STM JOURNALS

International Journal of
Molecular Biotechnological Research

Volume 1, Issue 1, 2023
January—June
DOI (Journal): 10.37591/IJMBR

https://journals.stmjournals.com/ijmbr/

Research IJMBR

# Integrating Network Pharmacology and Molecular Docking to Assess Rutin's Pharmacological Properties

Govardhan R.[1,*]

***Abstract***

***Objectives****: In the present research, the network pharmacology process was applied to determine the underlying mechanism of the pharmacological properties of Rutin. Network pharmacology was utilized to reveal the interactions between medications and the targets of illnesses, and it is capable of completely articulating the complexity between diseases and medications. The identification of diverse drug-target interactions using network pharmacology may be utilized to discover novel medications for difficult conditions like Parkinson's and Alzheimer's diseases. Rutin's considerable antioxidant capabilities have led to a broad range of pharmaceutical uses.* ***Methods****: In this research, the binding affinity of Rutin corresponding to the targeted proteins was analyzed (TNF, ALOX5, PTGS2, IL2 and TERT). Pathway evaluation for these targets showed a nitrogen metabolism pathway. An online tool called PyRx was used to perform molecular docking. The study was done virtually using input and structure of protein and phytocompounds from UniProtKB and PubChem. With the help of BIOVIA Discovery Studio software, the protein structure was analyzed. ADMET screening was used to evaluate the Rutin's pharmacological properties.* ***Results****: The results from this research showed that the proteins ALOX5 and PTGS2 had the best binding affinity to Rutin in performing molecular docking.* ***Conclusion:*** *According to the results of molecular docking, these target proteins have appropriate pharmacological effects, which offers a theoretical foundation and a hint for the investigation of the pharmacological mechanism of Rutin.*

**Keywords:** Parkinson's and Alzheimer's disorders, network pharmacology, molecular docking, nitrogen metabolism pathway

## INTRODUCTION

Around the world, both conventional and contemporary treatment systems rely heavily on plants as natural resources. For thousands of years, people have utilized plants and products derived from plants as medicines. Alkaloids, flavonoids, essential oils, and phenolic compounds are just a few of the numerous active ingredients found in medicinal plants that contribute to their healing properties. Due to the existence of bioactive substances, plants are used as a phytomedicine to treat several diseases [1].

*Author for Correspondence
Govardhan R
**E-mail:** ravigovardhan0209@gmail.com

Student, Department of Biotechnology, Ramaiah university of applied sciences, Bengaluru, Karnataka, India

Received Date: March 02, 2023
Accepted Date: March 15, 2023
Published Date: March 30, 2023

**Citation:** Govardhan R. Integrating Network Pharmacology and Molecular Docking to Assess Rutin's Pharmacological Properties. International Journal of Molecular Biotechnological and Research. 2023;1(1): 20–33p.

A citrus flavonoid glycoside, rutin is a polyphenolic molecule with a low molecular weight. In both the human body and those of other animals and plants, rutin and related flavonoids have a number of physiological roles. Plants produce more flavonoids in response to stressful conditions such as fungal or bacterial infection or UV radiation doses. Rutin is one of the most well-known natural antioxidants in its category. Very little rutin is absorbed since it is metabolized by gut bacteria after intake into other chemicals. Rutin, a

substance made by higher plants and found in many fruits and fruit rinds, especially citrus fruits, and berries like *Morus alba* and *Ruta graveolens*, is present. Large amounts of vitamin P, also known as rutin, are found in several medicinal fruits, vegetables, and herbs, such as buckwheat and asparagus. Additionally, buckwheat, or *Fagopyrum esculentum*, is one of the best dietary sources of rutin.

Rutin has several medicinal effects, including those that are antibacterial, antiprotozoal, anticancer, anti-inflammatory, antiallergenic, antiviral, cytoprotective, hypolipidemic, antiplatelet, antispasmodic, and antihypertensive. In food, rutin can be used as a UV absorber, colorant, antioxidant, preservative, stabilizer, and/or preservative. In addition, it is a constituent in a number of herbal medicines, multivitamin supplements, goods for the cosmetic and chemical industries, animal feed, and other things [2].

Rutin is frequently used for things like severe introversion, aging skin, exercise-related illnesses that affect the aviation route, and many other things, however, there is not any logical evidence to support any of these uses. Figure 1. Shows flowchart of in-silico analysis of Rutin.

