CAPITAL UNIVERSITY OF SCIENCE AND
TECHNOLOGY, ISLAMABAD

In Silico Investigation of *Berberis lycium* Phytochemicals against Metastatic Breast Cancer

by

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A thesis submitted in partial fulfillment for the
degree of Master of Science

in the

Faculty of Health and Life Sciences
Department of Bioinformatics and Biosciences

2026

CERTIFICATE OF APPROVAL

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Acknowledgement

All praise and gratitude are due to Almighty Allah, whose countless blessings, mercy, and guidance granted me the strength and determination to complete this research.

I sincerely thank my respected supervisor, Dr. Sohail Ahmad Jan, for his invaluable guidance, encouragement, and continuous support throughout this research. I am grateful to the Dean, the Head of the Department of Bioinformatics and Biosciences, and all the faculty members and staff of CUST, Islamabad, for their support and guidance.

My heartfelt thanks go to my beloved parents and family for their unconditional love, prayers, and encouragement. I am especially grateful to my brother, Raja Shahzaib Ahmed, for his constant support throughout this journey.

I would especially like to thank my closest friend, Hira Tahreem, for her exceptional support throughout this journey. Her assistance in converting my thesis into the university's LaTeX template, along with her constant guidance and help during every stage of my thesis, was truly invaluable. I am deeply grateful for her kindness and encouragement.

I am deeply thankful to my fiancé, Usama Hameed, for his constant motivation, belief in me, and encouragement. Finally, I sincerely thank my dear friend Rameen Ahmad, my colleagues, and everyone who supported me, directly or indirectly, in completing this thesis.

(Faryal Naz Tabassum)

Abstract

Breast cancer is the most frequently diagnosed malignancy among women and continues to be a leading cause of cancer-related mortality worldwide. Metastatic breast cancer (MBC) represents the most aggressive form of the disease, characterized by recurrence, therapeutic resistance and poor survival outcomes. Although advances in targeted therapy and immunotherapy have improved treatment options, their effectiveness remains limited, underscoring the urgent need for new, safe, and affordable therapeutic agents.

Berberis *lycium*, a medicinal plant indigenous to South Asia, has long been utilized in traditional medicine for managing infections, inflammation, diabetes and cancer. Its roots, bark and stem are rich in alkaloids such as berberine, oxyberberine, palmatine, koumidine, stilbene and favipiravir, which possess diverse pharmacological activities. These phytochemicals were identified through the PubChem database, and their physicochemical properties were assessed to determine drug-likeness.

Mutations in BRCA1 are strongly associated with breast cancer progression, recurrence and resistance to therapy, making it an important molecular target. To explore the therapeutic relevance of *Berberis lycium* compounds, ADMET profiling was performed using SwissADME and pkCSM, predicting absorption, distribution, metabolism, excretion and toxicity. The analysis revealed that berberine and oxyberberine demonstrated favorable pharmacokinetics and low toxicity, while palmatine and koumidine showed moderate drug-likeness, and stilbene and favipiravir exhibited supportive potential.

The anticancer activity of these compounds was further investigated through molecular docking using CB-Dock2, targeting the BRCA1 protein. Docking simulations indicated that berberine displayed the strongest binding affinity (Vina score = -7.0), forming stable hydrogen bonds and hydrophobic interactions at the BRCA1 active site. Oxyberberine also showed high affinity (Vina score = -7.0), whereas the remaining compounds produced moderate interactions. To validate and visualize these molecular contacts, Discovery Studio was employed, confirming the binding orientations and inhibitory potential of the phytochemicals.

This study fills an important research gap by presenting the first comprehensive *in silico* evaluation of *Berberis lycium* phytochemicals against metastatic breast cancer. The findings highlight berberine as the lead compound, supported by oxyberberine as a secondary candidate. Overall, *Berberis lycium* represents a promising natural source of anticancer agents, offering a cost-effective and innovative strategy to overcome drug resistance in breast cancer.

