EXPLORING PHYTOCHEMICAL COMPOUNDS AGAINST GPC3 FOR OVARIAN CANCER TREATMENT: AN IN-SILICO APPROACH

Thesis submitted in partial fulfilment of the requirements for the degree of

MASTERS OF SCIENCE

in

BIOTECHNOLOGY

by

Vaishnavi Juneja

(2K23/MSCBIO/55)

Under the Supervision of

Dr. ASMITA DAS

Associate Professor

Department of Biotechnology

Delhi Technological University

Department Of Biotechnology

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Shahbad Daulatpur, Main Bawana Road, Delhi-110042, India

June 2025

ACKNOWLEDGEMENT

As I submit my M.Sc. Dissertation, I acknowledge that the success of this project has greatly depended on the support and guidance of many other, beyond my own efforts. I would like to take this opportunity to express my sincere gratitude to everyone who played a key role in the successful completion of this project. Your encouragement, advice, and assistance have been invaluable, and I am deeply appreciative of the contributions each of you made to this endeavor.
My initial thank is addressed to my mentor Dr. Asmita Das, Department of Biotechnology, Delhi Technological University, who gave me this opportunity to work in a project under her. It was her enigmatic supervision, constant encouragement and expert guidance which enable me to complete this work. I humbly seize this opportunity to express my gratitude to her. Words are inadequate in offering thanks to PhD scholars Who like a family, shown confidence in me and helped me in my project.
I extend my thanks to technical staff Mr. Jitendra Singh and Mr. C.B Singh who have been an aid whenever required. Lastly, I wish to extend my thanks to my family and friends who have supported me through the entire process.

Vaishnavi Juneja
2k23/MSCBIO/55

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-42

CANDIDATE'S DECLARATION

I, Vaishnavi Juneja, 2K23/MSCBIO/55 student of M.Sc. Biotechnology, hereby certify that the thesis entitled, **"Exploring Phytochemical Compounds against GPC3 for Ovarian Cancer Treatment: An In-Silico Approach"** in partial fulfilment of the requirement for the award of the Degree of Masters of Sciences submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from May 2024 to May 2025 under the supervision of Dr. Asmita Das

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Candidate's Signature

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-42

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Certified that Ms. Vaishnavi Juneja, 2K23/MSCBIO/55 has carried out their research work presented in this thesis entitled "**Exploring Phytochemical Compounds against GPC3 for Ovarian Cancer treatment: An In-Silico Approach**" from Department of Biotechnology, Delhi Technological University, Delhi under my supervision. The Thesis embodies result 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.

Dr. Asmita Das
Associate Professor
Department of Biotechnology
Delhi Technological University

Prof. Yasha Hasija
Head Of Department
Department of Biotechnology
Delhi Technological University

Date:

Place: Delhi Technological University

ABSTRACT

Glypican-3(GPC3), a heparan sulfate is a membrane bound proteoglycan which has emerged as a promising therapeutic target because of its expression in various cancers including ovarian cancer. In this study, five phytochemicals were selected and Molecular Docking technique was used in order evaluate the binding affinity of these phytochemicals with GPC3. For benchmarking the efficiency of these phytochemicals, a control compounds which is a known inhibitor of GPC3 was also used for comparative evaluation as a reference compound. The docking was performed using PyRx platform which have an integrated AutoDock Vina. Post docking visualization and analysis was done using BIOVIA Discovery Studio Visualizer. To assess the efficiency of these phytochemicals and to predict the drug likeliness of these phytochemicals' pharmacokinetic properties of all these compounds were analyzed using a online tool called SwissADME in which Absorption, Digestion, Metabolism, Excretion and Toxicity was assessed. The aim was to identify among the phytochemicals which is superior in binding affinity as compared to control compound and it was observed that Berbamine was having the highest binding affinity even surpassing the reference compound. Limonin and Liquoric acid were also promising compounds in terms of binding affinity. Also, they had balanced pharmacokinetic properties. The integrated docking and Pharmacokinetic analysis suggest that Berbamine holds potential as a lead compound for the development of novel therapeutics which can help in the treatment of ovarian cancer.

Keywords-GPC3, Ovarian Cancer, Wnt/ β- catenin, SwissADME, Binding affinity, Molecular Docking

LIST OF PUBLICATIONS

Title of the Paper: In silico analysis of Phytochemical Compounds for targeting GPC3 for Ovarian Cancer Treatment

Author Name: Vaishnavi Juneja, Dr. Asmita Das

Name of the Conference: 2nd International Conference on Advances in Engineering and Medical Sciences 2025

Date and Venue: 14th and 15th April 2025 at International School of Science for Women, Rajhamundry East Godavri, India

Indexing: Scopus Indexed

Registration: Done

Status of Paper: Acceptance Received

Date of Paper Communication: 11th April 2025

Paper Acceptance Date: 12th April 2025

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LIST OF ABBREVIATIONS

➢ ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicity
➢ BMP	Bone Morphogenetic Proteins
➢ BRCA1 & BRCA2	Breast Cancer Gene 1 and 2
➢ CAR	Chimeric Antigen Receptor
➢ EMT	Epithelial to Mesenchymal Transition
➢ EGFR	Estimated Glomerular Filtration rate
➢ GPC3	Glypican-3
➢ HER2	Human Epithelial growth factor
➢ OCCC	Epithelial Ovarian Cell Carcinoma
➢ PARP	poly(ADP-ribose) polymerase
➢ PDB	Protein Data Bank
➢ VEGF	Vascular Endothelial growth factor

CHAPTER 1

INTRODUCTION

Ovarian cancer is one of the most lethal gynecological malignancies affecting women worldwide. Ovarian cancer is heterogenous in nature and this disease progresses through several molecular level changes and have a very limited targeted therapeutic option. Ovarian cancer is a serious cause of public health concern. The disease burden varies significantly by geography, developed countries in Eastern and Central Europe, North America and Australia have higher incidence and mortality rates, while Asia and Africa have relatively lower incidence but still faces challenges in early diagnosis and also limited access to care[1]. The incidence of ovarian cancer generally increases with age; it predominantly affects postmenopausal women with median age of around 63 years.

Ovarian cancer development is influenced by a complex interplay of genetic, hormonal, reproductive and environmental factors. Familial history and inherited mutations in genes such as BRCA1 and BRCA2 is one of the most significant risk factors. BRCA1 and BRCA2 are tumor suppressor genes which are involved in DNA repair, Women who carry mutations in BRCA1 have up to a 40-60% lifetime risk while those who carry BRCA2 mutations face a 10-25% risk[2].

Glypican-3(GPC3) is a cell surface heparan sulfate proteoglycan is overexpressed in ovarian cancer. GPC3 have been extensively studied in Hepatocellular Carcinoma[3]. GPC3 contributes to ovarian cancer by enhancing Wnt/β- catenin signalling. GPC3 plays a significant role in cell growth, differentiation and migration. While GPC3 is silenced in most adult tissues, it becomes re-expressed in several cancers making it an oncofoetal antigen and a promising therapeutic target. Its aberrant expression has been observed in ovarian clear cell carcinomas and other ovarian cancer subtypes, where it promotes tumorigenesis by enhancing cell proliferation, angiogenesis and resistance to apoptosis[4]. GPC3 facilitates binding of Wnt ligand to frizzled receptors and activate β- catenin signalling pathway which ultimately leads to transcription of genes involved in proliferation and invasion. It inhibits apoptotic pathways and promotes epithelial to mesenchymal transition which leads to increase in cancer cell

motility and invasiveness which facilitates metastasis[5]. Targeting GPC3 has emerged as a rational strategy for immunotherapy and molecular targeted therapy in GPC3 expressing tumors.

Phytochemicals are bioactive compounds derived from plants; they are anti-proliferative, pro-apoptotic, anti-inflammatory, antioxidant, and anti-angiogenic due to which they have immense scope of utilization in oncology[6]. These compounds are naturally occurring in nature and target multiple cancer associated pathways which makes them ideal for managing several heterogenous and drug-resistant cancers like ovarian cancer. There are different classes of phytochemicals such as flavonoids, alkaloids, terpenoids, saponins and polyphenols and all these classes exhibit potential cancer activity by modulating signalling pathways including P13K/Akt, MAPK, Wnt/β- catenin and NF- κB.

In ovarian cancer phytochemicals have demonstrated immense potential and have been proven to inhibit cell proliferation, induce apoptosis, suppress metastasis and enhance sensitivity to chemotherapy[7]. Phytochemicals have demonstrated immense ability to inhibit cell proliferation, induce apoptosis, suppress metastasis and enhance sensitivity to chemotherapy. Some promising phytochemicals which have shown in silico affinity for GPC3 are Curcumin, Ginsenosides. Their low toxicity, broad spectrum activity makes them attractive candidates for drug development and complementary therapy.

In this study we aimed to explore the therapeutic potential of naturally occurring phytochemicals against Glypican-3(GPC3), that is overexpressed in ovarian cancer. It is a heparan sulfate and plays a critical role in tumor growth, metastasis and chemoresistance. It activates the Wnt/β- catenin signalling pathway. We employed in silico molecular docking techniques to overcome limitations of current treatment strategies for example chemoresistance, adverse effects, high costs. Using the in silico molecular docking technique we evaluated the binding affinity and interaction profiles of five structurally diverse phytochemicals – Berbamine, Limonin, Liquoric acid, Piperlongumine and Mimosine against GPC3 protein. To assess their comparative efficacy a known GPC3 inhibitor 20(S) Ginsenoside Rh2 was used a reference compound. PyRx platform was used to perform Molecular docking and interaction analysis were carried out using BIOVIA Discovery Studio. Additionally, we performed pharmacokinetic and drug likeness evaluations using SwissADME to assess their suitability as oral therapeutic candidates. This study highlights the promise of phytochemicals as safe, accessible, efficient and biologically active agents.

