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Network Pharmacology and Molecular Docking Approach to Reveal the Underlying Mechanisms of Isorhamnetin in the Treatment of Lymphangitis Carcinomatosis

Harsimran Kaur Hora[1,*]

Abstract

Objective: *Lymphangitic carcinomatosis (LC) is a tumor that spreads through lung lymphatics and is most commonly seen secondary to adenocarcinoma. In the majority of cases, LC is a result of tumor cells spreading through the lymphatic system. However, approximately 20% of LC cases can be attributed to lymphangitis caused by tumors obstructing lymphatic drainage. This blockage commonly occurs near the lung hilum, where a centrally located mass can cause inflammation of the lymphatic tubules.* ***Materials and methods:*** *Using, Drugbank and PPI network, stitch identification of the ligands and target proteins was done. Using PyRx the ligands and proteins were docked and to identify the potential ligand, visualization of the docked complexes was done on BIOVIA. ADMET analysis was done for the same ligands to study their pharmacological properties.* ***Results:*** *The proteins AKT1, CYP2C8, and PTK2 were identified as target proteins expressed in LC which docked effectively with the ligand Icaritin respectively. The study's findings indicate that the derivatives of Isorhamnetin such as Icaritin and some others like Quercetin-3'-O-phosphate, and Morin had potential benefits in the prognosis of LC.* ***Conclusion:*** *According to investigation findings, Isorhamnetin itself and Icaritin, Quercetin-3'-O-phosphate, and Morin could be therapeutic for treating LC.*

Keywords: Lymphangitis carcinomatosis, Isorhamnetin, AKT1, CYP2C8, PTK2, Icaritin, Quercetin-3'-O-phosphate, Morin

INTRODUCTION

Lymphangitic carcinomatosis (LC) is a tumor that spreads through lung lymphatics and is most commonly seen secondary to adenocarcinomas (cancers that have spread from their original sites to other areas of the body). Usually, LC develops due to the spread of tumor cells through the lymphatic system. The cells travel along the lymphatic vessels and can form secondary tumors in other parts of the body. It is a rare form of pulmonary metastasis. Most often, LC affects people between the ages of 40 and 49 [1]. Lymphangitis (inflammation of the lymphatic tubules) is caused by tumors that block the drainage of the lymph ducts in about 20% of cases. Lymphatic drainage in the lung is often blocked by a mass near the hilum. In LC, the tumor grows in and obstructs lymphatics in the lungs, resulting in radiologically similar patterns to pulmonary interstitial edema from heart failure including Kerley B lines, thickening of the fissures, and Pleural effusions, especially laminar effusions [2].

***Author for Correspondence**
Harsimran Kaur Hora
E-mail: harsimrankaurhora@gmail.com

[1]Student, Department of Bioinformatics, BioNome, Bengaluru, Karnataka, India

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The infiltration of cancerous cells into the lymphatic vessels is commonly referred to as pulmonary lymphangitic carcinomatosis (PLC), and it typically occurs in the pulmonary interstitial lymphatics. This condition is characterized by the spread of cancer cells through the lymphatic vessels in the lung, leading to inflammation and obstruction of the lymphatic vessels. PLC describes the clinical form of LC. PLC is a type of metastatic cancer that grows diffusely or focally in the lymphatic vessels of the lung. The most frequent cause of PLC is adenocarcinomas, a type of cancer that originates in glandular cells. This type of cancer is often found in the lungs and can lead to the development of PLC through the spread of cancerous cells along the lymphatic vessels [3]. Although PLC is most associated with lung cancer, rare reports have described instances of non-pulmonary lymphangitic carcinomatosis affecting other organs. Some of these cases have involved LC of the skin, duodenum, and kidney [4]. LC is the tumor's spread to the lungs' lymphatic system. Cancer cells can accumulate in the lymphatic vessels, resulting in blockage of the vessels. This obstruction can cause inflammation and damage to the lymphatic system and prevent the normal drainage of fluids and immune cells. The accumulation of cancer cells within the lymphatic vessels is a hallmark feature of PLC. LC's initial lesions (primary lesions) include the breast, stomach, lung, pancreas, colon, and prostate [5].

PLC is a disease that is associated with a high mortality rate. Due to its aggressive nature and the difficulty in treating it, patients with PLC often have a poor prognosis. The severity of the disease, along with its tendency to spread rapidly, can make it challenging to manage and can result in a high mortality rate. Lungs, livers, bones, and brains can be affected by this disease. LC in the lungs can lead to significant respiratory distress, and in severe cases, it can be fatal. The cancerous cells can cause obstruction and inflammation of the airways and can affect the lungs' ability to function properly. As a result, patients with LC may experience symptoms such as coughing, shortness of breath, and chest pain, which can be debilitating and even life-threatening in some cases [6]. So, patients with LC develop respiratory failure at the time of diagnosis, and emergency treatment is required in such cases [7]. It is important to determine whether there is smooth or nodular interstitial thickening, lymphadenopathy, alveolar opacities, and pericardial effusions in order to narrow down the diagnosis of LC. LC may also arise from choriocarcinoma, melanoma, or metastatic adenocarcinoma from an unknown primary cancer [8].

The fruits of Hippophae rhamnoides and the leaves of Ginkgo biloba contain various active ingredients, with isorhamnetin being one of the most important. Isorhamnetin is a flavonoid that has been studied for its potential health benefits, including its anti-inflammatory and antioxidant properties. It is found in high concentrations in these plants and is commonly used in traditional medicine. It is an important monomethoxy flavonol. It is a naturally occurring compound in vegetables and fruits. It is a tyrosinase inhibitor, a metabolite, an antitumor agent, and an anticoagulant. Isorhamnetin possesses extensive pharmacological activities, including anti-tumor, anti-inflammatory, anti-oxidation, etc that may defend against many chronic diseases. As well as having pharmacological effects on cardiovascular diseases and tumors, isorhamnetin may also prevent neurodegenerative diseases such as Alzheimer's. Additionally, it has pharmacodynamic effects against hyperuricemia and pulmonary fibrosis [9].

Isorhamnetin is also known as 3-methyl quercetin. In terms of lipid molecules, isorhamnetin is considered a flavonoid. As a water-insoluble, relatively neutral molecule, isorhamnetin is very hydrophobic. Isorhamnetin is a flavonoid that is present in all eukaryotes, from yeast to humans. This compound is known for its bitter taste and is commonly found in fruits and vegetables such as onions, grapes, and broccoli. Although isorhamnetin is present in a variety of plants, its concentrations can vary widely depending on the plant species and part of the plant.

There are a few foods that contain the greatest amount of Isorhamnetin outside of the human body, such as parsley, green bell peppers, and dill, and fewer foods, like romaine lettuces, Chinese cabbages, and pears [10].

METHODOLOGY

Retrieval of Ligands

The DrugBank database is an online resource that provides comprehensive and freely accessible information on drugs and their targets. This database offers a range of bioinformatics and cheminformatics tools, making it a valuable resource for researchers and clinicians alike. With its detailed drug information and advanced search capabilities, DrugBank is a valuable tool for those seeking to better understand the properties and effects of various medications. The canonical SMILES of the compound name "Isorhamnetin" was entered at DrugBank (https://go.drugbank.com/structures/search/small_molecule_drugs/structure#results) [11]. All 22 potential experimental ligands were selected. And their canonical SMILES and PubChem IDs were gathered via PubChem (https://pubchem.ncbi.nlm.nih.gov/) [12]. Also .sdf format and .pdb format of all 22 ligands were downloaded from PubChem.

Identified and Predicted Targets

Identifying the potential targets for drug-like compounds is challenging in the drug-discovery process. TargetNet is used for predictions of Drug–target interactions (DTIs) [13].

A file was created of Canonical SMILES of all 22 ligands and was added to TargetNet (http://targetnet.scbdd.com/calcnet/index/) [14] for which 623 targts were predicted out of which the top 100 genes were selected. An excel sheet was created with these top 100 proteins and their Uniprot_ID and GeneID were also collected from UniProt (https://www.uniprot.org/) [15].

Network of protein-chemical interactions, allowing researchers to better understand the relationships Another database STITCH (http://stitch.embl.de/) [16] was also used for the target proteins identification for (LC) and the canonical smiles of all 22 ligands were used to build the ligand-protein interactions. STITCH, which stands for "Search Tool for Interacting Chemicals," is a database that integrates information on protein-chemical interactions from a variety of sources. These sources include experimental databases, pathway databases, drug-target databases, and text mining tools, as well as drug-target predictions. By combining these different data sources, STITCH creates a unified between various molecules and their effects on biological processes. [17]. In the result, 10 Predicted Functional Proteins are seen in the STITCH databases for which the list is made in excel.

