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Integrative Approach to Identify Pyrrolidine-Based Phytocompounds as Potential Hypoglycemic Lead Through Network Pharmacology, Molecular Docking, and Dynamics Simulation.

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ABSTRACT

The present study aims to identify the hypoglycemic mechanism pathway of the bioactive molecule of *Fagopyrum esculentum* network pharmacology, molecular docking, and dynamic simulation. The potential bioactives of Fagopyrum esculentum were procured from IMPPAT 2.0. The genes were identified using the superPRED program, and the genes associated with glycation conditions were obtained from the Gene Card database. Further, the common gene was recognized from the Venny-2.0 tool. The STRING database created the PPI network of common genes. Further, the relationship of genes with different physiological pathways was described with the help of the KEGG enrichment pathway and Gene Ontology study. After finding the potential genes from network pharmacology, they were correlated with the protein they expressed. This PDB ID was used for molecular docking with selected potential bioactives using Molegro Virtual Docker version 6.0 software. The iMODS server was used for dynamic simulation of the docked complex. The findings of present network pharmacology strategy contributed to identifying the pyrrolidine (Pyrosaccharopine) and polyhydroxy compounds from *Fagopyrum esculentum* as major structural features of ligands for managing glycemic complications.

Keywords: Network pharmacology, Molecular docking, Advanced glycation end products, Pyrrolidine, Polyhydroxy.

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INTRODUCTION

The Advanced glycation end products (AGEs) are developed by the non-enzymatic process of reducing sugar with N-terminal amino (phospholipids, amino acids, nucleic acids, and proteins) and lysine side chains also known as glycation [1]. Different degenerative diseases may result from protein glycation conjugate [2]. This conjugation alters the lipoprotein and glycoprotein intermediate, causing some significant anomalies in functional bioactive i.e. nucleic acid, protein, and lipids. The AGEs are also responsible for the development of late diabetes complications, renal failure, Alzheimer's disease, retinopathy, and atherosclerosis [3-5]. Oxidative stress and hyperglycemia enhance the production of AGEs; however, different synthetic and herbal antioxidants and antiglycation agents may prevent this event.

The *Fagopyrum esculentum*, also named "Buckwheat", is one of the earliest herbs in the Polygonaceous family. Buckwheat is considered a pseudocereal with a high nutritional profile because of its balanced amino acid composition, flavonoids, D-chiro-inositol, soluble carbohydrates, high levels of vitamin B1 and B2, fagopyritols, lysine, and thiamin-binding proteins. [6]. Antioxidant components such as flavonoids, tocopherols, phenolic acids, inositol phosphates, reduced glutathione, and melatonin are also abundant in *Fagopyrum esculentum*. [7-8]. Compared to most vegetables, fruits, and grain crops, it comprises more rutin (quercetin-3-arabinoside), potent flavanol glucoside having anti-inflammatory, anti-carcinogenic, antioxidant, and antiglycative properties [9-12]. The above promising activity due to different phytochemicals found in another study of *Fagopyrum esculentum,* pioneer the path for exploring its broad therapeutic value. In the same sequence of exploration of biological profiling, novel computational approaches such as network pharmacology, molecular docking, and ADME study may play a significant role with more accuracy.

Network pharmacology is an innovative, cost-efficient, and promising approach to drug development. It constructs a biological network using bioinformatics and systems biology. A "network-target, multiple-components" approach should be employed as a substitute for the existing "one-target, one-drug mode," as network pharmacology can offer an extensive understanding of the core concepts of systems biology. It has been considered the next drug development paradigm [13-14]. The present work includes a network pharmacology and molecular docking study to investigate the antiglycation effects of Fagopyrum esculentum. Our results reveal potential mechanisms that may provide a therapeutic effect in glycation. Figure 1 depicts the detailed workflow of this study.

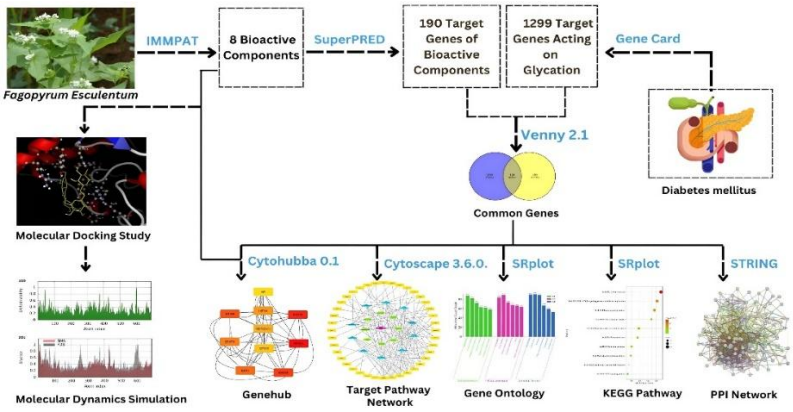

Figure 1. Network pharmacology workflow to identify *Fagopyrum esculentum* targets in the glycemic disorder.