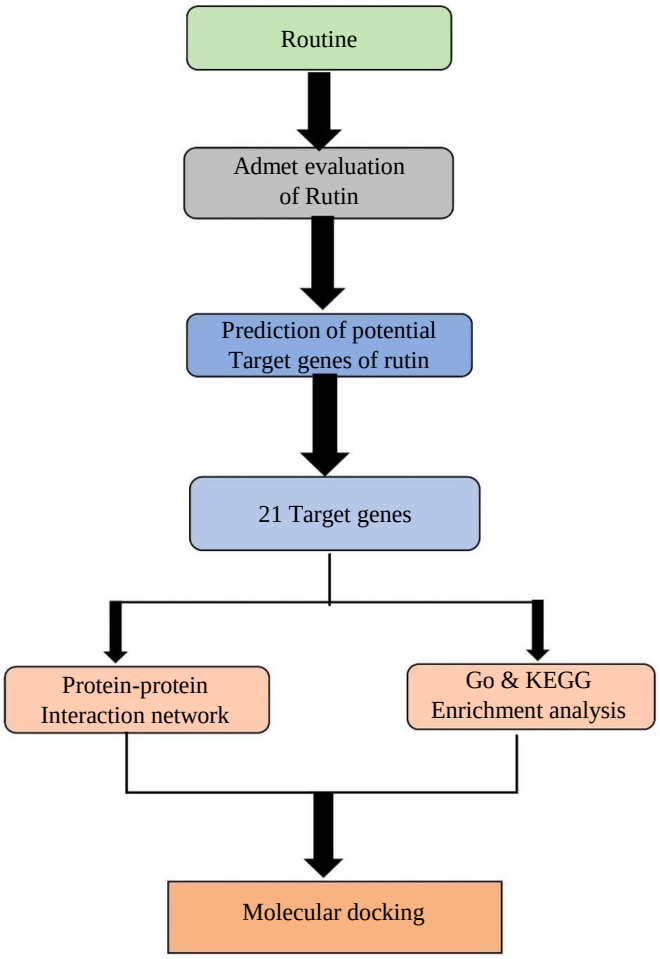



**Figure 1.** The overall flowchart of in-silico analysis of Rutin.

## METHODOLOGY

### LIGAND RETRIEVAL: PubChem Compound Library

PubChem (https://pubchem.ncbi.nlm.nih.gov) is a website that was made available to the public in 2004 as part of the Molecular Libraries Roadmap Initiatives of the US National Institutes of Health (NIH). It contains data about chemicals and their biological effects. Substance, Compound, and BioAssay are three interconnected databases that make up PubChem. Independent data contributors to

PubChem have contributed chemical data to the substance database, and the Compound database has extracted specific chemical structures from the substance database [3]. The canonical SMILES and PubChem CID of rutin was extracted by entering rutin in the query box.

**Target Gene Screening: SwissTargetPrediction**

An online program called SwissTargetPrediction (www.swisstargetprediction.ch) seeks to forecast the most likely protein targets for small compounds. Through reverse screening, predictions are based on the similarity principle. Non-experts are protected from methodological mistakes and professionals from tiresome technical tasks by the user-friendly graphical interface. This makes it possible for anybody to do reverse screening on chemical libraries that have been meticulously created in the past [4]. The canonical smiles were pasted in the given box and the species was set to Homo sapiens. In the end, the predict targets option was selected.

**Protein-Protein Interaction Network Construction: STRING DATABASE**

Protein-protein interactions, including both physical and functional interactions, are carefully gathered and integrated into the STRING database (https://string-db.org/). The information comes from several sources, including computerized text mining of scientific literature, computational interaction predictions from co-expression, conserved genomic context, databases of interaction studies, and recognized complexes/pathways from vetted sources [5].

Now the database has 21 additional capable rutin targets. To form a protein interaction network, the species was set to Homo sapiens and the minimum interaction score was set at 0.4.

**Gene Functional Analysis: Go Function and KEGG Pathway**

ShinyGO (ShinyGO 0.76.3 (sdstate.edu) )was developed by considering 59 plant, 256 animal, 115 archeal, and 1678 bacterial species which were represented in the extensive annotation database for ShinyGO, which was taken from Ensembl and STRING-DB. The innovative features of ShinyGO include a graphical representation of enrichment findings and gene properties as well as access via application program interface to KEGG and STRING for the retrieval of route diagrams and protein-protein interaction networks. With the aid of the user-friendly, graphical online program ShinyGO, researchers may extract useful information from gene sets [6]. This was done to annotate the interaction of rutin with target proteins and determine their role.

**Molecular Docking and Visualization**

Molecular docking is performed using PyRx. The proteins are purified in BIOVIA and then docked in PyRx software. PyRx works on Windows, Mac OS X, or Unix/Linux computer clusters. PyRx can perform AutoDock jobs internally, or on a cloud with the help of the Opal Web Services Toolkit [7].

Visualization is performed using BIOVIA Discovery Studios. BIOVIA Discovery Studio combines the transcription of small compounds and macromolecules. It is created by BIOVIA by Dassault Systems (Accelrys). Advanced drug design and research on protein modeling are both handled by Discovery Studio, a single integrated graphical user interface. This program offers a variety of plot viewers and data visualization viewers [8]. The docked ligands and purified proteins are loaded into BIOVIA and 2D, and 3D structures are downloaded.

**ADMET SCREENING: ADMET LAB 2.0**

The pharmacokinetic factors that determine whether a medication molecule will reach the body's target protein and how long it will remain in circulation are covered by ADME. To lower the attrition rate, thorough investigations of ADMET processes are now frequently conducted at the early stages of drug development. These studies evaluate the efficiency and biopharmaceutical features of drug candidates in parallel and have become standardized. This is because the majority of clinical trial failures have been brought on by ADMET problems rather than insufficient effectiveness. If

ADMET-related research can deflect even one clinical trial failure, which is the most expensive place to have a failure, it might save a lot of time and money. An online platform called ADMET lab (https://admetmesh.scbdd.com/) was created based on a well-compiled database that incorporates the current ADMET and fundamental physicochemical-related endpoints as much as the feasibility to ease the ADMET evaluation. Drug-likeness evaluation, ADMET prediction (31 endpoints assessment), systematic ADMET evaluation for single chemical, and database/similarity searching based on the ADMET database with 288,967 entries are the four primary modules created to efficiently examine ADMET features [9]. The SMILES of Rutin were considered for ADMET analysis.

The pharmacological properties of rutin were evaluated.

## RESULTS

### Retrieval of Ligand

Due to their extensive biological activity, large safety margins, and cheaper cost, natural chemicals like bioflavonoids have found use in the healthcare system. Due to its powerful antioxidant capabilities, rutin, a polyphenolic bioflavonoid, has demonstrated a wide range of therapeutic uses. Rutin has excellent antioxidant effects, making it a very powerful chemical. This intriguing natural chemical will soon advance to the forefront of therapy for the treatment of many chronic human illnesses by increasing its bioavailability by utilizing revolutionary drug delivery techniques with minimal side effects. The canonical SMILES and PubChem ID of Rutin were obtained (Table 1) along with their 2D structure (Figure 2).