Keywords: Breast cancer, *Berberis lycium*, Phytochemicals, *In silico* drug discovery, ADMET profiling, Lipinski's Rule of Five, Molecular docking, BRCA1 protein, Computational pharmacology.

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Abbreviations

AACR	American Association for Cancer Research
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
AI	Artificial Intelligence
BRCA1/BRCA2	Breast cancer gene 1 / Breast cancer gene 2
ctDNA	Circulating tumor DNA
EMT	Epithelial–Mesenchymal Transition
ER	Estrogen receptor
HER2	Human epidermal growth factor receptor 2
HR+	Hormone receptor positive
Ki-67	Cellular proliferation marker (used as biomarker)
MAPK	Mitogen-activated protein kinase pathway
NCCN	National Comprehensive Cancer Network
PARP	Poly (ADP-ribose) polymerase inhibitors
PI3K/Akt	Phosphoinositide 3-kinase / Protein kinase B signaling pathway
PR	Progesterone receptor
SERDs	Selective estrogen receptor degrader

Chapter 1

Introduction

Cancer refers to a group of disorders characterized by abnormal and unchecked cell proliferation, often resulting in tumor formation. These tumors can infiltrate surrounding tissues and, in many cases, metastasize to distant parts of the body [1]. Many cancers can be cured if identified early and treated promptly, since early detection improves survival and reduces the need for aggressive therapy. Malignant tumors differ from benign ones because they spread, while benign tumors remain localized. More than 100 distinct cancer types affect humans [2].

Breast cancer represents the most common malignancy among women and ranks as the second major cause of cancer related mortality worldwide [3]. Anatomically, the breasts are paired glands situated over the pectoralis major muscle, varying in size and density. They consist of milk secreting cells grouped into lobules, which collectively form lobes interspersed with adipose tissue. Milk is synthesized in acini and conveyed through lactiferous ducts that terminate at the nipple. Structural support is provided by connective tissue strands referred to as Cooper's ligaments. [4].

The prognosis of BC depends on its stage. Stage 0 and Stage I both carry a 100% five year survival rate. Stage II has a survival rate of about 93%, Stage III about 72%, and Stage IV only 22% [5]. Women who have previously had BC face a higher probability of recurrence. A second cancer may develop in the same breast

or in the opposite one. Although most women with ductal carcinoma in situ do not experience recurrence, they remain at increased risk [6].

Breast cancer in close relatives suggests familial involvement. In some families, cases occur more often than expected by chance. This may result from shared lifestyle factors, inherited genes, or both [7, 8]. A genetic mutation is a change in a gene that can raise cancer risk. Such mutations may be inherited from parents. Females carrying BRCA gene mutations have an elevated susceptibility to developing malignancies in both breasts. In cases where cancer arises in one breast, the probability of occurrence in the opposite breast is significantly increased. BRCA mutations also increase the likelihood of ovarian cancer at any age [9].

Hormonal fluctuations contribute to breast cancer susceptibility. During menopause, ovarian production of estrogen and progesterone ceases, leading to the termination of the menstrual cycle. Women who undergo menopause beyond the age of 55 remain exposed to these hormones for an extended duration, which elevates their likelihood of developing breast cancer. Early menopause reduces hormone exposure and lowers risk [10].

Breast density is another factor. Breasts with dense tissue possess a greater proportion of ducts, glands, and connective structures than fatty tissue, and this characteristic is often genetically determined. Women exhibiting dense breast composition have an elevated susceptibility to breast cancer. In mammographic imaging, dense tissue is displayed as white areas resembling tumors, whereas fatty tissue appears darker, which complicates interpretation and may obscure malignant growths [11].

By 2026, breast cancer treatment has become increasingly personalized and multimodal. Depending on tumor subtype and stage, therapy may involve surgery, radiotherapy, chemotherapy, hormone therapy, targeted therapy and immunotherapy. Therapeutic agents aimed at HER2 receptors and CDK4/6 pathways have markedly enhanced progression free survival outcomes in patients with hormone receptor–positive metastatic breast cancer [12]. Advances in precision medicine,

including circulating tumor DNA (ctDNA) testing, help clinicians predict treatment response and tailor therapy more effectively [13].

