Molecular Docking-Based Evaluation of Quercetin as a Multi-Target Therapeutic Agent in Estrogen-Associated Gallbladder Carcinogenesis.

Thesis Submitted

in Partial Fulfilment of the Requirements for the Degree of

MASTERS OF SCIENCE

IN BIOTECHNOLOGY

by

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May, 2026

ACKNOWLEDGEMENT

I would like to express my gratitude to my supervisor, Dr. Navneeta Bharadvaja, for giving me the opportunity to do research and providing invaluable guidance throughout this research. Her dynamism, vision, sincerity, and motivation have deeply inspired me. She has motivated me to carry out the research and to present my work as clearly as possible. It was a great privilege and honour to work and study under her guidance. I am extremely grateful for what she has offered me. Her insightful feedback pushed me to sharpen my thinking and brought my work to a higher level.

I also take the opportunity to acknowledge the contribution of Prof. Yasha Hasija, Head of the Department of Biotechnology, Delhi Technological University, for allowing us to use the department facilities and for his full support and assistance during the development of the project. I would also like to acknowledge the contribution of all faculty members of the department for their cooperation and assistance during the development of the project.

I am highly thankful to Mr. Chhail Bihari and Mr. Jitendra Singh for their support. I am extremely grateful and wish to express my wholehearted thanks to respected lab seniors Ms. Harshita Singh, Mr. Sidharth Sharma, and Ms. Anuradha for their kind support. I would also like to express my gratitude to my parents for their love, prayers, care, and sacrifices in educating and preparing me for my future. I would also like to thank the institution, Delhi Technological University, Delhi, for providing me with opportunities throughout the tenure of my study.
Finally, my thanks go to my constant guide Harshita Singh for her help through the work and all the people who have supported me to complete the research work directly or indirectly.

Victoria Siro

CANDIDATE'S DECLARATION

I **Victoria Siro**, 24/MSCBIO/065, student of M.Sc. Biotechnology, hereby declares that the work which is being presented in the thesis entitled "**Molecular Docking-Based Evaluation of Quercetin as a Multi-Target Therapeutic Agent in Estrogen-Associated Gallbladder Carcinogenesis "**in partial fulfilment of the requirements for the award of the Degree of Master of science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from January 2026 to May 2026 under the supervision of Dr. Navneeta Bharadvaja.

Title of the paper: Multi target therapeutic potential of quercetin in estrogen-driven gall bladder pathogenesis: A network pharmacology and molecular docking
Author names: Victoria Siro, Teena Bhardwaj & Navneeta Bharadvaja
Name of conference: International conference on cognitive informatics engineering and technology -2026
Conference venue and date: Vidya Vikas college of engineering and technology autonomous Tiruchengode, Tamil Nadu, India (online mode), on 28 & 29 March, 2026.
Registration: done
Status of paper: Acceptance received
Date of paper acceptance: 27th March, 2026
The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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This is to certify that the student has incorporated all the corrections suggested by the examiner in the thesis and the statement made by the candidate is correct to the best of our knowledge.

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CERTIFICATE BY THE SUPERVISOR

Certified that Victoria Siro(24/MSCBIO/065) has carried out their search work presented in this thesis entitled "**Molecular Docking-Based Evaluation of Quercetin as a Multi-Target Therapeutic Agent in Estrogen-Associated Gallbladder Carcinogenesis**" for the award of Master of science from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

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Molecular docking-based evaluation of quercetin as a multi target therapeutic agent in estrogen associated gallbladder carcinogenesis

Victoria Siro

ABSTRACT

Gallbladder carcinoma (GBC) is an intense and highly invasive malignant cancer of the biliary tract associated with unfavourable prognosis and high mortality due to late-stage diagnosis. Chronic inflammation, oxidative stress, hormonal imbalance, and dysregulation of signaling pathways such as PI3K/AKT, MAPK, and NF-κB play major roles in the progression of estrogen-associated gallbladder carcinogenesis. Estrogen-mediated signaling further contributes to tumour development by promoting gallstone formation, abnormal cell proliferation, angiogenesis, and resistance to apoptosis. Considering the complex and multi-factorial nature of gallbladder cancer, there is a growing need for therapeutic agents capable of targeting multiple molecular pathways simultaneously. The present study aimed to evaluate the multi-target therapeutic potential of quercetin, a naturally occurring flavonoid, against estrogen-associated gallbladder carcinogenesis using molecular docking and network pharmacology approaches. Potential protein targets of quercetin were identified using SwissTargetPrediction, while gallbladder disease-associated genes were collected from GeneCards. Key hub proteins including AKT1, PIK3CA, EGFR, SRC, and BRAF were selected for further investigation. Molecular docking studies were performed using PyRx integrated with AutoDock Vina. Among the selected proteins, AKT1 exhibited the strongest binding affinity with quercetin, showing a docking score of approximately −9.7 kcal/mol, indicating stable and favourable interaction. Interaction analysis demonstrated the presence of multiple hydrogen bonds and hydrophobic interactions within the active binding site, confirming structural compatibility between quercetin and the target protein. Visualization studies using PyMOL and Discovery Studio further validated the docking results. The findings of this study suggest that quercetin possesses significant multi-target therapeutic potential by modulating critical signalling pathways involved in gallbladder carcinogenesis, particularly the PI3K/AKT pathway. Therefore, quercetin may serve as a promising natural compound for the development of future therapeutic strategies against estrogen-associated gallbladder cancer. Further in vitro, in vivo, and clinical studies are recommended to validate its therapeutic efficacy and safety.

Keywords: Gallbladder carcinoma; Quercetin; Molecular docking; PI3K/AKT pathway; Estrogen signalling; Network pharmacology; AKT1; Flavonoids; Autodocking Vina; Multi-target therapy; Gallbladder cancer; Protein–ligand interaction; Computational biology; Anticancer activity; Therapeutic targets

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List of abbreviations

Abbreviation	Full Form
ADME	Absorption, Distribution, Metabolism and Excretion
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
Akt	Protein Kinase B
Å	Angstrom
Abbreviation	Full Form
ATP	Adenosine Triphosphate
AutoDock Vina	Automated Docking Virtual Screening Software
Bax	Bcl-2 Associated X Protein
Bcl-2	B-Cell Lymphoma 2
CA 19-9	Carbohydrate Antigen 19-9
CEA	Carcinoembryonic Antigen
CT	Computed Tomography
CYP19A1	Cytochrome P450 Family 19 Subfamily A Member 1
DNA	Deoxyribonucleic Acid
EGFR	Epidermal Growth Factor Receptor
ER	Estrogen Receptor
ESR2	Estrogen Receptor Beta
GBC	Gallbladder Cancer / Gallbladder Carcinoma

HMG-CoA	3-Hydroxy-3-Methylglutaryl-Coenzyme A
IL-6	Interleukin-6
INSR	Insulin Receptor
kcal/mol	Kilocalorie per Mole
MAPK	Mitogen-Activated Protein Kinase
MRI	Magnetic Resonance Imaging
mTOR	Mechanistic Target of Rapamycin
NF-κB	Nuclear Factor Kappa-B
OIA	Office of International Affairs
PDB	Protein Data Bank
PI3K	Phosphoinositide 3-Kinase
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
PyMOL	Python Molecular Graphics System
PyRx	Python Prescription Virtual Screening Tool
ROS	Reactive Oxygen Species
SDF	Structure Data File
SMILES	Simplified Molecular Input Line Entry System
SRC	Proto-Oncogene Tyrosine-Protein Kinase Src
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
TBtools	Toolkit for Biologists Integrating Various Biological Data Handling Tools
TNF-α	Tumor Necrosis Factor Alpha
WHO	World Health Organization

Molecular Docking-Based Evaluation of Quercetin as a Multi-Target Therapeutic Agent in Estrogen-Associated Gallbladder Carcinogenesis.