**Table 1.** The ligand name, canonical SMILES, and PubChem ID of Rutin.

| Ligand Name | Canonical SMILES | PubChem ID |
|---|---|---|
| Rutin | CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O)O | 5280805 |

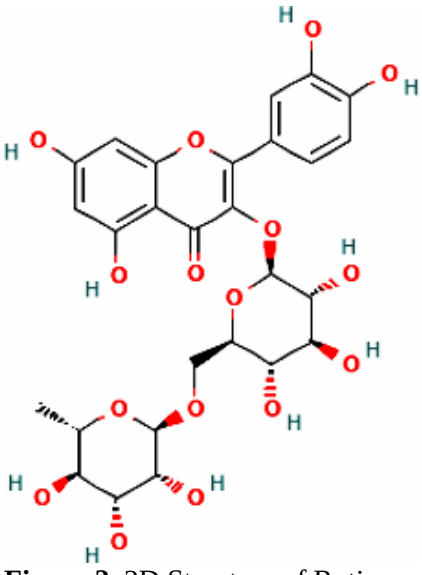


**Figure 2.** 2D Structure of Rutin.

## TARGET PREDICTION

SwissTargetPrediction was used for this process. The canonical SMILES and species were selected as Homo sapiens. Further, the query was run to predict targets. Based on green probability bars from the result page 21 targets were found. These green bars represent the likelihood that the proteins mentioned will behave as biotargets for a query molecule that is presumed to be bioactive. It is crucial to remember that they DO NOT represent the likelihood that the given molecule will be active. Of

course, it is highly recommended to thoroughly go through the ligands of each expected target before doing any further research. Table 2 shows potential genes targeted by Rutin.

**Table 2.** Potential genes targeted by Rutin.

| S.N. | Gene | Uniprot ID | Description |
|---|---|---|---|
| 1 | NMUR2 | Q9GZQ4 | Neuromedin-U receptor 2 |
| 2 | ADRA2A | P08913 | Alpha-2a adrenergic receptor |
| 3 | ADRA2C | P18825 | Adrenergic receptor alpha-2 |
| 4 | ACHE | P22303 | Acetylcholinesterase |
| 5 | AKR1B1 | P15121 | Aldose reductase |
| 6 | CA7 | P43166 | Carbonic anhydrase VII |
| 7 | CA12 | O43570 | Carbonic anhydrase XII |
| 8 | CA4 | P22748 | Carbonic anhydrase IV |
| 9 | NOX4 | Q9NPH5 | NADPH oxidase 4 |
| 10 | CA2 | P00918 | Carbonic anhydrase II |
| 11 | NQO2 | P16083 | Quinone reductase 2 |
| 12 | RPS6KA3 | P51812 | Ribosomal protein S6 kinase alpha 3 |
| 13 | XDH | P47989 | Xanthine dehydrogenase |
| 14 | CD38 | P28907 | Lymphocyte differentiation antigen CD38 |
| 15 | PTGS2 | P35354 | Cyclooxygenase-2 |
| 16 | PDE5A | O76074 | Phosphodiesterase 5A |
| 17 | TNF | P01375 | TNF-alpha |
| 18 | IL2 | P60568 | Interleukin-2 |
| 19 | ADORA1 | P30542 | Adenosine A1 receptor (by homology) |
| 20 | ALOX5 | P09917 | Arachidonate 5-lipoxygenase |
| 21 | TERT | O14746 | Telomerase reverse transcriptase |

## PROTEIN-PROTEIN NETWORK CONSTRUCTION AND INTERACTION

Initially, the 21 discovered targets were loaded to the STRING database, where the screening criteria were set to a combined score > 0.400 being the medium confidence score and the PPI network was formed by KMEAN grouping. Moving forward, 18 targets were found in the group. (Figure 2) Then, referring to the edges and nodes of interactions, TNF and PTGS2 were found to have maximum interaction. Other targets like ALOX5, IL2 and TERT have more interactions after the above-mentioned ones. There was a direct correlation found between these targets. Figure 3 shows the PPI network attained from KMEAN clustering.

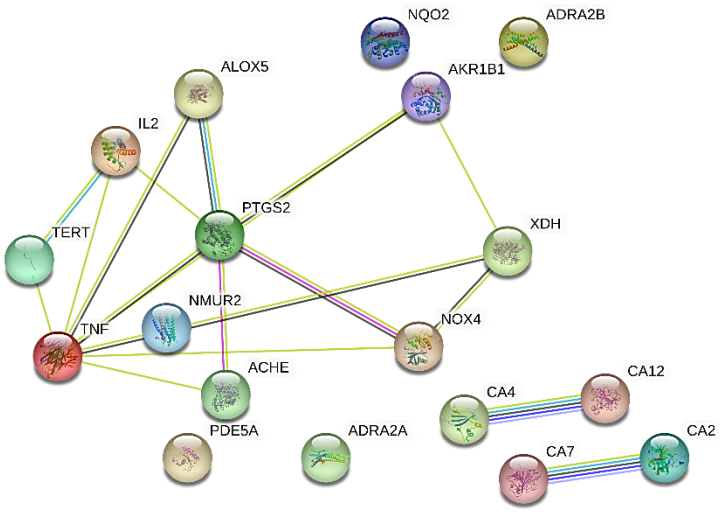



**Figure 3.** PPI network attained from KMEAN clustering.

**NETWORK STATS**

- Number of nodes: 18
- Number of edges: 18
- Average node degree: 2
- Avg. local clustering coefficient: 0.571
- PPI enrichment p-value: 8.86e-08

**GO ENHANCEMENT EVALUATION**

The ShinyGo tool was used for performing GO enhancement evaluation on the 21 identified targets. The Benjamini–Hochberg procedure was used to correct p-values, and the best 10 significantly enhanced items in the BP, MF, and CC categories were picked based on $P < 0.05$, as shown in Figure 4. BP (18 hits), MF (19 hits), and CC (5 hits). Target proteins in the BP section were mostly occupied in Neg. reg. of smooth muscle contraction One-carbon metabolic process, bicarbonate transport Regulation of smooth muscle contraction, regulation of vasoconstriction Smooth muscle contraction, Regulation of tube diameter, blood vessel diameter maintenance regulation of peptide transport, Regulation of blood circulation. The target proteins in the MF category were mostly involved in Alpha2-adrenergic receptor activity heterotrimeric G-protein binding, alpha-adrenergic receptor activity, carbonate dehydratase activity, epinephrine binding, and adrenergic receptor activity. The target proteins in the CC category were engaged in the Basolateral plasma membrane, Basal plasma membrane, Basal part of the cell, and Perinuclear region of the cytoplasm.