New drugs, such as oral selective estrogen receptor degraders (SERDs) like imlunestrant, are expanding options for resistant or recurrent disease [14]. Combination therapies and innovative approaches such as cancer vaccines and RNA-based treatments are also showing promising results in clinical trials for advanced breast cancer [15]. Overall, current research highlights precision-guided, combination treatment strategies aimed at improving survival and reducing recurrence [16].

Breast cancer treatment today is far more advanced and personalized than in the past. Physicians now apply a combination of therapies depending on cancer's type and stage. These involve surgery to remove tumors, chemotherapy & radiotherapy to avoid malignant cells and hormone therapy for cancers influenced by hormonal activity. Current research emphasizes targeted therapy, which specifically attacks cancer cells such as HER2-positive cells with few side effects and immunotherapy which strengthens immune system to fight cancer particularly in aggressive forms like triple negative breast cancer [17].

Modern drugs such as CDK4/6 inhibitors and newly developed hormone therapies (SERDs) are improving survival outcomes in advanced cases [18]. One widely used medication is trastuzumab (Herceptin), a targeted therapy for HER2 positive breast cancer. It works by blocking cancer cell growth and enabling the immune system to eliminate them. Recent studies confirm that trastuzumab enhances survival and is more effective with fewer side effects compared to conventional chemotherapy [19].

Berberis lycium is a medicinal plant native to South Asia, particularly found in Pakistan and Kashmir, and has long been recognized in traditional medicine for its therapeutic value. It has been used to manage infections, inflammation, diabetes, and cancer. The plant contains several bioactive alkaloids, which are known for their strong pharmacological properties. These compounds are of particular interest in cancer research because they can interact with molecular targets that play a role in tumor growth, progression, and resistance to therapy. The ability

of natural phytochemicals to influence such pathways makes *Berberis lycium* a promising candidate for anticancer drug discovery and highlights its potential in the development of targeted therapies[20, 21].

Molecular docking serves as a computational approach to predict how chemical compounds interact with cancer-associated proteins such as enzymes, receptors, and signaling molecules that drive tumor development and resistance. Docking studies involving *Berberis lycium* phytochemicals have demonstrated strong binding tendencies, suggesting that these compounds may inhibit cancer proteins by attaching to their active sites and interfering with their function. When combined with pharmacokinetic and toxicity assessments, docking provides a systematic framework to evaluate whether plant-derived molecules possess drug-like characteristics and therapeutic potential. *In silico* investigations of *Berberis lycium* therefore establish a foundation for further experimental validation, offering a cost-effective and reliable pathway to identify natural compounds with anticancer activity against metastatic breast cancer[22, 23].

1.1 Problem statement

Metastatic Breast Cancer is a highly intense disease with limited effective treatments. Natural compounds show anticancer potential, phytochemicals of *Berberis lycium* are not well studied against this cancer. Therefore, this study uses *in silico* methods to evaluate their potential as therapeutic agents.

1.2 Hypothesis

Berberis lycium phytochemicals may show strong binding affinity and anticancer potential against metastatic breast cancer targets and could act as potential therapeutic agents in *in silico* studies.

1.3 Research Aim

The aim of study is to explore *Berberis lycium* phytochemicals through computational approaches for developing natural solutions against drug resistance in metastatic breast cancer.

1.4 Objectives

i. To explore the bioactive compounds obtained from *Berberis lycium* as potential BRCA1 inhibitors.

ii. To study binding interaction of selected compounds with metastatic breast cancer target protein using molecular docking.

iii. To evaluate the pharmacological suitability and safety profiles of phytochemicals through ADMET analysis, aiming to highlight promising candidates for natural anticancer therapy.