Chapter 1 - Introduction

Gallbladder cancer (GBC) is a highly invasive malignancy of the biliary system, an organ responsible for storing bile juice and aiding in the digestion and absorption of fats in the small intestine. Unfortunately, this disease is often diagnosed in its late stages and is associated with a notably poor prognosis (1,2). Among these risk factors, estrogen plays a pivotal and distinct role in gallbladder pathogenesis. Estrogen significantly alters cholesterol metabolism through the activation of HMG-CoA reductase, which increases the cholesterol supersaturation of bile. This process enhances mucin-mediated crystal nucleation and impairs gallbladder motility, ultimately resulting in gallstone formation and biliary stasis. Beyond stone formation, estrogen receptor-mediated signalling activates both the PI3K/AKT and MAPK pathways. This activation initiates cellular proliferation, anti-apoptosis, and angiogenesis, facilitating the epithelial transformation and multistep carcinogenesis that ultimately contribute to the development of gallbladder cancer (9,11).

Quercetin, a naturally occurring bioactive flavonoid, presents a promising solution. It is widely recognized for its significant anti-inflammatory, antioxidant, and anti-cancer properties, notably demonstrating the ability to target multiple biological pathways at once (12,13,19). However, its specific multi-target therapeutic efficacy and underlying mechanisms in the context of estrogen-driven gallbladder pathogenesis remain poorly understood.

Gallbladder cancer (GBC) is an aggressive malignancy arising from the epithelial lining of the gallbladder and represents the most common cancer of the biliary tract, although it remains relatively rare globally. The incidence of GBC shows marked geographic variation, with high prevalence reported in regions such as northern India, Chile, and parts of East Asia, suggesting a strong association with environmental, dietary, and genetic factors.

Increasing age is another significant risk factor, with most cases occurring after the age of 50. Chronic inflammation of the gallbladder, particularly due to gallstones (cholelithiasis), is the most important predisposing factor, leading to continuous epithelial irritation and dysplasia. Additional risk factors include obesity, infections (e.g., *Salmonella typhi* carriers), porcelain gallbladder, exposure to carcinogens, and certain genetic predispositions (6,7).

Due to its asymptomatic nature in early stages and lack of specific biomarkers, GBC is often diagnosed at advanced stages, contributing to its poor prognosis and high mortality rate (1,2).

Objectives of the Study

Primary Objective

1. To evaluate the multi-target therapeutic potential of quercetin in estrogen-associated gallbladder carcinogenesis using molecular docking approach.

Secondary Objectives

2. To investigate the binding interactions and affinity of quercetin with key target proteins using molecular docking, thereby validating its potential role in modulating critical signaling pathways.

Chapter 2- Literature review

Gallbladder carcinoma (GBC) is a malignant tumour arising from the epithelial lining of the gallbladder. It is the most common cancer of the biliary tract and is known for its aggressive nature and poor prognosis, mainly because it is often diagnosed at an advanced stage. Gallbladder carcinoma is a primary cancer that originates in the mucosal cells of the gallbladder, most commonly as adenocarcinoma (≈90–95%) (1,2).

Adenocarcinoma forms gland-like structure and secrete mucus or from secretory structures from the glandular epithelial cells, it is developed in many organs that have glandular tissues and gall bladder is one of the organs. It is a malignant tumor and there is dysregulation with the major three pathways PI3K/Akt, MAPK and NF-κB which results in faster growth of tumor and uncontrolled cell proliferation. Targeting this pathway with quercetin can help restore control (18). It has a higher incidence in regions such as Northern India, Chile, and Japan, and is strongly associated with gallstones (cholelithiasis), which represent the most important risk factor. Other contributing factors include chronic inflammation such as chronic cholecystitis, porcelain gallbladder (calcification of the gallbladder wall), gallbladder polyps larger than 1 cm, infections like *Salmonella typhi*, and lifestyle factors such as obesity and a high-fat diet (6,7).

The development of gallbladder cancer usually happens in a gradual, step-by-step manner starting with long-term irritation of the gallbladder lining, most commonly due to gallstones. These stones repeatedly injure the inner lining, causing chronic inflammation, which forces the normal cells to adapt for survival. As a result, the normal gallbladder epithelial cells change into a different type of cells (called metaplasia), which are better able to tolerate stress but are more unstable. Over time, these altered cells begin to show abnormal features such as irregular shape, enlarged nuclei, and loss of normal organization—this stage is called dysplasia and is considered precancerous. With continued damage and accumulation of genetic mutations, these dysplastic cells lose control over their growth and start invading surrounding tissues, leading to carcinoma (cancer) (3).

Microscopically, most gallbladder cancers are adenocarcinomas, meaning they arise from gland-forming cells, while less commonly they can be squamous cell carcinomas or adenosquamous carcinomas, which tend to be more aggressive (3).

Clinically, early stages are often asymptomatic, but as the disease progresses, patients may present with right upper abdominal pain, nausea, vomiting, jaundice (a late sign), weight loss, and sometimes a palpable mass in advanced stages. Diagnosis is primarily based on imaging techniques such as ultrasound, followed by CT scan or MRI, with tumor markers like CA 19-9 and CEA providing supportive but non-specific information; definitive diagnosis is confirmed by biopsy. The prognosis is generally poor due to late detection, with a low 5-year survival rate, although outcomes improve significantly if the cancer is identified early and surgically removed (1,2,3).

2.2 Relation with Gallbladder Carcinoma and Estrogen

Estrogen increases the risk of gallbladder carcinoma indirectly, mainly by promoting gallstone formation and chronic inflammation. Estrogen plays a particularly significant role in driving the molecular mechanisms of gallbladder cancer. Estrogen alters cholesterol metabolism by activating the enzyme HMG-CoA reductase, which leads to the cholesterol supersaturation of bile. This promotes mucin-mediated crystal nucleation and impairs normal gallbladder motility, ultimately resulting in gallstone formation and biliary stasis (9).

Beyond the physical formation of stones, estrogen receptor-mediated signaling actively drives cancer progression by stimulating the PI3K/AKT and MAPK pathways. This activation promotes abnormal cell proliferation, prevents natural cell death (anti-apoptosis), and encourages the formation of new blood vessels (angiogenesis), all of which facilitate epithelial transformation and multistep carcinogenesis (10).

Gallbladder cancer occurrence is higher In women, these gallstones lead to chronic inflammation of the gallbladder (chronic cholecystitis), which can progress to carcinoma, and the disease is often detected relatively earlier. In contrast, men have a lower incidence because they lack this estrogen-related mechanism; their cases are more often linked to

non-hormonal factors such as chronic inflammation or infections, and they typically present later with more aggressive disease and a poorer prognosis (6,7).

