molecular mechanism and therapeutic effect of *B.rapa* for the treatment of DM were determined by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses using the Enrichr Platform. Fifty-two active ingredients were screened from *B. rapa*, and 1528 putative target genes were identified from these ingredients. Four hundred and fifty-four overlapping targets matched with DM were considered potential therapeutic targets. First, ten key targets (ALB, AKT1, TNF, GAPDH, MAPK3, EGFR, VEGFA, CTNNB1, CASP3, and STAT3) were found by topological analysis. Then, the results of GO and KEGG suggested that the anti-diabetes effect of *B. rapa* was strongly associated with the AGE-RAGE signaling pathway in diabetic complications, Neuroactive ligand-receptor interaction, Lipid and atherosclerosis, PI3K-Akt signaling pathway, and Calcium signaling pathway. The AKT1 (Serine/Threonine Protein Kinase) enzyme is targeted by major bioactive constituents of *B.rapa*. Molecular Docking studies revealed that Liquiritin (docking score -6.1 Kcal/mol) showed the highest binding affinity with AKT1. These results suggest that *Brassica rapa* may play a role in regulating several pathways that are involved in the development of Diabetes Mellitus.

Keywords: Network pharmacology, molecular mechanisms, *Brassica rapa*, Diabetes Mellitus (DM)

INTRODUCTION

Over the past few decades, the prevalence of diabetes mellitus has increased, making it a common, albeit potentially devastating, medical condition¹. Diabetes is considered a global health issue, with high morbidity and mortality rates. Nearly 463 million people are affected by DM, and it is estimated to increase to 700 million by 2045 globally². Both environmental and genetic factors play a major role in the development of Diabetes mellitus. Environmental factors, such as inactivity, drugs and toxic agents, obesity, viral infections, and geographical location, can contribute to the onset of diabetes. While type I diabetes is not a genetically predestined disease, an increased susceptibility can be inherited. Genetic

susceptibility plays a crucial role in the etiology and manifestation of type II diabetes, with concordance rates in monozygotic twins approaching 100%³. Oral hypoglycemic drugs such as biguanides, sulfonylureas, glitazones, DPP-4 inhibitors, and insulin therapy are commonly used to treat diabetes. However, long-term use of these drugs can lead to decreased efficacy and hyperinsulinemia, while major side effects associated with DM include weight loss, lactic acidosis, ketoacidosis, anemia, bone fractures, and gastrointestinal and cardiovascular complications. Therefore, there is a need to explore medicinal plants that have potential anti-diabetic activity⁴.

Computational investigations have gained new insights into identifying the phytoconstituents found in medicinal plants for therapeutic purposes. Network pharmacology is an emerging frontier in the drug discovery process, as it integrates the principles of systems medicine with those of information science⁵. Network Pharmacology plays a major role in exploring the biological network of drug candidates, and helps in constructing poly-target drug molecules to optimize medication efficacy⁶.

Brassica rapa is an edible plant that belongs to the brassicaceae family. The plant contains flavanoids such as isorhamnetin, kaempferol, and quercetin glycosides, as well as glucosinolates, phenylpropanoid derivatives, indole alkaloids, organic acids, and various minerals such as copper, manganese, and calcium. It possesses antioxidant, anti-inflammatory, anti-arthritis, antimicrobial, antifungal, hepatic protective, and nephroprotective properties⁷. Studies have shown that *Brassica rapa*, in combination with *Eleocharis dulcis*, has a hypoglycemic effect with no reported side effect⁸.

Several studies have investigated the anti-diabetic activity of *Brassica rapa*, but the exact mechanism of action has not been systematically elucidated. In this study, we used bioinformatic analysis, including network pharmacology and molecular docking, to explore the mechanisms of *Brassica rapa* in the treatment of diabetes.

MATERIALS AND METHODS

Collection and Screening of Active Compounds

A comprehensive review of previous phytochemical investigations on *Brassica rapa* was conducted, resulting in the compilation of a list of 52 compounds isolated or identified. The literature search was carried out through the utilization of the Scopus and Web of Science-Clarivate databases, employing the keyword "*Brassica rapa*" and covering literature up until December 2022. The compounds were then converted to the Canonical SMILES format utilizing the PubChem database.

Drug-Likeness Prediction

Lipinski's Rule of Five (RO5) is a widely accepted criterion for assessing the suitability of a molecule as an oral drug in humans. The evaluation of drug-likeness is based on several parameters, including molecular weight, octanol-water partition coefficient (XLogP3), topological polar surface area, rotatable bond count, hydrogen bond acceptor count, and hydrogen bond donor count. To determine the drug-likeness of the phytoconstituent, the SMILES format was uploaded into the SwissADME server (<http://www.swissadme.ch>), a web-based platform that evaluates the pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of small molecules. Subsequently, a screening process was carried out using the default parameters⁹.

Screening for Potential Target Genes for *Brassica rapa* Active Constituents against Diabetes Mellitus

The prediction of targets for phytoconstituents was conducted using two databases: PharmMapper (<http://lilab.ecust.edu.cn/pharmmapper/>) and SwissTargetPrediction (<http://www.swisstargetprediction.ch/>). PharmMapper, a web-based platform, determines potential drug targets by comparing the query compound to its internal pharmacophore model database through a reverse pharmacophore process¹⁰. SwissTargetPrediction, a web server, predicts targets of bioactive small molecules based on a combination of 2D and 3D similarity measures with known ligands¹¹. The 3D molecular structure file of phytoconstituents was imported into PharmMapper, while its canonical SMILES was entered

into SwissTargetPrediction. The resulting candidate targets were then normalized through the UniProt database (<http://www.uniprot.org/>).

Screening of Targets for DM

The next phase in comprehending the molecular mechanism of herbs utilized to treat medical conditions involves the prediction of genes associated with diseases. In this context, the target genes for Diabetes Mellitus were obtained from the Human Gene Database (GeneCards, <https://www.genecards.org/>). GeneCards is a comprehensive database that integrates information on all annotated and predicted human genes from around 150 different databases. The target genes related to diabetes were retrieved by searching for keywords such as "diabetes" or "diabetes mellitus" (Yang et al., 2019). The score ranks diseases based on their proximity to the gene and takes into account the reliability of the sources, with a criteria score of greater than 5. This approach is commonly used in network pharmacology research and is applied for further screening of the target genes¹².

Acquisition of Intersection Target

The ingredient targets of *Brassica rapa* and the Diabetes mellitus -related targets were uploaded to Venny 2.1.0 (<https://bioinfo.p.cn.csc.es/tools/venny/>) for intersection to obtain the potential targets of *Brassica rapa* for the treatment of DM.

Protein-Protein Interaction

Protein-protein interaction (PPI) network analysis is an effective method for comprehending the molecular mechanisms of diseases and identifying novel drug targets. In this study, the researchers constructed a PPI network for the protein targets of *Brassica rapa* using the String database (<https://string-db.org/>), which is a widely utilized resource for searching and predicting protein interactions. The String database comprises a vast amount of experimental data, text mining results from PubMed abstracts and other databases, as well as bioinformatic predictions, making it a comprehensive and informative website for PPI analysis. The thickness of the lines in the network symbolizes the level of data support, or confidence. The minimum interaction score used in this analysis was medium confidence (0.400), which is also the default threshold¹³.

Network Analysis

After obtaining the PPI network from STRING, the researchers utilized the CytoHubba plugin of Cytoscape, a software for visualizing and analyzing complex networks, to analyze the core regulatory genes of the network and identify crucial targets. The CytoHubba plugin is specifically designed for hub analysis and identification of significant proteins in PPI networks. It applies various centrality measures to detect highly connected proteins, or "hubs," in the network, which are believed to play a vital role in regulating protein interactions. By recognizing these key targets, researchers can attain a deeper understanding of the molecular mechanisms of the disease and potentially discover new drug targets for further investigation¹⁴.

Gene Ontology Enrichment Analysis

The Gene Set Enrichment Analysis (GSEA) is a robust analytical tool utilized to interpret gene expression data obtained from genome-wide experiments. These enrichment analysis tools aid in linking prior knowledge with newly generated data and discovering the nature of genes in both health and disease conditions^{15, 16}. The Enrichr platform was utilized for the KEGG and GO analysis of targets predicted for

Diabetes mellitus (DM). Enrichr is a comprehensive web server for gene enrichment analysis and annotation, integrating a variety of databases such as KEGG, GEO, GO, PPI databases, ChEA 2016, OMIM, etc¹⁷. The following steps were taken to perform the enrichment analysis and gene annotation: Firstly, the list of target genes related to phytoconstituents of *Brassica rapa* was input into the Enrichr web server. Secondly, the signaling pathway information was selected based on clinical and pathological data.