MATERIALS AND METHODS

Screening and target prediction of bioactive compounds

Information on active constituents of *Fagopyrum esculentum* was retrieved from different databases like the Indian Medicinal Plants, Phytochemistry, and Therapeutics 2.0 (IMPPAT 2.0) [15]. The ADME (Absorption, Distribution, Metabolism, and Excretion) parameters were employed to determine the potential bioactive component in *Fagopyrum esculentum* as per the IMPPAT suggested. According to this, the active ingredients with drug-likeness (DL) ≥ 0.18% were biologically appropriate for drug development.

In comparison, bioactive compounds with oral bioavailability (OB) ≥ 30% have good absorption and significant metabolism after oral administration [16-17]. The SuperPRED 3.0 database was used to predict targets using the Simplified Molecular Input Line Entry System (SMILES) of selected bioactive compounds [18]. The Uniport Protein Database (https://www.uniport.org/) was used to standardize the targets [19].

Disease targets gene finding

The Gene Cards Database (https://www.genechttp://ards.org/) and the OMIM Database (http://www.onim.org/) are recognized as disease-target databases that provide a variety of genetic information via online retrieval [20]. Using "glycation" as the keyword, the above database generated the action targets associated with *Fagopyrum esculentum*.

Screening of common targets of disease and bioactive compounds

The genes involved in "Glycation" diseases and the preferred genes for which the selected bioactive components have affinity were mapped using the online Venny 2.1 tool (http://bioinfogp.cnb.csic.es/tools/venny/index.html). This process results from the intersection targets (common gene hub) [21].

Protein-protein interaction (PPI) network construction

From the previous step 139 intersect target genes were found and imported into the STRING database (https://string-db.org/) [22]. The Homosapies species were selected to construct a protein interaction network. After continuation, the result was saved in “TVS” format, which was then imported into Cytoscape v3.6.0 software for visual analysis and screening of the core target [23]. The customization of node's color and size was done according to the degree value, and the edge's thickness according to the combination score.

Gene Ontology (GO) functional annotation and KEGG pathway analysis

The Gene Ontology (GO) database was created by the Gene Ontology Federation. The GO database is divided into three categories: molecular function (MF), biological process (BP), and cellular components (CC) which characterize the cellular environment where the gene product is found. Different input data like; the top 10 target pathways, P value, enrichment score, and count genes, were retrieved from the STRING database. These input data were imported into the SRplot tool to generate the Kyoto Encyclopedia Gene and Genomes (KEGG) enrichment bubble chart and GO functional annotation [24-25].

Molecular Docking Study

Ligand Preparation

The 2D structure of the potential bioactive compounds was obtained from Chem Draw 22.2.0 64-bit software, and Chem Draw 3D 22.2.0 64-bit software was used to convert the 2D to 3D structure. The MM2 force field approach in the same software was used for energy minimization of the ligands. These steps are crucial before ligand import for the docking process. The algorithm used in MVD Version 6.0 software performs hydrogen atom insertion and the required valency evaluations [26].

Protein Preparation

The RCSB Protein Data Bank was used to obtain the 3D crystal structure of the protein targets, It can be accessed directly at the following URL (https://www.rcsb.org/pdb). The proteins with PDB ID: 3DO7, 6NJS, and 1I09 were used for the docking study [27]. Co-factors and water molecules appeared explicitly excluded from a PDB after loading it into the MVD Version 6.0 program. Furthermore, the allocation of polar hydrogen and missing charges was executed using the Molegro algorithm.

Cavities detection

The Molegro Virtual Docker version 6.0 software has an integrated cavity discovery algorithm that automatically identifies potential binding cavities, within the 30×30×30 Å3 cube, it is employed in ligand

binding. The algorithm concentrates on pursuing a specific area or volume during the imitation phase that follows the cavity identification technique. Five distinct binding sites on protein targets PDB ID: 3DO7, 6NJS, and 1I09 were identified. The greatest volume and surface area were considered for the ligand binding procedure shown in Table 1. (Figure 2) [28].

Table 1. Coordinates values of the active site in a target protein.

Protein Target	PDB ID	Volume Å3	Surface Area Å2	X-Center	Y-Center	Z-Center
NFKB1	3DO7	319.488	1241.6	19.89	51.28	78.70
STAT3	6NJS	95.232	302.08	-4.48	17.39	25.75
GSK3β	1I09	642.56	1873.93	28.79	44.88	65.84