CHAPTER 2

REVIEW OF LITERATURE

2.1. OVARIAN CANCER

Ovarian cancer is one of the most lethal gynecologic malignancies among women. Ovarian cancer is the 18th most common cancer worldwide and 8th most common cancer in women. In 2024, Approximately 19680 women were diagnosed with ovarian cancer and about 12740 women were projected to die from this disease in the same year. Patients with this fatal disease have only 45.6% five-year survival rate[8]. The survival rate generally increases if effective early-stage detection is possible. Ovarian cancer is a heterogenous disease which develops through a complex interplay of genetic mutations, disrupted signaling pathways, epigenetic alterations, tumor microenvironmental changes. There are certain types of ovarian cancers in which germ cell tumors are more common in younger women. Around 90% of ovarian cancers are epithelial in origin of which high grade serous carcinoma (HGSC) is the most prevalent and most aggressive subtype. The other types of Ovarian cancers include Germ cell tumors which arise from primordial germ cells of ovary and constitutes 5% of ovarian cancer[9]. The other type of Ovarian cancer is Sex-cord stromal tumors which originate from the stromal or connective tissue of the ovaries. This type contributes to about 5-8% of ovarian cancers. Other rare types of cancers include Primary Peritoneal Carcinoma and Small Cell Carcinoma of ovary. Each type of ovarian cancer differs in prognosis, clinical presentation and treatment responsiveness.

2.1.1 Molecular Pathogenesis and Mechanisms

Most high-grade ovarian cancer follow the dualistic model of ovarian carcinogenesis with distinct genetic alterations and origins. The development of ovarian cancer is by genomic instability, defective DNA repair, oncogene activation, tumor suppressor gene inactivation. These molecular mechanisms interact with tumor microenvironment to support progression, metastasis. The treatment strategies for ovarian cancer includes surgical cytoreduction which can be followed by platinum-based chemotherapy[10]. Chemoresistance and relapse can also

be used as treatment strategies. In recent years targeted therapies such as PARP inhibitors have also been introduced. Additionally anti-angiogenic agents lie bevacizumab have been incorporated into treatment regimens. Understanding the mechanisms, role of molecular targets opens new avenues for targeted therapy. Among the most common molecular targets for Ovarian Cancer treatment are BRCA1 and BRCA2 which are essential for DNA repair and serve as predictive markers for PARP inhibitor therapy[11]. Another central target is TP53 mutated in over 95% of high grade serious ovarian carcinomas leading to genomic instability and resistance to apoptosis. Also, VEGF which promotes angiogenesis and tumor growth can be very beneficial target and its inhibition by agents like bevacizumab has already shown several clinical benefits[12]. Another signaling pathways which can be used for targeting include P13K/AKT/mTOR, EGFR, and HER2 which contributes to proliferation and chemoresistance.

2.1.2 Stages of Ovarian cancer

Ovarian cancer classifies into four stages that is Stage I, Stage II, Stage III, Stage IV. This classification is based on how far the cancer has spread from the ovaries. In Stage I cancer is mostly confined to one or both the ovaries and early detection at this stage is possible with prognosis. Stage II is called when cancer has spread to nearby pelvic organs for example uterus, fallopian tubes or bladder. In Stage III this disease extends beyond pelvis and spreads to abdominal cavity which can possibly affect the lining of the abdomen or nearby lymph nodes. Stage IV, the most advanced stage and in this stage the cancer metastases to distant organs such as lungs, liver etc[13]. The stage of cancer is detected by imaging and surgical evaluation and staging is necessary for determining prognosis and for selecting the treatment strategy.

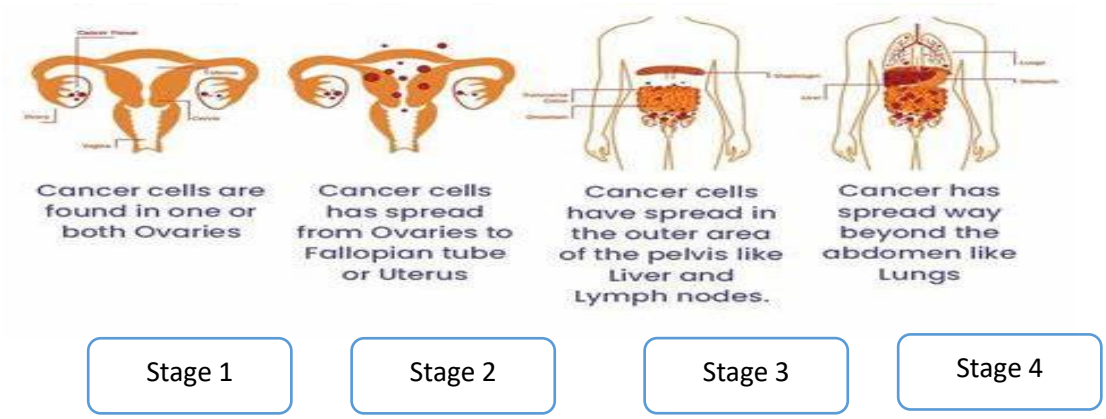


Fig 2.1 Stages of Ovarian Cancer

2.1.3 Symptoms of Ovarian Cancer

Ovarian cancer often known as a silent killer is one of the most lethal diseases because its early symptoms are subtle and are often not detected. The early symptoms are mistaken for common urinary issues and hence can go unnoticed. Common early symptoms of Ovarian cancer include pelvic or abdominal pain, abdominal bleeding, persistent feeling of fullness, frequent or urgent urination. As and when disease progress more symptoms develop for example unexplained weight loss, fatigue, change in bowel habits, back pain and menstrual irregularities. These symptoms develop at benign conditions due to which this cancer is often diagnosed at an advanced stage[14]. Early prognosis and clinical evaluation play a major role for timely diagnosis and good outcomes.

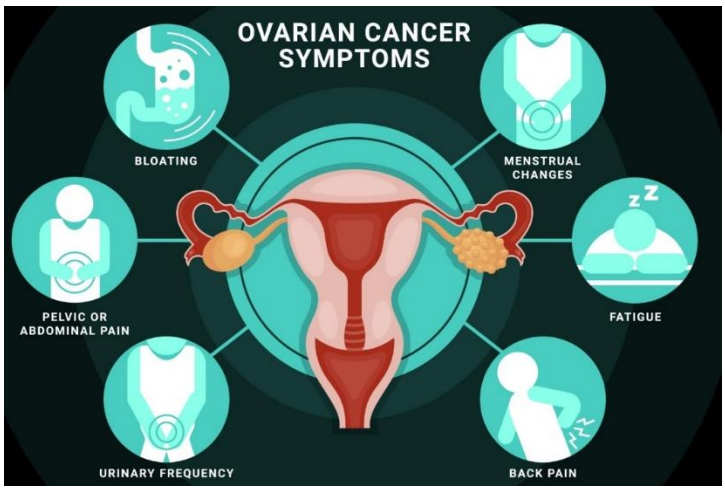


Fig 2.2 Symptoms of Ovarian Cancer

2.2 Introduction to Phytochemicals and its potential Therapeutic Properties

Phytochemicals, naturally occurring bioactive compounds which are found in plants are not essential nutrients like vitamins or minerals but serves significant health promoting benefits. The phytochemicals are secondary metabolites produced by plants as a part of their defense mechanisms against environmental stress, pests and pathogens. Apart from these benefits they also exert beneficial effects in humans. Phytochemicals broadly classify in major categories of Flavonoids, alkaloids, terpenoids, phenolic acids, saponins, lignans[15]. Each class contains numerous compounds having distinct mechanisms of action and different therapeutic effects.

Phytochemicals have a vast therapeutic potential. The phytochemicals are anti-inflammatory, antioxidant, anticancer, antimicrobial, antidiabetic and cardioprotective. For example, Flavonoids such as quercetin are oxidants that have the capability to scavenge free radicals and protect cells from oxidative stress and hence, they can play a very important role in prevention of chronic diseases like cancer and cardiovascular disorders[16]. Similarly, Alkaloids such as vincristine and vinblastine prevent microtubule polymerization which ultimately triggers apoptosis in rapidly dividing cancer cells and hence, they are well established chemotherapeutic agents used in treatment of cancers. In cancer therapy, phytochemicals have shown promise to prevent tumor initiation and progression. In this study, five phytochemicals – Berbamine, Limonin, Liquoric acid, Piperlongumine and Mimosine were selected for in silico evaluation against GPC3.

Berbamine is a bisbenzylisoquinoline alkaloid which is derived from *Berberis* species, Berbamine is known for its potent anticancer, anti-inflammatory and antioxidant activities[17]. It has the ability to interfere with key oncogenic pathways such as Wnt/ β- catenin.

Limonin is a triterpenoid limonoid derived from citrus fruits which is extensively studied for its antiproliferative and pro-apoptotic effects in cancer[18].

Liquoric acid is a flavonoid compound which is extracted from the root of Glycyrrhiza glabra demonstrates strong anti-inflammatory and anticancer effects. Liquoric acid also have the capacity to modulate molecular signalling pathways and to regulate oxidative stress[19].

Piperlongumine is an alkaloid which is obtained from long pepper, it is widely recognized for promoting apoptosis and inducing oxidative stress in cancer cells which makes it a promising agent in cancer therapeutics[20].

Mimosine, a non-protein amino acid found in non-protein amino acid found in members of Mimosoideae family. Mimosine is known for its ability to arrest cell cycle progression and the ability to inhibit DNA replication[21].

The selection of these phytochemicals aims to explore a range of bioactive scaffolds for their potential inhibitory interactions with GPC3 for potential treatment of ovarian cancer.

2.3 Molecular Biology of GPC3

Glypican-3(GPC3), a member of glypican family which comprises heparan sulfate proteoglycans which are attached to the cell surface via a glycosylphosphatidylinositol (GPI) anchor. The GPC3 gene is located on X chromosome (Xq26), this gene encodes a core protein of approximately 70 kDa which then undergoes post-translational modifications including cleavage by furin-like convertases and addition of heparan sulfate side chains at its C terminal region. GPC3 is functionally involved in the regulation of cell proliferation, differentiation and apoptosis during embryogenesis with its expression being downregulated in most tissues[22]. Aberrant reexpression of GPC3 is observed in various cancers such as hepatocellular carcinoma, melanoma, ovarian cancer. It plays a major role in tumor progression. The key mechanism of GPC3 is its interaction with Wnt/ β- catenin signalling pathway. GPC3 serves as a co-receptor which enhances Wnt ligand binding to the Frizzled receptor and LRP5/6 which leads to the stabilization and nuclear translocation of β- catenin, which then activates the transcription of genes and promote cell proliferation and survival[23]. Also, GPC3 can bind to growth factors such as Fibroblast growth factor (FGFs), Bone morphogenetic proteins (BMPs) and modulate their signalling. GPC3 expression leads to increased invasiveness, epithelial to mesenchymal transition (EMT) and resistance to chemotherapy by inhibiting apoptotic pathways and by enhancing drug efflux transporters. The most critical component of GPC3 is the heparan sulfate chains which plays a major role in binding capability and also influence the spatial orientation of signalling complexes. GPC3 has emerged as a very valuable therapeutic target due to its membrane bound nature and tumor specific overexpression. Monoclonal antibodies, peptide vaccines, chimeric antigen receptor (CAR) T cells and small molecule inhibitors are the strategies being explored[24]. Understanding the molecular biology of GPC3 provides insights into its role in cancer and also aids in rational design of targeted therapies.