Ligand preparation

The Pubchem database was utilized to obtain the compound structures. Avogadro, a free molecular editor, was used to optimize the geometric structures of the compounds using the GAFF force field and the steepest descent algorithm with 5000 steps per update. The energy-optimized phytochemicals were then used for docking¹⁸. Out of the 42 identified phytochemical compounds from *Brassica rapa*, only ten were selected for the docking study. The 3D structures of the compounds were prepared, minimizing energy, adding hydrogen atoms, and assigning charges as needed, using the UCSF Chimera structure build module. These structures were saved in the PDB format and optimized for docking using UCSF Chimera tools¹⁹.

Retrieval and Preparation of target protein

The Crystal Structure of AKT1 in Complex with Covalent-Allosteric AKT Inhibitor 27 (PDB ID: 6HHG) was retrieved from the RSCB PDB (<https://www.rcsb.org/>). It was solved using X-ray diffraction with a resolution of 2.30 Å²⁰. The initial PDB protein structure cannot be utilized for molecular docking as it lacks crucial information such as bond orders, formal atomic charges, and correct alignment of terminal amide groups.

The raw X-ray crystal structure of the 6HHG protein was purified by removing all the solvent molecules. The implicit hydrogen atoms were then added to the atoms to meet their appropriate valences. Unimportant ligands and ions were deleted from the protein structure. Subsequently, the structure was optimized by defining the bond orders, bond angles, and topology, and formal atomic charges were assigned to the amino acid residues. The energy minimization of the protein was performed using SPDBV 4.1, and the computation was carried out in a vacuum with the GROMOSS96 43B1 parameter set.

Active site analysis

The probable binding sites found in the three-dimensional structure of AKT1 was searched using the Computed Atlas of Surface Topography of Proteins (CASTp) server²¹. The website was used to identify the binding sites, active sites, surface structural pockets area, shape, and volume of every pocket and internal cavity of proteins. It also shows the number, boundary of mouth openings of each pocket, molecular reachable surface, and area of each pocket.

Docking

Molecular docking experiments were carried out using the AutoDock Vina tool, which is included in the UCSF Chimera software version 1.10.2, with the default parameters and a grid box (30.085 -36.412 -16.653)²². The View Dock tool was used to explore the predicted score values (19) (23). UCSF Chimera was used to check docking results, binding sites, and image processing. The 2D and 3D protein-ligand interactions were visualized in Discovery Studio Visualizer.

RESULTS

Screening of chemical compounds in *Brassica rapa* and selection for the potential active compounds

A comprehensive literature review was conducted, yielding a compilation of 52 compounds present in *Brassica rapa*, as depicted in Table 1 (Supplementary File 1). The predominant compounds identified were Glucosinolates, Isothiocyanates, Flavonoids, Sulfur Compounds, Indoles, Phenylpropanoids, Lignin, Diarylheptanoids, and Volatiles. The drug-like potential of the compounds was assessed using Lipinski's rule of five, with the relevant parameters provided in Table 2 (Supplementary File 2). Out of the 52 compounds that fulfilled the established criteria, their potential targets were explored via the Swiss Target Prediction databases, leading to the acquisition of information for 52 compound.

Target Identification and Analysis

The active constituents of *Brassica rapa* were subjected to target prediction utilizing the PharmMapper and Swiss Target Prediction databases. The merged target data revealed 1528 potential target genes of the 52 active constituents. Upon identifying the promising targets of the compounds, a comprehensive search was conducted on the GeneCards databases, resulting in the retrieval of 2803 genes associated with Diabetes mellitus (DM). A Venny tool was employed to predict the common targets of both DM and the compound-related genes, leading to the selection of 454 potential genes of *Brassica rapa* as key targets. Further information on these targets can be found in Table 3 (Supplementary File 3) and Figure 1.

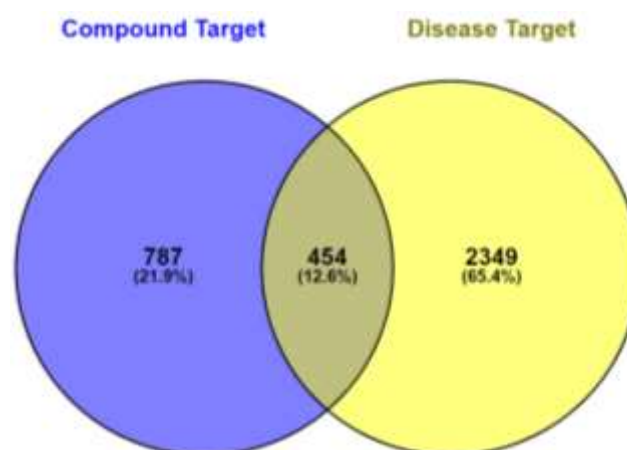


Figure 1: Common targets in Venn diagram.

Integration of Protein-Protein Interaction Network

The 454 potential genes of *Brassica rapa* were utilized in the construction of a protein-protein interaction (PPI) network using the STRING database. The PPI network, depicted in Figure 2, demonstrates the interrelationship among multiple targets in the context of disease development through the representation of nodes and their interactions. The network comprises 450 nodes and 7897 edges. A network analyzer tool was employed to analyze the PPI network of overlapping genes. According to the results of the CytoHubba calculation, ALB was found to be at the center of the network with the highest degree, followed by AKT1, TNF, GAPDH, MAPK3, EGFR, VEGFA, CTNNB1, and CASP3, which were designated as hub targets that may play a pivotal role in the advancement of Diabetes Mellitus. The degree values were represented by color changes from red to yellow in Figure 3 and Table 3, where higher degree values are indicated by more intense yellow hues.