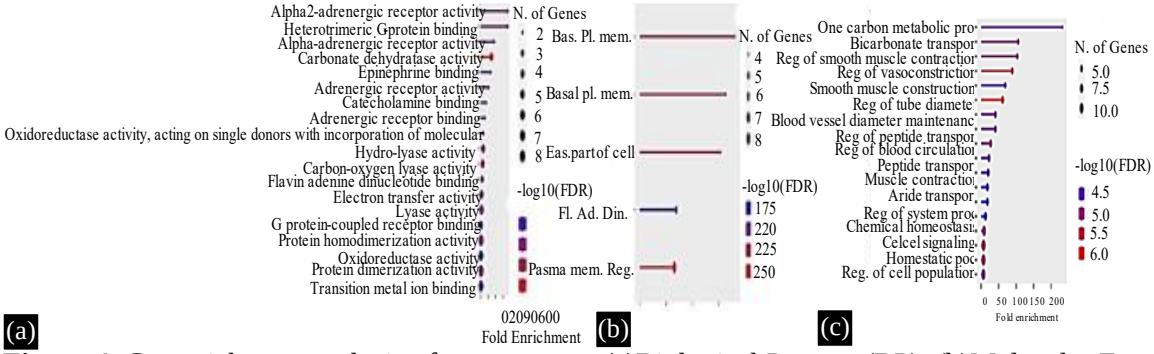



**Figure 4**. Go enrichment analysis of target genes, (a)Biological Process (BP), (b)Molecular Function (MF), (c) Cellular Component (CC).

**KEGG Enhancement Evaluation**

KEGG enhancement evaluation was run on these possible genes using the ShinyGO program. 20 potential target genes from 21 target genes were found to be enriched in the KEGG pathway enhancement study, and 10 signal pathways were directly linked to the target genes ($P < 0.05$). The 10 pathways are represented in Figure 5, along with their enrichment ratios. The pathways that were highly enhanced included nitrogen metabolism, Proximal tubule bicarbonate reclamation, Allograft rejection, and Graft-versus-host disease.

**MOLECULAR DOCKING**

TNF (uniprotKB-Q9UNG2), PTGS2 (UniProtKB- D9MWI3), ALOX5 (UniProtKB-P09917), IL2(uniprotKB-P60568) and TERT (uniprotKB-Q14746) were the potential targets whose crystal structure was downloaded in PDB format. Essential information about proteins was downloaded from The RCSB Protein Data Bank. The structures of the following were downloaded, TNF-3B94, ALOX5-3V92, PTGS2-3NTG, IL2-7RAA and TERT-7TRD. As mentioned earlier Rutin's 2D structure was extracted and docking was performed against 5 proteins. The binding affinities of the proteins towards Rutin are shown in Table 3. 3NTG showed the highest binding affinity.