Protein-Protein Interaction (PPI) Network Construction and Analysis

STRING (*Search Tool for the Retrieval of Interacting Genes/Proteins*) (https://string-db.org/) [18] is a database of known and predicted protein–protein interactions.

Both the predicted proteins that are the top 100 proteins from TargetNet databases and 10 proteins from STITCH databases were added to the STRING database. The protein interactions were restricted to the highest confidence score of 0.900 and were subjected to KMEAN Clustering. Totally three major cluster were identified and by seeing the networks one was selected based on the one having the major interactions with the proteins. This step was done to fetch that protein which is highly connected to one another. AKT1, CYP2C8, and PTK2 were identified as primary target genes. And then three lists were made of all three clusters having all the proteins interacting with one another.

Protein Retrieval and Purification

The three-dimensional crystal structure of AKT1, CYP2C8, and PTK2 were downloaded from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (https://www.rcsb.org/) in the .pdb format [19]. All the proteins were resolved using the X-RAY diffraction method. The resolution of each protein is as follows: Human AKT1 2.70 Å, Human CYP2C8 2.70 Å and Human PTK2 2.80 Å. Using the Biovia Discovery Studio visualizer, the proteins AKT1, CYP2C8, and PTK2 were purified before docking, and the following procedure was followed: Water molecules were completely removed before docking since they have the potential to negatively impact docking scores. To hasten binding with the chosen ligands for the inquiry, the prebound ligands are

removed from the crystal structures. To make the protein structures simpler, extra chains were eliminated; however, chain A was left in place for analysis. Structures that have been refined are improved by the addition of polar hydrogen atoms.

Molecular Docking

Molecular docking is a computational method that predicts the optimal orientation of a ligand molecule when it is bound to a macromolecular target to form a stable complex. This method involves exploring the various conformations that the ligand can adopt within the binding site of the target molecule. By predicting the preferred orientation of the ligand, molecular docking can provide insights into the binding affinity and specificity of a potential drug candidate, helping researchers to identify promising compounds for further study [20].

The RCSB Protein Data Bank was used to gather the crystal structures of the target proteins, which were then purified by removing the ligands and water motifs and adding hydrogen. From PubChem, the 3D chemical structure of all 22 ligands was gathered. The purified proteins (AKT1, CYP2C8, and PTK2) were loaded as the macromolecule one at a time and phytocompounds were loaded as the ligands. The ligands were docked independently against AKT1, CYP2C8, and PTK2. The loaded ligands were converted to pdbqt format after energy minimization. The docking interactions of the ligand and target protein were evaluated based on the binding affinity. In PyRx (https://pyrx.sourceforge.io/) [21], ligands show 9 different conformations, out of which the best docking confirmation having zero roots mean square deviation RMSD is taken as it shows the best binding complex.

Caflanone, Icaritin, Quercetin-3'-O-phosphate, Isorhamnetin, Morin, Rhamnetin were discovered to be the most effective compounds binding with AKT1, while Morin, Quercetin-3'-O-phosphate, Fisetin, Myricetin, Icaritin, Kaempherol were found to be the most effective compounds binding with PTK2, Diosmetin, Isorhamnetin, Quercetin, Baicalein, Icaritin, Tricetin were found to be the most effective compounds binding with CYP2C8. These compounds were chosen for visualization and pharmacological studies.

Visualization

The docked structures from PyRx was visualized using the structure visualization tool BIOVIA discovery studio software (https://discover.3ds.com/discovery-studio-visualizer-download) [22]. The best-binding conformations were retrieved in The molecular structures were analyzed in both two-dimensional and three-dimensional models using the BIOVIA Discovery Studio Visualizer software, which allows the visualization and manipulation of molecular structures in various formats, including the widely used PDB format. In addition to examining the covalent bonding between atoms, the software was used to explore the non-bonded interactions between different parts of the molecules. These analyses provided insights into the structural features of the molecules and their potential interactions with other molecules or biological systems.

ADMET Screening

The main reasons for the failure of drug development are the pharmacokinetics and toxicity of candidate compounds. The evaluation of a molecule's absorption, distribution, metabolism, excretion, and toxicity (ADMET) is a critical step in drug discovery and development. By assessing these factors as early as possible, researchers can identify potential issues and focus their efforts on the most promising compounds. One approach for early ADMET evaluation is to perform screening studies on visually selected molecules prior to chemical synthesis and biochemical assays. This strategy allows researchers to quickly identify molecules with favorable ADMET profiles and prioritize them for further study, increasing the efficiency of the drug discovery process [23].

ADMET analysis was performed using ADMETlab2.0, an online server (https://admetmesh.scbdd.com/) [24]. Canonical SMILES of all 22 ligands were entered at ADMETlab2.0 and the results were downloaded in .csv format.