2.3 Quercetin as an Anticancer Compound

Quercetin is a natural plant-derived flavonoid with significant anticancer potential, primarily due to its ability to act through multiple biological mechanisms. It promotes apoptosis (programmed cell death) in cancer cells by regulating proteins like Bax and Bcl-2, inhibits uncontrolled cell division by inducing cell cycle arrest, and reduces oxidative damage through its antioxidant action against Reactive Oxygen Species. Additionally, it suppresses inflammation by inhibiting pathways such as NF-κB, and prevents tumor growth by blocking angiogenesis and metastasis (12,13).

Although most evidence comes from laboratory and animal studies, quercetin is widely regarded as a promising chemopreventive agent, supporting cancer prevention rather than serving as a standalone therapeutic drug (13).

Research over the years has increasingly focused on Quercetin as a promising natural anticancer agent. In various studies, scientists observed that quercetin can push cancer cells toward apoptosis, essentially triggering a self-destruct mechanism that prevents their uncontrolled growth. As the investigations deepened, it became clear that this compound does more than just kill cancer cells—it also slows down and suppresses overall tumor development. A key part of this effect lies in how quercetin interferes with major cellular signaling systems, particularly the PI3K/AKT signaling pathway, MAPK signaling pathway, and the inflammatory regulator NF-κB. By dampening these pathways, which normally help cancer cells survive, multiply, and spread, quercetin effectively disrupts the internal machinery that tumors rely on (15,19).

2.4 Quercetin and PI3K-AKT-mTOR Signaling

The Role of PI3K/AKT in Gallbladder Cancer: In estrogen-driven gallbladder pathogenesis, estrogen receptor-mediated signaling activates the PI3K/AKT pathway. This activation drives

abnormal cell proliferation, prevents natural cell death (anti-apoptosis), and encourages angiogenesis, facilitating carcinogenesis (19).

mTOR stands for “Mechanistic Target Of Rapamycin.” It is a protein inside cells that acts like a master regulator of growth and metabolism.

Quercetin's Therapeutic Modulation: Quercetin demonstrates significant therapeutic potential by modulating the PI3K/AKT pathway. By actively targeting this signaling cascade, quercetin helps reduce cellular proliferation and induces apoptosis (13,16).

Identification of Key Targets: Network pharmacology analysis reveals that genes associated with PI3K/AKT signaling, specifically AKT1 and PIK3CA, are major hub genes overlapping between quercetin targets and gallbladder disease mechanisms (17).

Strong Binding Affinity: Molecular docking studies show strong binding affinity of quercetin with PIK3CA and AKT1 (20).

2.5 Estrogen Receptor and PI3K/AKT Crosstalk

“Estrogen receptor and PI3K/AKT crosstalk” means that the estrogen receptor and PI3K/AKT pathway interact and influence each other. When estrogen binds to its receptor, it activates signals that promote cell growth and also activates PI3K/AKT. At the same time, PI3K/AKT can activate estrogen receptors even without estrogen, forming a continuous loop that enhances tumor growth and survival (30,31,32,33).

2.6 Quercetin in Hormone-Related Disorders and Cancer

Acting as a phytoestrogen, it can bind to estrogen receptors and influence proliferation and apoptosis (34). It also inhibits PI3K activity and reduces AKT phosphorylation, thereby preventing abnormal activation of estrogen signaling. Additionally, quercetin regulates downstream targets like mTOR and apoptotic proteins, promoting apoptosis and inhibiting tumor growth (34).

2.7 Flavonoids Targeting PI3K/Akt/mTOR

Flavonoids such as quercetin, kaempferol, luteolin, and apigenin act as modulators of the PI3K/Akt/mTOR pathway. These compounds inhibit kinase activity, reduce phosphorylation events, and regulate downstream targets, resulting in reduced tumor growth and increased apoptosis (35).

They also exhibit antioxidant and anti-inflammatory effects by reducing ROS and inhibiting NF-κB signaling, further contributing to anticancer activity (35).

2.8 Docking Methodology

Computational approaches for docking molecular compounds are used to predict ligand–protein interactions. It involves conformational sampling and scoring functions to estimate binding affinity (37).

The workflow includes protein preparation, ligand preparation, active site identification, docking simulation, and interaction analysis. Tools such as PyRx and AutoDock Vina are commonly used for docking studies (44).

Chapter 3 - Methodology

3.1 Computational resources and database used

This thesis aims to explore a methodological approach using molecular docking analysis for evaluating the therapeutic potential of quercetin in gallbladder disease.

3.1.1 Database and tool Utilized:

Chemical and Ligand Databases:

A). PubChem: (**https://pubchem.ncbi.nlm.nih.gov/**) used for Retrieval of 3D structure of quercetin and acquisition of SDF format and SMILES notation. Application is widely used as the primary source for ligand preparation (47).

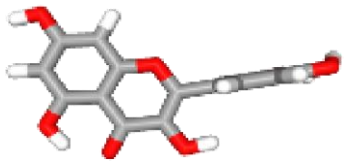


Fig.1: 3D image (ball and stick) of quercetin obtained from pubchem. Co-ordinates are also downloaded in sdf format.

B). Structural Databases

Protein Data Bank (PDB):(https://www.rcsb.org/) Retrieval of 3D structures of target proteins (e.g., AKT1, PIK3CA, INSR). Here AKT1 is chosen as the protein target with the PDB ID-3O96.

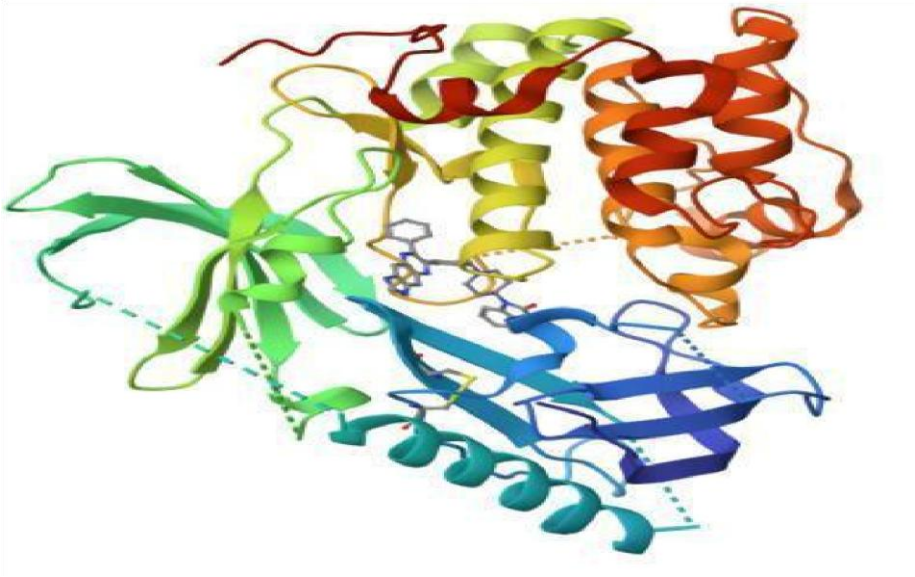


Fig.2: 3D structure of AKT1(PDB ID-3O96) retrieved from protein data bank.

C). Target Prediction Tools : SwissTargetPrediction : (http://www.swisstargetprediction.ch/) Prediction of potential protein targets of quercetin.