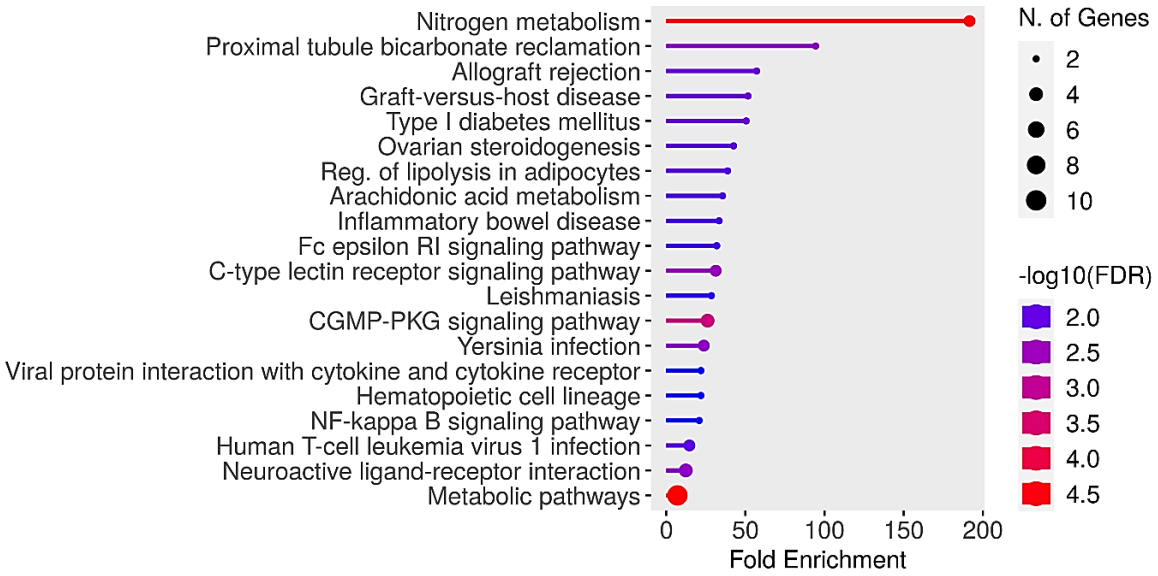


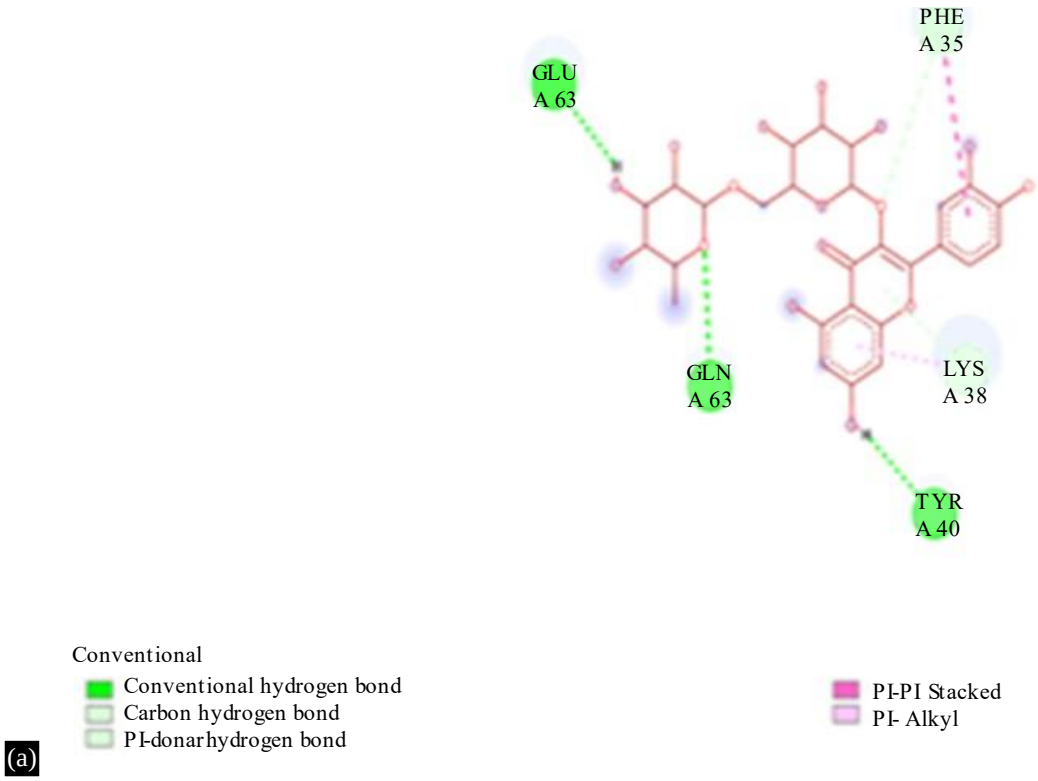

**Figure 5**. KEGG enhancement evaluation of target genes.

**Table 3.** Binding affinity of target proteins towards Rutin.

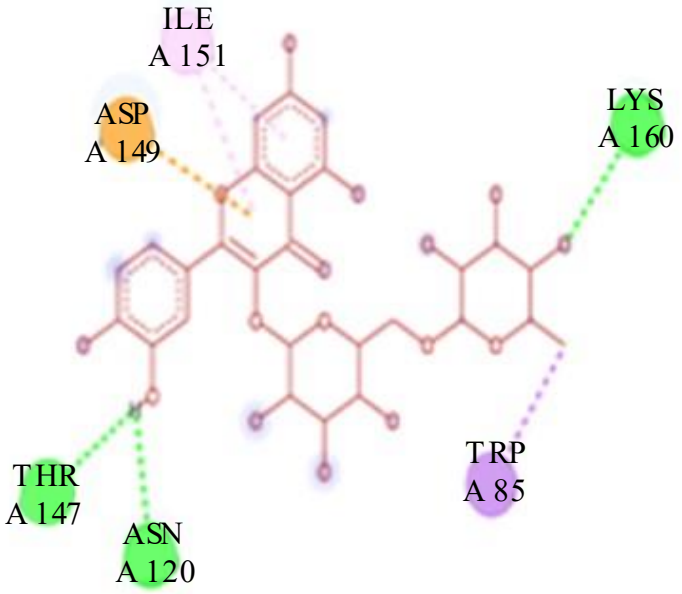
| **Ligand** | **Binding Affinity** | | | | |
|---|---|---|---|---|---|
| Rutin | ***3NTG*** | ***3V92*** | ***7TRD*** | ***3B94*** | ***7RAA*** |
| | -9.9 | -9.5 | -7.8 | -6.9 | -6.6 |

## VISUALIZATION

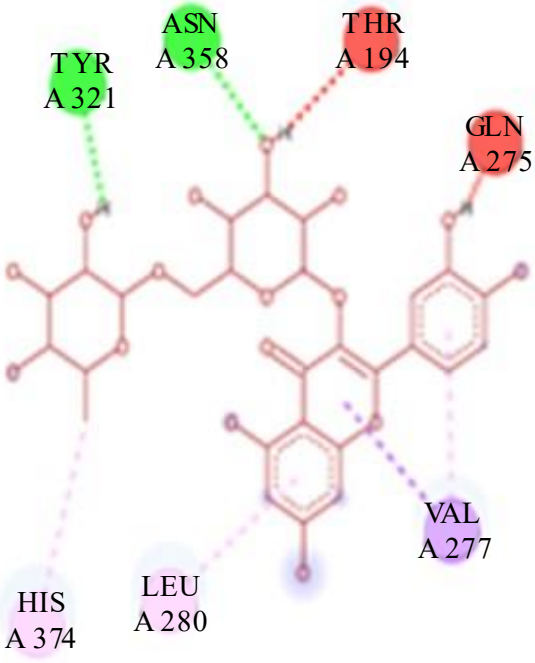
Details about the interactions, bond distances, and amino acid residues were obtained by 3D and 2D visualization of the selected top 5 proteins using BIOVIA Discovery Studio (Figures 6 and 7).