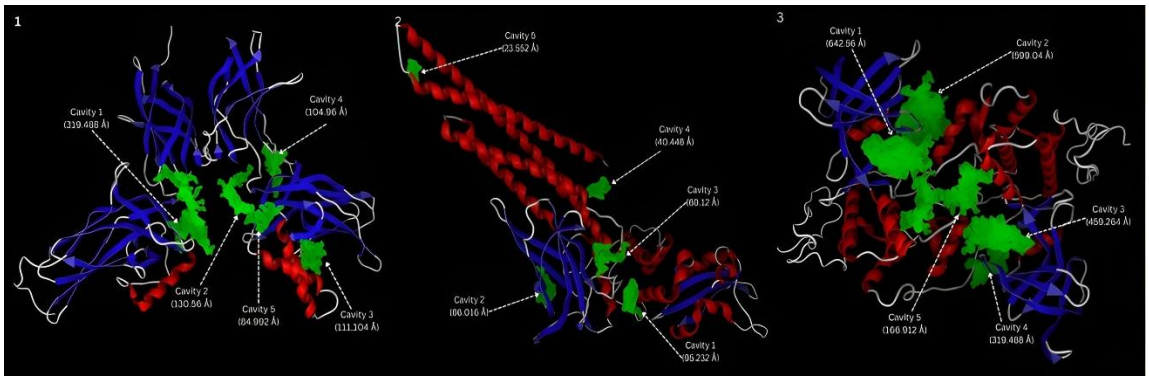

Figure 2. Detected cavities in protein target (1) 3DO7, (2) 6NJS, and (3) 1I09, (Å represented the volume of the cavity, cartoon model of target protein represented in blue and red color and cavities represented in green color).

Molecular docking study

The Molegro Virtual Docker (MVD) version 6.0 software was employed for the docking simulation study. The MolDock scoring was performed at 0.30 Å grid resolution and MolDock SE was used as the search algorithm. A restricted sphere with a radius of 15.0 Å, centered at coordinates values (X, Y, and Z) encircled this active site shown in Table 1. The number of runs was ten times associated with each ligand with 1500 iterations and a population size of 50 to cover the whole surface of the detected cavities binding site. Among the different scoring functions, MolDock score hydrogen bond, and interaction were employed for finding the optimal pose [28]. PyMol 2.4.0 software was used for the visualization [29].

Molecular Dynamics Simulations

The protein stability and mobility of the docked ligand-protein complex were evaluated using the iMODS server (http://imods.chaconlab.org). The MDS was accomplished in Normal Mode Analysis (NMA) to generate the internal coordinates of the target protein. This server was used to evaluate stability and provide a range of results, including B-factors, deformability, eigenvalues, variance maps, co-variance, elastic networks in the atoms, and residue indexes in magnitude and direction. The results were obtained according to the default preset parameters after uploading the docked PDB files as input data to the iMODS server [30-31].

RESULTS

Screening of potential bioactive compounds

In the IMPPAT database, "*Fagopyrum esculentum*" was used as a keyword to search bioactive compounds, and 42 compounds were obtained. A total of 8 potential effective compounds were selected based on drug-likeness parameter (DL) and oral bioavailability (OB) (Table 2).

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Table 2. The potential bioactive compounds of Fagopyrum *esculentum* plant.

S.N.	Bioactive Compounds	Class	CF	MW (g/mol)	OB %	DL%
1.	3,4-dihydroxy-2-piperidinomethanol	Alkaloids	$C_7H_{15}NO_3$	161.20	32.55	0.37
2.	Cianidanol	Flavonoids	$C_{15}H_{14}O_6$	290.27	34.54	0.21
3.	Fagomine	Alkaloids	$C_6H_{13}NO_3$	147.09	31.46	0.25
4.	Cyanidin	Flavonoids	$C_{15}H_{11}O_6$	287.25	32.87	0.31
5.	Quercetin	Flavonoids	$C_{15}H_{10}O_7$	302.24	34.17	0.28
6.	Rutin	Flavonoids	$C_{27}H_{30}O_{16}$	610.52	31.95	0.14
7.	Pyrosaccharopine	Amino acids	$C_{11}H_{18}N_2O_5$	258.12	33.12	0.13
8.	Cyanidin-3-O-rutinoside	Flavonoids	$C_{27}H_{32}O_{16}$	258.27	36.50	0.19

CF: Chemical Formula, MW: Molecular weight, OB: Oral bioavailability, DL: Drug-likeness

Table 3. Result of KEGG pathway enrichment.