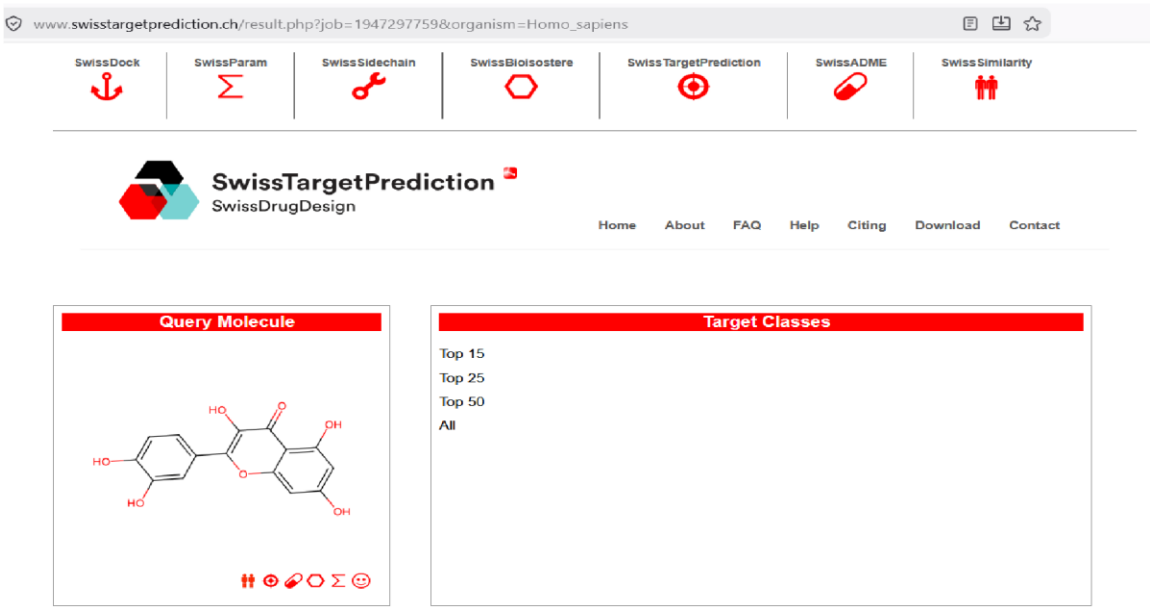


Fig. 3: SwissTargetPediction

C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O (smile notation) from the pubchem to the SwissTargetPrediction website (https://www.swissadme.ch/index.php#).

Parameters used are -Organism: *Homo sapiens, and*

Output:List of probable targets based on chemical similarity.

PyRx: Virtual screening and molecular docking. It is an user-friendly interface and Integration of AutoDock Vina. This tool is used in this thesis for docking purposes as it is simpler, faster and more user friendly (37,44).

AutoDock Vina (alternatively used for this study): Docking simulations give an output as binding affinity values in kcal/mol and binding energy. Their role is to predict ligand–protein interactions to show its affinity and energy (44).

E). Visualization Tools

PyMOL: Visualization of docking results and structural analysis of protein–ligand complexes (37).

Discovery Studio: Detailed interaction analysis and Identification of Hydrogen bonds and Hydrophobic interactions (20,37).

3.1.2 Software Utilized

PyMOL:

PyMOL is a widely used molecular visualization tool in computational biology and structural bioinformatics. It enables detailed visualization of protein–ligand complexes, allowing researchers to analyze structural conformations and binding interactions. In this study, PyMOL was used to visualize docking results and examine the spatial orientation of quercetin within the active sites of target proteins (37).

PyRx (with AutoDock Vina):

PyRx is an integrated virtual screening software that provides a user-friendly graphical interface for molecular docking studies. It incorporates AutoDock Vina as its docking engine, which utilizes a scoring function and efficient optimization algorithms to predict ligand–protein binding affinities. In this study, PyRx was employed for ligand preparation, energy minimization, and docking simulations, while AutoDock Vina calculated binding energies and generated multiple binding conformations (37,44).

Discovery Studio:

Discovery Studio is a comprehensive molecular modeling and visualization software used for analyzing protein–ligand interactions. It provides detailed insights into binding mechanisms, including hydrogen bonding, hydrophobic interactions, and other non-covalent interactions. In this study, it was used to validate docking results and analyze interaction profiles of quercetin with target proteins (20,37).

3.2 Objective

3.2.1 Objective 1: Evaluation of Multi-Target Therapeutic Potential of Quercetin

Data Extraction

The 3-dimensional (3D) structure of the bioactive compound quercetin was retrieved from the PubChem database in SDF format. The canonical SMILES notation of quercetin was also obtained for further analysis. To identify potential protein targets of quercetin, the SMILES notation was submitted to the SwissTargetPrediction web server with Homo sapiens selected as the target organism. Additionally, disease-associated genes related to gallbladder carcinoma and estrogen signaling were collected from the GeneCards database using relevant keywords. The retrieved genes were filtered based on their relevance score to ensure strong association with disease pathogenesis (47,48,36).

Identification of Common Targets

To identify potential therapeutic targets, the predicted targets of quercetin were intersected with gallbladder disease-associated genes. This intersection analysis was performed using TBtools-II software, which generated a Venn diagram to identify overlapping genes. These common targets were considered as candidate targets through which quercetin may exert its therapeutic effects (36).

Target Protein Preparation

The 3D structures of selected hub proteins such as AKT1, EGFR, and PIK3CA were retrieved from the Protein Data Bank (PDB). The protein structures were prepared by removing water molecules and any co-crystallized ligands. Hydrogen atoms were added, and the structures were optimized for docking. The prepared protein structures were saved in appropriate formats for further docking analysis (50).

Ligand Preparation

The ligand molecule (quercetin) obtained from PubChem was prepared using PyRx software. Energy minimization was performed to achieve a stable conformation. The ligand

file was then converted into the required docking format, ensuring proper torsion and charge assignment (44).

Molecular Docking Studies

Molecular docking was carried out using PyRx integrated with AutoDock Vina. The prepared protein was set as the receptor, and quercetin was treated as a flexible ligand. A defined grid box is created around the active site of the protein ensuring accurate docking. The docking simulation generated multiple binding conformations, each associated with a binding affinity score. Lower binding energy values indicated stronger ligand–protein interactions (37,44).

Analysis and Visualization

The docking results were analyzed based on binding affinity values and interaction profiles. The best docking poses were selected and visualized using PyMOL and Discovery Studio software(20,37). Here interactions such as hydrogen bonding strength and bond number can be viewed for further confirmation other than binding affinity.

3.2.2 Objective 2: Molecular Docking-Based Validation of Target Proteins

Selection of Target Proteins

Key hub proteins identified from the network analysis were chosen for docking studies. The selection was based on their central role in pathways such as PI3K/AKT and estrogen signaling and taken from swisstargetPrediction, where potential targets are predicted (18).

Protein Structure Retrieval and Preparation

The 3D structures of selected proteins were retrieved from the Protein Data Bank (PDB). The structures were prepared by removing water molecules and co-crystallized ligands, followed by the addition of hydrogen atoms and optimization of the protein structure to ensure stability for docking (50).

Ligand Preparation

The quercetin ligand was prepared using PyRx software. Energy minimization was carried out to obtain a stable conformation, and the ligand file was converted into the required docking format with appropriate charge assignments (44).