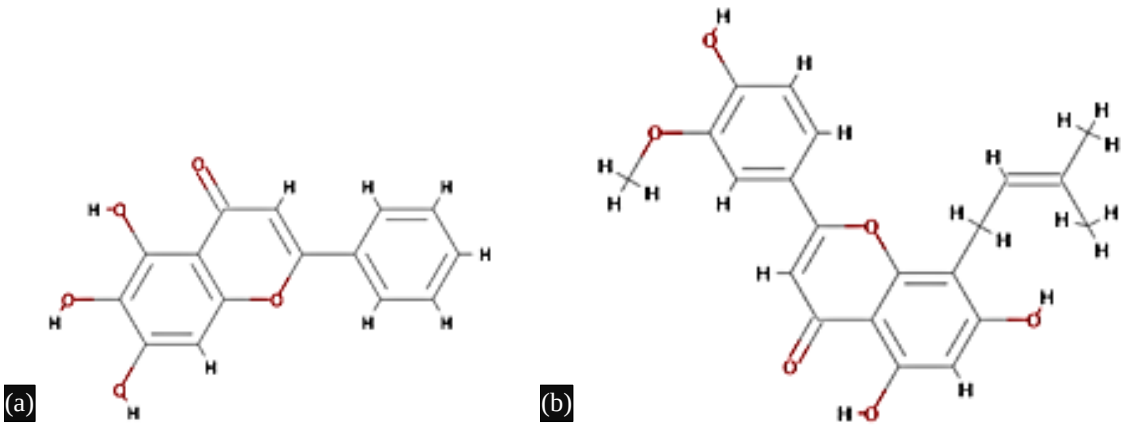
RESULTS

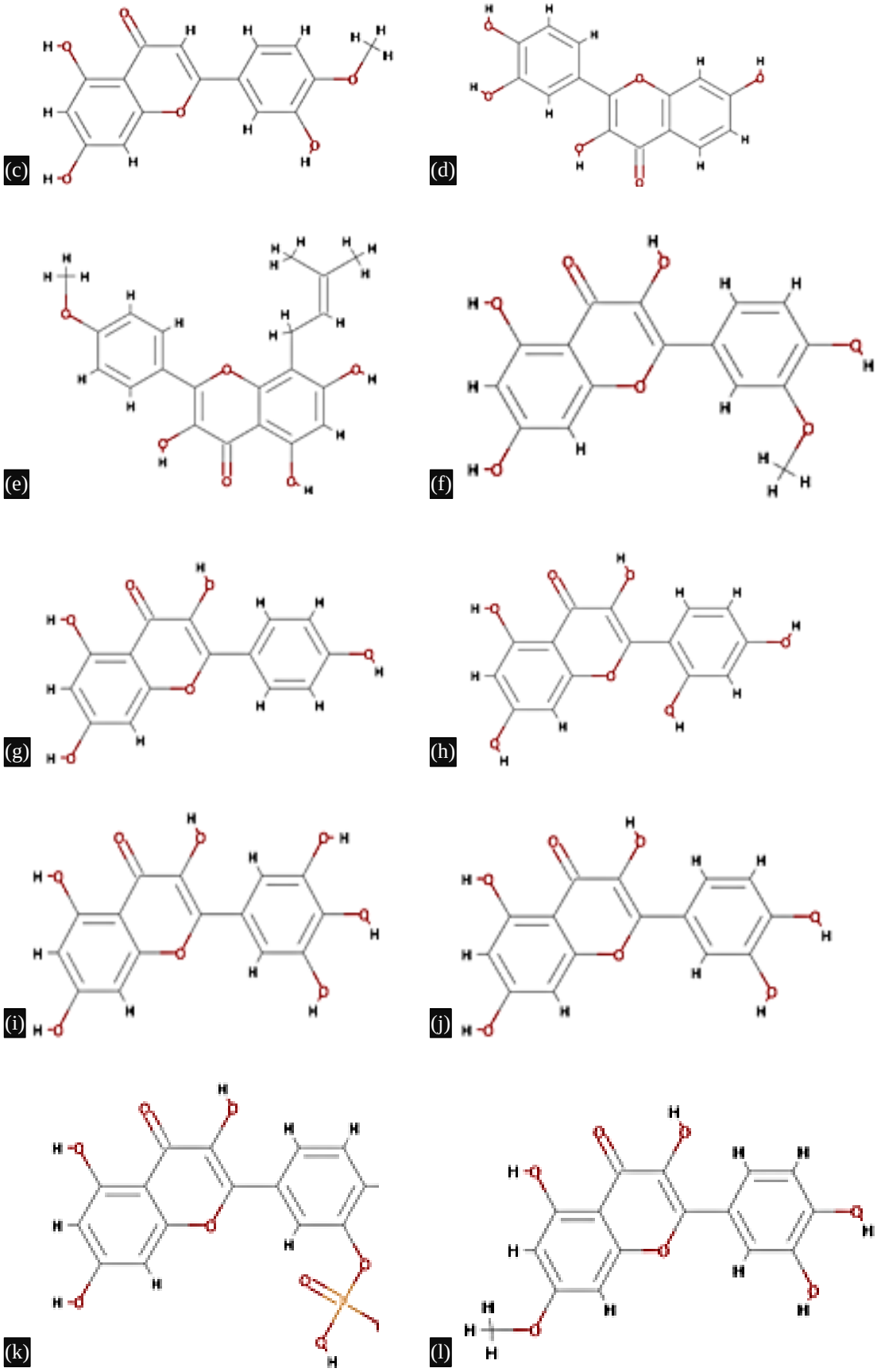
Retrieval of Ligand

Isorhamnetin possesses extensive pharmacological activities. Numerous studies have explored the potential health benefits of isorhamnetin, including its anti-inflammatory, anti-oxidant, anti-tumor, and cardiovascular and cerebrovascular protective effects. In addition, isorhamnetin has been investigated for its ability to protect against organ damage and prevent obesity. These findings suggest that isorhamnetin may have a wide range of therapeutic applications and highlight the importance of continued research into its pharmacological properties. Recent research is focusing on Mechanisms of Isorhamnetin in the treatment of LC. All 22 potential experimental ligands and their canonical SMILES and PubChem IDs were obtained (Table 1) along with their 2D structure (Figure 1).

Table 1. The retrieval data of 22 ligands.

DrugBank Accession Number	Generic Name	PubChem ID
DB16767	Isorhamnetin	5281654
DB16772	Rhamnetin	5281691
DB02375	Myricetin	5281672
DB04216	Quercetin	5280343
DB07795	Fisetin	5281614
DB01852	Kaempherol	5280863
DB17283	Chrysoeriol	5280666
DB16885	Eupatilin	5273755
DB11259	Diosmetin	5281612
DB16770	Morin	5281670
DB08230	Tricetin	5281701
DB15584	Luteolin	5280445
DB14008	Hispidulin	5281628
DB13983	5,7,2′-trihydroxy-6,8-dimethoxyflavone	9818878
DB12672	Icaritin	5318980
DB12937	Quercetin-3'-O-phosphate	20833257
DB17263	Caflanone	44562555
DB16101	Baicalein	5281605
DB07352	Apigenin	5280443
DB12058	Recoflavone	9952125
DB15581	Chrysin	5281607
DB17042	PD-98059	4713
DB16767	Isorhamnetin	5281654
DB16772	Rhamnetin	5281691





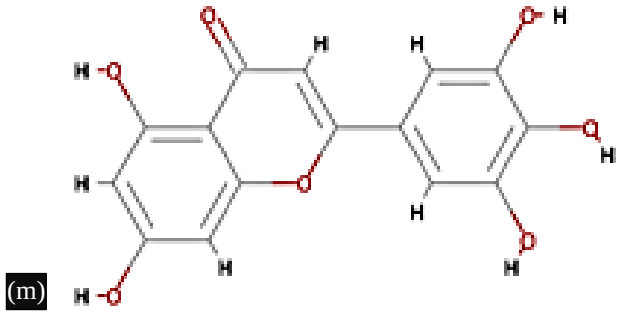


Figure 1. (a–m) 2D-Structure of top ligands. (A) Baicalein (B) Caflanone (C) Diosmetin (D) Fisetin (E) Icaritin (F) Isorhamnetin (G) Kaempferol (H) Morin (I) Myricetin (J) Quercetin (K) Quercetin-3'-O-phosphate (L) Rhamnetin (M) Tricetin

Identified and Predicted Targets

Top 100 target proteins and 10 target proteins were obtained from the TargetNet database, and STITCH database, respectively. All these were then chosen for further research. Figure 2 shows Prediction of molecular targets for Isorhamnetin and its analogs.

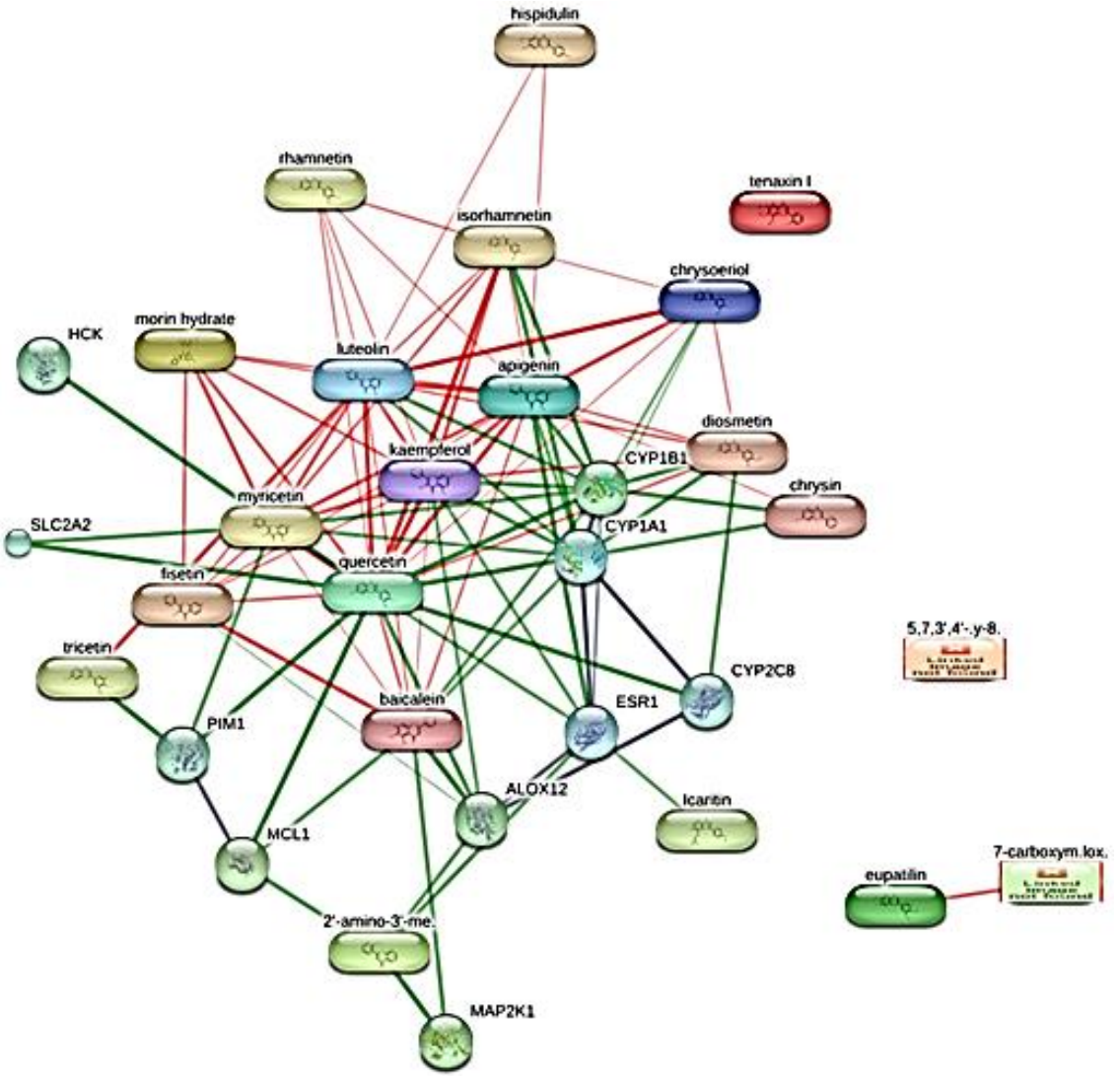


Figure 2. Prediction of molecular targets for Isorhamnetin and its analogs.