Pathway Code	Pathway	Enrichment	P-value	Matching genes in the network
hsa04931	Insulin resistance	4.80726	3.04E-19	NFKB1, PIK3CA, STAT3, NOS3, GSK3B, PRKCD, ACACB, PDPK1, PRKAA1, MTOR, PTPN1, PIK3CD, PRKCZ, RPS6KA3, CPT1B, SLC2A1, PIK3R1, RPS6KA1, PRKCB, PIK3CB
hsa04933	AGE-RAGE signaling pathway in diabetic complications	4.56926	5.04E-15	MAPK1, SERPINE1, NFKB1, CDK4, PIK3CA, STAT3, NOS3, PRKCD, STAT1, NOX1, PIK3CD, PRKCZ, PRKCA, PIK3R1, PRKCB, PIK3CB
hsa04910	Insulin signaling pathway	4.41462	2.13E-13	MAPK1, GCK, PIK3CA, PDE3B, PRKACA, GSK3B, ACACB, PDPK1, PRKAA1, MTOR, PTPN1, PIK3CD, PRKCZ, PIK3R1, ACACA, PIK3CB
hs05418	Fluid shear stress and 0atherosclerosis	3.45418	2.44E-11	PLAT, NFKB1, PIK3CA, NOS3, HSP90AA1, PRKAA1, CHUK, NOX1, PIK3CD, PRKCZ, NFE2L2, GSTP1, PIK3R1, PIK3CB
hsa05212	Pancreatic cancer	3.36811	1.53E-10	MAPK1, NFKB1, CDK4, PIK3CA, STAT3, STAT1, MTOR, CHUK, PIK3CD, PIK3R1, PIK3CB
hsa04930	Type II diabetes mellitus	4.2121	1.16E-09	MAPK1, GCK, PIK3CA, PRKCD, MTOR, PIK3CD, PRKCZ, PIK3R1, PIK3CB
hsa01522	Endocrine resistance	3.01004	1.56E-09	MAPK1, CDK4, PIK3CA, PRKACA, CARM1, ESR2, MTOR, PIK3CD, ESR1, PIK3R1, PIK3CB
hsa04922	Glucagon signaling pathway	2.95703	2.96E-07	GCK, EP300, PDE3B, PRKACA, ACACB, PRKAA1, CPT1B, SLC2A1, ACACA
hsa04922	Carbohydrate digestion and absorption	2.74132	4.54E-06	PIK3CA, SLC5A1, PIK3CD, PIK3R1, PRKCB, PIK3CB
hsa04668	TNF signaling pathway	2.67522	6.57E-06	MAPK1, NFKB1, PIK3CA, PTGS2, CHUK, PIK3CD, PIK3R1, PIK3CB

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Screening of Gene Targets

The OMIM and Gene Card databases were used to screen reported glycation-related genes with the keyword "glycation." After duplicates were eliminated, 1299 genes were identified. A Venn diagram was constructed to anticipate the common target genes for both the glycation and bioactive components. A total of 139 potential anti-glycation genes for *Fagopyrum esculentum* were identified and considered key targets (**Figure 3a**).

Construction and analysis of the PPI Network

The protein-protein interaction (PPI) network was constructed by contributing the 139 antiglycation overlapping target genes of *Fagopyrum esculentum* to the STRING database. The 1040 edges, 139 nodes, and 15 average node degrees were involved in the PPI network depicted in **Figure 3b**. Cytoscape 3.6.0 software was used to analyze the PPI network data obtained from the STRING database (**Figure 3c**). Later, a network analyzer tool (Cytohubba v 0.1 tool) was employed for analyzing and scrutinizing genes from the PPI network that result in the top 10 genes such as NFKB1 (98.52), STAT3 (94.41), GSK3β (93.66), MTOR (92.25), ESR1 (92.08) STAT1 (91.83) HIF1A (91.67) HSP90AA1 (90.01) EP300 (89.67), and AR (83.50) showed the highest degrees of interaction (**Figure 3d**). The highest degree indicates that the targeted genes have a great correlation with each other; hence, all of these genes might be crucial targets. NFKB1, STAT3, and GSK3β were identified as the antiglycation targets of *Fagopyrum esculentum* and were selected for the investigation of molecular docking study.

GO (Gene Ontology) and KEGG pathway analysis

The SRPlot database was employed to analyze the KEGG enrichment pathway and Gene Ontology for the target of *Fagopyrum esculentum*. According to the results, the predicted target genes of *Fagopyrum esculentum* were mostly enriched in 132 molecular functions (MF), 90 cellular components (CC), and 219 biological processes (BP). The top 6 enriched situations of Gene Ontology investigation are shown in **Figure 3e**. The metabolic processes, biological regulation, regulation of the Cellular processes, cellular responses to stimuli, and metabolic process regulation are all involved in the biological process (BP). The cell periphery, plasma membrane, protein-containing complex, intracellular organelle lumen, vesicle, and intrinsic component of membrane were the primary components of the cell. Enzyme binding, protein binding, carbohydrate derivative binding, ion binding, catalysis activity, and transferase activity are involved in molecular functions (MF). The KEGG pathway analysis was performed to identify the enrichment pathway of the significant signaling pathway linked to the antiglycation effect of *Fagopyrum esculentum.* Out of 186 enrichment pathways, the first top 10 pathways were selected based on P-value and gene count (**Figure 3f).** Notably, most of the genes were involved in the following pathways: Insulin resistance, Type II diabetes mellitus, the AGE-RAGE signaling pathway in diabetic complications, the insulin signaling pathway, and the TNF signaling pathway. Finally, the KEGG pathway analysis revealed that NFKB1, STAT3, GSK3β, MAPK1, PIK3R1, and PIK3CB were prominently enriched genes (Table 3).