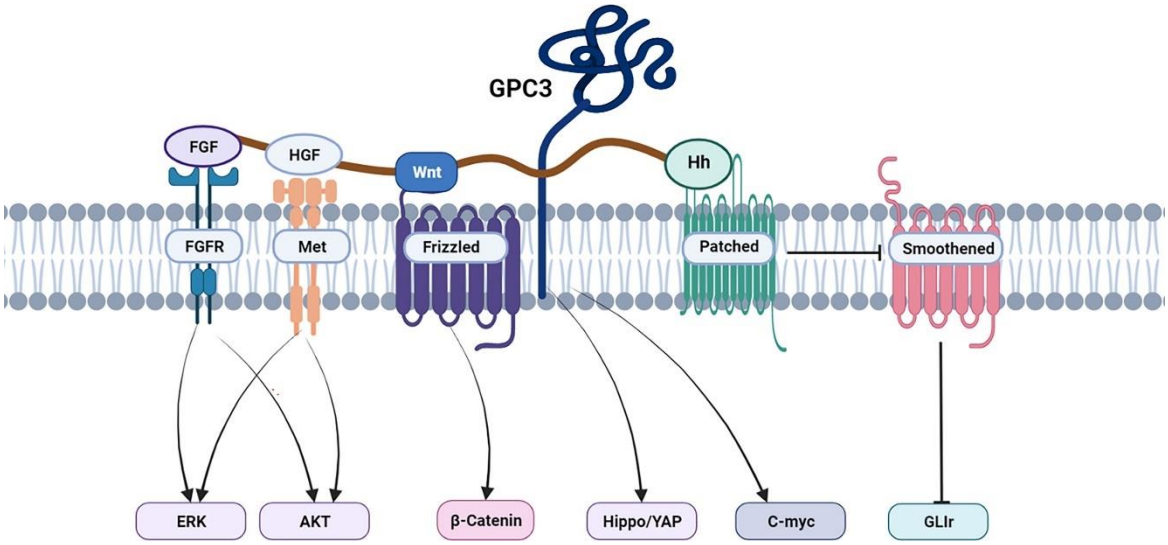


Fig 2.3 Role of GPC3 in multiple signalling pathways

2.4 Mechanism of GPC3

GPC3 a heparan sulfate whose overexpression causes cell proliferation follows different pathways which include WNT signalling pathway which regulates tumorigenesis and leads to tumor. Also, another pathway followed by GPC3 is Hedgehog pathway which leads to tumor progression.

2.4.1 WNT signalling pathway

It directly regulates embryonic development, cell differentiation, tumorigenesis and metastasis. The WNT signalling pathway is composed of ligand WNT's protein family, membrane receptor protein, cytoplasmic signal transduction protein and the downstream transcription pathway. 18 WNT family members have been identified in humans and all these different WNT members activate different signalling pathway. When WNT1,2,3,3a, and 8 activate the receptors and then the downstream signal β- catenin and hence it is called as canonical WNT signalling pathway (WNT/ β- catenin pathway)[25]. LRP5/6 is a single transmembrane protein, consists of 1600 amino acids and have a extracellular domains which is composed of four tandem β- propeller domains which can bind with different WNT and form a complex with FZD protein. The membrane complex then transmits signals and activates the dishevelled in cytoplasm which then mediates WNT pathway activation. GPC3 help in activation of WNT signalling by promoting formation of membrane surface complexes in cancers, it plays a role of signal recruiter in initial activation of WNT signalling[26]. Also, GPC3 stabilize the bindings of WNTs to FZD and positively regulate WNTs downstream signal transduction.

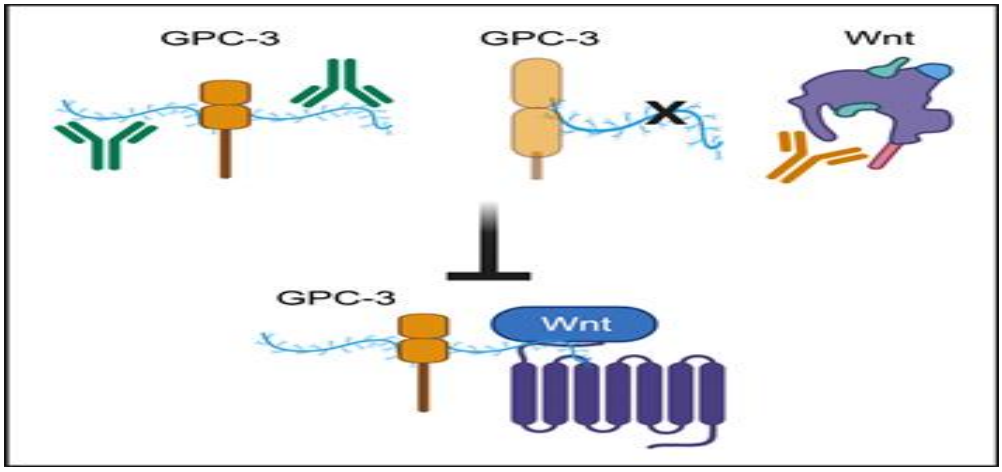


Fig 2.4 WNT/ β- catenin signalling pathway

2.4.2 Hedgehog signalling pathway

This pathway plays a key role in embryo morphogenesis, the abnormal activation of this pathway in adults can lead to progression of tumors. Three Hhs have been identified win mammals which include Sonic (Shh), Indian (Ihh) and Desert (Dhh). The Hh signalling is triggered by ligand binding to cell surface receptor called Patched which is transmembrane protein , after the binding it will abrogate its inhibitory effect on G protein coupled receptor Smo which further triggers signalling cascade and cause accumulation of transcription factors and regulate the expression of genes for cell proliferation, migration and differentiation[27]. GPC3 is a potent negative regulator in this pathway, there is a GPC3 loss of function mutation which leads to excessive activation of Hh signalling pathway. GPC3 inhibit Hh downstream signalling by binding to Shh and Ihh with high affinity. The binding causes endocytosis and degradation of GPC3-Hh complex.

2.5 Expression and Function of GPC3 in Ovarian Cancer

Glypican-3(GPC3) is being extensively studied as a critical oncogenic factor in ovarian cancer. It is normally expressed during embryonic development and is largely silenced in adult tissues but becomes aberrantly re-expressed in several malignancies like in a subset of ovarian cancer particularly clear cell and endometrioid subtypes. GPC3 expression is significantly elevated in malignant ovarian tissues as compared to normal ovarian epithelium when compared using Immunohistochemical studies[28]. The restricted expression in tumor cells with virtually no expression in other normal adult tissues and localization in membrane make GPC3 a very interesting target in Endometrial-associated ovarian clear cell carcinoma (OCCC) which is the second most common histotype of ovarian carcinoma[29].

2.6 Existing Studies Targeting GPC3 in Silico

There are several in silico studies which explore GPC3 as a molecular target due to its overexpression in various cancers. 20(S)- Ginsenoside Rh2 is a bioactive compound has already shown significant therapeutic potential and have efficiency against GPC3. In a study by Zhang et al.(2022) 20(S)-Ginsenoside Rh2 was docked using molecular docking with GPC3[30]. It demonstrated strong binding affinity and have stable interactions within the protein's active site and this suggests its role as a promising GPC3 inhibitor. The compound forms Hydrogen bonds and hydrophobic interactions with key residues which are responsible for GPC2's functional activity which indicates its potential that it can interfere with GPC3 mediated signalling pathways[31]. The binding affinity was formulated using molecular docking and visualized using visualization tools and the computational findings supported further investigation of Ginsenoside Rh2 as a therapeutic agent and hence can be used in drug development strategies targeting GPC3- positive malignancies[32].

2.7 Molecular Docking

Molecular Docking, an essential bioinformatics based theoretical modelling technique used to investigate the profiles of ligand-protein interactions, determine affinity and forecast binding conformers. Developed in 1980s these computational techniques have been proven to be essential in transforming the drug development process[33]. Early molecular modelling methods provided a strict interpretation of ligand-protein interactions due to their limited computational power. However, with the advancements in Computational techniques it is now feasible to model dynamic interactions between proteins and ligands. This approach entails examination of arrangement and direction of molecules which is called pose inside a macromolecular target binding site[34]. There are several possibilities generated by a variety of searching algorithms which are then assessed and then ranked using scoring methods. Molecular docking developments include creation of scoring functions which is essential for grading the ligand poses that are created to assess the binding energy and contact stability between the ligand and the protein target. Researcher are trying to raise the predictability of molecular docking simulations by increasing the accuracy and consistency of scoring functions. A number of softwares are available for molecular docking like GOLD, PyRx, Auto Dock, Instadock[35].

Furthermore, molecular docking has been used for purposes other than conventional drug development it has also been used in various fields like virtual compounds library screening,

structure-based medication creation, and comprehending the molecular underpinnings of ligand selectivity and specificity[36]. It has been included into more extensive computational workflows that include pharmacophore modelling and molecular dynamics simulations. This has made it possible to investigate ligand-protein interactions in greater details and in several dimensions. Thus, molecular docking remains a fundamental component of contemporary computational biology and drug discovery. Molecular docking is becoming more relevant day by day as the technology progress speeding up drug discovery initiatives.