Protein-Protein Interaction (PPI) Network Construction and Analysis

Based on the 100+10 (TargetNet + STITCH) discovered targets, the STRING database was screened with a score > 0.900 for the highest confidence and the PPI network was created by KMEAN Clustering. As a result, 47 targets were found in the cluster (Figure 3). Then according to the edges and nodes of the interactions, Isorhamnetin's effects on LC are caused mainly by AKT1, CYP2C8, and PTK2 (Figure 4).

Molecular Docking Results

Table 2 lists, respectively, the binding affinities of all the chosen ligands towards the AKT1, CYP2C8, and PTK2 proteins as determined by PyRx.

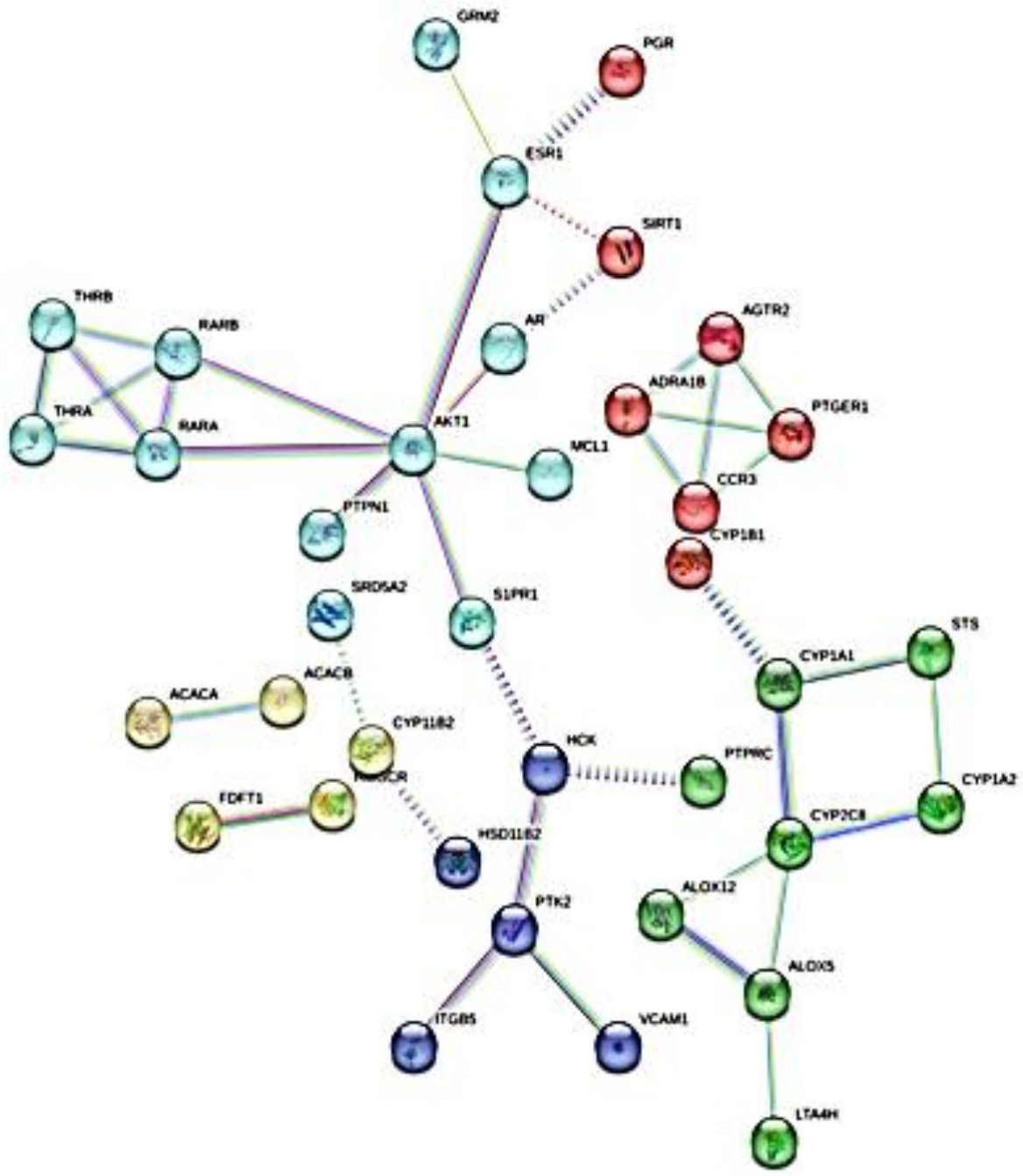


Figure 3. PPI network obtained for the queried protein by applying KMEAN clustering.

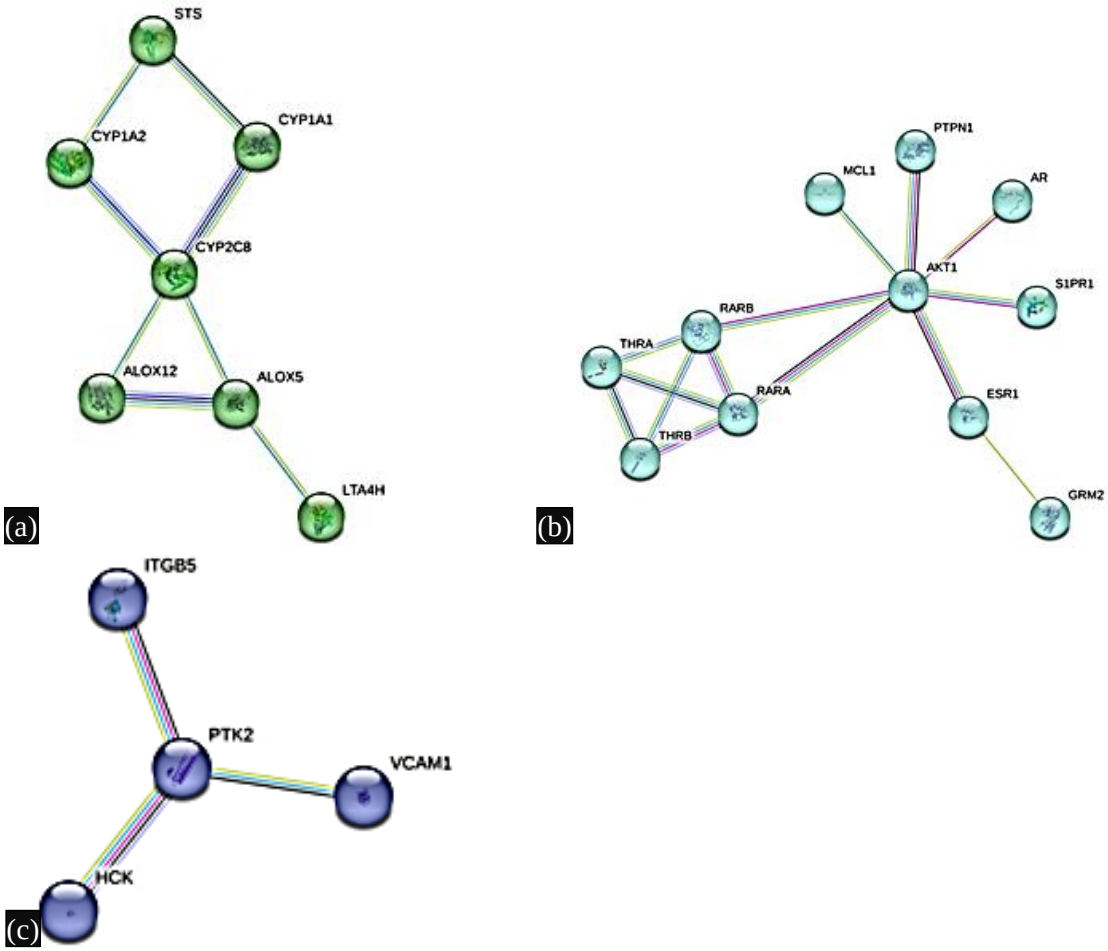


Figure 4. (a–c) Major clusters identified through K-mean clusterin. (A) CYP2C8 - interaction network (B) AKT1 - interaction network (C) PTK2 - interaction network

Table 2. Docking score of proteins AKT1, CYP2C8, and PTK2 with selected ligands.