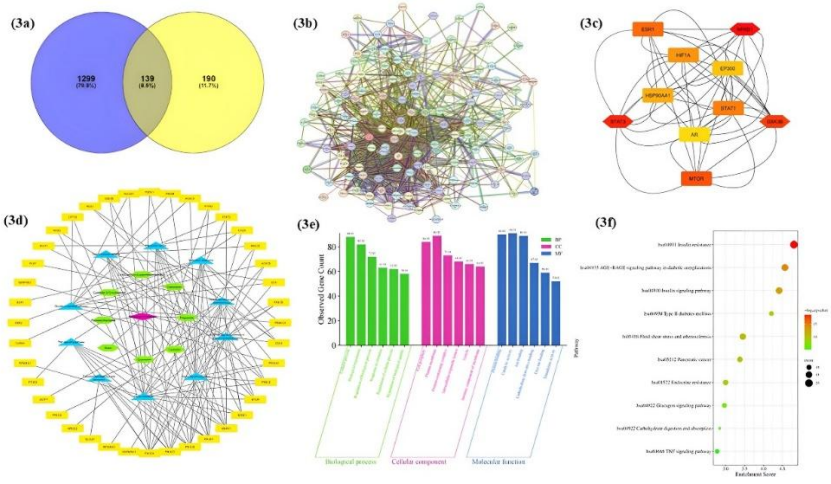

Figure 3. (3a) Venn diagram of disease and bioactive compounds target genes. (3b) Protein-protein interaction network (PPI) of target genes. (3c) Top 10 genes ranked by degree method

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generated by Cytohubba v 0.1 tool. (3d) Effective bioactive compounds target the network of *Fagopyrum esculentum* generated Cytoscape 3.6.0. software. (The pink diamond node represents the selected plant, the green hexagonal node represents potential bioactive compounds, the blue triangular node represents the pathway associated with core targets and the yellow square node represents the gene hub). (3e) GO functional enrichment results of the target genes. (The highest bar represents the number of observed gene counts). (3f) The top 10 KEGG enrichment bubble charts were generated by the SRplot tool. (The X-axis represents the enrichment score; the Y-axis represents the name and code of the selected pathway and the color of the dot represents the significance of enrichment).

Molecular docking study

The top three protein targets, named NFKB1 (PDB ID: 3DO7), STAT3 (PDB ID: 6NJS), and GSK3β (PDB ID: lI09), were obtained from the Protein Data Bank (PDB) selected for molecular docking study. The binding energy for all 8 ligands for binding affinity ranged from -64.808 to -161.18 kcal/mol. Out of 24 protein-ligands complexes, nine complexes i.e. **C_8, C_6, A_8, B_8, A_6, C_7, B_7, B_6**, and **A_7** revealed the highest MolDock score as compared to the control drugs (Table 4). This MolDock score of interaction suggested the significant affinity of Rutin, Pyrosaccharopine, and Cyanidin-3-O-rutinoside bioactive for proteins responsible involved in glycation. However, bioactive compounds and the control drugs formed hydrogen bonds with similar amino acids (Glu280(A), Gln247, Gln185(A), Tyr222(A)), and also shared the same steric interaction amino acid residues (Ser279(A), Tyr222(A), Gln185(A), Tyr221(A), Arg220(B), lle258, Leu260) which depicted in **Figure 4** (Table 4).