Docking Simulation

Molecular docking simulations were performed using PyRX integrated with AutoDock Vina. The prepared protein structures were set as receptors, and quercetin was treated as a flexible ligand. Multiple docking poses were generated along with binding affinity scores (37,44).

Analysis of Docking Results

Docking results were evaluated based on binding affinity values (kcal/mol), where lower values indicated stronger binding interactions. The best docking conformations were selected for further analysis (37).

Visualization of Protein–Ligand Interactions

The selected docking poses were visualized using PyMOL and Discovery Studio. These tools were used to analyze hydrogen bonding, hydrophobic interactions, and other non-covalent interactions between quercetin and target proteins. This analysis helped validate the binding stability and therapeutic potential of quercetin (20,37).Chapter 4-

Results and Discussion

4.1 Results and Discussion for Objective 1

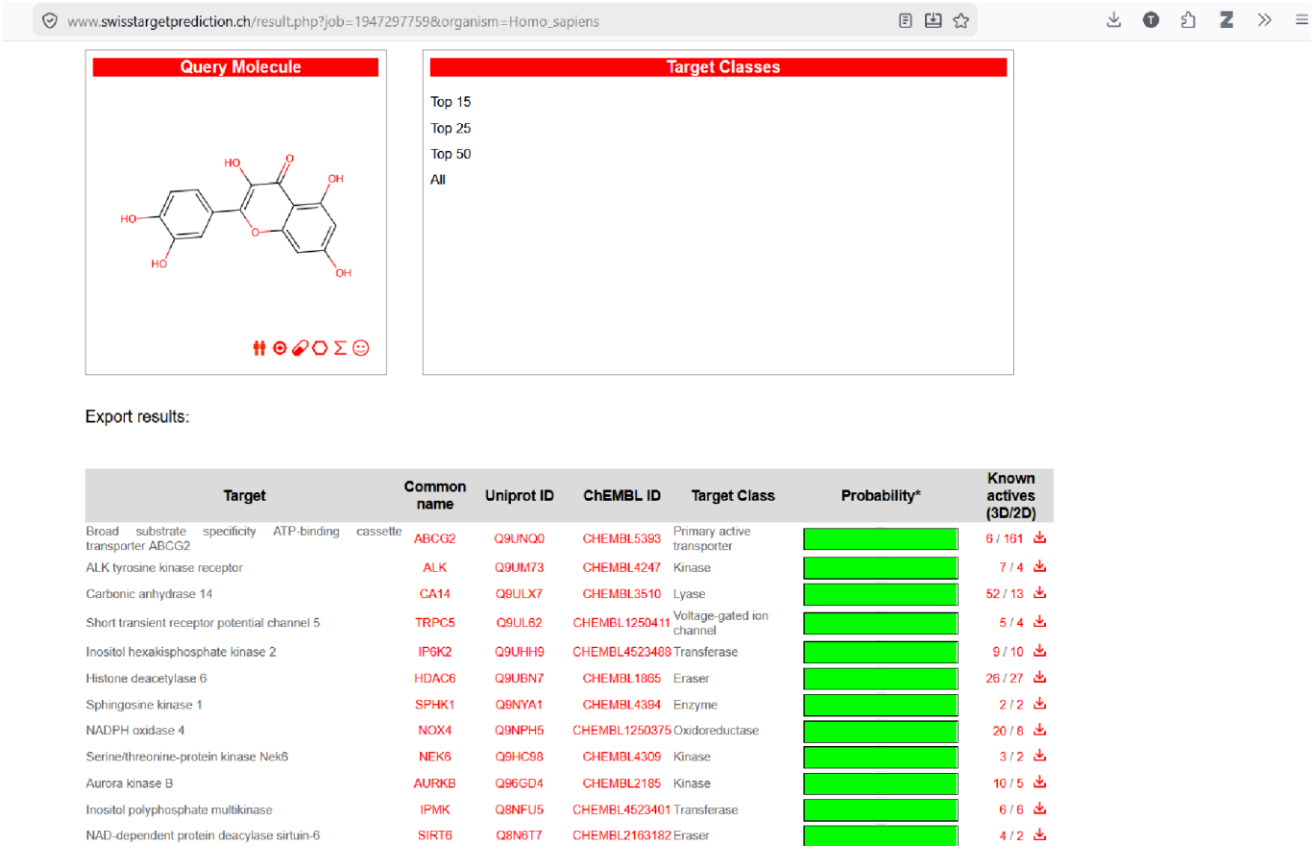
Evaluation of the Multi-Target Therapeutic Potential of Quercetin

4.1.1 Results

Identification of Potential Targets

The target prediction analysis of quercetin using SwissTargetPrediction resulted in a total of 96 potential protein targets. Concurrently, 102 gallbladder disease-associated genes were retrieved from the GeneCards database based on relevance scores. Intersection analysis

between these datasets identified 7 overlapping genes, which were considered as potential therapeutic targets involved in estrogen-associated gallbladder carcinogenesis.



Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Broad substrate specificity ATP-binding cassette transporter ABCG2	ABCG2	Q9UNQ0	CHEMBL5393	Primary active transporter		6 / 161
ALK tyrosine kinase receptor	ALK	Q9UM73	CHEMBL4247	Kinase		7 / 4
Carbonic anhydrase 14	CA14	Q9ULX7	CHEMBL3510	Lyase		52 / 13
Short transient receptor potential channel 5	TRPC5	Q9UL62	CHEMBL1250411	Voltage-gated ion channel		5 / 4
Inositol hexakisphosphate kinase 2	IP6K2	Q9UHH9	CHEMBL4523488	Transferase		9 / 10
Histone deacetylase 6	HDAC6	Q9UBN7	CHEMBL1865	Eraser		26 / 27
Sphingosine kinase 1	SPHK1	Q9NYA1	CHEMBL4394	Enzyme		2 / 2
NADPH oxidase 4	NOX4	Q9NPH5	CHEMBL1250375	Oxidoreductase		20 / 8
Serine/threonine-protein kinase Nek6	NEK6	Q9HC98	CHEMBL4309	Kinase		3 / 2
Aurora kinase B	AURKB	Q96GD4	CHEMBL2185	Kinase		10 / 5
Inositol polyphosphate multikinase	IPMK	Q8NFU5	CHEMBL4523401	Transferase		6 / 6
NAD-dependent protein deacylase sirtuin-6	SIRT6	Q8N6T7	CHEMBL2163182	Eraser		4 / 2

Fig.4: SwissTargetPrediction of Quercetin showing 90 potential protein targets.

In the export results section, there are 100 potential protein targets and the “Probability” column indicates how likely the molecule is to interact with that protein. A value close to **1.0** means a very strong prediction based on similarity to known bioactive compounds. For the whole information of the exported result, the top hit along with the known active 3D/2D can be seen in the URL link https://www.swisstargetprediction.ch/result.php?job=1947297759&organism=Homo_sapiens

From these target predictions the biologically important cancer-related targets also known as hub genes are selected, they are AKT1, PIK3CA, EGFR, BRAF and SRC.For gallbladder cancer / PI3K-AKT pathway work, the AKT1 target is prioritized.