1.5 Scope

This study focuses on the *in silico* analysis of *Berberis lycium* phytochemicals against metastatic BC using molecular docking and ADMET prediction to evaluate their therapeutic potential. It provides a fast, cost effective and reliable way to identify promising phytochemicals that may interact with key targets like BRCA1 gene.

Chapter 2

Literature Review

2.1 Cancer Overview

Cancer is a disease where cells grow and spread without control. In healthy conditions, cells follow a normal cycle they grow, divide and die which keeps the body's tissues balanced. In cancer, this regulation fails, leading to excessive and abnormal cell proliferation. Cells can invade nearby tissues and spread to distant organs through the blood or lymphatic systems, process called metastasis. Cancer can arise in almost any body part and varies in severity depending on type and stage. It is a leading cause of death over worldwide, with millions of new cases diagnosed each year. Research have improved early detection and treatment, but cancer remains a major health burden. Environmental, genetic, and lifestyle factors all contribute to its development. In addition, socioeconomic conditions, limited access to healthcare, and lack of awareness in many regions worsen the global burden of cancer [24].

Cancer is a disease in which abnormal cells divide uncontrolable form and invade surrounding tissues. Normally, the body regulates cell division through the cell cycle and programmed cell death. In cancer, genetic changes disrupt this system, allowing cells to multiply continuously. These abnormal cells may form tumors, although some cancers like leukemia do not produce solid masses. A key feature of cancer is metastasis, spreading from its original site to another parts of the

body, which makes treatment more difficult. Cancer cells can evade the immune system, allow them to survive longer. The disease can vary widely in severity and progression. Some cancers grow slowly, while others are highly aggressive. Tumors are abnormal growths of tissue resulting from excessive cell division. Benign tumor are non cancerous and do not spread to the other parts of body. They grow slowly and are less harmful. Malignant tumors are cancerous and can invade nearby tissues. They also have ability to metastasize to distant organs. Malignant tumors grow rapidly and are more dangerous. Diagnosis is typically confirmed through biopsy and imaging techniques. Early detection of malignant tumors improves treatment outcomes. Chemotherapy uses the drugs to kill rapidly dividing cancer cells.

These drugs move throughout the body, making them effective for widespread cancer. However, they also affect normal cells, causing side effects. Common side effects including hair loss, nausea and fatigue. Chemotherapy may be used before or after surgery. It can also be combined with other treatments. Advances have improved drug effectiveness and reduced side effects. Treatment schedules vary depending on the patient. Monitoring is essential during therapy. Despite challenges, chemotherapy remains a key treatment option. Radiotherapy uses high energy radiation to destroy cancer cells. It works by damaging DNA of cancer cells preventing growth. It is a localized treatment targeting specific areas. Radiotherapy is often combined with surgery or chemotherapy. Side effects may include fatigue and skin irritation. Advanced techniques improve precision and effectiveness. Treatment duration depends on cancer type. It is used for both curative and palliative purposes. Radiotherapy helps relieve symptoms in advanced cancer. Continuous research improves outcomes. It remains an important treatment method. In addition, newer approaches like targeted therapy and hormone therapy are being integrated into treatment plans to improve survival rates [25].

Cancer is caused by mutations in DNA of cells, which alter normal cellular functions. These mutations may occur due to internal factors such as inherited genetic changes or errors during cell division. External factors, including tobacco smoke, radiation, harmful chemicals, and infections, are major contributors.