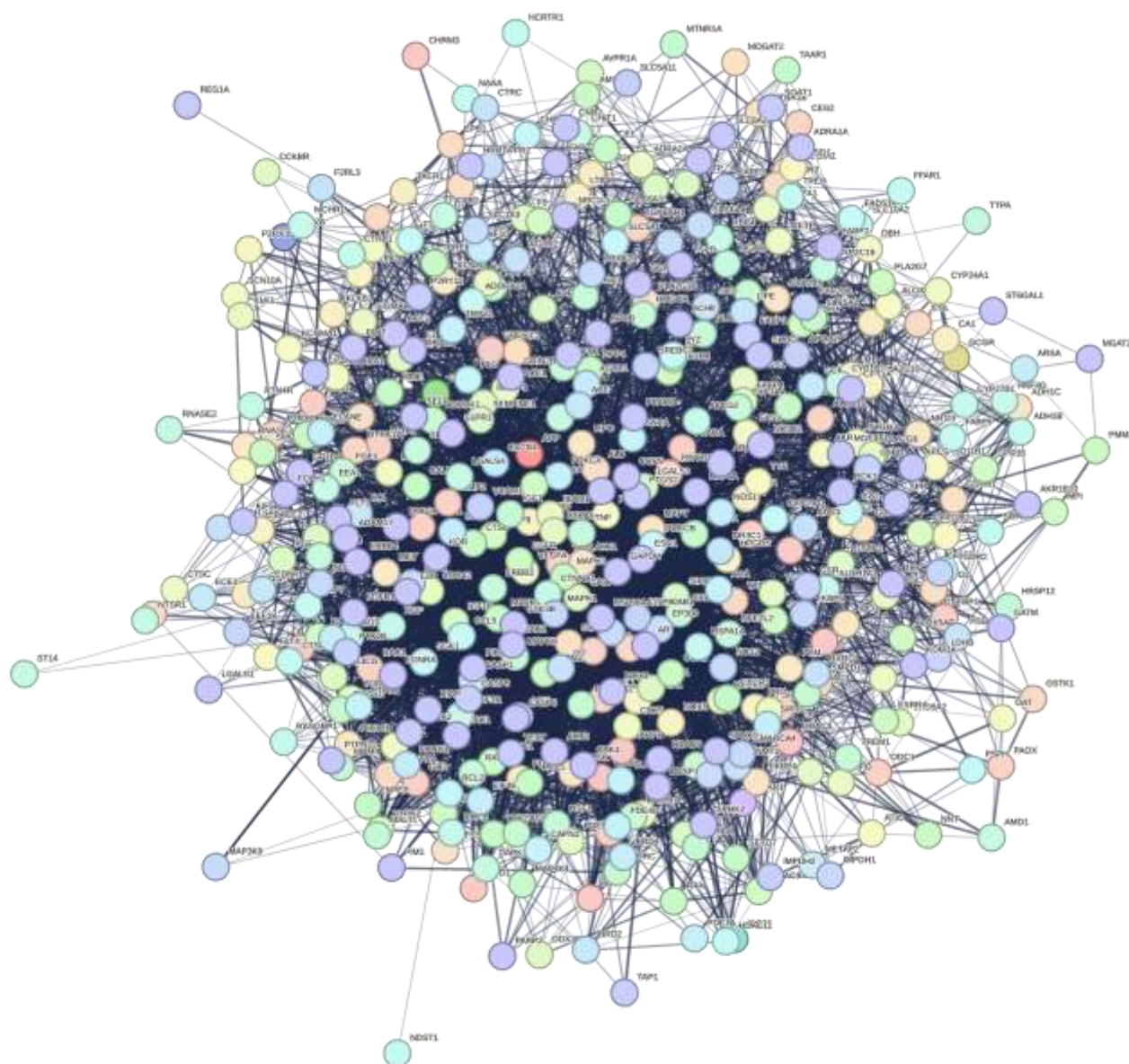


Figure 2: Protein-protein interaction (PPI) network and Hub gene analysis. (a) PPI network performed by STRING database. Contains 197 common DEGs. (<http://string-db.org/>; version 11.5).

Table 4: Active compounds of *Brassica rapa* related to hub targets.

Gene Symbol	Gene Name	Uniprot ID	Degree
ALB	Albumin	P02768	241
AKT1	RAC-alpha serine/threonine-protein kinase	P31749	232
TNF	Tumor necrosis factor ligand	Q9UNG2	230
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	P04406	217
MAPK3	Mitogen-activated protein kinase	L7RXH5	171
EGFR	Epidermal growth factor receptor	P00533	169
VEGFA	Vascular endothelial growth factor A	P15692	166
CTNNB1	Catenin beta-1	P35222	159
CASP3	Caspase-3	P42574	149
STAT3	Signal transducer and activator of transcription 3	P40763	147

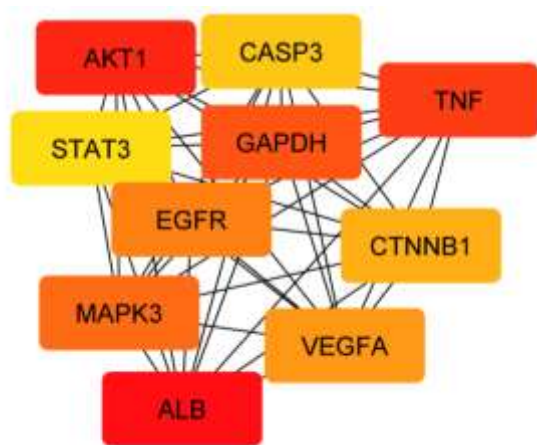


Figure 3: PPI network of the top 10 hub genes generated by (Cytoscape plugin cytoHubba; <https://apps.cytoscape.org/apps/cytohubba>; version 0.1) (the redder the colour, the more important it is).

GO and KEGG Pathway Enrichment Analyses

GO Enrichment Analysis

To clarify the multiple mechanisms of *Brassica rapa* on Diabetes mellitus from a systematic level, we performed an enrichment analysis for the biological process (BP), molecular function (MF), and cellular component (CC) of the retrieved protein targets of *Brassica rapa*. The significantly enriched BP terms were mainly involved in Positive Regulation Of Intracellular Signal Transduction (GO:1902533), Proteolysis (GO:0006508), Protein Phosphorylation (GO:0006468), Regulation Of Cell Population Proliferation (GO:0042127),

Positive Regulation Of Cellular Process (GO:0048522), Cellular Response To Cytokine Stimulus (GO:0071345), Positive Regulation Of MAPK Cascade (GO:0043410), Positive Regulation Of Cell Population Proliferation (GO:0008284), Regulation Of Apoptotic Process (GO:0042981) and Negative Regulation Of Apoptotic Process (GO:0043066) (Figure 4 and Table 5). The most frequently occurring protein targets were AKT1, PTPN6, GPER1, VEGFA, BCL2, RELA, SIRT1, TNF, KDR, EGFR, FLT4, IGF1R, IGF1, PTK2B, AKT2, ADAM10, FGFR1, FLT1, PDGFRA and CSF1R.

The significantly enriched MF terms of these targets are shown in (Figure 5 and Table 6). The results suggested that targets of *Brassica rapa* were strongly correlated with the molecular functions such as Endopeptidase Activity (GO:0004175), G-Protein-Coupled Receptor Activity (GO:0004930), Protein Homodimerization Activity (GO:0042803), Transition Metal Ion Binding (GO:0046914), Serine-Type Peptidase Activity (GO:0008236), Serine-Type Endopeptidase Activity (GO:0004252), Protein Serine/Threonine Kinase Activity (GO:0004674), Protein Tyrosine Kinase Activity (GO:0004713), Heme Binding (GO:0020037) and Histone Deacetylase Activity (GO:0004407). As shown in (Figure 6 and Table 7), the top ten cellular components were Integral Component Of Plasma Membrane (GO:0005887), Secretory Granule Lumen (GO:0034774), Intracellular Organelle Lumen (GO:0070013), Membrane Raft (GO:0045121), Vacuolar Lumen (GO:0005775), Neuron Projection (GO:0043005), Ficolin-1-Rich Granule Lumen (GO:1904813), Focal Adhesion (GO:0005925), Ficolin-1-Rich Granule (GO:0101002) and Cell-Substrate Junction (GO:0030055). These above mentioned observations are valued in improved understanding of the mechanism of DM.

Table 5: Gene Ontology Term, Biological Process, Direct (Top 10).

S.No	Name	p-value	Adjusted p-Value	Odd Ratio	Combined Score
1	Positive Regulation Of Intracellular Signal Transduction (GO:1902533)	2.551e-35	9.858e-32	7.73	615.41
2	Proteolysis (GO:0006508)	9.472e-30	1.830e-26	10.08	673.86
3	Protein Phosphorylation (GO:0006468)	1.168e-26	1.505e-23	6.67	398.55
4	Regulation Of Cell Population Proliferation (GO:0042127)	7.113e-26	6.873e-23	5.23	302.74
5	Positive Regulation Of Cellular Process (GO:0048522)	4.303e-25	3.326e-22	5.67	317.84
6	Cellular Response To Cytokine Stimulus (GO:0071345)	7.602e-25	4.897e-22	6.46	358.74
7	Positive Regulation Of MAPK Cascade (GO:0043410)	9.213e-25	5.087e-22	9.01	498.80
8	Positive Regulation Of Cell Population Proliferation (GO:0008284)	2.130e-24	1.029e-21	6.44	350.95
9	Regulation Of Apoptotic Process (GO:0042981)	8.929e-24	3.835e-21	5.03	266.74
10	Negative Regulation Of Apoptotic Process (GO:0043066)	4.186e-23	1.618e-20	6.13	315.76