4.2 Results and Discussion for Secondary Objectives.

4.2.1 Molecular Docking Analysis (Objective 2)

Molecular docking studies were performed to validate the interaction between quercetin and selected hub proteins. Docking simulations using PyRx integrated with AutoDock Vina generated multiple binding conformations.

Among the selected targets, AKT1 showed the strongest binding affinity with quercetin, with a docking score of approximately −9.7 kcal/mol. Other proteins such as EGFR, PIK3CA, and INSR also demonstrated favorable binding affinities.

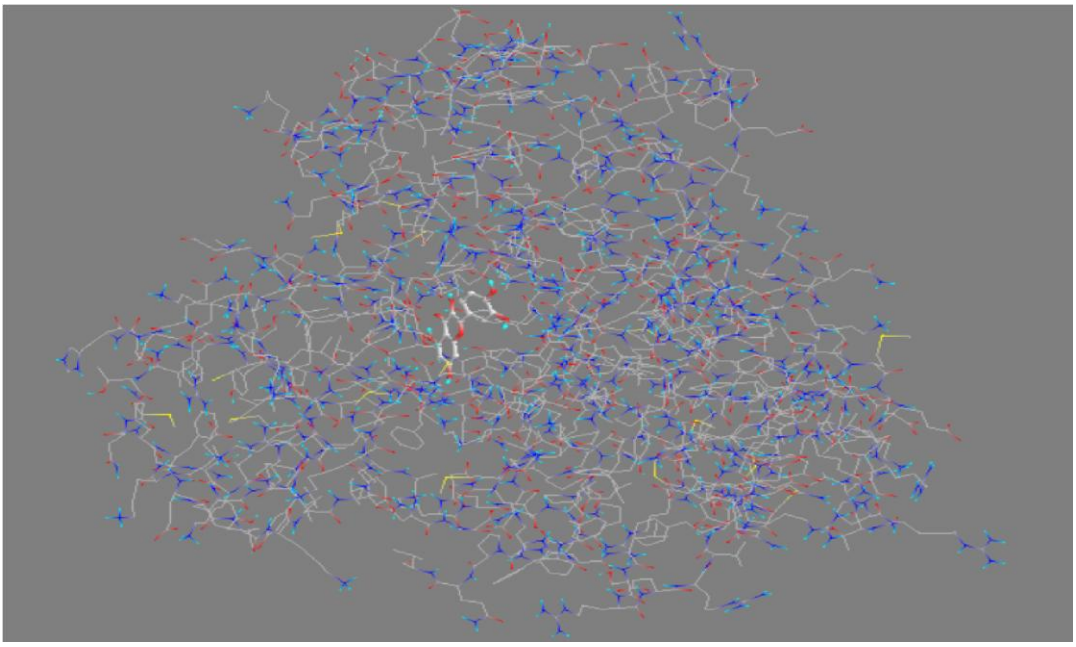


Fig.5: Snapshot of protein-ligand interaction of quercetin and 3096 (AKT1) target .

The interaction analysis revealed: Formation of hydrogen bonds with key amino acid residues, strong hydrophobic interactions within the binding pocket and Stable ligand orientation within the active site.

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
3096_3096ligand_uff_E=379.98	-9.7	0	0
3096_3096ligand_uff_E=379.98	-9.6	3.415	1.775
3096_3096ligand_uff_E=379.98	-9.6	5.133	2.74
3096_3096ligand_uff_E=379.98	-9.4	3.936	2.122
3096_3096ligand_uff_E=379.98	-8.9	6.768	2.367
3096_3096ligand_uff_E=379.98	-8.8	9.014	5.226
3096_3096ligand_uff_E=379.98	-8.7	5.809	3.675
3096_3096ligand_uff_E=379.98	-8.5	12.545	7.838
3096_3096ligand_uff_E=379.98	-7.9	12.882	9.605

Table 1: From PyRx showing binding affinities between quercetin and AKT1, lower the negative value stronger is the binding affinity. -9.7kcal/mol has the highest binding affinity showing strong interaction between the target protein and the ligand.

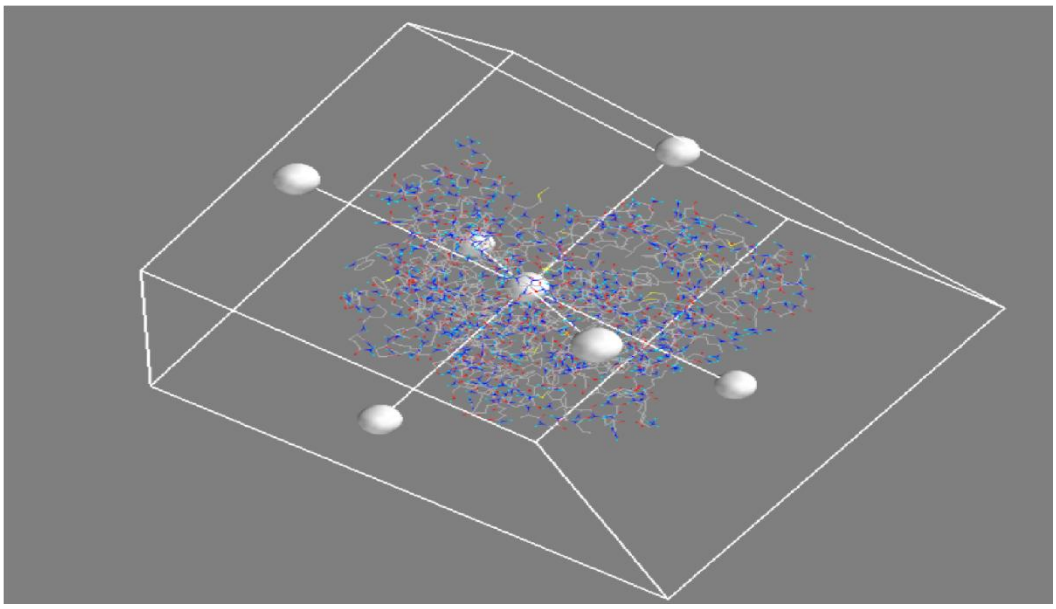


Fig.6: Grid box generation with center X: 7.1909, Y: -3.4246 and Z: 11.3926, and Dimensions (Angstrom) X: 65.4634, Y: 80.3505 and Z: 73.6662. Making sure the macromolecule and ligand is inside the box.

Fig.7: visualization of protein target (pink) and 3O96/ AKT1 in blue showing interaction through PyMoL. Ligand is inside the cavity (binding site), not floating outside and properly oriented.