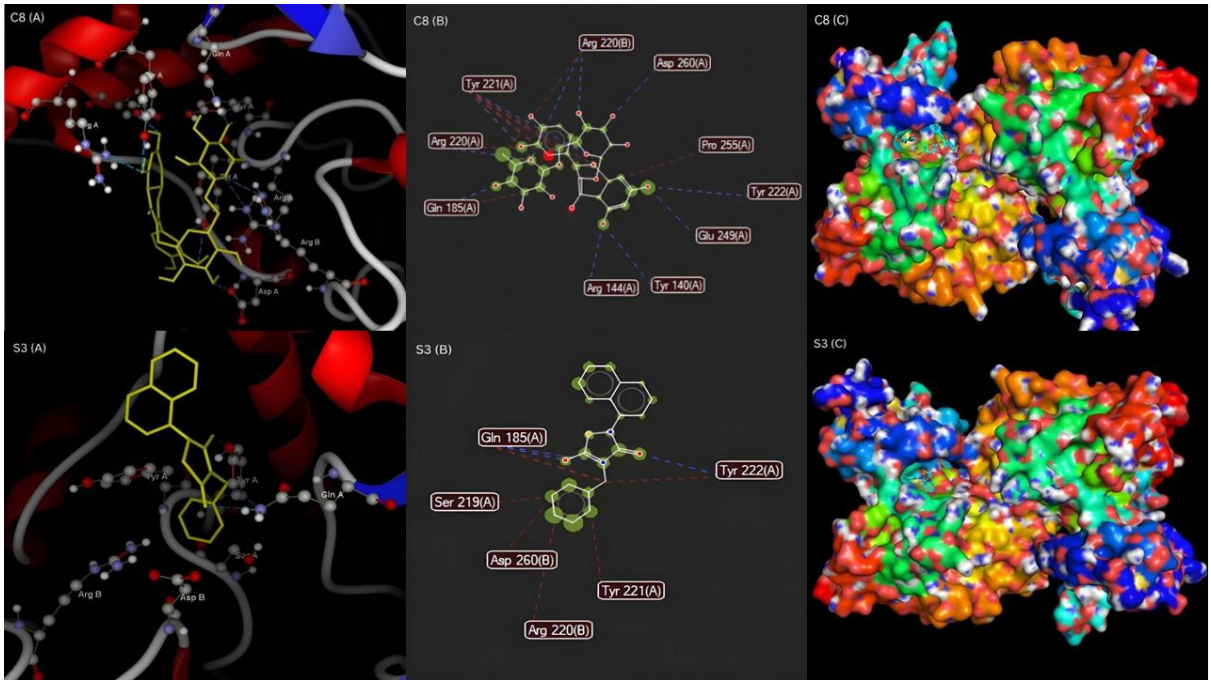

Figure 4: The protein interaction view of bioactive compound (C_8) and control drug (S_3) as ligands with the selected target protein from Molegro virtual docker v 6.0. software. (A) The amino acids of the active site are presented in ball and stick form, ligands are presented in thick lines with fixed color, (B) H-bond interaction is shown in a blue dot line, and steric interaction is represented in a red dot line of ligands with protein target, (C) Solid surface interaction of ligands in the binding pockets of respective protein targets.

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Table 4. Residual interaction of the best pose of selected bioactive compounds and control drugs (H-Bond and steric bond interaction generated by MVD v.6.0 software).

Complex ID	MolDock Score (kcal/mol)	Interaction (kcal/mol)	H-Bond Interaction	Steric Bond Interaction
A_6	-139.492	-151.936	Val208(A), Lys219(A), Asn242(A), Lys274(A), Ser275(A), Gln307(A)	Cys207(A), Val208(A), Asn242(A), Lys274(A), Gln3037(A)
A_7	-100.036	-93.0652	Gln280(A)	Ser279(A), Glu280(A)
A_8	-146.349	-128.747	Arg117(A), Lys274(A), Thr276(A), Ser279(A), Glu280(A), Lys305(A)	Lys273(A), Lys274(A), Asp304(A)
B_6	-143.759	-127.741	Glu247, Ala250, Cys251, Glu254, Glu324, Arg325, Gln326, Lys348, Ser514	Gln247, Ala250, lle258, Glu324, Arg325, Pro333,
B_7	-101.728	-88.9792	Arg246, Cys259	Arg246, Arg262, leu263
B_8	-147.853	-143.086	Gln247, Ala250, Cys251, lle258, leu260, Glu324	Gln247, Ala250, lle258, leu260
C_6	-148.374	-148.374	Tyr140(A), Arg144(A), Ser147(A), Arg148(A), Gln185(A), Tyr221(B), Tyr222(A), Glu249(A)	Ala143(A), Tyr140(A), Arg144(A), Arg148(A), Tyr221(A), Tyr222(A), Glu249(A)
C_7	-103.552	-103.552	Arg223(A), lle228(B)	Tyr216(A), Cys218(A), Arg220(B), Arg223(B), Gly262(B), Gln265(A), Gln265(B)
C_8	-161.18	-161.18	Tyr140(A), Arg144(A), Gln185(A), Arg220(A), Arg220(B), Tyr221(A), Glu249(A), Asp260(A)	Gln185(A), Arg220(A), Arg220(B), Tyr221(A), Pro255(A)
S_1	-69.8379	-70.207	Ser279(A), Glu280(A)	Ser279(A)
S_2	-81.8427	-94.951	Gln247, Cys259, leu260, Cys328, Pro333, Arg350	lle258, leu260, Glu324
S_3	-95.2692	-103.149	Gln185 (A), Tyr222 (A)	Gln185 (A), Ser219 (A), Arg220(B), Tyr221(A), Tyr222(A), Arg220(B)

Molecular Dynamics Simulations

Pyrosaccharopine is a pyrrolidine ring containing bioactive molecule that was determined as the new promising anti-glycemic compound for molecular dynamic simulation (MDS) on the selected protein target glycogen synthase kinase-3 beta (PDB ID: 1IO9) based on the results of the molecular docking study. In the study, Normal Mode Analysis (NMA) is a computational approach to studying and characterizing protein flexibility. The direction of arrow illustrates the orientation of amino acid residue within the target protein (**Figure 5a**). A deformability graph depicts the displacement of residues with 'Hinges' in the major amino acid chain (**Figure 5b**). The subsequent graph of B-factors represents the favorable atomic displacement during thermal vibration and flexibility in different protein regions during molecular dynamics simulation. Experimental B-factors were calculated based on the NMA and PDB hinges in a graph (**Figure 5c**). The energy required for the protein's deformability was associated with the eigenvalue. The motion's stiffness was demonstrated by the obtained eigenvalue 4.977971e-05. This lower eigenvalue suggested easy deformation of protein structure (**Figure 5d**). The eigenvalue exhibits an inverse relationship with the normal mode of variance. The cumulative (green) and individual (purple) variances were depicted in the colored bar chart (**Figure 5e**). The covariance matrix demonstrated the correlation of residues after protein complexation. The correlated motions are represented by the red dot, uncorrelated motions by the white dot, and anti-correlated motions in the blue dot of different amino residues within the protein (**Figure 5f**). The elastic network model showed pairs of atoms that were linked or spring-

connected. Each dot indicates a spring connecting a pair of atoms, with darker gray dots indicating stiffer springs. The combined effect of these dots indicates increased stability (**Figure 5g**).