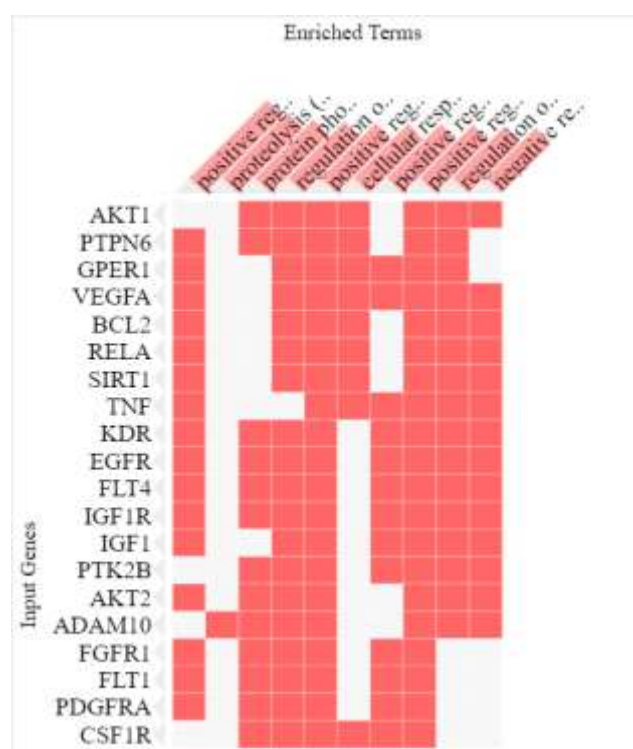
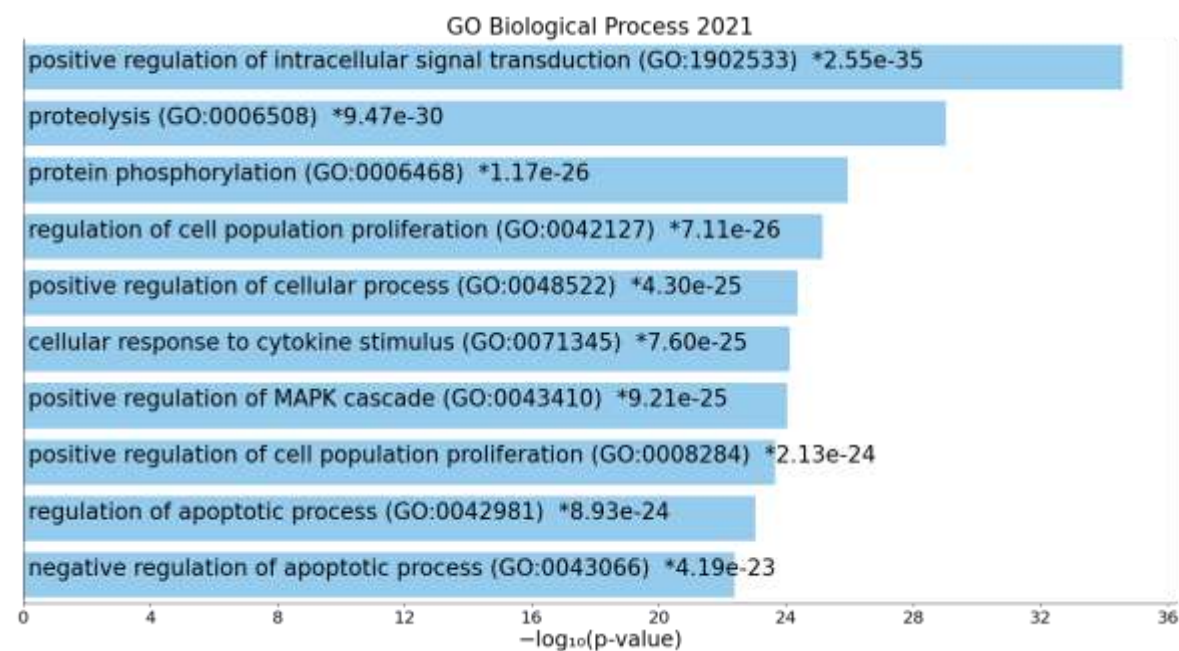
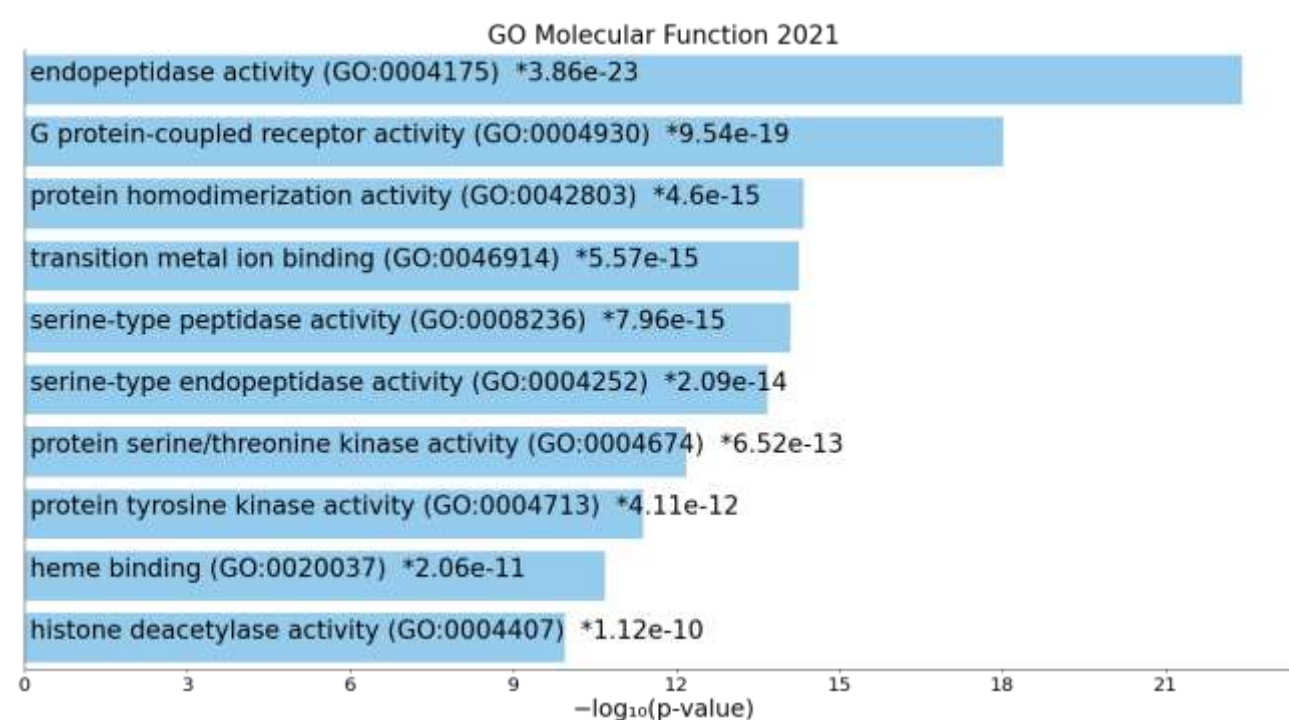


Figure 4: Functional enrichment analysis of the GO in terms of biological processes. (network, with combined scores in descending order. (A) Bar graph showing the pathways and their combined scores. (B) A clustergram of related genes.

Table 6: Gene ontology term, molecular function, direct (Top 10).

S.No	Name	p-value	Adjusted p-Value	Odd Ratio	Combined Score
1	Endopeptidase Activity (GO:0004175)	3.86E-23	2.52E-20	7.85	405.38
2	G Protein-Coupled Receptor Activity (GO:0004930)	9.54E-19	3.11E-16	7.2	298.6
3	Protein Homodimerization Activity (GO:0042803)	4.60E-15	9.08E-13	4.1	135.41
4	Transition Metal Ion Binding (GO:0046914)	5.57E-15	9.08E-13	4.84	158.93
5	Serine-Type Peptidase Activity (GO:0008236)	7.97E-15	1.04E-12	10.17	330.24
6	Serine-Type Endopeptidase Activity (GO:0004252)	2.09E-14	2.27E-12	11.24	353.96
7	Protein Serine/Threonine Kinase Activity (GO:0004674)	6.52E-13	6.08E-11	5.02	140.94
8	Protein Tyrosine Kinase Activity (GO:0004713)	4.11E-12	3.35E-10	9.55	250.34
9	Heme Binding (GO:0020037)	2.06E-11	1.49E-09	10.24	251.88
10	Histone Deacetylase Activity (GO:0004407)	1.12E-10	6.98E-09	39.51	905.41

**Figure 5:** Functional enrichment analysis of the GO in terms of molecular function. (network, with combined scores in descending order. (A) Bar graph showing the pathways and their combined scores.**Table 7: Gene ontology term, Cellular Component, direct (Top 10).**

S.No	Name	p-value	Adjusted p-Value	Odd Ratio	Combined Score
1	Integral Component Of Plasma Membrane (GO:0005887)	2.16E-28	5.44E-26	4.17	265.4
2	Secretory Granule Lumen (GO:0034774)	2.68E-16	3.38E-14	6.13	219.7
3	Intracellular Organelle Lumen (GO:0070013)	1.62E-14	1.36E-12	3.55	112.75
4	Membrane Raft (GO:0045121)	3.52E-13	2.22E-11	7.79	223.46
5	Vacuolar Lumen (GO:0005775)	1.12E-10	5.64E-09	6.72	154.04
6	Neuron Projection (GO:0043005)	4.89E-10	2.05E-08	3.46	74.16
7	Ficolin-1-Rich Granule Lumen (GO:1904813)	2.74E-09	9.87E-08	7.13	140.65
8	Focal Adhesion (GO:0005925)	5.49E-09	1.73E-07	3.8	72.34
9	Ficolin-1-Rich Granule (GO:0101002)	7.89E-09	1.94E-07	5.45	101.61
10	Cell-Substrate Junction (GO:0030055)	8.29E-09	1.94E-07	3.73	69.38