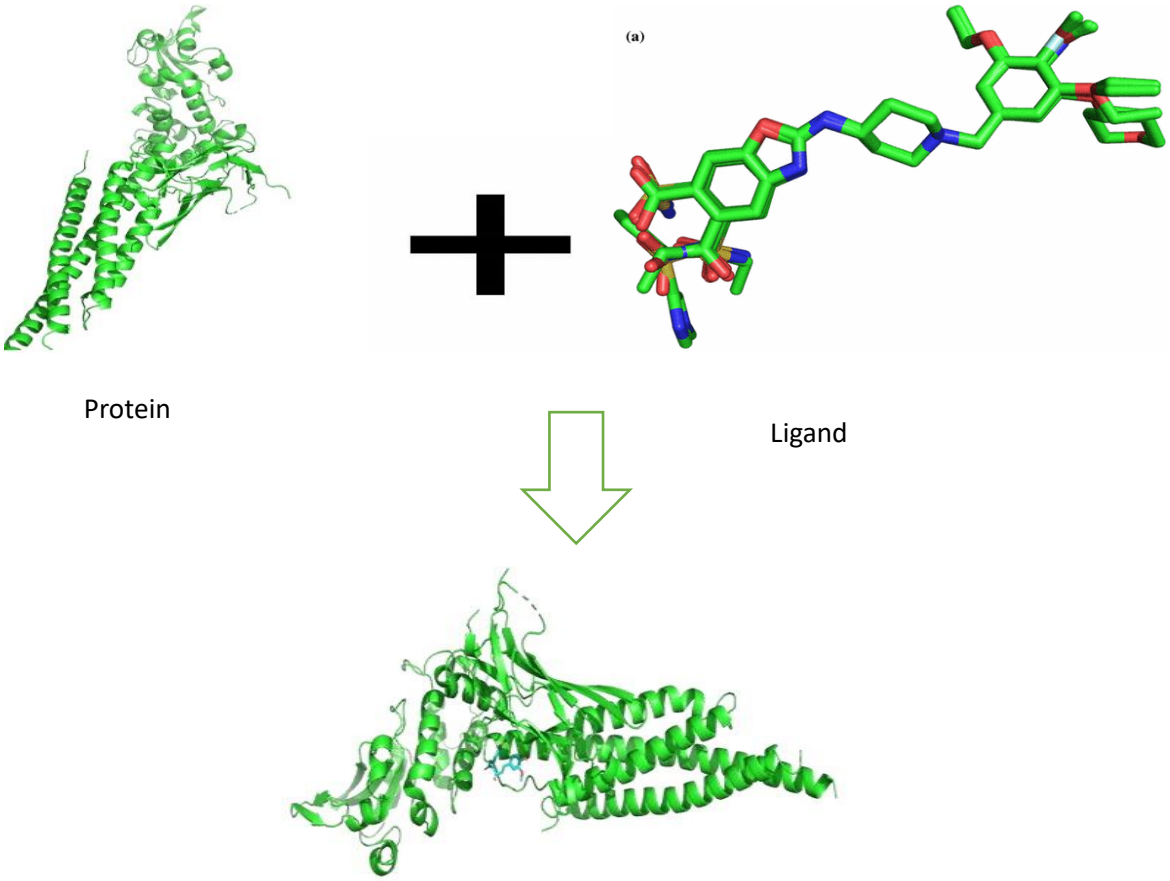


Fig 2.5. Elements involved in Molecular Docking: Proteins and Ligands

2.8 Visualization

BIOVIA Discovery Studio a widely used computational platform is designed for simulation, molecular modelling and visualization in drug discovery and structural biology. It is developed by Dassault Systems and integrates a variety of tools that help in accomplishing various tasks such as protein-ligand interaction analysis, pharmacophore modelling and ADMET prediction. This platform has become a very important component of in silico workflows in academic and industrial research settings[37]. Visualization of docking results is a essential component in structure-based drug designing. In order to understand the nature and quality of ligand binding the visualization of docking results play a very important role.\

Discovery Studio helps researchers to visualize and interpret docking poses through both 2D and 3D interaction maps by highlighting non covalent bonds like Hydrogen bonds, van der Waals interactions, salt bridges, hydrophobic contacts. This mapping of interactions enables researchers to identify amino acid residues which are responsible for stabilizing ligand within the binding pocket.

BIOVIA Discovery Studio also provides researchers with the tools which assist them in receptor preparation, binding pocket analysis, molecular dynamics simulations, active site identification[38]. The versatile nature of BIOVIA Discovery studio helps in exploring molecular behaviour at atomic level. This platform also allows to analyse the structural compatibility of ligands post docking and to refine models by minimizing energy and also helps to visually validate binding conformations. The most important role played by this platform is Lead optimization, it helps in identifying regions of molecular interactions. Discovery studio is mostly used as a follow-up tool for visualization and interpretation of docking results obtained from docking software like PyRx, AutoDock. Interactive interface and detailed analytical capabilities of this software have made the software used extensively[39].

2.9 Pharmacokinetics Parameters

The pharmacokinetic properties are the properties which describes how a compound behaves in a body how it is absorbed, distributed, metabolized and excreted in the body. These properties allow researchers to predict a molecule's bioavailability, toxicity and overall suitability as a drug candidate[40]. The properties which are analysed are

- **Gastrointestinal (GI) Absorption**- It predicts if a compound is well absorbed through the intestinal lining when the drug is taken orally, High GI absorption is very important for orally administered drugs.

- **Lipinski's Rule of five**- there is a set of rules which predict bioavailability, LogP, H bond donors, H bond acceptors. If compounds violate mor ethan one rule they are less preferred as a candidate for drug
- **Distribution-** This assessment is very important in order to identify how the drug is distributed in the body
- **Blood Brain Barrier Permeability**- It estimates the ability of a compound to cross the blood-brain barrier which is important for Central nervous system
- **Toxicity-** To measure hepatotoxicity, mutagenicity, cardiotoxicity

2.9.1 SWISS- ADME(T) analysis

A drug needs to be at its target concentration in the body and in a bioactive state for a long enough interval of time which causes desired biological reaction in order to be effective. ADMET analysis include Absorption, distribution, metabolism, excretion and toxicity of a drug[41]. This is the most important evaluation done early in drug development process. In order to guarantee that only promising candidates proceed through the development pipeline, ADMET property analysis is essential. It helps in predicting the drug's pharmacokinetic and toxicological profile. Computer simulations provide a detailed explanation for ADMET analysis. SwissADME is a free analysis tool which provides a variety of fast and precise predictive models for pharmacokinetic and physiochemical properties of drugs[42]. The tool incorporates cutting edge criteria like Bioavailability Radar. Absorption, Digestion Metabolism of the drug, Lipinski Rules of drug. We can access SwissADME through SwissADME, This tool makes it simple for experts and novices in computational biology to analyse the drug effectivity during drug development process. By facilitating the assessment of ADMET features, the tool enables scientists to spot any challenges due to drug during drug discovery process, this boosts overall effectiveness and also increases success rate of creating new drugs for treating more diseases[43].

CHAPTER 3

METHODOLOGY

3.1 Computational Resources

Databases Utilized: Several databases were used for the In silico study. For various purposes like Literature Review, Protein Target acquisition, small molecules(ligands) library acquisition, data retrieval and analysis different databases were referred.

- **PubMed (https://pubmed.ncbi.nlm.nih.gov/) :** Several medical sources are cited extensively in PubMed which can be referred for literature review. Medline, scientific publications, digital books are some of the examples which can be accessed on PubMed
- **Drug bank (https://go.drugbank.com/):** it is an indispensable tool for all the biopharmaceutical research. It has comprehensive and trustworthy drug data arranged for easy access.
- **PubChem(https://pubchem.ncbi.nlm.nih.gov/):** PubChem is world's largest collection of chemical information. It is an open access chemistry database run by National Institute of Health(NIH) which allows people to submit and share the scientific data. Several informational entries is regularly given by PubChem periodically since its founding which solidify its position further as an indispensable tool for researchers.
- **PDB (https://www.rcsb.org/)-** it is a platform which contains experimentally determined 3D structures for important biological molecules including proteins, genetic material. Its US data centre is RCSB PDB. The major goals of RCSB PDB is undertaking research and offering instructions in the domains of basic biological sciences, wellness, power and biotechnology
- **Swiss ADME(http://www.swissadme.ch/)** – During drug development process ADME analysis is the most important process and this is the online free accessible platform which helps users predict ADME variables, pharmacokinetic profiles, medicinal chemistry, drug likeliness

Software Utilized:

- **PyRx-** It is an open-source program which operates on every main operating system. It has an easy-to-use interface and is available for free use online. It provides a GUI and enables users to perform molecular docking using AutoDock and AutoDock Vina. Also, it helps in assisting in preparation of ligands and receptors by converting the files into PDBQT format which is required for molecular docking and this minimizes the energy. It also provides advantage of batch docking of multiple ligands against one target protein.
- **BIOVIA Discovery Studio-** This software provides a comprehensive set of reliable tools that help computational chemists, structural biologists to create innovative biotherapeutics and small molecule medications which are stable, optimum and have attractive safety profiles.

3.2 Workflow

A comprehensive analysis and survey of the literature revealed a correlation between GPC3 overexpression and Endometrial associated ovarian cell carcinoma (OCCC) which indicates GPC3 as a promising target in Ovarian cancer treatment. To find drugs for the treatment of Ovarian cancer natural compounds such as Phytochemicals were selected from library and Five phytochemicals were selected for the comparison along with a reference compound which was a known inhibitor of GPC3 selected through literature survey. Ligand structures were created using platform called PubChem and Open Babel in PyRx was used to convert them to. pdbqt format. Similarly, protein structure was downloaded from RCSB PDB and was prepared for utilization in molecular docking. PyRx was used for molecular docking. The best affinity compounds were then visualized using BIOVIA Discovery Studio Visualizer. After the visualization and interaction analysis, the compounds were assessed using Swiss-ADME for assessing their pharmacokinetic properties which play a very important role in drug discovery process. The best candidates were then selected and this opens new avenues for further evaluation using experimental studies so that they can be used in treatment of ovarian cancers. Molecular docking provided a comprehensive analysis of binding and visualization provided key residues by which molecules were interacting with each other. This structured in silico workflow ensured the identification of potential lead compounds based on their binding efficiency and pharmacokinetic property analysis.

3.2.1 Ligand Selection

For this study. a list of phytochemical compounds was selected based on their anti-cancer properties and based on their potential to interact with GPC3 which is overexpressed in ovarian cancer. The selection was made by an extensive literature survey of natural compounds with known cytotoxic, anti-proliferative or apoptotic effects[44].

After an extensive literature survey five phytochemicals were shortlisted that were Berbamine, Limonin, Liquoric acid, Piperlongumine and Mimosine. A reference compound was also included that is 20(S)-Ginsenoside Rh2 which is known for its anti-cancer properties with prior in silico evidence of GPC3 inhibition. PubChem database was used to retrieve 2D structures of these ligands. The filed were obtained in SDF format and their 3D structures were generated or optimized using Open Babel and ligand preparation tool within the PyRX software. Prior to docking all ligands were prepared by energy minimization to obtain stable conformations.