Protein	Ligand	Binding Affinity
AKT1	Caflanone	-10.4
	Icaritin	-10.2
	Quercetin-3'-O-phosphate	-9.9
	Isorhamnetin	-9.7
	Morin	-9.7
	Rhamnetin	-9.7
CYP2C8	Diosmetin	-9.1
	Isorhamnetin	-9
	Quercetin	-9
	Baicalein	-8.9
	Icaritin	-8.9
	Tricetin	-8.8
PTK2	Morin	-8.4
	Quercetin-3'-O-phosphate	-8.4
	Fisetin	-8.3
	Myricetin	-8.3
	Icaritin	-8.2
	Kaempherol	-8.2

The docking conformation with the highest binding energy was taken into account for further analysis. The phytocompounds above -9.0 including the compounds Caflanone, Icaritin, Quercetin-3'-O-phosphate, Isorhamnetin, Morin, and Rhamnetin were shown to have the highest binding energy with AKT1 whereas compounds such as Diosmetin, Isorhamnetin, Quercetin, Baicalein, Icaritin and Tricetin with binding energy greater than -8.6 showed highest binding affinity with CYP2C8 whereas compounds such as Morin, Quercetin-3'-O-phosphate, Fisetin, Myricetin, Icaritin and Kaempherol with binding energy greater than -8.5 showed highest binding affinity with PTK2 in this investigation. These three options will be selected for further analysis.

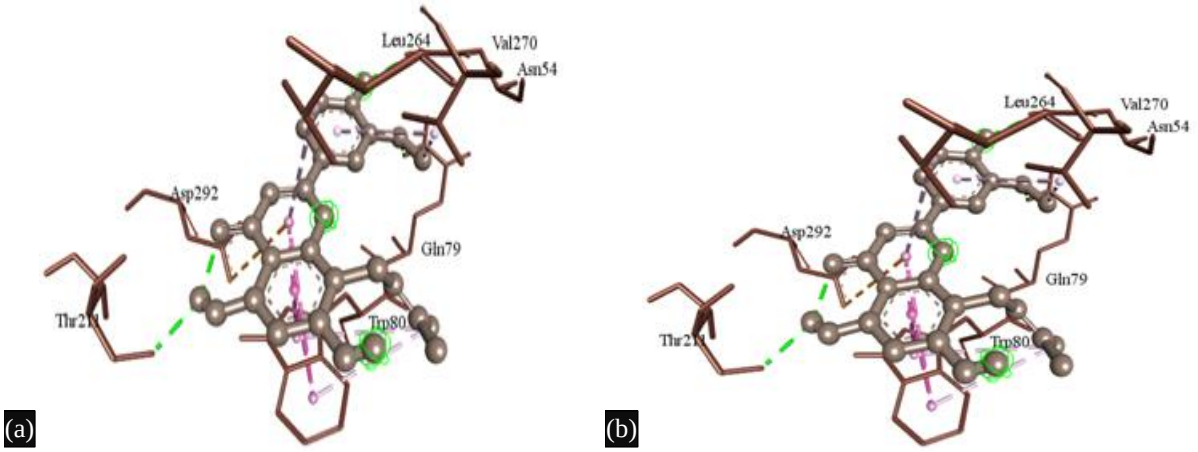
Visualization

Molecular Interactions Of The Top Ligands With Akt1

As shown in Figure 5 AKT1 showed the maximum number of interactions including amino acids such as Leucine, Valine, Asparagine, Glutamine, Tryptophan, Threonine, and Aspartic acid with the ligand (A) Caflanone with a binding energy of -10.4 (Table 2). (B) Icaritin showed the next highest interactions with amino acids Lysine, Leucine, Valine, Asparagine, Tryptophan, Glutamine, Isoleucine, Aspartic acid and Threonine with AKT1. AKT1 shared interactions with (C) Isorhamnetin involving amino acids Valine, Tryptophan, Asparagine, Glutamine, and Aspartic acid (D) Haematoxylin involving amino acids Valine, Tryptophan, Asparagine, Glutamine, Aspartic acid, and Leucine, Threonine, Aspartic acid, Tryptophan, and Serine respectively. (E) Quercetin-3'-O-phosphate interacted with Tryptophan, Serine, Leucine, Threonine, Tyrosine and Aspartic acid. The least interactions of AKT1 were seen with (F)Rhamnetin involving Lysine, Asparagine, Tyrosine, Arginine, Leucine, Aspartic acid, Leucine and Tryptophan. Among all the amino acid interactions with the above ligands, Tryptophan was found to most commonly interact with all the ligands.

Molecular Interactions of the Top Ligands with CYP2C8

As shown in Figure 6 CYP2C8 showed the maximum number of interactions including amino acids such as Leucine, Valine, Histidine, Serine, Arginine, Cysteine, Alanine and Glutamine with the ligand (B) Diosmetin with a binding energy of -9.1 (Table 2). (D) Isorhamnetin showed the next highest interactions with amino acids Cysteine, Serine, Valine, Leucine and Alanine with CYP2C8. CYP2C8 shared interactions with (A)Baicalein involving amino acids Valine, Arginine, Histidine, Serine, Leucine, Phenylalanine and Cysteine (C) Icariin involving amino acids Isoleucine, Glycine, Asparagine, Leucine, and Valine respectively. (E) Quercetin interacted with Valine, Leucine, Threonine, Alanine, Cysteine, Phenylalanine, Serine and Arginine. The least interactions of CYP2C8 were seen with (F) Tricetin involving Valine, Alanine, Threonine, Alanine, Phenylalanine, Cysteine, Serine and Arginine. Among all the amino acid interactions with the above ligands, Valine was found to most commonly interact with all the ligands.