For example, smoking is strongly linked to lung cancer, while ultraviolet radiation can cause skin cancer. Certain viruses, such as human papillomavirus (HPV), are also known to increase cancer risk. Often, cancer develops due to a combination of multiple risk factors over time. Lifestyle such as poor diet and poor exercise further increase susceptibility. Environmental pollution plays role in cancer development. Not all mutations lead to cancer, but when critical genes are affected, the risk increases significantly. Tobacco use is most significant risk factor, responsible for a large proportion of cancer related deaths. Age is another important factor, as the risk increases with the accumulation of genetic damage over time. Lifestyle factors like, unhealthy diet, obesity and poor physical activity contribute significantly. Environmental exposures, including pollution and radiation, also play a major role. Genetic predisposition can increase the likelihood of certain cancers. Chronic infections and inflammation are additional contributing factors. Hormonal imbalances may influence cancers like breast and prostate cancer. Awareness and prevention strategies are essential in managing cancer risk. Cancer prevention involves reducing exposure to risk factors and adopting healthy habits. Avoiding tobacco is the most effective preventive measure. A balance diet rich in fruits and vegetables supports overall health. Apply regular physical activity reduces cancer risk. Limiting alcohol consumption also helps prevent cancer. Protecting skin from excessive sunlight reduces skin cancer risk. Vaccination against HPV and hepatitis B prevents certain cancers. Environmental protection is also important. Public awareness campaigns promote preventive measures. Prevention is more cost effective than treatment. Healthy lifestyle choices significantly reduce cancer risk. Additionally, workplace safety regulations and reduction of occupational hazards like asbestos exposure are important preventive measures [26].

Cancer develops through a multistep process involving the accumulation of genetic mutations over time. These mutations affect genes responsible for cell growth, division, and death. As mutations increase, cells begin to grow uncontrollably and avoid programmed cell death. These abnormal cells form tumors and may stimulate new blood vessel formation, known as angiogenesis. Eventually, some cancer cells spread to other parts of body. This process can take many years and involves multiple stages. Environmental factors and genetic factors both involve to this

progression. The complexity of cancer development makes it difficult to treat. Understanding this process helps in developing targeted therapies. Continuous research is improving knowledge in this area. DNA mutations are central to cancer development as they disrupt normal cellular regulation. Mutations can activate oncogenes which promote cell growth or deactivate tumor suppressor genes. This imbalance leads to uncontrolled cell division. Mutations may result from environmental exposures or errors during cell replication. Some mutations are inherited and increase cancer risk. Accumulation of multiple mutations is required for cancer to develop. Advances in genetic research have identified specific mutations linked to different cancers. This has led to targeted treatment approaches. Not all mutations are harmful, but critical ones can lead to cancer. Understanding DNA mutations is essential for diagnosis and therapy. Immunotherapy enhances body's immune system to fight cancer. It helps immune system to recognize and destroy cancer cell. Types include monoclonal antibodies and checkpoint inhibitors. It has shown success in treating certain cancers. Immunotherapy is more targeted than traditional treatments. Side effects may include immune reactions. Not all patients respond to this therapy. Research is ongoing to improve effectiveness. It is often used with other treatments. Personalized medicine is important in this approach. It represents a major advancement in cancer treatment. Furthermore, stem cell therapy and gene editing technologies are being explored as future strategies to correct genetic defects and improve treatment outcomes [27].

Cancer diagnosis involves various techniques to detect and confirm the disease. Imaging methods such as X rays, CT scans and MRIs help to identify abnormal growths. Blood tests can detect tumor markers associated with certain cancers. A biopsy is the most reliable method, where tissue is examined under a microscope. Molecular and genetic testing provide additional information about mutations. Early and accurate diagnosis is crucial for effective treatment. Screening programs help detect cancer before symptoms appear. Advances in diagnostic technology have improved accuracy. Diagnosis also helps determine cancer stage and type. This information guides treatment decisions. Early detection significantly improves cancer survival rates. Screening tests such as mammography, Pap smears, and colonoscopy help identify cancer early. Detecting cancer before

symptoms appear allows for more effective treatment. Early stage cancer is easier to treat and less likely to spread.

Public awareness plays a key role in early detection. Regular medical check ups are essential for high risk individuals. Technological advancements have improved screening accuracy. Early diagnosis reduces treatment costs and improves quality of life. Lack of awareness remains a challenge in many regions. Promoting early detection is key strategy in cancer control. Artificial intelligence and also machine learning are being applied to diagnostic imaging, improving accuracy and speed in detecting cancerous changes [28].