3.2.2 Target Protein Preparation

Glypican-3(GPC3) is the target protein which is selected for this study. It is a membrane bound heparan sulfate proteoglycan which is known to play a significant role in cell proliferation and tumor progression in ovarian and liver cancers. The three-dimensional structure of Glypican-3 was retrieved from the RCSB Protein Databank (PDB) with PDB Id [7ZA1]. The selected structure was evaluated for resolution, chain completeness and biological relevance. The protein file which was downloaded from RCSB PDB is in PCB format and is subjected to preparation prior to docking. The protein structure preparation includes the following steps:

- **Removal of non-essential compounds**- cleaning all non-essential components which can include water molecules co crystallized ligands and the ions that could interfere with docking simulations. This cleaning step was done by BIOVIA Discovery Studio Visualizer.
- **Addition of Hydrogen bonds**- The next step in the protein preparation is to add Hydrogen atoms in order to stabilize the protein structures and then incomplete residues or missing side chains were corrected if necessary.
- **Energy Minimization** – Energy minimization of structure is done to reduce steric clashes and in order to improve the geometry of structure.

The optimized GPC3 model was then saved in a docking compatible format that is PDBQT format.

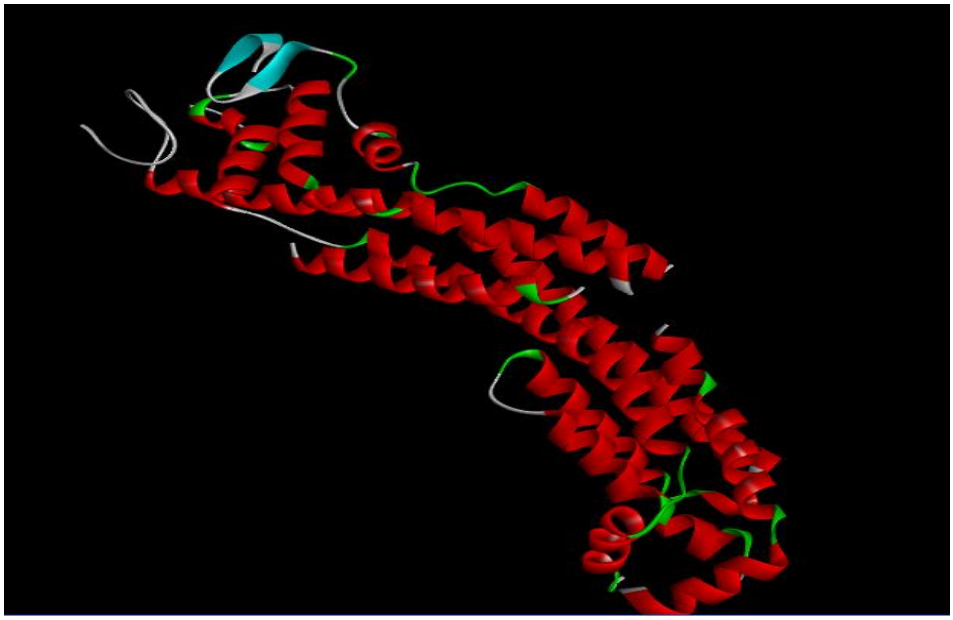


Fig 3.1 Prepared Structure of Target protein (GPC3)

3.2.3 Molecular Docking

Molecular docking was done to predict the binding affinity of selected test phytochemical ligands and reference compounds with the target Glypican-3(GPC3) using the platform known as PyRx (Python Prescription) Virtual Screening tool. It is an open source platform which have an integrated AutoDock Vina for an efficient ligand-protein docking[45]. Molecular docking plays a major role in allowing researchers to identify the potential inhibitors by stimulating the best binding orientation of a ligand within the active site of a target protein.

The steps followed in Molecular docking were:

1) The prepared 3D structure of GPC3 which was cleaned and optimized was imported into PyRx, using the in build AutoDock tool it was converted into PDBQT format. All non-polar Hydrogens ere merged and in order to ensure the compatibility with docking algorithms Gasteiger charges were added.

2) Ligands which were retrieved from PubChem in SDF format were also converted into PDBQT format and also, they underwent energy minimization using UFF that is Universal Force Field in PyRx so that stable conformations can be achieved for docking

3) Defining grid box was the next step, grid box was of maximum size so that it can contain the active or binding site of GPC3. The dimensions of grid box was X:90.99, Y: 105.07, Z:25.

4) AutoDock Vina was then used to perform the docking simulations and exhaustiveness was set to a standard value in order to balance speed and thoroughness

5) Each ligand was docked independently and the binding affinities were recorded. The best pose for each ligand was selected on the basis of lowest binding energy for post docking analysis

3.2.4 Visualization

PyRx provided preliminary visualization, in order to get detailed analysis the best scoring poses were exported to BIOVIA Discovery studio Visualizer (https://discover.3ds.com/discovery-studio-visualizer). This software was used for detailed interaction analysis and provided automated identification and classification of non-covalent interactions which included Hydrogen bonds, Hydrophobic interactions, π-π stacking and salt bridges. This software facilitated generating 2D interaction diagrams which clearly depicted the network of interactions between each phytochemical and GPC3 residues. It also allowed researchers to explore the distance measurements and interaction types were color coded for clarity[46]. The interactions analysis focused on identifying key protein residues within 4Å of docking ligands Hydrophobic interactions were identified between non polar atoms with a cutoff of 4Å. Hydrogen bonds were defined with a distance cutoff of 3.5Å.

3.2.5 ADMET analysis and Drug- Likeness Prediction

Pharmacokinetic properties play a major role in determining the efficacy of selected phytochemical ligands. In order to identify the drug likeness of the phytochemical compounds SwissADME software was used which is a widely recognized free online tool developed by Swiss Institute of Bioinformatics. The canonical SMILES of each selected compound was taken from PubChem database and was entered in the SwissADME portal to get a detailed report on parameters like Lipophilicity (LogP), water solubility, gastrointestinal absorption, blood- brain barrier permeability, bioavailability score, Gastrointestinal absorption, cytochrome P450 interactions. Also, Lipinski's rule of five were used to assess drug likeness. This in silico analysis of ADME helped in prioritizing phytochemicals with favourable pharmacokinetic behaviour and to assess bioavailability of the phytochemical and also to predict the drug likeness of phytochemicals which aids in reducing the risk of failure in later stages of drug development as they indicate if there are any challenges in the initial phase of drug development process[47].