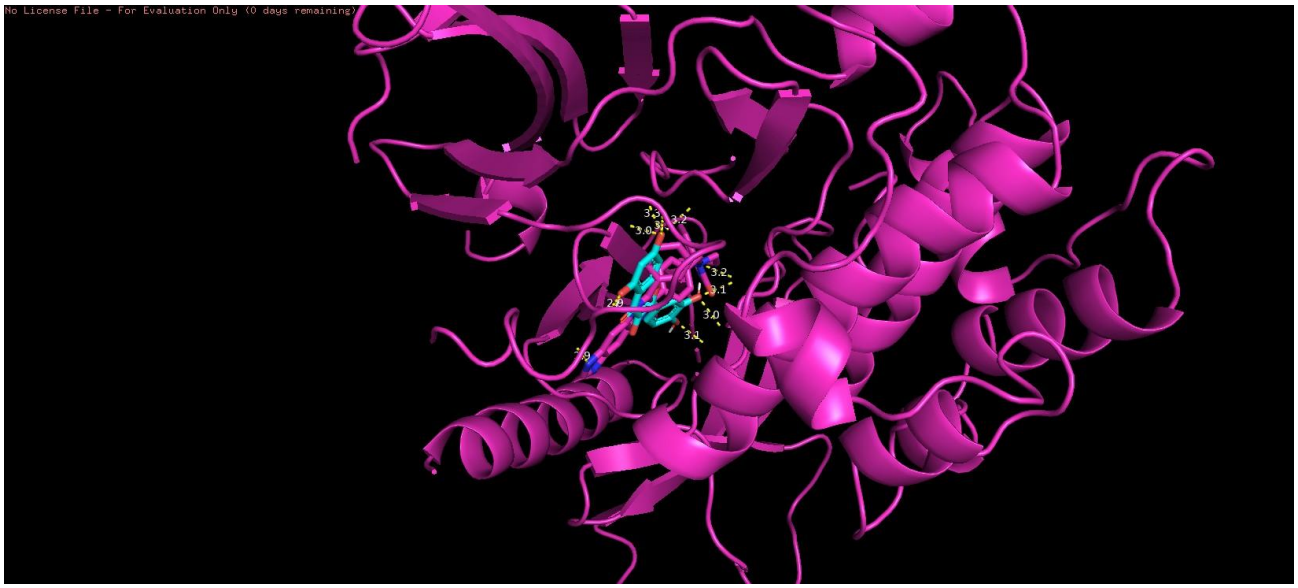


Fig.8: There are 10 Hydrogen bonds having distances of 2.9,2.9,3.0,3.0,3.1,3.1,3.2,3.23.3 and 3.3 Å. Showing strong bonding interaction with strong H-bong strength.

These results indicate a strong and stable interaction between quercetin and the selected target proteins.

4.2.2. Validation of Therapeutic Potential through Docking

The molecular docking results provide strong evidence supporting the therapeutic potential of quercetin. The high binding affinity observed with key target proteins, particularly AKT1, indicates that quercetin can effectively interact with and potentially inhibit these proteins (19,20). The presence of stable hydrogen bonding and hydrophobic interactions further confirms the structural compatibility of quercetin with the active sites of these proteins. Such interactions are critical for effective inhibition of protein function and subsequent modulation of signalling pathways (20,37).By targeting proteins involved in PI3K/AKT and estrogen signalling pathways, quercetin may regulate key processes such as cell proliferation, apoptosis, and inflammation. This multi-target interaction profile enhances its potential as a therapeutic agent compared to conventional drugs that typically act on a single target (13,19,34).

CHAPTER – 5

CONCLUSION, FUTURE PERSPECTIVES AND SOCIAL IMPACT

5.1 Conclusion

The present study successfully employed a molecular docking approach to evaluate the multi-target therapeutic potential of quercetin in estrogen-associated gallbladder carcinogenesis. The findings highlight the complex molecular mechanisms underlying gallbladder cancer and emphasize the importance of targeting multiple pathways for effective treatment (19,20,36). Through the SwissTargetPrediction tool which acts as the network pharmacology tool, a total of seven overlapping gene targets were identified between quercetin and gallbladder disease-associated genes. Among these, key hub genes such as EGFR, AKT1, SRC, ESR2, and CYP19A1 were found to play crucial roles in disease progression. These genes are involved in critical biological processes including cell proliferation, apoptosis, inflammation, and hormone-mediated signalling
(36,48). Functional enrichment analysis further revealed that these targets are significantly associated with major signalling pathways such as the PI3K/AKT pathway, estrogen signalling pathway, and cancer-related pathways, which are known to contribute to tumour growth and progression (18,19). The presence of stable hydrogen bonding and hydrophobic interactions confirmed the structural compatibility and potential inhibitory action of quercetin on these targets (20,37). Overall, the results indicate that quercetin exhibits significant potential as a multi-target therapeutic agent, capable of modulating key molecular pathways involved in estrogen-driven gallbladder carcinogenesis. This study provides a strong computational foundation for further experimental validation and drug development (13,19).

5.2 Future Perspectives

Although the present study provides valuable insights, it is primarily based on dry lab analysis, and further research is required to validate and expand these findings. Future studies should focus on validating the findings of this research through multiple experiments such as wet labs and clinical approaches. Initially, in vitro studies using appropriate cell lines are necessary to confirm the biological activity and anti-cancer effects of quercetin. Furthermore, clinical investigations are essential to assess the effectiveness and applicability of quercetin in human subjects suffering from gallbladder carcinoma (13,21).In addition to validation, emphasis should also be placed on the development of advanced drug delivery systems. Exploring combination therapy approaches, where quercetin is used alongside existing chemotherapeutic agents, may improve treatment outcomes and help reduce drug resistance (29). Lastly, further computational and experimental studies should be conducted to identify additional molecular targets and signaling pathways influenced by quercetin, thereby expanding its therapeutic potential (36).

5.3 Social Impact

Gallbladder cancer remains a highly aggressive malignancy with poor prognosis, particularly in regions with high prevalence such as northern India (40,41). The findings of this study have important social and healthcare implications. Quercetin demonstrates significant potential as an affordable therapeutic agent due to its natural occurrence in commonly consumed fruits and vegetables, making it a cost-effective and accessible alternative to expensive conventional cancer treatments (43).The use of multi-target therapeutic agents such as quercetin may also improve treatment efficacy and reduce recurrence rates, ultimately lowering the overall disease burden associated with gallbladder cancer (13,19). In addition, this study underscores the growing importance of plant-derived compounds in modern drug discovery, thereby promoting further research in the field of nutraceuticals and natural products for therapeutic applications (43).