(b)


(c)

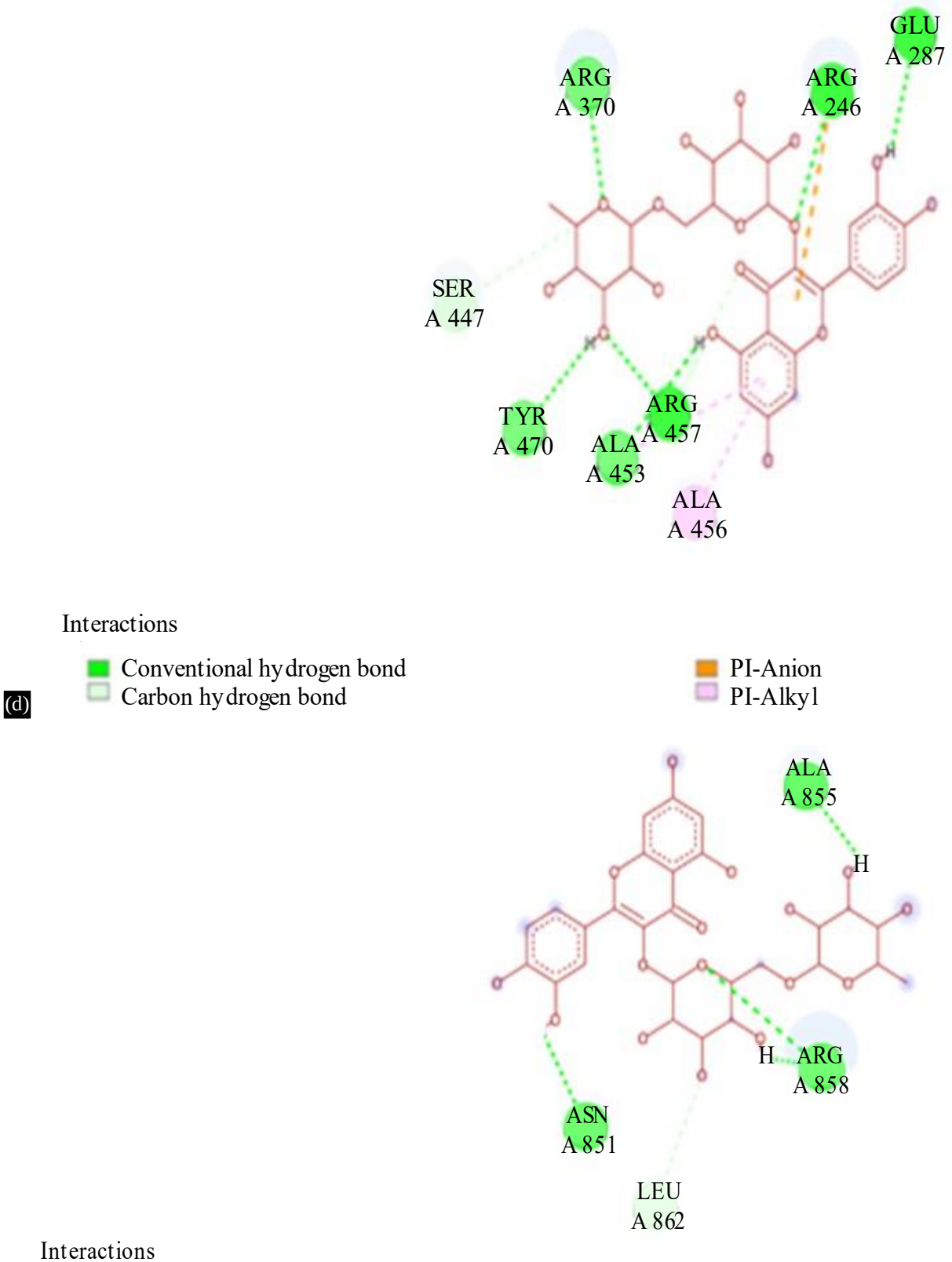
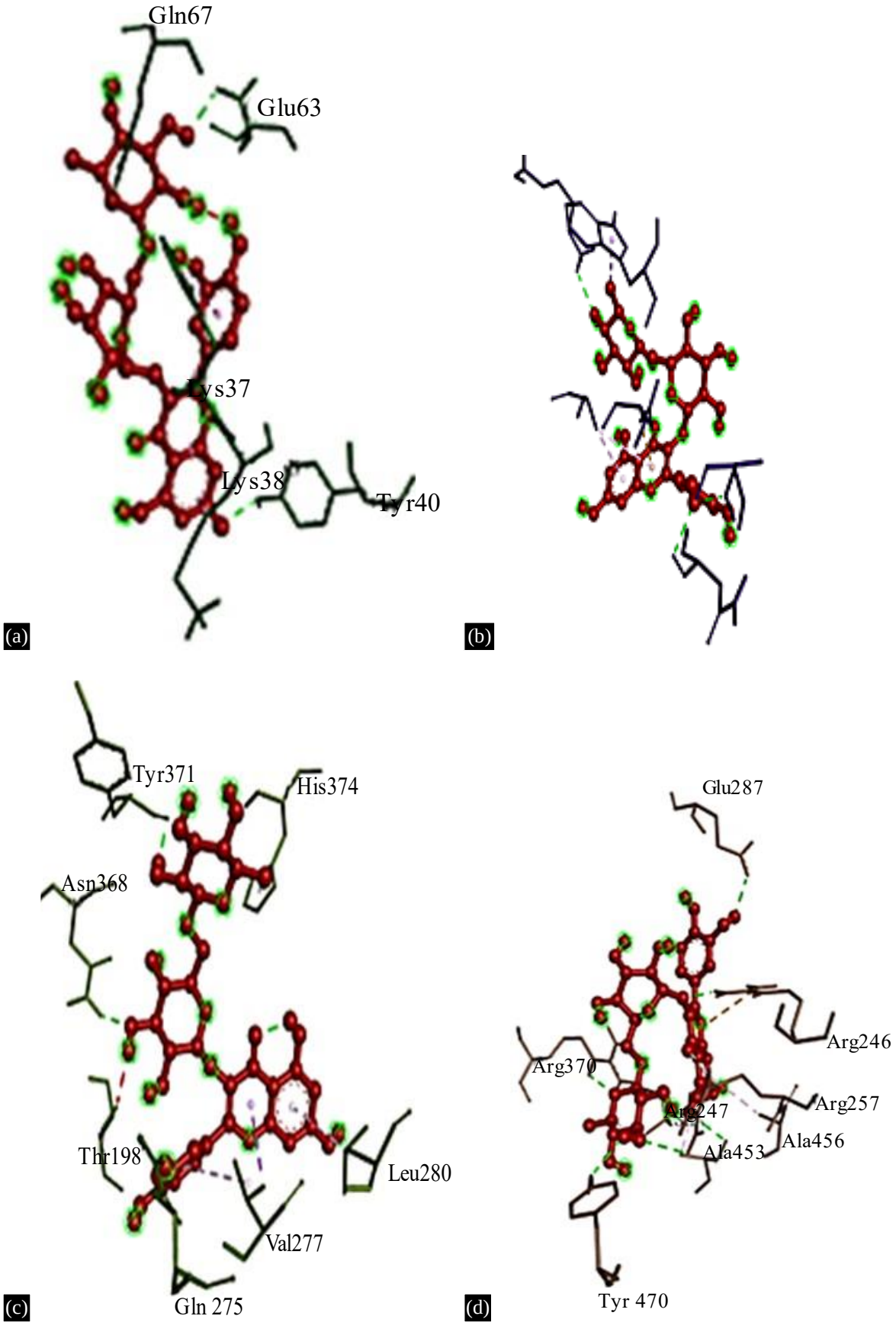
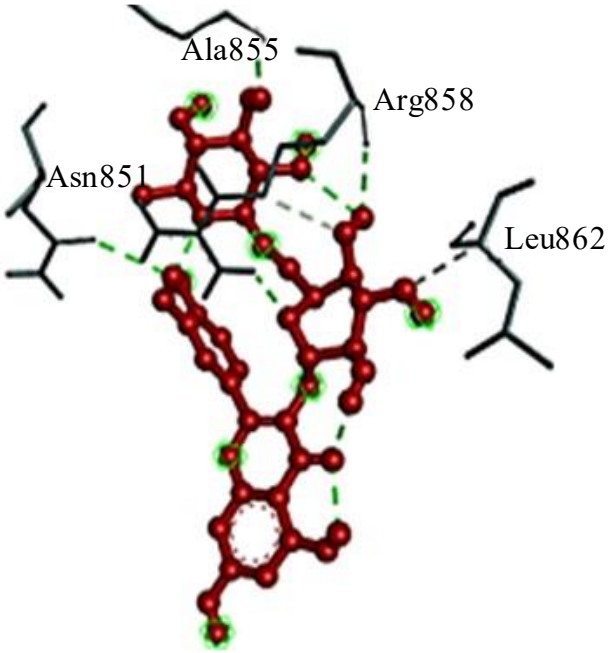