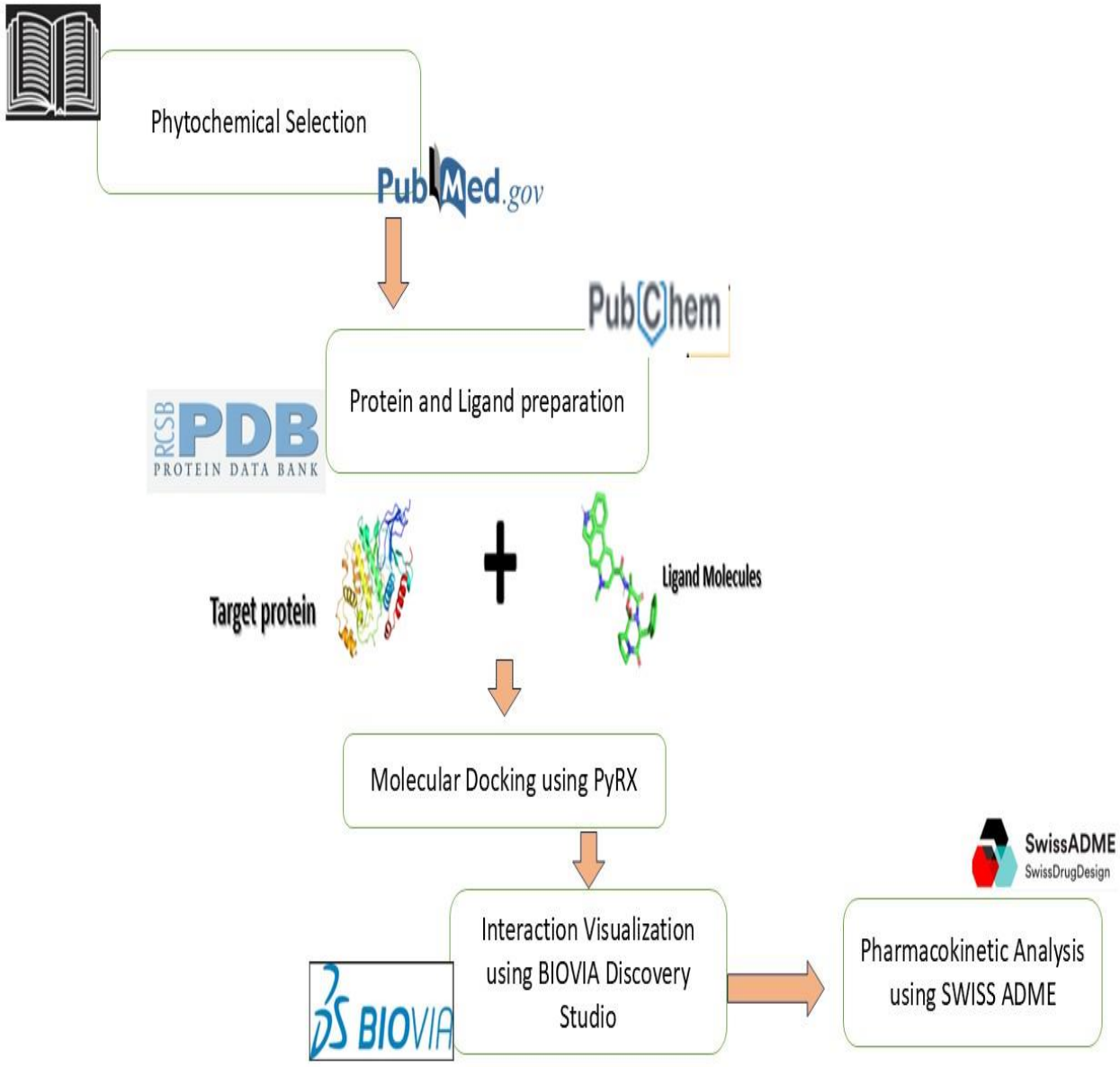


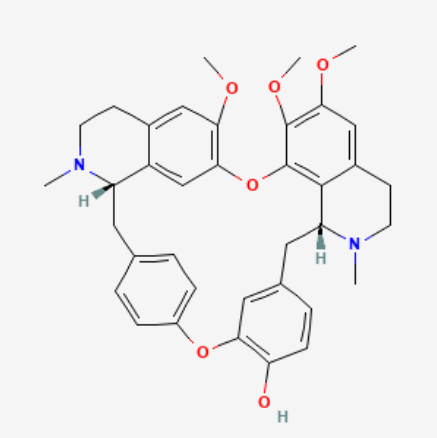
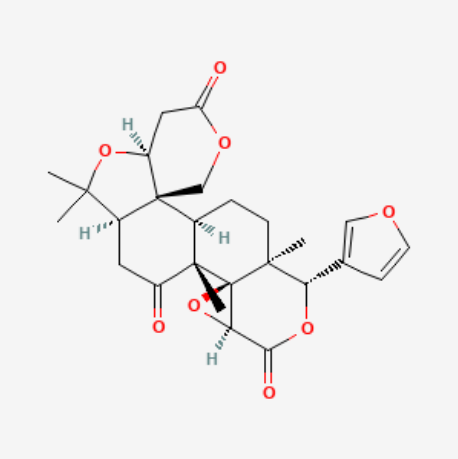
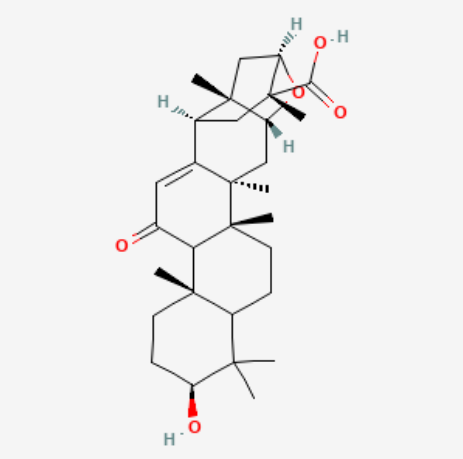
Fig 3.2 Overview of Methodology

CHAPTER 4

RESULT AND DISCUSSION

4.1 Molecular docking based virtual screening

Virtual screening is a process in computational biology to find possible drugs against predetermined biological targets. To minimize empirical effort and in order to save time , this approach has become very important. Using molecular docking based virtual screening we aim to reduce the amount of in vitro work which is required, compounds were molecular docked and binding affinity of each compound was analysed[48]. The reference compound 20(S)-Ginsenoside Rh2 has a binding affinity of -7.0 kcal/mol which was found to be lower than Berbamine that have binding affinity with GPC3 as -7.5 kcal/mol. This suggests than Berbamine possess a stronger potential to inhibit GPC3 as compared to standard reference compound which is taken as a control in this study. Limonin and Liquoric acid which had binding affinity of -7.1 kcal/mol. Piperlongumine and Mimosine which were also docked against GPC3 showed binding affinity of -6.2 kcal/mol and -4.7 kcal/mol which is lower than other compounds. The results indicate the potential of Berbamine as a superior candidate to interact with and inhibit GPC3 in ovarian cancer. Berbamine binding affinity was better than control compound and out of all five phytochemicals Berbamine showed the most promising results.

S. No.	PubChem ID	Name of ligands	Structure of Ligands	Binding affinity (kcal/mol)
1	275182	Berbamine		-7.5
2	179651	Limonin		-7.1
3	131751571	Liquoric acid		-7.1

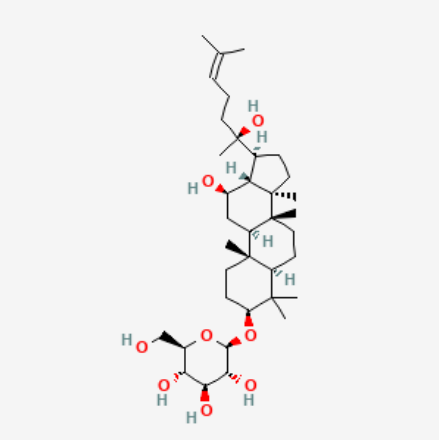
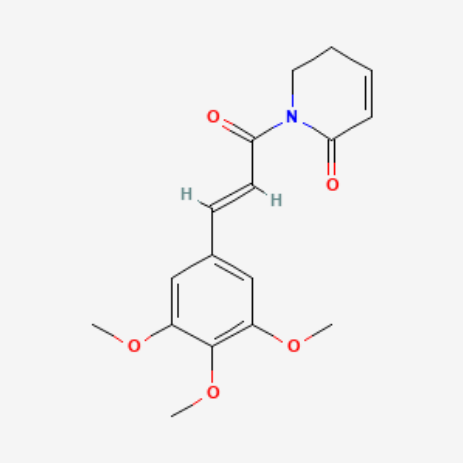
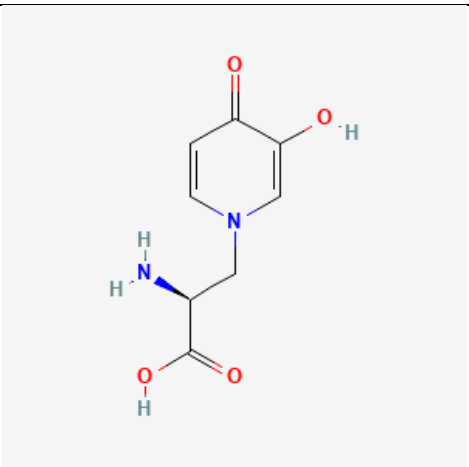
4	119307	20(S)-Ginsenoside Rh2(Control)		-7.0
5	637858	Piperlongumine		-6.2
6	440473	Mimosine		-4.7