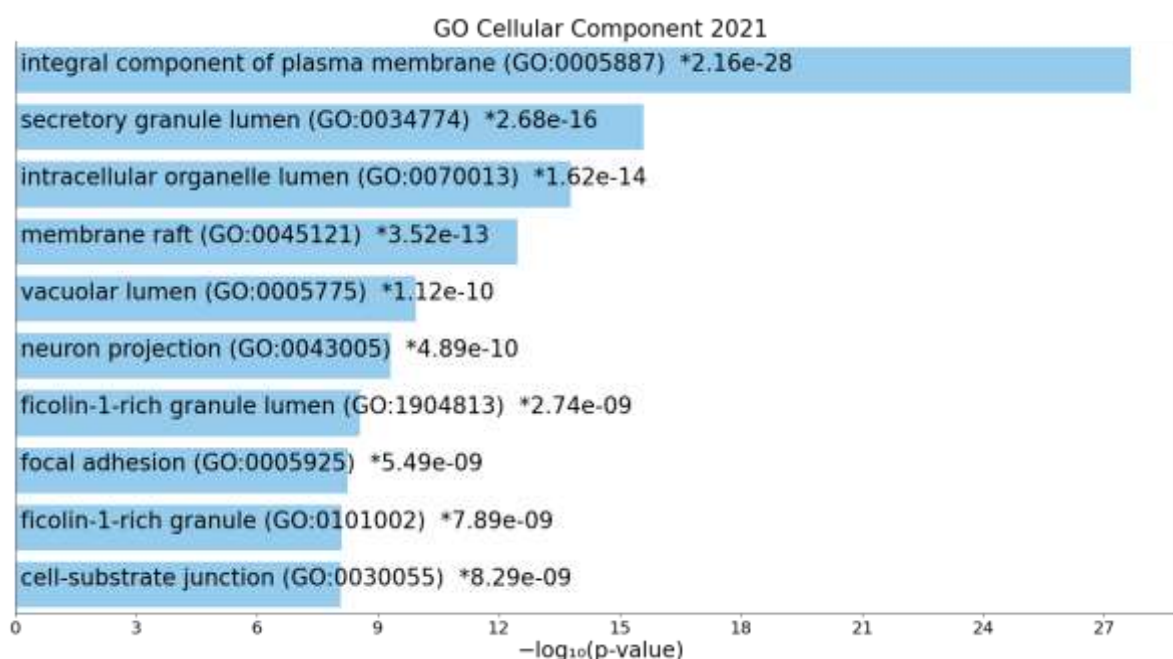


Figure 6: Functional enrichment analysis of the GO in terms of Cellular processes. (network, with combined scores in descending order. (A) Bar graph showing the pathways and their combined scores.

Pathway Enrichment Analysis

In terms of KEGG analysis, The top 10 significantly enriched pathways were screened out based on the threshold of p Value (Figure 7 and Table 8). The results indicated that these genes were mainly associated with the Pathways in cancer, AGE-RAGE signaling pathway in diabetic complications, Neuroactive ligand-receptor interaction, Lipid and atherosclerosis, Proteoglycans in cancer, Prostate cancer, HIF-1 signaling pathway, PI3K-Akt signaling pathway and Calcium signaling pathway. These results suggest that *Brassica rapa* may play a role in regulating several pathways that are involved in the development of Diabetes Mellitus. For example, the PI3K-Akt signaling pathway is a critical signaling pathway that is involved in insulin signaling, glucose uptake, and regulation of cell growth and survival. The enrichment of this

pathway in the targets of *Brassica rapa* suggests that it may have a positive effect on insulin signaling and glucose metabolism. The AGE-RAGE signaling pathway in diabetic complications is also involved in the development of diabetic complications and oxidative stress. The enrichment of this pathway in the targets of *Brassica rapa* suggests that it may have a protective effect against the development of diabetic complications. The Neuroactive ligand-receptor interaction pathway is involved in the regulation of neurotransmitter release and synaptic plasticity, and its enrichment in the targets of *Brassica rapa* suggests that it may have a positive effect on neuronal function. These observations provide a foundation for further investigation into the potential therapeutic effects of *Brassica rapa* in the treatment of Diabetes Mellitus.

Table 8: KEGG pathway enrichment (Top 10).

S.No	Name	p-value	Adjusted p-Value	Odd Ratio	Combined Score
1	Pathways in cancer	1.437e-49	4.167e-47	10.20	1147.00
2	AGE-RAGE signaling pathway in diabetic complications	8.586e-34	1.245e-31	26.22	1996.09
3	Neuroactive ligand-receptor interaction	7.445e-32	7.196e-30	9.51	681.57
4	Lipid and atherosclerosis	1.516e-31	1.099e-29	12.93	917.36
5	Proteoglycans in cancer	3.667e-29	2.127e-27	12.52	819.66
6	Prostate cancer	9.516e-29	4.600e-27	22.73	1466.37
7	HIF-1 signaling pathway	3.192e-28	1.322e-26	20.08	1271.36
8	PI3K-Akt signaling pathway	3.890e-28	1.410e-26	8.45	533.34
9	Calcium signaling pathway	3.123e-27	1.006e-25	10.59	646.61
10	Human cytomegalovirus infection	2.323e-26	6.736e-25	10.79	636.66

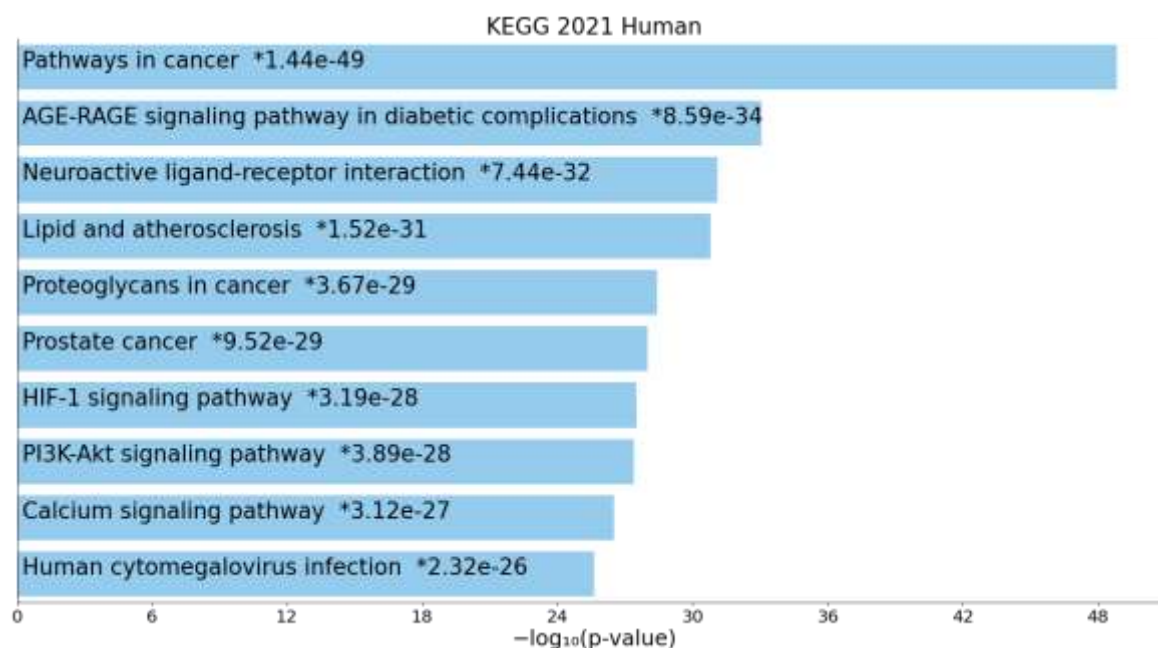


Figure 7: Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of 454 common targets of *Brassica rapa* and DM, (A) histogram of the top 10 KEGG pathways.

Molecular Docking

Molecular docking is an essential computational tool in the drug discovery domain. It is done to further select the potential compounds and study the bond formation in the protein-ligand complex at the binding site. Through systematic analysis of the PPI network, the gene AKT1 (Serine/ Threonine Protein Kinase) is selected for molecular docking. The crystal structure of target proteins (PDB ID: 6HHG) were retrieved from PDB. Structural refinement was completed by using the UCSF chimera tool. Energy minimization was completed at 1000 decent steps, while the non-standard residues were also removed from the receptors of the protein to avoid clashes and incorrect configurations.