**Figure 6.** 2D visualization images of target proteins with Rutin, (a) IL2, (b)TNF, (c) PTGS2, (d)ALOX5, (e) 7TRD.






(e)

**Figure7.** 3D visualization images of target proteins with Rutin, (a) IL2, (b) TNF, (c) PTGS2, (d) ALOX5, (e)-7TRD

## ADMET ANALYSIS

These results indicate that Rutin contains all drug-likeness properties confirmed through ADMET analysis as shown in Table 4.

**Table 4.** ADMET properties of Rutin.

| Molecular Weight | Absorption | | Distribution | Metabolism | | Excretion | Toxicity | | |
|---|---|---|---|---|---|---|---|---|---|
| 610.15 | ***WS*** | ***SP*** | ***BBB*** | ***CYP3A4-sub*** | ***CYP2C19-inh*** | ***TC*** | ***ORAT*** | ***HT*** | ***AMES*** |
| | -3.928 | -10.26 | 0.111 | YES | NO | 1.349 | 0 | 0.092 | 0.805 |

*BBBP = blood brain barrier permeability, logBBB, ORAT = oral rat acute toxicity (LD50), SP = skin permeability (log Kp), TC = total clearance (logml/min/kg), WS = water solubility (logmol/L)*

## DISCUSSION

A new field known as network pharmacology (NP) has evolved to study pharmacological effects and interactions with numerous targets. It uses computing capacity to catalog the chemical interactions of a pharmacological molecule in a live cell in a systematic manner. NP emerged as a critical tool for comprehending the underlying complicated interactions between botanical formulas and the entire organism. It also tries to find novel drug leads and targets, as well as repurpose current drug molecules for alternative therapeutic diseases, by allowing unbiased exploration of prospective target areas. New interests in systems biology have led to the recognition that single-protein interventions cannot successfully cure complicated disorders. This forced drug researchers to acknowledge the idea of polypharmacology, which they previously considered an undesirable quality that needed to be eliminated or decreased to generate clean medications functioning on single targets. Modifying phenotypes requires the simultaneous manipulation of several targets, according to network biology. Methods for aiding polypharmacology can assist in boosting effectiveness and forecasting undesired off-target effects. miRNA-based biomarkers were developed with the help of NP. To do this, a network of miRNAs and their targets was built and improved to investigate the data for specific disorders. This method, together with literature mining, proved beneficial in developing powerful miRNA markers for illnesses. NP was also employed in the development of a drug-gene-disease comodule. Initially, a drug-disease network was constructed using information acquired from

databases, which was then supplemented with gene data. A mathematical model was used to investigate gene proximity. This network detected the linkage of several genes for the majority of disorders, as well as medication and disease target sharing. These networks provide information on novel therapeutic associations and their biological links [10].

In the United States, interest in buckwheat-derived rutin may be traced back to the 1940s, when buckwheat was grown for rutin as a source of medicine. Rutin's poor bioavailability is its principal drawback, which is mostly brought on by its low water solubility, poor stability, and restricted membrane permeability. Rutin may exhibit measurable bioactivity in several in vitro systems, however, this is a key feature that inhibits its biological effects when applied to living organisms. Its limited liposolubility further restricts its usefulness for topical applications.

Recent studies have nonetheless demonstrated Rutin's broad-spectrum pharmacological advantages for the management of several chronic illnesses, including cancer, diabetes, hypertension, and hypercholesterolemia. Because it is a harmless, non-oxidizable molecule, its usage is favorable compared to that of other flavonoids. Reviewing the current techniques for rutin extraction and purification is crucial given the rising trend in demand for this flavonoid [11].