2.2 Breast Cancer Introduction

BC is a malignant disease in which the breast cells grow and multiply without control, most often starting in ducts or lobules of breast tissue. Under normal physiological conditions, cell growth and division are carefully regulated, but in breast cancer these mechanisms fail, leading to tumor formation and the possibility of metastasis. BC is one of the most common cancer affecting women over worldwide and men can also develop it in rare cases.

The disease is influenced by genetic mutations, hormonal changes, environmental exposures, and lifestyle factors. Family history, reproductive patterns, and age further increase risk, while obesity and alcohol consumption are also linked to higher incidence. Detecting breast cancer early greatly improves survival chances, which makes screening programs, self examination, and public awareness essential parts of effective management.

The top section shows the anatomy of the breast, including fat, muscle, ribs, lobules, ducts, and the nipple. It highlights where ductal cancer (originating in the milk ducts) and lobular cancer (originate in lobules) begin. The bottom section explains the five stages of cancer progression starting from normal cells, moving through atypical hyperplasia and in situ carcinoma, then becoming invasive as cancer breaks through the duct wall, and finally metastatic, when cancer cells

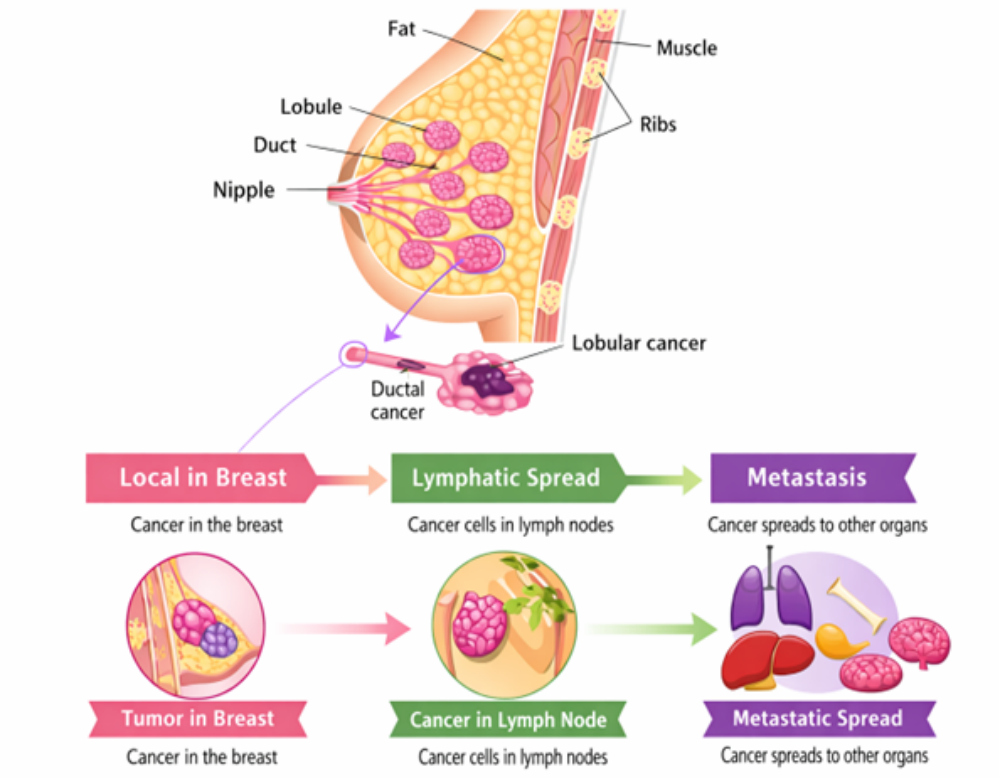


FIGURE 2.1: Breast cancer's transformation from a localized condition to a systemic disease [29].

travel through the blood and lymph vessels to the other parts of body. According to recent global data, breast cancer has surpassed all other cancers in incidence with the millions of new cases diagnosed annually. Advance in molecular biology, imaging technologies, and therapeutic approaches have improved prognosis and patient outcomes. However, mortality remains high in low and middle income countries due to late diagnosis and limited healthcare access. Continuous research is necessary to improve prevention, early diagnosis, and treatment strategies. This study aims to provide a comprehensive overview of BC biology, risk factors and therapeutic approaches [29, 30] (Fig. 2.1).