Table 4.1 Binding affinity of Five phytochemical compounds and Control compound

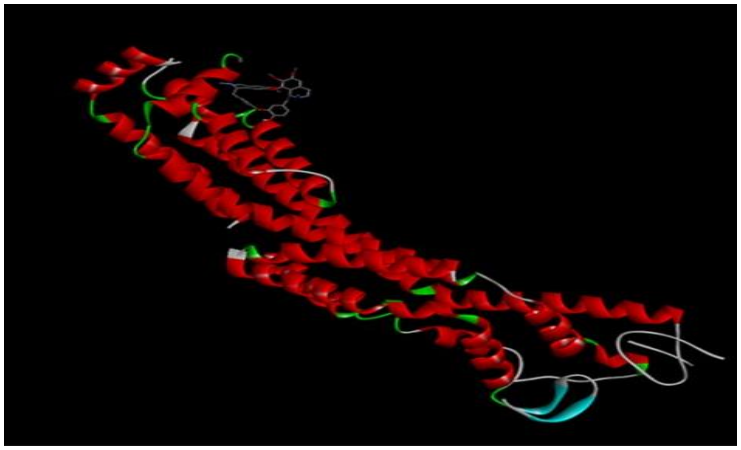


Fig 4.1 (a) Molecular docked complex GPC3-Berbamine

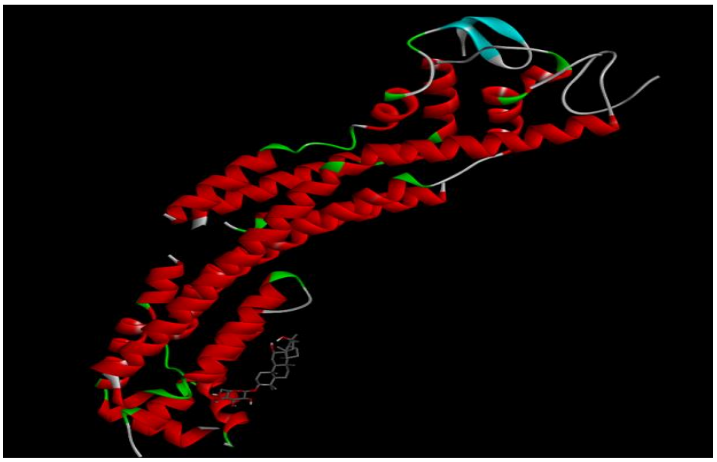


Fig 4.1 (b) Molecular docked complex GPC3-20(S)-Ginsenoside Rh2

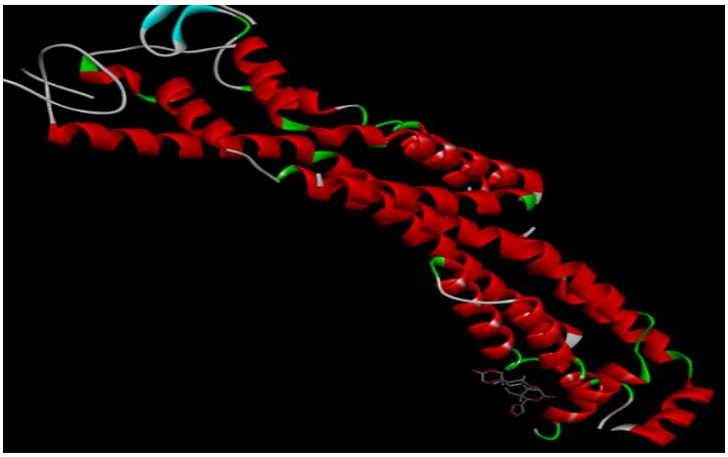


Fig 4.1 (c) Molecular docked complex GPC3-Limonin

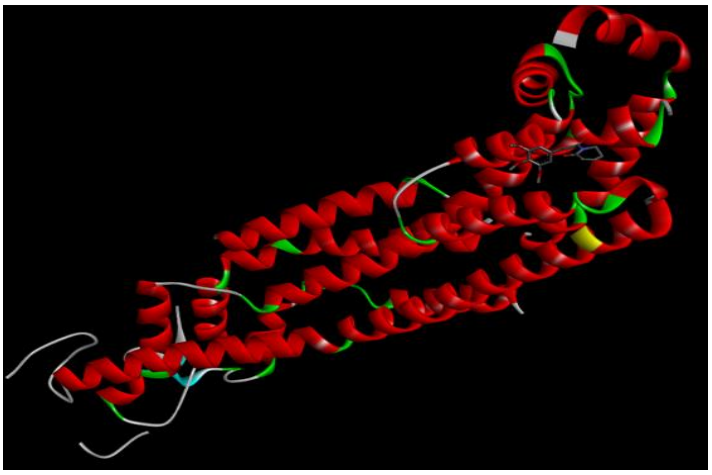


Fig 4.1 (d) Molecular docked complex GPC3-Piperlongumine

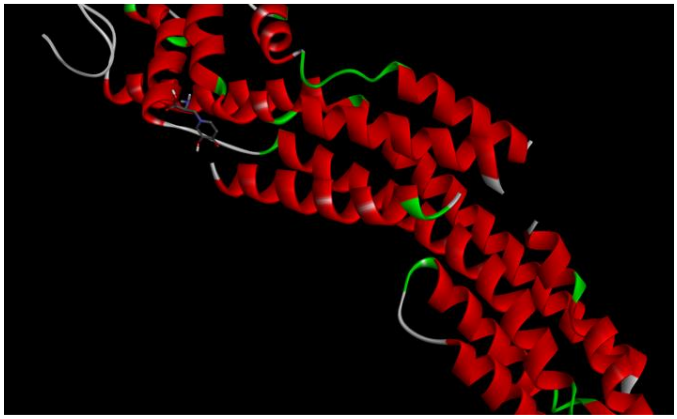


Fig 4.1 (e) Molecular docked complex GPC3-Mimosine

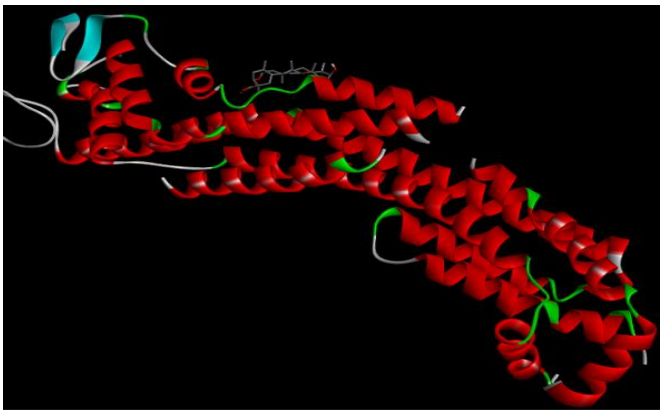


Fig 4.1 (f) Molecular docked complex GPC3-Liquoric acid

4.2 Interaction Analysis

The analysis of interactions of docked phytochemicals with GPC3 was performed using BIOVIA Discovery Studio Visualizer which provided insights into the nature of molecular binding. Among all the tested and control compounds Berbamine demonstrated most stable and extensive interaction analysis, It formed strong hydrogen bonds with polar residues GLN A145, THR A120, ASP A350. It also engages in key hydrophobic interactions with residues like PHE A144, LEU A351 which contributes to stabilization of aromatic ring of the ligand within the GPC3 binding cavity. The control compound 20(S)- Ginsenoside Rh2 exhibited several interactions notably with GLN A145, GLU A149, ARG A325 which suggests its effective anchoring. Limonin showed an extensive network of interactions including hydrogen binding with ASN A 195, also it formed hydrophobic contacts with PHE A144, PRO A374, GLN A145 which indicate strong receptor engagement. Liquouric acid similarly showed binding within a pocket comprising residues like THR A152, LEU A400, SER A403 which contributes to both polar and hydrophobic contacts. Piperlongumine exhibit unique π-π stacking interactions with PHE A130 and conventional hydrogen bonds with PHEA142

Mimosine shows least binding affinity displayed minimal interactions limited to GLU A251, SER A 234, CYS A232. These interaction profiles shows that these compounds have potential as lead compounds for further experimental validation. The interaction analysis was done using BIOVIA Discovery studio Visualizer which provided with the 2D and 3D interaction diagrams for detailed analysis.

S. No.	PubChem ID	Name of ligands	Amino acids are involved in different types of binding interactions	
			Hydrogen bonds	Other interactions
1.	275182	Berbamine	GLNA145, THR A120, ASP A350	PHE 144, LEU A351
2.	179651	Limonin	ASN A195	PHE A144, GLN A145, THR A150, PRO A374, VAL A 177
3.	131751571	Liquoric acid	ASN A195	THR A152, SER A403, LEU A400, ALA A404
4.	119307	20(S)-Ginsenoside Rh2 (Control)	ARG A 325, GLN A145, GLU A149	THR A152, SER A403, THR A150 , PRO A374, VAL A177
5.	637858	Piperlongumine	PHE A142, PHE A130	VAL A145, ALA A122, ALA A128
6.	440473	Mimosine	SER A234	GLU A251, CYS A323(minimal contacts)

Table 4.2 Interaction Analysis

2D Diagrams of interactions

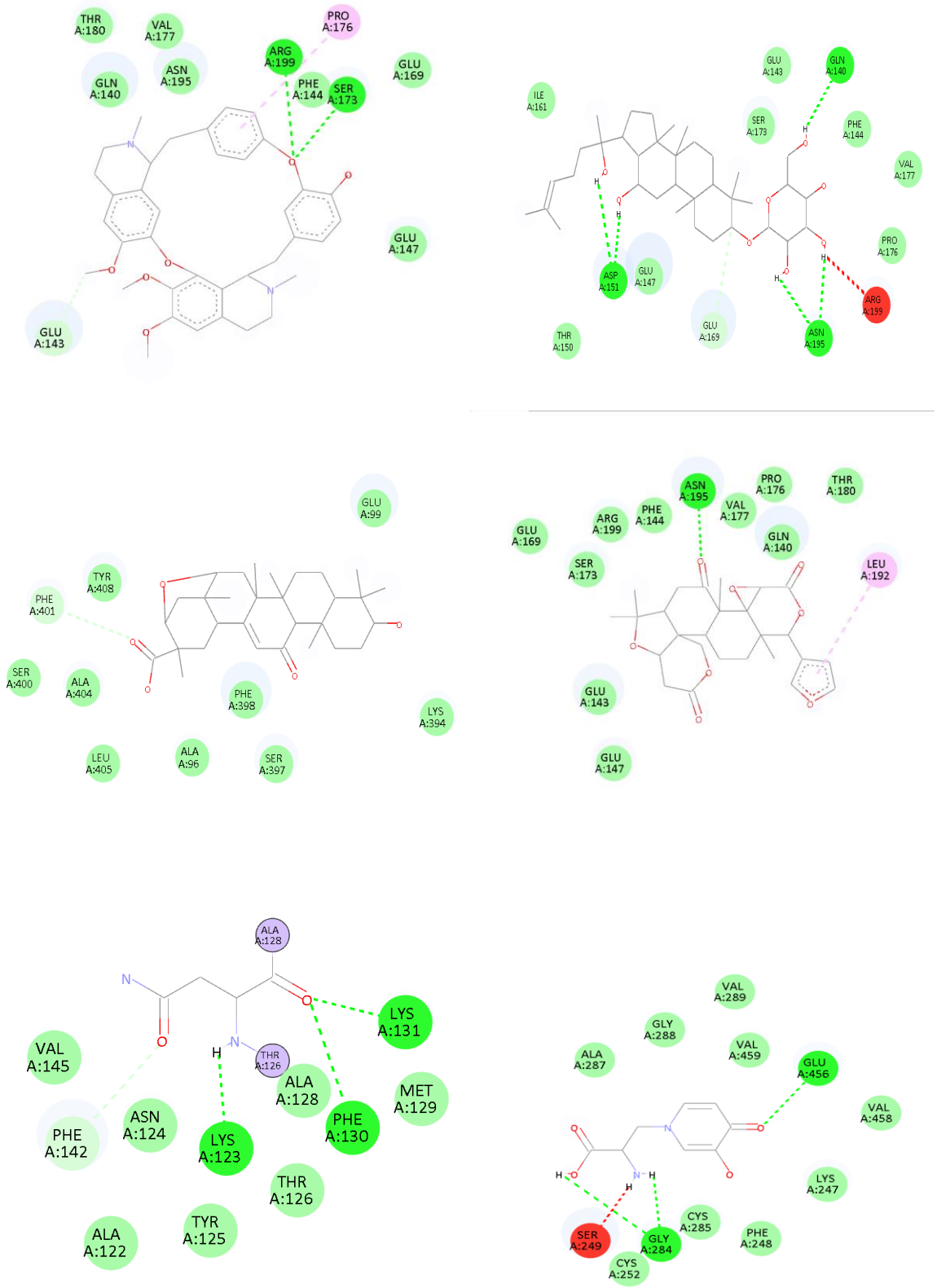
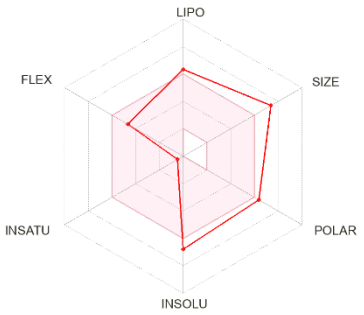
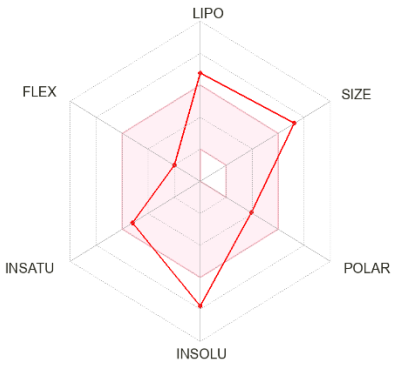
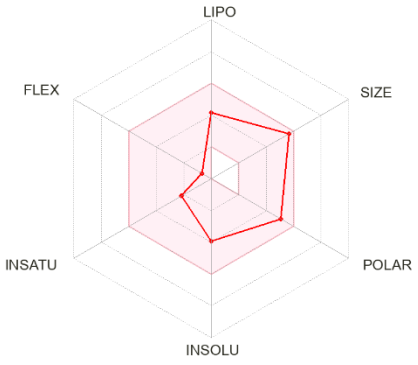


Fig 4.2 2D interaction diagrams (a) Berbamine (b) 20(S)-Ginsenoside (c) liquoric acid (d)Limonin (e)Piperlongumine (f) Mimosine

4.3 ADMET Property Analysis

SwissADME software is the software used to evaluate their pharmacokinetic behaviour and drug likeness properties. The ADMET analysis include Absorption, Distribution, Metabolism, Excretion, Toxicity profiling of the phytochemicals. All the five test compounds that are Berbamine, Limonin, Liquoric acid, Piperlongumine and Mimosine along with the control 20(S)-Ginsenoside Rh2 were analysed using SwissADME tool. They exhibited high gastrointestinal (GI) absorption and also indicated good oral bioavailability potential. Notably, Piperlongumine was the only compound which is predicted to have blood- brain barrier (BBB) permeability which suggests its potential to be used in CNS related cancers. On the other hand, Berbamine did not cross the BBB which makes them advantageous for non-CNS targeted therapies in cancers such as ovarian cancer.