GO enhancement evaluation had three subgroups namely MF, biological function, and cellular components. BP has been involved in the bicarbonate transport Regulation of smooth muscle contraction which is an essential function of the bicarbonate ion in critical systems. Immune disorders, cystic fibrosis, carcinogenesis, renal conditions, brain dysfunction, tooth fracture, ischemia-reperfusion damage, hypertension, impaired reproductive system, and systemic acidosis are some of the illnesses caused by poor bicarbonate transport [12]. One more was the Regulation of blood circulation which regulates the microcirculation, a network of blood arteries made up of arterioles, capillaries, and venules, which serves as the location of restricted blood flow control and facilitates the exchange of chemicals between blood and tissue [13]. MF was occupied in Carbonate dehydratase activity, Epinephrine binding means when epinephrine binds to the liver cell's surface receptor, a conformational shift might take place. This alteration in receptor shape enables the G-protein to bind and activate. Adenylate cyclase binds to the molecule as a result of a conformational shift brought about by the activation G-protein [14].

When KEGG analysis was performed nitrogen metabolic pathway was found to be the major pathway associated with Rutin. In cyanobacterial cells, the element nitrogen (N) makes up between 5 and 10% of the dry weight. Different extracellular N-sources are connected through assimilatory routes in free-living cyanobacteria to cellular N-containing elements. The glutamine synthetase/glutamate synthase cycle converts ammonium, inorganic nitrogen, into glutamine and glutamate. Although urea and amino acid usage has not received enough attention up to this point, some amino acids, such as glutamine and arginine, can also give N to some cyanobacteria. How the input about the N status of the cell is detected and sent to the protein(s) responsible for regulating gene expression will be a central focus of future study [15]. With available results from the study, it is noticed that Rutin has a broad-spectrum of pharmacological properties. Researchers also think rutin can help protect against brain damage and neurodegenerative illness due to its anti-inflammatory and antioxidant capabilities. The effectiveness of rutin in the treatment of Alzheimer's and Parkinson's diseases are being investigated in studies. Rutin, according to experts, defends the brain by lowering pro-inflammatory cytokines and boosting antioxidant enzyme activity [16].

## CONCLUSION

As the research progressed it was determined that Rutin has a broad range of pharmacological effects. Meanwhile, investigations had taken place to see whether Rutin's mechanism of action could be changed to form high-potential anticancer and anti-diabetic medications. This evidence gives way to a different perspective on Rutin research, its development and therapeutic usage.

**Acknowledgment**

I would like to acknowledge BioNome in Bengaluru, India for providing computing facilities and assistance with research studies. I would also like to thank Ms. Susha Dinesh for her assistance with the project.

**Abbreviations**

*TNF:* tumor necrosis factor

*ALOX5:* arachidonate 5-lipoxygenase

*PTGS2:* prostaglandin-endoperoxide synthase 2

*IL2:* interleukin 2

*TERT:* telomerase reverse transcriptase

**REFERENCES**

1. Atanassova M, Bagdassarian V. Rutin content in plant products. J Univ Chem Technol Metall. 2009;44(2):201–3.
2. Patel K, Patel DK. The beneficial role of rutin, a naturally occurring flavonoid in health promotion and disease prevention: a systematic review and update. Bioact Food Diet Interv Arthritis Relat Inflam Dis. 2019:457–79.
3. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, et al. PubChem substance and compound databases. Nucleic Acids Res. 2016;44(D1):D1202–13. doi: 10.1093/nar/gkv951, PMID 26400175.
4. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res. 2019;47(W1):W357–64. doi: 10.1093/nar/gkz382, PMID 31106366.
5. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic acids research, gkac1000; 2022.
6. Ge SX, Jung D, Yao R. ShinyGO: a graphical gene-set enrichment tool for animals and plants. Bioinformatics. 2020;36(8):2628–9. doi: 10.1093/bioinformatics/btz931, PMID 31882993.
7. Jacob RB, Andersen T, McDougal OM. Accessible high-throughput virtual screening molecular docking software for students and educators. PLoS Comput Biol. 2012;8(5):e1002499. doi: 10.1371/journal.pcbi.1002499, PMID 22693435.
8. Pawar SS, Rohane SH. Review on Discovery Studio: an important tool for molecular docking. Asian J Res Chem. 2021;14(1):1–3. doi: 10.5958/0974–4150.2021.00014.6.
9. Dong J, Wang NN, Yao ZJ, Zhang L, Cheng Y, Ouyang D et al. ADMETlab: a platform for systematic ADMET evaluation based on a comprehensively collected ADMET database. J Cheminform. 2018;10(1):29. doi: 10.1186/s13321–018–0283-x, PMID 29943074.
10. Chandran U, Mehendale N, Patil S, Chaguturu R, Patwardhan B. Network pharmacology. Innov Approaches Drug Discov. 2017:127–64. doi: 10.1016/B978–0–12–801814–9.00005–2.
11. Rutin. A review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability.
12. Lee D, Hong JH. The fundamental role of bicarbonate transporters and associated carbonic anhydrase enzymes in maintaining ion and pH homeostasis in non-secretory organs. Int J Mol Sci. 2020;21(1):339. doi: 10.3390/ijms21010339.

13. Pittman RN. Chapter 2, The Circulatory System and Oxygen Transport. Available from: https://www.ncbi.nlm.nih.gov/books/NBK54112/. Regulation of tissue oxygenation. San Rafael, (CA): Morgan & Claypool Life Sciences; 2011.
14. CHEM; n.d. Available from: https://www.chem.udel.edu/.
15. Flores E, Herrero A. Assimilatory nitrogen metabolism and its regulation. In: The molecular biology of cyanobacteria. Dordrecht: Springer; 1994. p. 487–517.
16. Alzarea SI, Qasim S, Uttra AM, Khan YH, Aljoufi FA, Ahmed SR, et al. Network pharmacology and molecular docking based prediction of mechanism of pharmacological attributes of Glutinol. Processes. 2022;10(8):1492. doi: 10.3390/pr10081492.