Molecular docking was performed to screen out the putative targets of constituents with the ability to lower the risk of DM. Docking analysis successfully predicted the strong binding affinity between constituents and the binding pockets of target proteins. Docking scores, along with binding energy, were employed as key criteria for compound screening (Table 9). Clusters having a maximum absolute value of binding energy and highest conformation were selected.

Out of 52 phytoconstituents present in *Brassica rapa*, the Liquiritin, Licochalcone A, Liquiritigenin, 4,4'-Dihydroxy-3'-Methoxychalcone, Arvelxin, Syringin, Dihydroxyrangingin, Triandrin, Glucoberteroin, 5-Methylthiopentyl Isothiocyanate, Trans-6-Shogaol, 6-Paradol, Hexylacetate And Dihydroxybenzoquinone were docked against AKT1 protein to calculate binding energy and inhibitory constant (K_i) values. The docking results showed that all the phytochemicals expressed different behaviour while making interactions with the protein.

Liquiritin from *Brassica rapa* showed binding affinity of -6.1 kcal/mol and interacted with Asn 31 and Val 7 residues of AKT1 protein. Asn 31 forming Pi-sigma with bond distance of 2.84 and Val 7 interacted with Pi-alkyl with bond distance of 3.73 respectively.

The molecular interaction analysis of AKT1 protein with Liquiritigenin utilizes the least energy of -5.5 kcal/mol. The

AKT1- Liquiritigenin conformation depicts 2 pi-alkyl bond with Val 106 and Val 7 aminocids residues. AKT1 protein docking analysis with Licochalcone showed Four alkyl bonds with Val 4; two conventional bond with Leu 110; and one pi-alkyl bond with Val 106; One Carbon bond with Lys 112; and amide pi stacked with Thr 105 [Figure 8 (A)-(J)] with a binding energy value of -5.6 kcal/mol.

The docking study of AKT1 protein with 4,4'-Dihydroxy-3'-Methoxychalcone has utilized a energy value of -5.3 kcal/mol and four conventional bond with Ser 56, Asn 31 and Asn 53; and one Pi-alkyl bond with Pro 51; and one alkyl bond with Ala 58 aminoacid residues.

AKT1 protein with Syringin a binding energy of -5.0 kcal/mol and showed seven conventional bonds with ile 6, Asn 31, Lys 112 and Gly 109 ;and two carbon bond interactions with Asp 3; and one pi -alkyl bond with Val ;and one Alkyl bond with Ala 5 aminoacid residues.

The AKT1 protein - 6-Paradol complex formation utilized a energy value of -4.4 kcal/mol and results in two alkyl bond with Val 106 and Ala 5 ; and two conventional bond with Ile 6 and Asn 31 aminoacid residues.

AKT1 protein with Dihydroxyrangingin utilizes a binding energy of -4.9 kcal/mol and showed five conventional bond interactions with Gly 109, Asn 31, Ile 6, Val7; and one alkyl bond interactions with Val7; and one alkyl bond with Ala 5 aminoacid residues.

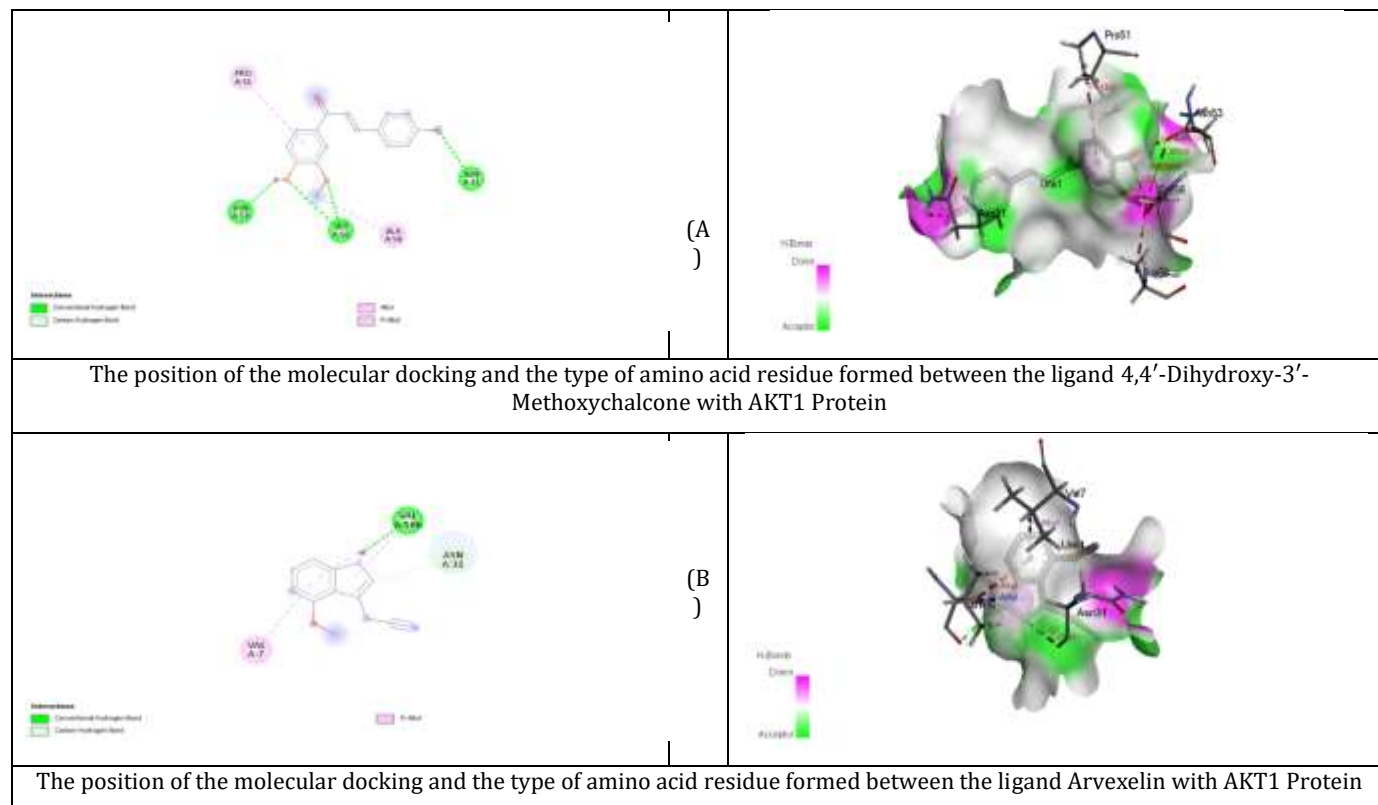
Arvelxin showed binding affinity of -4.5 kcal/mol and displays three Pi-Alkyl interactions with Val 7 and Val 106; and one conventional bond interactions with Val 7 aminoacid residues. Glucoberteroin shows three alkyl interactions with Val 7; and two conventional bond interactions with Lys 112 and Gly 109; and two carbon bond interactions with Gly 109 and Asp 3 aminoacid residues with binding energy -4.7 kcal/mol. Hexylacetate docked with AKT1 protein with binding affinity of -3.5 kcal/mol and possess three alkyl interactions with Val 7 and Val 106; and one conventional bond with Asn 31 amino acid.