2.3 Molecular Basis of Breast Cancer

BC develops through genetic and molecular changes that interfere with normal cell cycle regulation. Signaling pathways i.e, HER2, PI3K/Akt and MAPK frequently disrupted leading to uncontrolled cell growth and enhanced survival of abnormal

cells. Tumor suppressor genes like p53 lose their protective function, allow cancer cells to avoid programme cell death. These molecular alterations also enable invasion into the surrounding tissues and metastasis to distant organs. They explain why tumors often resist therapy and recur after treatment. Advances in molecular biology have identified biomarkers that aid in diagnosis and prognosis. Personalized medicine relies heavily on understanding these molecular changes. Targeted therapies are designed based on these pathways, improving treatment outcomes. Continuous research is exploring new molecular targets, including epigenetic regulators and immune checkpoints. Understanding molecular mechanisms is essential for modern cancer research and clinical applications [29].

2.4 Breast Cancer Epidemiology

The epidemiology of BC varies across regions due to differences in lifestyle, genetics, and healthcare systems. Developed countries report higher incidence rates, largely because of widespread screening and advanced diagnostic facilities. In contrast, developing countries show higher mortality rates since many cases are detected at later stages. Age is a major risk factor, with most cases occurring in women over 50. Genetic predisposition, especially mutations in *BRCA1* and *BRCA2*, further increases susceptibility. Epidemiological studies help identify high risk groups and guide awareness campaigns. They also assist policymakers in allocating resources for screening and treatment programs. Trends over time reveal how environmental and lifestyle changes affect disease occurrence. Monitoring epidemiology supports early detection strategies and clinical research. It plays a vital role in designing preventive interventions. Global collaborations and registries are increasingly important to reduce disparities in outcomes [29, 30].

2.5 Breast Cancer Risk Factors

Risk factors for BC include both modifiable and non modifiable elements. Genetic mutations such as *BRCA1* and *BRCA2* are strong hereditary risk factors.

Hormonal influences, including early menarche, late menopause, and prolonged estrogen exposure, significantly raise risk. Lifestyle factors like obesity, alcohol use, smoking and physical inactivity also contribute to disease development. Environmental exposures like radiation and pollutants further increase risk. Reproductive history, such as delayed childbirth or not breastfeeding, is linked to higher susceptibility. Chronic inflammation and certain benign breast conditions may predispose individuals to cancer. Awareness of these risk factors supports preventive measures and early screening. Lifestyle changes can reduce risk in many people. Identifying risk factors is crucial for clinical management and research. Understanding them helps design effective prevention strategies, and integrating genetic counseling with lifestyle interventions provides a more comprehensive approach [30].

2.6 Genetic Alterations

BRCA1 and *BRCA2* genes are vital for DNA repair and maintaining genome stability. Mutations in genes greatly increase the risk of breast cancer and ovarian cancers. These mutations can be inherited, making family history an important risk indicator. Faulty DNA repair leads to mutation accumulation, driving cancer development. Genetic testing identifies high risk individuals, enabling preventive strategies such as enhanced screening or preventive surgery. BRCA mutations are also important for selecting targeted therapies like PARP inhibitors. Genetic counseling is essential for affected individuals and families. Research on BRCA genes has advanced understanding of hereditary breast cancer. These mutations serve as biomarkers for risk assessment. Studying BRCA genes remains a critical focus in cancer genetics, and new studies are exploring how BRCA mutations interact with other genetic and environmental factors [30].