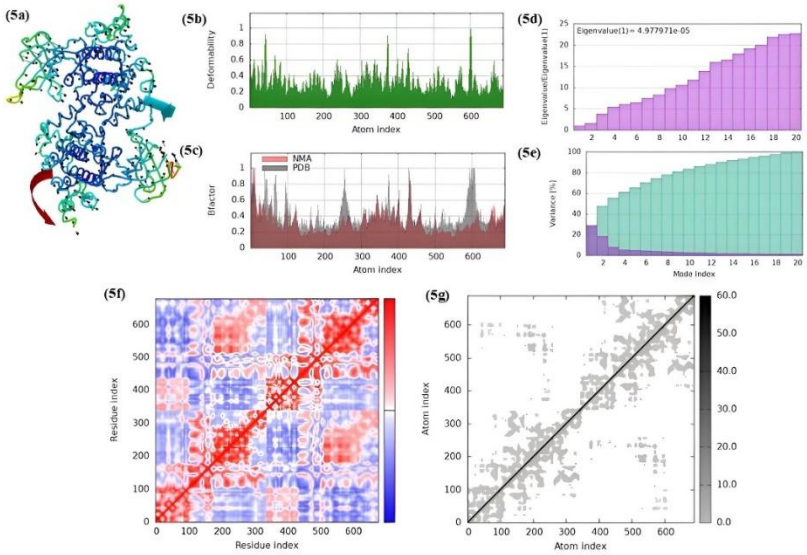

Figure 10. Molecular dynamic simulation (MDS) of Pyrosaccharopine with the protein target (1IO9). (5a) The direction of residues was shown by the cyan and red color. (5b) Deformability. (5c) B factor/mobility. (5d) The stiffness of the motion is represented by the eigenvalue. (5e) The normal mode variance. (5f) This graph elucidates the covariance matrix for the ligand-protein complex (The red region shows the correlation, and the blue region shows uncorrelated motion between residues). (5g) A stiffer mode of the residues was revealed by the elastic network model.

DISCUSSION

The IMPPAT database revealed that the eight potentially effective compounds (3,4-dihydroxy-2-piperidinomethanol, cianidanol, fagomine, cyanidin, quercetin, rutin, pyrosaccharopine, and cyanidin-3-O-rutinoside) were selected based on oral bioavailability and drug-likeness parameters. Cyanidin has demonstrated a protective effect by complexing with methylglyoxal (MGO), a reactive Di-carbonyl glucose metabolite. Thus, cyanidin prevents the formation of the protein-carbonyl complex (advanced glycation end product, AGEs). Cyanidin also decreased the production of hydroxyl radicals and superoxide anions in the lysine/MG system [32]. Fagomine is a naturally occurring polyhydroxylated piperidine that has shown α-glucosidase inhibitory effects. Its structure is similar to the standard α-glucosidase inhibitor compound deoxynojirimycin (DNJ) [33]. Advanced glycation end products (AGEs) are also inhibited by quercetin, a polyphenolic flavonoid molecule that appeared to be an effective anti-glycation agent. To produce a hypoglycemic effect, it can inhibit disaccharidase activity [34-35]. Other research has demonstrated that quercetin can reduce INS-1 beta cell (rat cell lines that store and release insulin in response to glucose concentration) activity and promote insulin release. Long-term administration of quercetin has also been shown to inhibit cell growth and trigger apoptosis, primarily by blocking PI3K/Akt interaction [36]. On the other hand, Rutin inhibits the formation of fructosamine and α-dicarbonyl compounds to lower the level of AGEs. Thus, the rutin may be effective as an anti-glycation agent [37]. The protein-protein network shows an interaction between the target of *Fagopyrum esculentum*. Among them, NFKB1 (98.52), STAT3 (94.41), and GSK3β (93.66) were found to be the most promising three genes based on the highest degree value. It was speculated that they may be the main target genes of *Fagopyrum esculentum* for expressing antiglycation effects.

Gene ontology (GO) enrichment analysis of bioactive molecules of *Fagopyrum esculentum* affects mainly a biological response to a stimulus*;* which helps to attain blood glycemic index by aggravating the glucagon-dependent liver glucose anabolism. The selected bioactive is also correlated to cellular processes within GO enrichment analysis. Cellular process regulation in hyperglycemic conditions advances the defective apoptosis condition of pancreatic β-cells due to oxidative stress caused by enhanced reactive oxygen species (ROS) formation [38]. The' protein-containing complex' is another aspect of the cellular component. This also contributes to type 2 diabetes conditions by stimulating the release of different