Moreover, All the compounds adhered well tov Lipinski's Rule of Five with only one minor violation in Berbamine which is acceptable and does not rule it out. Also, Bioavailability scores of all the compounds were within the acceptable limits which supported their potential as orally active agents. Next property which was analysed was metabolism and none of the compounds inhibited major cytochrome P450 enzymes such as CYP1A2 and CYP3A4 which indicates a low risk of drug-drug interactions and favours the metabolic profiles. Additionally, all compounds showed low hepatotoxicity and mutagenicity which suggests good safety profiles. All compounds showed low predicted hepatotoxicity and mutagenicity and this suggests that they have good safety profiles. ADMET analysis revealed that Berbamine had a highly favourable pharmacokinetic profile despite one Lipinski violation and hence it is a good candidate as a lead compound. Strong GPC3 binding affinity and favourable ADMET parameters makes Berbamine a very promising therapeutic molecule for further in vitro and in vivo evaluation. Limonin and liquoric acid also demonstrate balanced ADMET properties which opens gate for further experimental studies.

S.No.	Name of Ligands	ADMET Properties			Bioavailability radar Illustration
		GI Absorption	Lipinski Rule of Five	BBB permeability	
1.	20(S)-Ginsenoside Rh2(Control)	Low	2 violations	No	
2.	Berbamine	High	Only 1 violation	No	
3.	Limonin	High	Yes, 0 violation	No	

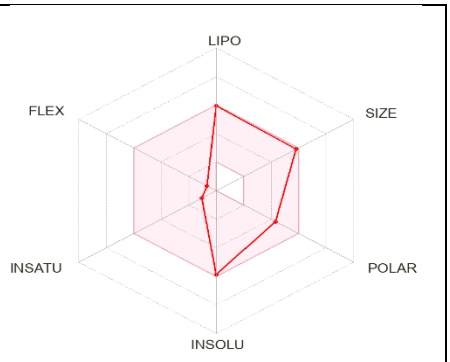
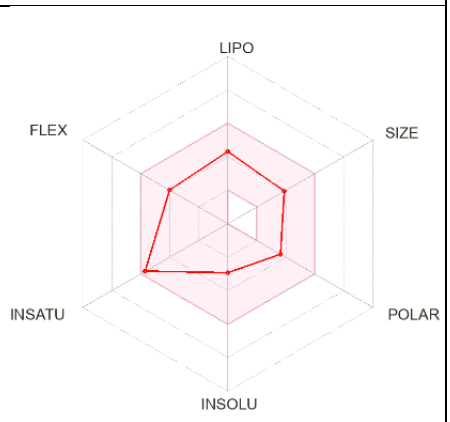
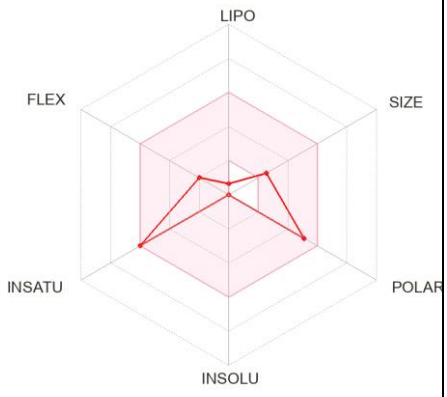
4.	Liquoric acid	High	Yes, 0 violation	No	
5.	Piperlongumine	High	Yes, 0 violation	Yes	
6.	Mimosine	High	Yes, 0 violation	No	

Table 4.3 Pharmacokinetic (ADMET) Analysis

Hence, Among the compounds, Berbamine exhibited most desirable properties and with its strong binding affinity against GPC3 it becomes a strong candidate for becoming a drug. The compound has shown a high Gastrointestinal absorption and moderate solubility and a high bioavailability score of 0.55. Followed by Limonin and Liquoric acid , they also show balanced pharmacokinetic properties and have a high binding affinity for GPC3 and with further experimental studies they can also be proven as good candidates for a drug against GPC3 for the treatment of ovarian cancer.

4.4 Discussion

The analysis of interactions of docked phytochemicals with GPC3 was gave insightful insights regarding the nature of molecular binding. Among all the tested compounds Berbamine was the most promising candidate which demonstrated the most stable and extensive interaction profile it formed very strong Hydrogen bonds and had the binding affinity higher than that of the control compound which is already proven to inhibit GPC3. Berbamine have surpassed the known GPC3 inhibitor with a binding affinity of -7.5 kcal/mol and have Hydrogen bonds and significant hydrophobic interactions which indicate a robust binding conformation with active site of GPC3. Moreover, Limonin and Liquoric acid also have high binding affinity of -7.1 kcal/mol and interactions included hydrogen bonds and hydrophobic interactions. Structural compatibility of the compounds with the GPC3 active site plays a very important role in affinity. Also, the ability of compounds to mimic or disrupt the interactions which are necessary for Wnt/ β- catenin signalling which is the mechanism responsible for cancer promotion. The interaction of Berbamine suggests a possible inhibitory role in GPC3 mediated signalling which helps in curbing ovarian cancer progression. The structures of Berbamine, Limonin and Liquoric acid indicate potential for lead optimization and derivatization in order to enhance their potential in drug development. Furthermore, ADME analysis which was done using SwissADME helped in validating the suitability of these compounds in terms of pharmacokinetics. All three compounds exhibited high gastrointestinal absorption which indicates their feasibility for oral administration and none of these compounds showed inhibitory effects on major cytochrome P450 enzymes which imply that there is a lower risk of metabolic complications and drug- drug interactions. They also showed a good drug likeness score. Only Berbamine showed one violation in Lipinski's rules which is acceptable in drug discovery process. Piperlongumine had a slightly lower binding affinity but showed excellent pharmacokinetic parameters including the Blood brain barrier permeability which makes it a perfect candidate for further investigation on other cancer types for example CNS related cancers.

Thus, the integration of molecular docking process using PyRx software and visualization using BIOVIA Discovery Studio Visualizer and ADMET analysis using SwissADME supports the potential of Berbamine as a lead compound in order to target GPC3 in ovarian cancer.

CHAPTER 5

CONCULSION AND FUTURE PROSPECTS

Cancer is one of the leading causes of mortality worldwide. It is one of the most lethal diseases with a complex etiology which involves genetic mutations, epigenetic alterations, dysregulation in signaling pathways, uncontrolled cell proliferation, apoptosis resistance, metastasis. Among different types of cancers, ovarian cancer remains one of the most lethal gynecological cancers which is often diagnosed at an advanced stage due to lack of early symptoms. Ovarian cancers fall under the category of solid tumors and hence the treatment of this cancer is difficult[49]. The disease also exhibits a high degree of heterogeneity which is also associated with resistance to standard chemotherapy which calls for the need of targeted and personalized therapeutic strategies.

A molecular target Glypican -3 which is a cell surface heparan sulfate proteoglycan is a target which is very less explored in cancers but have a significant effect as it is overexpressed in several cancers such as HCC and ovarian cancer. It plays a major role in tumor growth and progression by enhancing the Wnt/ β- catenin signalling pathway, also it inhibits apoptosis and promote epithelial to mesenchymal transition which helps in metastasis. It is selectively expressed in cancer cells and have no or minimal expression in normal adult tissues and this makes GPC3 a very promising candidate for therapeutic intervention.

Also, if we talk about the treatment opportunity using natural compounds, great amount of research has been going on around phytochemicals which are natural compounds which are bioactive in nature and is used in cancer treatment due to their multi targeting capabilities and relative low toxicity. These phytochemicals modulate various molecular targets and can target pathways which are involved in carcinogenesis. For this study we chose five phytochemicals namely – Berbamine, Limonin, Liquoric acid, Piperlongumine, Mimosine. Also, a control compound was chosen by literature review which is a known inhibitor of GPC3.

To investigate the binding affinity of these compounds against GPC3, Molecular docking was conducted using the PyRx platform which have an integrated AutoDock Vina engine which provides high throughput virtual screening. The protein and ligand preparation were done using Protein data bank and PubChem. The docking predicted binding affinities conformations. Post

docking analysis was done using BIOVIA Discovery Studio Visualizer which is a molecular modelling tool and enables in depth visualization. The visualization shown the crucial non covalent interactions such as Hydrogen bonds, hydrophobic contacts and π-π stacking between the ligands and specific amino acid residues within the GPC3 binding pocket. Berbamine demonstrated the strongest binding affinity and formed stable interactions. To further evaluate drug like potential of the phytochemicals, ADME analysis was done using SwissADME. Berbamine despite one minor rule violation exhibited a well-balanced pharmacokinetic profile which makes it potential to be a drug like candidate. The integration of docking scores, interaction analysis and ADME profiling led to identification of Berbamine as lead compound and this showed superior binding to GPC3 compared to control compound. Limonin and liquoric acid also proved to be promising candidates. This study demonstrates power of in silico approaches in drug discovery and enables rapid and cost-effective screening of phytochemical compounds to explore their therapeutic potential. The combination of molecular docking, visualization and ADME analysis provides opportunities for exploring natural compounds for process of lead identification and optimization.

In Conclusion, this work supports the potential of phytochemical based therapeutics in targeting GPC3 for the treatment of ovarian cancer. Berbamine stand out as a promising candidate for further investigation and development and findings of this study suggests that these phytochemicals can be key candidates for future research in the area of natural product based targeted cancer therapies[50].

Several directions for future research emerge from this study like we can experimentally validate the predicted binding of Berbamine, Limonin and Liquoric acid to GPC3 through techniques such as surface plasmon resonance. Also, Investigation of effects of these phytochemicals on GPC3 mediated signalling pathways in ovarian cancer cell lines can be done. One of the most efficient moves in future can be of using combination strategies in which investigation can be done for the potential synergistic effects of GPC3 targeting phytochemicals with conventional chemotherapeutic agents for treatment of ovarian cancer. Furthermore, structural optimization or derivatization of Berbamine can be done to improve its drug likeliness without compromising its binding efficacy. These findings open new avenues for developing Berbamine-based analogs as novel therapeutic strategies for ovarian cancer treatment.

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Author Name: Vaishnavi Juneja, Dr. Asmita Das

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