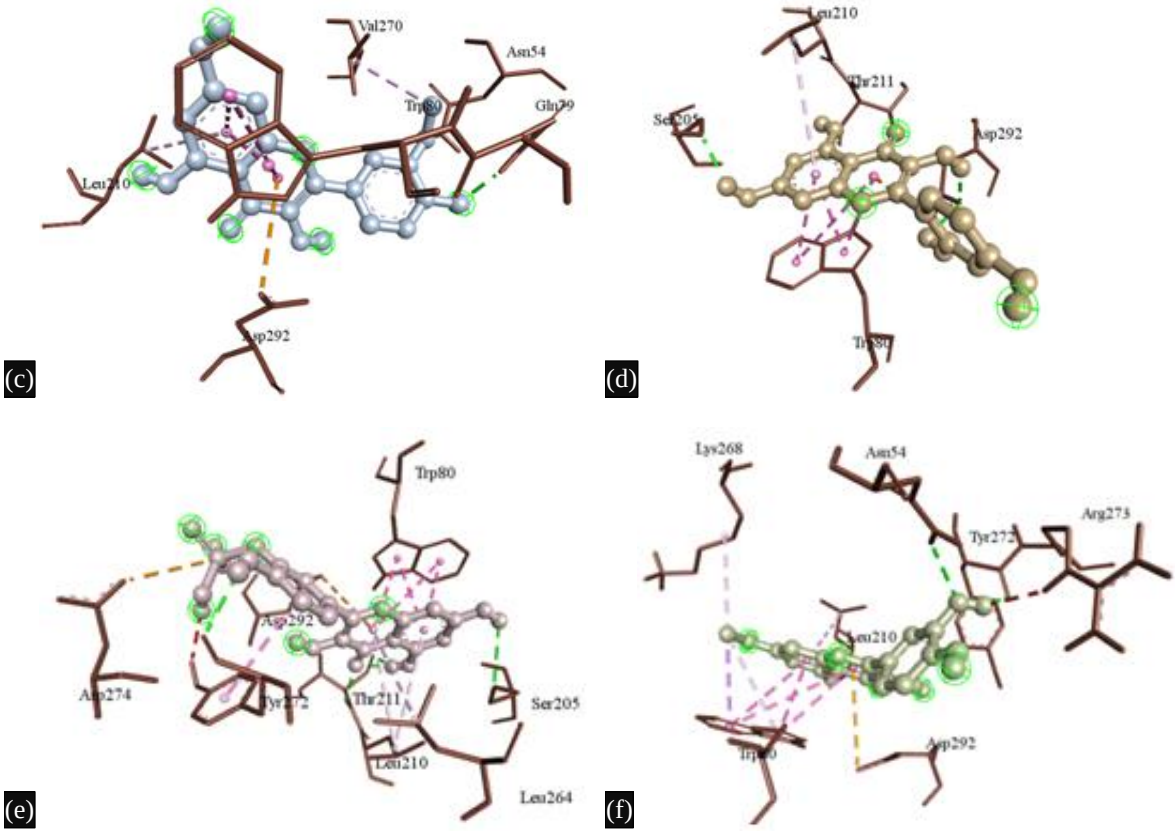
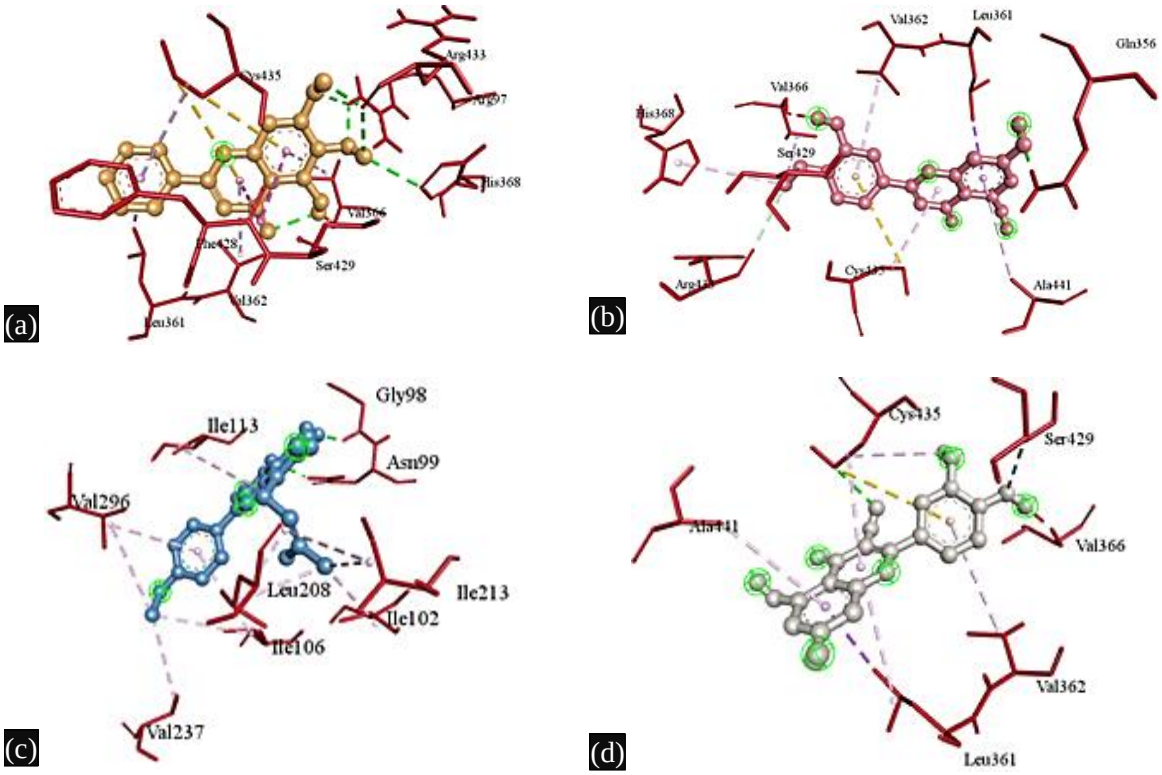


Figure 5. (a–f) Visualisation of molecular interactions of AKT1 with the top 6 ligands. (A) Caflanone - AKT1 (B) Icaritin - AKT1 (C) Isorhamnetin - AKT1 (D) Morin - AKT1 (E) Quercetin-3'-O-phosphate - AKT1 (F) Rhamnetin - AKT1



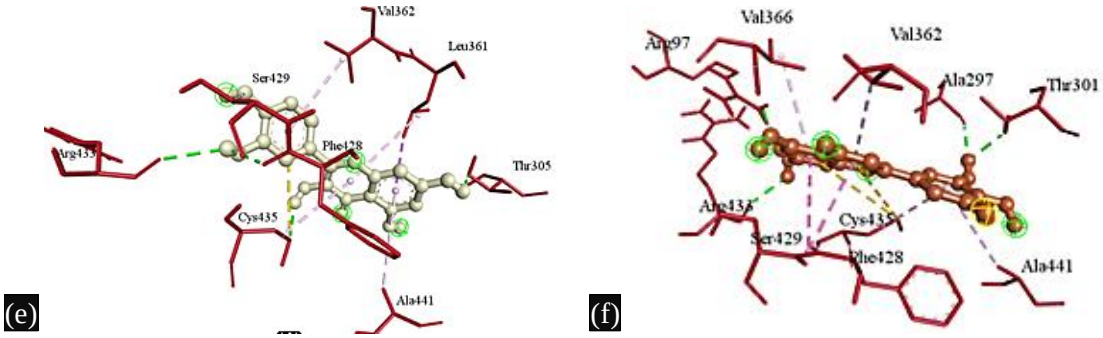
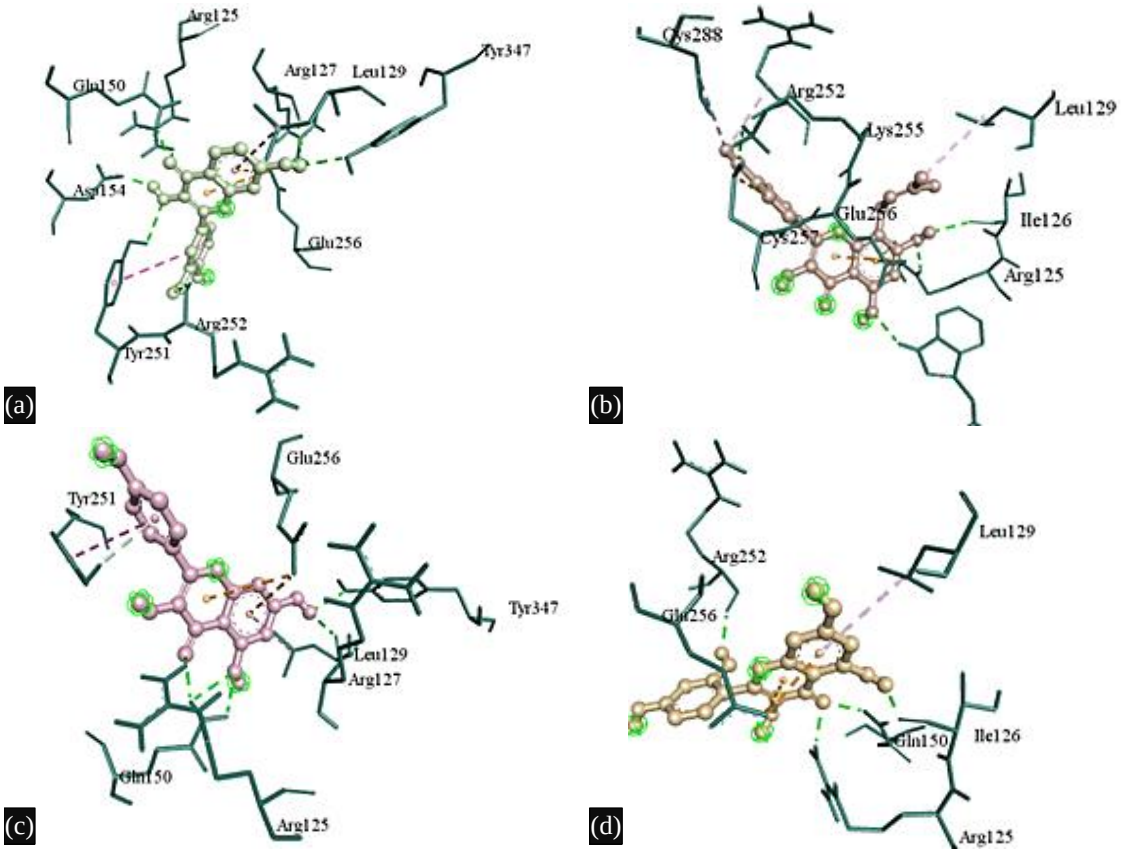


Figure 6. (a–f) Visualisation of molecular interactions of CYP2C8 with the top 6 ligands; (A) Baicalein - CYP2C8 (B) Diosmetin - CYP2C8 (C) Icaritin - CYP2C8 (D) Isorhamnetin - CYP2C8 (E) Quercetin - CYP2C8 (F) Tricetin - CYP2C8

Molecular Interactions of the Top Ligands with PKT2

As shown in Figure 7 PTK2 showed the maximum number of interactions including amino acids such as Arginine, Leucine, Isoleucine, Glutamine and Glutamic acid with the ligand (D) Morin with a binding energy of -8.4 (Table 2). (F) Quercetin-3'-O-phosphate showed the next highest interactions with amino acids Glutamic acid, Lysine, Tyrosine, Leucine, Arginine, Isoleucine, Glutamine, and Tyrosine with PTK2. PTK2 shared interactions with (A) Fisetin involving amino acids Arginine, Leucine, Tyrosine, Glutamic acid, Aspartic acid and Glutamine (C) Kaempherol involving amino acids Glutamic acid, Tyrosine, Leucine, Arginine, and Glutamine respectively. (B) Icritin interacted with Cysteine, Arginine, Lysine, Leucine, Glutamic acid, Isoleucine, and Tryptophan. PTK2 shared interactions with (E) Myricetin involving Aspartic acid, Cysteine, Glutamic acid, Glutamine, Arginine, and Tryptophan. Among all the amino acid interactions with the above ligands, Arginine was found to most commonly interact with all the ligands.