bioproteins i.e. leptin, TNF-alpha, and adiponectin, from adipocytic cells that enhance the secretion of insulin as well as insulin resistance [39]. Molecular function is another parameter found within the GO enrichment analysis, that suggested the quantum valuation of protein, carbohydrate, and enzyme binding process. The findings of above study show a significant quantum value, suggesting the beneficial contribution of bioactive components in tissue repair in diabetic inflammatory conditions (diabetic nephropathy) by affecting the protein-RNA complex [40]. In the same graphical observation, it was found that the selected bioactive of *Fagopyrum esculentum* affects the carbohydrate-binding efficiency in the biological system that may help to restore the metabolic defect of glucose along with the recovery from insulin resistance by preventing pancreatic β cell damage. In the same sequence of interpretation of Molecular function in the chart, significant values for 'enzyme binding' activity along with 'carbohydrate derivative binding' were observed suggesting the effect of selected bioactives from *Fagopyrum esculentum.* The aforementioned parameter's value aids in explaining how bioactives affect the activity of enzymes involved in the metabolism of carbohydrates, which may ultimately provide relief from diabetic conditions [41]. The KEGG enrichment analysis reveals, that the involvement of multiple signaling pathways, including insulin resistance, AGE-RAGE signaling pathway in diabetic complications, insulin signaling pathway, atherosclerosis, type II diabetes mellitus, endocrine resistance, glucagon signaling pathway, digestion and absorption of carbohydrates, fluid shear stress and TNF signaling pathway. (**Figure 5f**). When AGEs bind to RAGE, various pathways such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase pathway stimulate the ROS formation, downstream signaling pathway of p38, MAPK, and Jun N-terminal kinase (JNK) pathway. On the activation of the above-mentioned pathways, NF-κB signaling is modulated. When activated NF-κB enters the nucleus, it regulates the release of various inflammatory and immunological factors. The above-mentioned events stimulate the production of inflammatory mediators such as cytokines, (tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β)), and interleukin-6 (IL-6), hormones (angiotensin II (AngII), endothelin-1 (ET-1), aldosterone, and platelet-derived growth factor PDGF, and IKKβ (inhibitor of NF-κB kinase complex β). All these mediators lead to the formation of ROS that results the tissue damage, cell apoptosis, and inflammatory responses. These conditions trigger oxidative stress, which in turn causes pancreatic β-cells to degenerate, diabetic nephropathy, cardiovascular disease, and atherosclerosis [42-48].

Three different protein targets were selected for molecular docking study, related to NFKB1, STAT3, and GSK3β genes found from KEGG enrichment analysis and protein-protein network study. From the literature, it was found that the above genes significantly control the regulators of glycation and associated complications [49-51]. The eight bioactive molecules acquired from IMPPAT were docked with selected target proteins. The Molegro Virtual Docker (MVD) version 6.0 software was employed to obtain all of the docking results and visualized from PyMol 2.4.0 software. After the study of the MolDock score, it was found that Rutin, Pyrosaccharopine, and Cyanidin 3-O-rutinoside bioactive molecule of *Fagopyrum esculentum* formed a stable binding complex with the active pocket of the selected protein as compared to the standard drug molecules (Table 4). The efficiency of Pyrosaccharopine and protein target (1IO9) complex was assessed through molecular dynamic simulation with the help of the iMODS server. The different parametric results suggested profound stability of the ligand-protein complex. The B-factor graph with NMA and PDB hinges showed a significant deformity in amino acid residue, which depicted the functionally considerable mobility within the target protein. The lower eigenvalue suggests substantial deformation after binding the ligand. In the present observation, a minimal eigenvalue was found which signifies a stable deformed conformation by stiffness of residue motion. Further, amino acid residue's dot patterns in covariance (red) and elastic network graph (grey) confirm favorable deformation in the ligand-protein complex. However, the results were shown to be a satisfactory model for the development of antiglycation compounds by using the structural feature of selected bioactive molecules, which further required the validation of results through pharmacodynamics and another real-time experimental study to simplify the therapeutic efficacy of bioactive multiple pathway-based multitarget results.

CONCLUSION

Network pharmacology is a transformative and innovative virtual screening technique that bridges computational predictions and experimental validation. This technique helps to understand the relation of selected ligands with the genetic pathway involved in targeted protein expression. This strategy solves the explanation issue of the complex mechanism of ligand molecules involved in multi-target drug action for better therapeutic effects. Thus, in this present study, Molecular docking study followed by molecular dynamic simulation techniques and network pharmacology have been employed for virtual screening of potential natural leads and structural feature identification to develop more potent hypoglycemic

compounds. For this, eight potential bioactive components of the *Fagopyrum esculentum* plant from IMPPAT, and three protein targets were selected based on genes obtained from KEGG enrichment analysis and protein-protein network study. The outcome of present work revealed the high affinity of selected bioactive ligands for genes (NFKB1, STAT3, and GSK3β) which are involved in the regulation of protein targets that might contribute to the control of glycemic conditions in biological systems. An explanation of efficient binding and favorable conformation changes in targeted protein by pyrrolidine-containing bioactive molecule was found after a molecular dynamic simulation study evaluated the docked complex. Based on the above data analysis result further polyhydroxy and pyrrolidine-based synthetic compounds can be developed and validated for clinical experimental in-vivo hypoglycemic activity in the future.

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