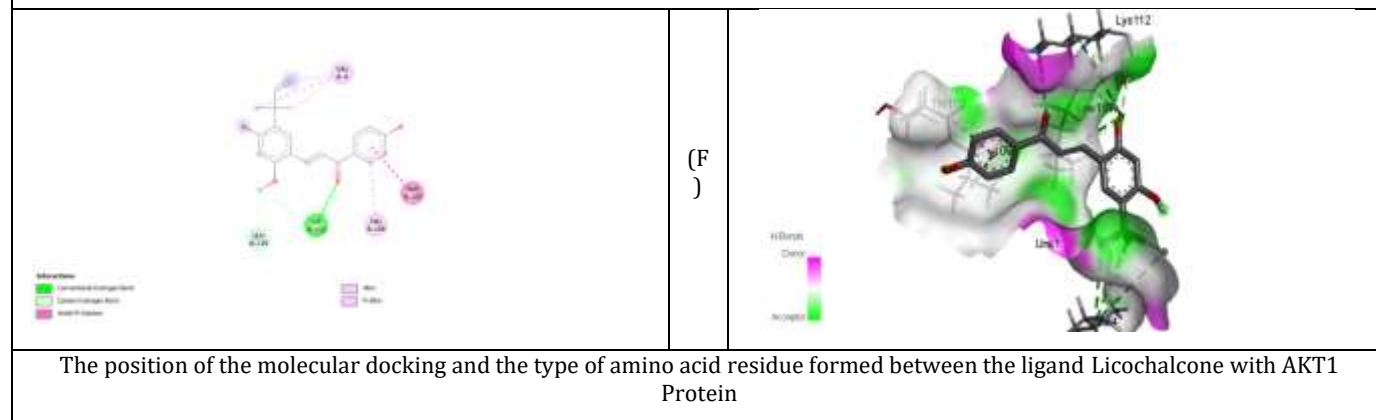
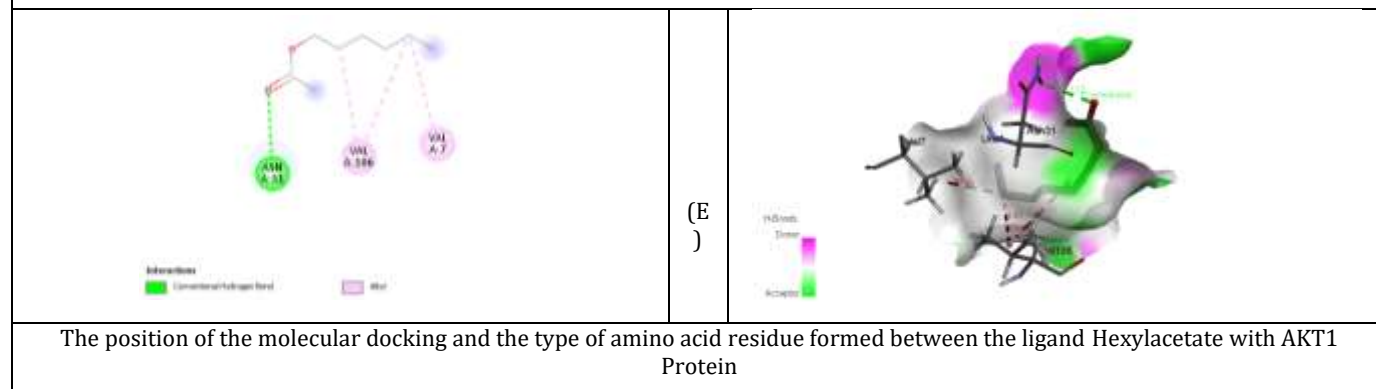
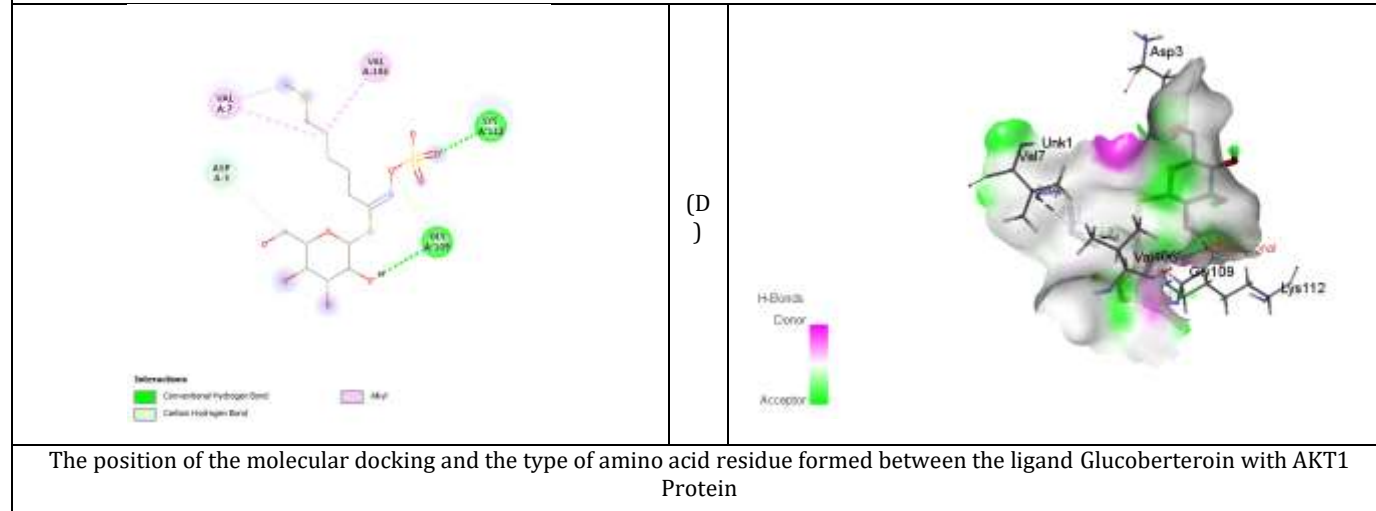
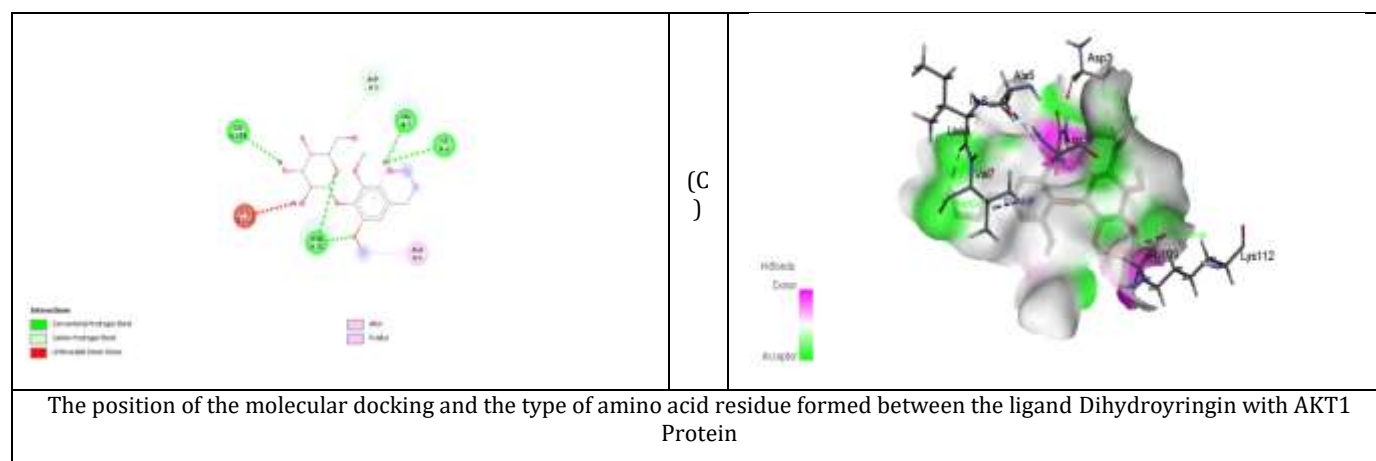
Table 9: The docking score of the bioactive constituents of *Brassica rapa* bound to the AKT1 Protein.

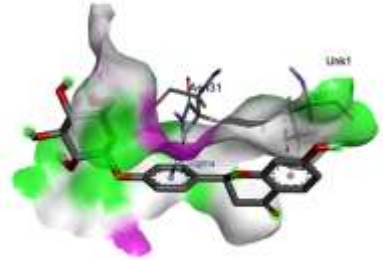
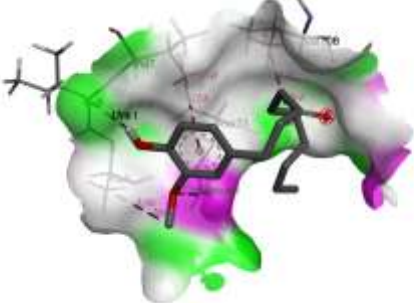
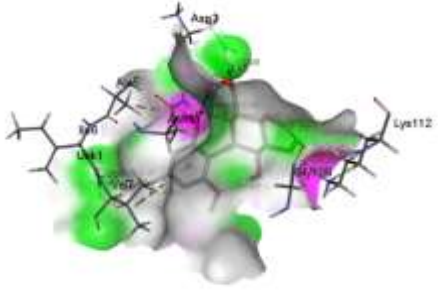
S.No	Phytochemical	Binding Affinity (Kcal/Mole)	Amino acid present in Binding Site	Type of bond	Bond Distance
1	4,4'-Dihydroxy-3'-Methoxychalcone	-5.3	Ala 58	Alkyl	3.75
			Ser 56	Conventional	2.22
				Conventional	2.99
			Asn 31	Conventional	2.71
			Asn 53	Conventional	1.93
				Carbon	3.64
			Pro 51	Pi - Alkyl	5.43
2	Arvexelin	-4.5	Val 7	Pi - Alkyl	4.82
			Val 106	Conventional	2.36
				Pi - Alkyl	4.88
				Pi - Alkyl	5.11
			Asn 31	Carbon	3.4
3	Dihydroxyringin	-4.9	Gly 109	Conventional	2.81
			Lys 112	Unfavorable Donor Donor	2.36
			Asn 31	Conventional	2.47
				Conventional	2.13
			Ala 5	Alkyl	4.2
			Ile 6	Conventional	2.59
			Val7	Conventional	2.12
				Pi Alkyl	4.86
			Asp3	Carbon Hydrogen Bond	3.44
4	Glucoberberoin	-4.7	Val7	Alkyl	3.74
				Alkyl	4.33
			Val 106	Alkyl	5.22
			Lys 112	Conventional	2.12
			Gly 109	Conventional	1.98
				Carbon	2.66
			Asp 3	Carbon	3.45
5	Hexylacetate	-3.5	Val 7	Alkyl	3.9
			Val 106	Alkyl	4.69
				Alkyl	4.47
			Asn 31	Conventional	2.72
6	Licochalcone	-5.6	Val 4	Alkyl	5.49
				Alkyl	4.89
				Alkyl	4.18
				Alkyl	4.97
			Leu 110	Conventional	3.63
				Conventional	3.55
			Lys 112	Carbon	2.34