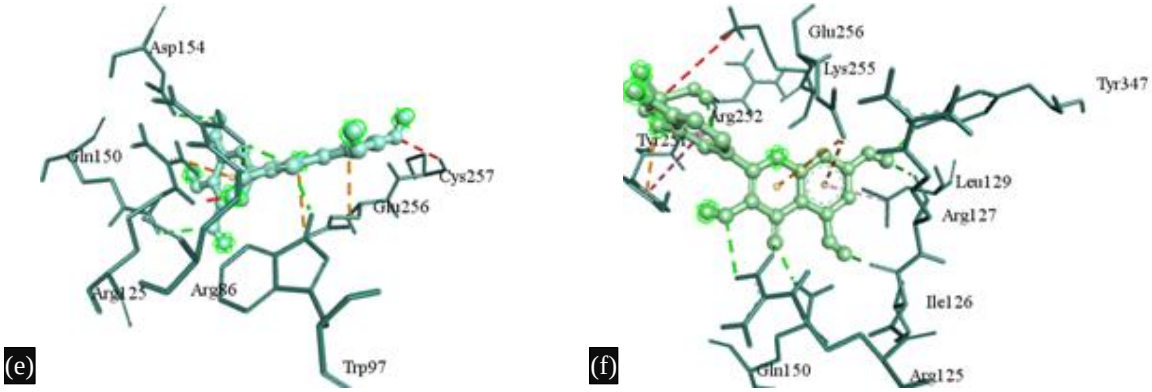


Figure 7. (a–f) Visualisation of molecular interactions of PTK2 with the top 6 ligands. (A) Fisetin - PTK2 (B) Icritin - PTK2 (C) Kaempherol - PTK2 (D) Morin - PTK2 (E) Myricetin - PTK2 (F) Quercetin-3'-O-phosphate -PTK2

ADMET Analysis

The 22 ligands were screened for their, physicochemical property (Table 3), medicinal chemistry property (Table 4), absorption property (Table 5), distribution (Table 6), and toxicity (Table 7) through a web tool called ADMETlab 2.0. The 13 top and conman ligands related to the target proteins are displayed the all the tables.

Table 3. Physicochemical properties of Isorhamnetin and its analogs

PubChemID	MW	nHA	nHD	nRot	nRing	MaxRing	nHet	nRig	Flex	TPSA	LogS
5281654	316.06	7	4	2	3	10	7	18	0.111	120.36	-3.748
5281691	316.06	7	4	2	3	10	7	18	0.111	120.36	-3.712
5281672	318.04	8	6	1	3	10	8	18	0.056	151.59	-3.665
5280343	302.04	7	5	1	3	10	7	18	0.056	131.36	-3.671
5281614	286.05	6	4	1	3	10	6	18	0.056	111.13	-3.704
5280863	286.05	6	4	1	3	10	6	18	0.056	111.13	-3.624
5281612	300.06	6	3	2	3	10	6	18	0.111	100.13	-3.704
5281670	302.04	7	5	1	3	10	7	18	0.056	131.36	-3.497
5281701	302.04	7	5	1	3	10	7	18	0.056	131.36	-3.59
5318980	368.13	6	3	4	3	10	6	19	0.211	100.13	-3.315
20833257	382.01	10	5	3	3	10	11	20	0.15	174.73	-3.433
44562555	368.13	6	3	4	3	10	6	19	0.211	100.13	-3.261
5281605	270.05	5	3	1	3	10	5	18	0.056	90.9	-3.441

MW: Molecular weight; *nHA:* Number of hydrogen bond acceptors; *nHD:* Number of hydrogen bond donors*; nRot:* Number of rotatable bonds; *nRing:* Number of rings; *MaxRing:* Number of atoms in the biggest ring; *nHet:* Number of heteroatoms; *nRig:*Number of rigid bonds: *Flex:* Flexibility; *TPSA:* Topological polar surface area; *logS:* The logarithm of aqueous solubility value; *logD:* The logarithm of the n-octanol/water distribution coefficients at pH=7.4; *logP:* The logarithm of the n-octanol/water distribution coefficient taken into consideration.

Table 4. Medicinal chemistry properties of Isorhamnetin and its analogs.

PubChem id	QED	PAINS	Lipinski	Fsp3	SA Score
5281654	0.572	0	Accepted	0.062	0.978
5281691	0.535	1	Accepted	0.062	0.98
5281672	0.371	1	Accepted	0	0.999
5280343	0.434	1	Accepted	0	0.997

5281614	0.511	1	Accepted	0	0.994
5280863	0.546	0	Accepted	0	0.993
5281612	0.672	0	Accepted	0.062	0.998
5281670	0.464	0	Accepted	0	0.998
5281701	0.434	1	Accepted	0	1
5318980	0.6	0	Accepted	0.19	0.315
20833257	0.421	0	Accepted	0	0.998
44562555	0.6	0	Accepted	0.19	0.991
5281605	0.591	1	Accepted	0	0.999

QED: A measure for drug-likeness based on the concept of desirability; *PAINS:* Pan Assay Interference Compounds; *Lipinski Rule of 5:* Molecular weight less than 500 Daltons, nHD<5, nHA<10, lipohilicity<4.15 and TPSA: 40-140 Å; *Fsp3:* the number of sp3 hybridized carbons/total carbon count; *SAScore:* Synthetic accessibility score.

Table 5. Absorption properties of Isorhamnetin and its analogs.

PubChemID	Caco-2	MDCK	Pgp-inh	Pgp-sub	HIA	F(30%)
5281654	-5.056	9.45E-06	0.008	0.042	0.024	0.978
5281691	-5.109	9.31E-06	0.032	0.013	0.02	0.98
5281672	-5.653	6.38E-06	0.004	0.006	0.035	0.999
5280343	-5.204	7.69E-06	0.004	0.005	0.014	0.997
5281614	-4.987	9.79E-06	0.005	0.008	0.009	0.994
5280863	-4.974	9.07E-06	0.004	0.011	0.008	0.993
5281612	-4.916	1.16E-05	0.004	0.965	0.032	0.998
5281670	-5.182	7.00E-06	0.005	0.007	0.012	0.998
5281701	-5.255	7.83E-06	0.003	0.107	0.107	1
5318980	-4.883	1.16E-05	0.599	0.01	0.021	0.315
20833257	-5.772	7.67E-06	0.009	0.002	0.163	0.998
44562555	-4.903	1.16E-05	0.126	0.522	0.12	0.991
5281605	-4.981	1.26E-05	0.068	0.157	0.018	0.999

Caco-2: Caco-2 Permeability; *MDCK:* Madin−Darby Canine Kidney cells (MDCK) Permeability; *Pgp-inh:* P-glycoprotein inhibitor; *Pgp-sub:* P-glycoprotein substrate; *HIA:* Human intestinal absorption; *F(30%):* the human oral bioavailability 30%

Table 6. Distribution properties of Isorhamnetin and its analogs.

PubChemID	BBB	PPB	VDss	Fu
5281654	0.005	96.23%	0.647	8.51%
5281691	0.005	96.30%	0.611	8.11%
5281672	0.006	92.77%	0.633	10.35%
5280343	0.008	95.50%	0.579	7.42%
5281614	0.009	97.04%	0.477	5.17%
5280863	0.009	97.86%	0.522	4.41%
5281612	0.007	96.04%	0.657	6.73%
5281670	0.011	96.25%	0.551	6.23%
5281701	0.006	92.23%	0.603	9.11%
5318980	0.004	93.59%	0.702	7.93%
20833257	0.007	95.55%	0.613	6.70%
44562555	0.002	91.46%	0.707	9.62%
5281605	0.013	98.99%	0.436	4.59%

BBB: Blood–brain barrier; *PPB:* Plasma protein binding; *VDss:* Volume Distribution; *Fu:* fraction unbound in plasma.