REFERENCES

1. Kumar V, Abbas AK, Aster JC. *Robbins and Cotran Pathologic Basis of Disease*. 10th ed. Elsevier; 2020.
2. Townsend CM, Beauchamp RD, Evers BM, Mattox KL. *Sabiston Textbook of Surgery: The Biological Basis of Modern Surgical Practice*. 21st ed. Elsevier; 2021.
3. O'Connell PR, Williams NS, McCaskie AW. *Bailey & Love's Short Practice of Surgery*. 28th ed. CRC Press; 2022.
4. World Health Organization. *WHO Classification of Tumours: Digestive System Tumours*. 5th ed. IARC; 2019.
5. National Cancer Institute. *Gallbladder Cancer—Patient Version*.
6. Stinton LM, Shaffer EA. Epidemiology of gallbladder disease: cholelithiasis and cancer. *Gut Liver*. 2012;6(2):172–187.
7. Hundal R, Shaffer EA. Gallbladder cancer: epidemiology and outcome. *Clin Epidemiol*. 2014;6:99–109.
8. Lazcano-Ponce EC, et al. Epidemiology and molecular pathology of gallbladder cancer. *CA Cancer J Clin*. 2001;51(6):349–364.
9. Randi G, et al. Gallbladder cancer worldwide: geographical distribution and risk factors. *Int J Cancer*. 2006;118(7):1591–1602.
10. Shukla VK, et al. Molecular biology of gallbladder cancer: potential targets for therapy. *J Surg Oncol*. 2008;97(6):543–548.
11. Wang HH, et al. Estrogen receptors and cholesterol gallstone formation. *Hepatology*. 2017;65(6):2051–2063.
12. D'Andrea G. Quercetin: A flavonol with multifaceted therapeutic applications? *Fitoterapia*. 2015;106:256–271.
13. Tang SM, Deng XT, Zhou J, et al. Pharmacological basis and new insights of quercetin action in cancer treatment. *Biomed Pharmacother*. 2020;121:109604.
14. Murakami A, Ashida H, Terao J. Multitargeted cancer prevention by quercetin. *Cancer Lett*. 2008;269(2):315–325.
15. Russo M, et al. (2012). Quercetin and cancer (general mechanistic review).
16. Reyes-Farias M, Carrasco-Pozo C. The anti-cancer effect of quercetin. *Nutrients*. 2019.
17. Li Y, et al. Network pharmacology analysis of quercetin. 2021.
18. Zhou Y, et al. Mechanisms of PI3K/AKT signaling in cancer. 2020.
19. Zhang Q, Wen F, Sun F, et al. Quercetin inhibits PI3K-AKT signaling. 2022.
20. Chen J, et al. Molecular docking validation studies. 2021.
21. Hasan AMW, et al. Quercetin anticancer activity via PI3K-AKT-mTOR pathway. 2025.
22. Eity TA, et al. Therapeutic efficacy of quercetin in cancer therapy. 2024.
23. Guo Z, et al. Mechanism of action of quercetin in cellular signaling. *Front Med*. 2024.
24. Biswas P, et al. Quercetin anticancer activities. *Int J Mol Sci*. 2022;23(19):11746.
25. Sangeetha D, et al. Therapeutic potentials of quercetin. *Phytomedicine*. 2024.
26. Carnazza M, et al. Natural compounds targeting PI3K/Akt signaling. 2025.
27. Silva-Pinto PA, et al. Mechanisms and therapeutic potential of quercetin. *Heliyon*. 2025.
28. Yang Y, et al. Quercetin regulates PI3K/Akt/mTOR pathway. *Viruses*. 2025;17(12):1637.

29. Raghav A, et al. Nanoquercetin therapeutic potential. *Cells*. 2024;13(7):638.
30. Osborne CK, Schiff R. Mechanisms of endocrine resistance in breast cancer. *Annu Rev Med*. 2011;62:233–247.
31. Miller TW, et al. PI3K/AKT/mTOR and endocrine resistance. *J Clin Oncol*. 2011;29(33):4452–4461.
32. Campbell RA, et al. PI3K/AKT-mediated activation of ER. *J Biol Chem*. 2001;276(13):9817–9824.
33. Fresno Vara JA, et al. PI3K/Akt signalling pathway and cancer. *Cancer Treat Rev*. 2004;30(2):193–204.
34. Jian X, et al. Therapeutic effects of quercetin in gynecological disorders. *Biomed Pharmacother*. 2024.
35. Zughaibi TA, et al. Targeting PI3K/Akt/mTOR pathway by flavonoids. *Int J Mol Sci*. 2021;22(22):12455.
36. Hopkins AL. Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol*. 2008;4(11):682–690.
37. Morris GM, Lim-Wilby M. Molecular docking. *Methods Mol Biol*. 2008;443:365–382.
38. Bastin A, Teimouri M, Faramarz S, et al. In vitro and molecular docking analysis of quercetin. *Curr Pharm Des*. 2023.
39. Hanahan D, Weinberg RA. Hallmarks of cancer. *Cell*. 2011;144:646–674.
40. Bray F, et al. Global cancer statistics. *CA Cancer J Clin*. 2018;68:394–424.
41. Siegel RL, et al. Cancer statistics. *CA Cancer J Clin*. 2023;73:17–48.
42. Aggarwal BB, et al. Inflammation and cancer. *Biochem Pharmacol*. 2006;72:1605–1621.
43. Newman DJ, Cragg GM. Natural products in drug discovery. *J Nat Prod*. 2020;83:770–803.
44. Trott O, Olson AJ. AutoDock Vina: docking software. *J Comput Chem*. 2010;31:455–461.
45. Szklarczyk D, et al. STRING database. *Nucleic Acids Res*. 2021;49:D605–D612.
46. Shannon P, et al. Cytoscape: network analysis tool. *Genome Res*. 2003;13:2498–2504.
47. Kim S, et al. PubChem database update. *Nucleic Acids Res*. 2021;49:D1388–D1395.
48. Stelzer G, et al. GeneCards database. *Curr Protoc Bioinformatics*. 2016;54:1.30.1–1.30.33.
49. Kuleshov MV, et al. Enrichr: gene set enrichment tool. *Nucleic Acids Res*. 2016;44:W90–W97.
50. Berman HM, et al. Protein Data Bank. *Nucleic Acids Res*. 2000;28:235–242.
51. Wishart DS, et al. DrugBank database. *Nucleic Acids Res*. 2018;46:D1074–D1082.
52. Hopkins AL. Network pharmacology concepts in drug discovery. *Nat Biotechnol*. 2007;25:1110–1111.

List of Publication and conference:

1. **Conference accepted and under publication after passing the initial review process**.

   Publication status; under review for publication

   Presented on; 27th March 2026 (online mode)

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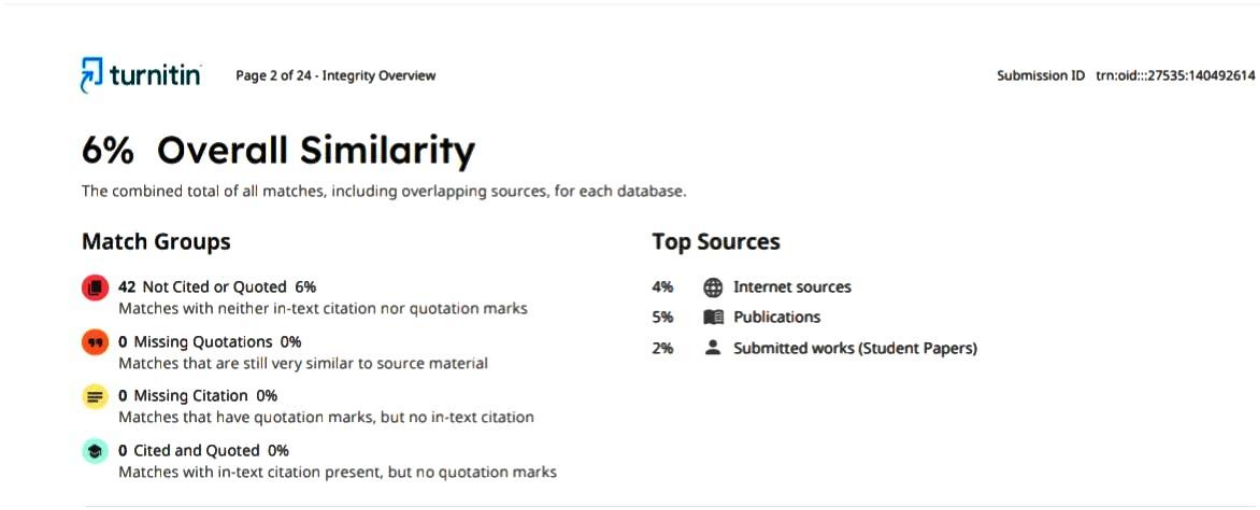

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