			Val 106	Pi Alkyl	4.87
			Thr 105	Amide Pi Stacked	4.42
7	Liquiritigenin	-5.5	Val 106	Pi Alkyl	5.23
			Val 7	Pi Alkyl	3.79
8	Liquiritin	-6.1	Asn 31	Pi Sigma	2.84
			Val 7	Pi Alkyl	3.73
9	6-Paradol	-4.4	Val 106	Alkyl	4.48
			Val7	Pi Alkyl	4.53
			Ala 5	Alkyl	3.9
			Ile 6	Conventional	2.44
			Asn 31	Conventional	2.57
10	Syringin	-5.0	ILE 6	Conventional	2.27
			Val 7	Pi -Alkyl	5.01
			Ala 5	Alkyl	3.89
			Asn 31	Conventional	2.52
				Conventional	2.44
				Conventional	2.33
			Asp 3	Carbon	3.57
				Carbon	3.45
			Lys 112	Conventional	2.22
			Gly 109	Conventional	2.42
				Conventional	2.13

Figure 8: Molecular docking of active compounds and AKT1 Protein





 Interactions: VAL A.183 VAL A.7	(G)	 H-Bonds: Donor Acceptor
The position of the molecular docking and the type of amino acid residue formed between the Liquiritigenin ligand with AKT1 Protein		
 Interactions: VAL A.7 ASP A.11	(F)	 H-Bonds: Donor Acceptor
The position of the molecular docking and the type of amino acid residue formed between the ligand Liquiritin with AKT1 Protein		
 Interactions: VAL A.199 VAL A.7 ASP A.11	(I)	 H-Bonds: Donor Acceptor
The position of the molecular docking and the type of amino acid residue formed between the ligand 6-Paradol with AKT1 Protein		
 Interactions: ASP A.11 VAL A.7 VAL A.183	(J)	 H-Bonds: Donor Acceptor
The position of the molecular docking and the type of amino acid residue formed between the ligand Syringin with AKT1 Protein		

DISCUSSION

This literature review focused on identifying the active compounds in *Brassica rapa* and their potential targets for the treatment of Diabetes Mellitus (DM). A total of 52 compounds were identified and subjected to target prediction using the PharmMapper and Swiss Target Prediction databases. The results showed that the active constituents of *Brassica rapa* had the potential to interact with 1528 potential target genes. After further analysis, 454 potential genes of *Brassica rapa* were selected as key targets for DM.

The 454 potential genes were then used to construct a protein-protein interaction (PPI) network using the STRING database. The PPI network demonstrated the interrelationships among the targets in the context of DM development. The network analysis tool revealed that ALB, AKT1, TNF, GAPDH, MAPK3, EGFR, VEGFA, CTNNB1, and CASP3 were the hub targets that could play a pivotal role in the advancement of DM.

To understand the mechanisms of *Brassica rapa* on DM from a systematic level, an enrichment analysis was performed for the biological process (BP), molecular function (MF), and cellular component (CC) of the protein targets of *Brassica rapa*. The results showed that the BP terms were mainly involved in Positive Regulation of Intracellular Signal Transduction, Proteolysis, Protein Phosphorylation, Regulation of Cell Population Proliferation, Positive Regulation of Cellular Process, and Cellular Response to Cytokine Stimulus. The most frequently occurring protein targets were AKT1, PTPN6, GPER1, VEGFA, BCL2, RELA, SIRT1, TNF, KDR, EGFR, FLT4, IGF1R, IGF1, PTK2B, AKT2, ADAM10, FGFR1, FLT1, PDGFRA, and CSF1R.

The significantly enriched MF terms indicated that the targets of *Brassica rapa* were strongly correlated with Endopeptidase Activity, G Protein-Coupled Receptor Activity, Protein Homodimerization Activity, Transition Metal Ion Binding, Serine-Type Peptidase Activity, Serine-Type Endopeptidase Activity, Protein Serine/Threonine Kinase Activity, Protein Tyrosine Kinase Activity, Heme Binding, and Histone Deacetylase Activity. The top five cellular components were Integral Component of Plasma Membrane, Secretory Granule Lumen, Intracellular Organelle Lumen, Membrane Raft, and Vacuolar Lumen.

The KEGG analysis revealed that the targets of *Brassica rapa* were significantly enriched for pathways such as Pathways in Cancer, AGE-RAGE signaling pathway in diabetic complications, Neuroactive ligand-receptor interaction, Lipid and Atherosclerosis, Proteoglycans in Cancer, Prostate Cancer, HIF-1 signaling pathway, PI3K-Akt signaling pathway, and Calcium Signaling Pathway. These results suggest that *Brassica rapa* may play a role in regulating several pathways involved in the development of Diabetes Mellitus, including the PI3K-Akt signaling pathway, which is critical for insulin signaling, glucose uptake, and regulation of cell growth and survival.

The AKT1 (also known as protein kinase B) protein plays a crucial role in the development of diabetes. This protein is involved in several signaling pathways that regulate glucose metabolism, insulin signaling, and cell survival. In individuals with diabetes, the insulin signaling pathway is disrupted, leading to high blood glucose levels and insulin resistance. AKT1 activation promotes glucose uptake by cells and inhibits insulin-stimulated gluconeogenesis.

Studies have shown that the activation of AKT1 is crucial in maintaining glucose homeostasis and that its loss or reduction leads to insulin resistance and glucose intolerance. The overactivation of AKT1, on the other hand, has been associated with the development of type 2 diabetes. This suggests that

the regulation of AKT1 activity is crucial in preventing and treating diabetes. In conclusion, AKT1 plays a significant role in the development of diabetes and its regulation may have therapeutic potential in the treatment of this disease.

The results of a molecular docking study performed on a set of phytochemicals found in *Brassica rapa* and their potential to interact with the AKT1 protein. The study utilized a crystal structure of AKT1 protein (PDB ID: 6HHG) and screened out the phytochemicals for their binding affinity with the protein. The study found that Liquiritin, Licochalcone A, Liquiritigenin, 4,4'-Dihydroxy-3'-Methoxychalcone, Arvelxin, Syringin, Dihydrosyringin, Triandrin, Glucoberteroin, 5-Methylthiopentyl Isothiocyanate, Trans-6-Shogaol, 6-Paradol, Hexylacetate, and Dihydroxybenzoquinone had different binding affinities with the AKT1 protein, as indicated by the binding energy and inhibitory constant (Ki) values.

The study found that Liquiritin had a binding affinity of -6.1 kcal/mol and interacted with Asn 31 and Val 7 residues of the AKT1 protein. Liquiritigenin had the lowest energy utilization of -5.5 kcal/mol, while Licochalcone A had a binding energy value of -5.6 kcal/mol. The study also found that Syringin had a binding energy of -5.0 kcal/mol, 6-Paradol had a binding energy of -4.4 kcal/mol, Dihydrosyringin had a binding energy of -4.9 kcal/mol, Arvelxin had a binding affinity of -4.5 kcal/mol, and Glucoberteroin had a binding energy of -4.7 kcal/mol. Hexylacetate had a binding affinity of -3.5 kcal/mol.

Overall, the molecular docking study provides valuable insights into the potential of these phytochemicals to interact with the AKT1 protein and suggests further research is needed to explore the relationship between these interactions and the risk of diabetes mellitus.

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