Table 7. Toxicity properties of Isorhamnetin and its analogs.

PubChemID	hERG	DILI	Ames	FDAMDD	Carcinogenicity	IGC50	LC50
5281654	0.061	0.978	0.596	0.462	0.047	4.28	5.158
5281691	0.067	0.979	0.693	0.471	0.058	4.438	5.364
5281672	0.145	0.982	0.482	0.557	0.028	3.885	4.982
5280343	0.099	0.98	0.657	0.31	0.05	4.231	5.222
5281614	0.043	0.978	0.73	0.259	0.147	4.714	5.305
5280863	0.07	0.979	0.672	0.109	0.097	4.386	5.223
5281612	0.044	0.861	0.508	0.742	0.055	4.344	5.075
5281670	0.157	0.979	0.616	0.386	0.035	4.189	5.27
5281701	0.112	0.936	0.392	0.86	0.034	4.005	4.989
5318980	0.009	0.982	0.701	0.048	0.09	4.637	6.163
20833257	0.023	0.979	0.433	0.905	0.029	4.309	4.698
44562555	0.011	0.941	0.521	0.303	0.13	4.772	5.884
5281605	0.044	0.958	0.497	0.084	0.318	4.016	4.767

hERG: The human ether-a-go-go related gene; *DILI:* Drug-induced liver injury; *AMES:* The Ames test for mutagenicity; *FDAMDD:* The maximum recommended daily dose, carcinogenicity, and 96-hour fathead minnow LC_{50} were examined.

DISCUSSION

Andral et al described the first case of LC secondary to uterine cancer in 1824. Within two months after developing respiratory symptoms, approximately 50% of patients die of PLC. Cancer cells multiply in the lymphatic system, causing obstructions in it. The condition, which is rare, is called lymphangitic carcinomatosis (LC). Pulmonary lymphangitis carcinomatosis (PLC) is a condition characterized by the spread of cancer cells through the lymphatic vessels of the lungs. This condition is most commonly associated with adenocarcinomas originating from the breast, lung, colon, stomach, pancreas, and prostate. Although PLC is typically restricted to the lungs, rare cases of non-pulmonary lymphangitic carcinomatosis have also been reported in other organs. Overall, the identification of the primary cancer site and early detection of PLC are crucial for effective management of this condition. In the literature, approximately 7.9% of PLCs had renal cancer. In most cases, dyspnea (shortness of breath) and dry cough are nonspecific and have a tendency to go undetected or delayed. In most cases, the disease is diagnosed after death, with a poor prognosis. If hypoxemia and progressive dyspnea are present without an obvious cause and there is a high suspicion of malignancy, then LC should be considered as a diagnosis [25].

Isorhamnetin has been demonstrated to have pharmacological effects in various studies including cardiovascular protection, anti-inflammation, anti-tumor, anti-oxidation, anti-bacterial, and anti-virus [9]. Therefore, for the current study phytocompounds of Isorhamnetin were investigated to assess their potential as LC drug candidates. Isorhamnetin is a flavonoid compound that is naturally present in a variety of plant-based foods. Parsley, green bell peppers, and dill are among the richest dietary sources of isorhamnetin, while romaine lettuce, Chinese cabbage, and pears contain lower concentrations of this compound. Isorhamnetin has gained significant attention in recent years due to its potential health benefits, including anti-inflammatory, anti-oxidant, and cardiovascular protective effects. Further research is needed to explore the full range of pharmacological properties of isorhamnetin and its potential applications in human health [10].

In PPI network pathway analysis, we found that AKT1, CYP2C8, and PTK2 are the major protein obtained from STRING database analysis. From the past research paper, we found that a pharmacological effect of isorhamnetin affects the signaling pathways and downstream factors such as NF- κB, PI3K/ AKT, MAPK, and other signaling pathways [9]. Based on the results of the current study, it is evident that the protein-protein interaction network in Figures 3 and 4 provided a clear

understanding of the identification of the target proteins implicated in LC, namely AKT1, CYP2C8, and PTK2. The interactions and their interactive value revealed the LC-target PPI network AKT1, CYP2C8, and PTK2 were identified as responsible for the pathogenesis of LC and Isorhamnetin.

The ligands Caflanone, Icaritin, Quercetin-3'-O-phosphate, Isorhamnetin, Morin, and Rhamnetin showed the highest binding affinity of -10.4,-10.2, -9.9, -9.7, -9.7, -9.7 in AKT1 whereas ligands Diosmetin, Isorhamnetin, Quercetin, Baicalein, Icaritin, and Tricetin showed the highest binding affinity of -9.1, -9, -9,-8.9, -8.9, -8.8 in CYP2C8 whereas ligands Morin, Quercetin-3'-O-phosphate, Fisetin, Myricetin, Icaritin, and Kaempherol showed the highest binding affinity of -8.4,-8.4,-8.3,-8.3,-8.2,-8.2 Hence, these ligands were chosen as the core ligands.

The interactions of these ligands with the target AKT1, CYP2C8, and PTK2 were studied by visualizing them in BIOVIA. An overall analysis of the 2D structure of the top 6 ligands involved in docking with AKT1 (Figure 5) revealed the implication of LEU, TRP, and ASP as the most common amino acids. Whereas the examination of the 2D structures of the top 6 ligands docked with CYP2C8 (Figure 6) indicated the presence of VAL and CYS as the most common amino acids. Finally, the analysis of the 2D structures of the top 6 ligands docked with PTK2 (Figure 7) demonstrated the presence of ARG, and GLU amino acids as the most prevalent amino acids.

Further AKT1, CYP2C8, and PTK2 were subjected to pharmacological studies for drug design.

In the current study, 22 phytocompounds from Isorhamnetin were used, and they were chosen because of their high binding energy. To evaluate their pharmacological potential, all 22 compounds underwent a rigorous screening process that involved assessing various parameters, including physicochemical properties, toxicity, number of hydrogen atom donors and acceptors, and molecular weight. The screening process was designed to identify the most promising compounds for further investigation, with the goal of identifying new drug candidates or pharmacological tools for basic research. The results of this study could contribute to the development of novel therapeutic agents with improved efficacy and safety profiles.

Lipinski's rule criteria: the number of hydrogen donors to be less than 5 and the number of hydrogen acceptors to be less than 10, with molecular weight in the range between 150–500g/mol. The results of the analyses showed that all of the selected compounds possessed druggable properties and passed Lipinski and ADMET analysis filters. These findings suggest that the compounds may have potential for further development as drug candidates. The successful identification of drug-like molecules with desirable properties could ultimately lead to the development of new therapeutic agents for the treatment of various diseases.

Icaritin has been shown to exhibit a variety of pharmacological and biological activities, including but not limited to [list some of the activities if relevant, e.g. anti-tumor, anti-inflammatory, antioxidant, etc.]. These properties make icaritin a promising candidate for further investigation and potential therapeutic development. Due to its use in breast cancer, hepatocellular carcinoma, lung cancer, oral squamous cell carcinoma, endometrial cancer, esophageal cancer, colorectal cancer, glioblastoma, ovarian cancer, and osteosarcoma [26] it may have therapeutic effects for LC. Therefore, it can be concluded that Icaritin has potential as a drug candidate for the treatment of LC. However, further investigation is required to fully understand its role in the treatment of LC. Also, some other ligands like Quercetin-3'-O-phosphate and Morin can also be considered for investigation and may be used in the treatment of LC.

CONCLUSION

LC is a rare condition in which cancer cells multiply in the lymphatic system, causing obstructions in it. Isorhamnetin is recognized for its biological properties, which include antioxidant, antiviral, anticancer, antimicrobial, and anti-inflammatory effects. The current study performed an in-silico

attempt to employ Isorhamnetin and its derivatives against LC and discovered that Icaritin and some others ligands like Quercetin-3'-O-phosphate, and Morin may be used in the treatment of LC.

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List of Abbreviations

LC	Lymphangitis carcinomatosis
PLC	Pulmonary Lymphangitic Carcinomatosis
AKT1	Ak strain transforming1(a serine/threonine protein kinase)
CYP2C8	Cytochrome P450, family 2, subfamily C, polypeptide 8
PTK2	Protein Tyrosine Kinase 2
PPI	Protein-Protein interaction

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