2.7 Types of Breast Cancer

BC is divided into subtypes based on molecular and receptor features. These include HER2 positive, hormone receptor positive (Luminal A and B), and triple

negative breast cancer. Subtype shows distinct biological behavior and treatment response. HER2 positive cancers are aggressive but respond well to targeted therapy. Triple negative breast cancer lacks receptors and is harder to treat. Subtype classification is essential for personalized medicine. Molecular profiling guides treatment choices and predicts outcomes. Different subtypes require different therapeutic strategies, and ongoing research is identifying additional subgroups that may benefit from novel therapies.

2.7.1 Hormonal Role of Breast Cancer

Hormones such as estrogen and progesterone plays major role in breast cancer development. These hormones stimulate breast cell growth, raising the chance of mutations. Hormone receptor positive cancers depend on these hormones for growth. Estrogen receptors (ER) and progesterone receptors (PR) are used to classify tumors and guide treatment. Hormone therapy targets these pathways to block tumor growth. Hormonal imbalance, especially in postmenopausal women, increases risk. Long term hormone replacement therapy also contributes to risk. Understanding hormonal influence is crucial for treatment planning and prognosis. Hormone based therapies have greatly improved survival rates. Research continues to refine these therapies with fewer side effects. Hormonal mechanisms are central to breast cancer biology, and new agents are being tested to overcome resistance to current hormone therapies [31].

2.8 Early Stage Diagnosis and Screening

Early stage detection is key to improving breast cancer survival. Screening methods include mammography, ultrasound, MRI, and self examination. Mammography is the most widely used technique for early detection. High risk individuals may need advanced imaging such as MRI. Early diagnosis reduces treatment complexity and improves prognosis. Public awareness campaigns encourage regular screening. Screening programs are vital for lowering mortality rates. Research

aims to improve diagnostic accuracy and develop biomarkers. Early detection remains a cornerstone of cancer control strategies. It plays a important role in reducing disease burden also digital health tools like AI assisted imaging are being developed to enhance accuracy [31].

2.9 Tumor Microenvironment in Breast Cancer

Tumor microenvironment consists of surrounding cells, immune cells, blood vessels and extracellular matrix. It plays key role in tumor growth, progression and metastasis. Cancer cells interact with stromal cells to promote survival and evade immune defenses. Angiogenesis supplies nutrients and oxygen to tumors. Immune cells may either suppress or support tumor growth. Targeting the tumor microenvironment has become a promising therapeutic strategy. Understanding these interactions helps predict treatment response. Research focuses on disrupting these supportive mechanisms. The microenvironment also contributes to drug resistance. Studying it is essential for developing advanced cancer therapies, and novel approaches such as immune modulation and nanotechnology are being explored to overcome its protective effects [32].

2.10 Metastasis in Breast Cancer

Metastasis refers to spread of breast cancer cells to the distant organs such as bones, lungs, liver and brain, and it is leading cause of the death among breast cancer patients. Cancer cells travels through blood and lymphatic systems to establish colonies in new tissues. Molecular processes like epithelial mesenchymal transition (EMT) play a critical role in enabling metastasis. Certain BC subtypes, such as triple negative and HER2 positive, show higher metastatic potential. Detecting metastasis early improves treatment outcomes and survival chances. Managing metastatic disease requires systemic therapies including chemotherapy, targeted therapy, and immunotherapy. Current research focuses on understanding

and blocking metastatic pathways. Despite progress, metastasis remains one of the greatest challenges in oncology, making it a central focus of ongoing cancer research [32].

2.11 Molecular Markers in Breast Cancer

Molecular markers are biological indicators use for diagnosis, prognosis and monitoring treatment response. Common biomarkers include HER2, estrogen receptor (ER), progesterone receptor (PR) and Ki–67. These markers help classify tumors and guide therapy decisions. Molecular assays are used to assess recurrence risk and predict treatment outcomes. Biomarkers enable personalized medicine approaches by tailoring treatment to individual patients. Ongoing research is dedicated to discovering new biomarkers for early detection and improved accuracy. They also enhance understanding of tumor biology and help track disease progression. Identifying biomarkers is crucial for developing targeted therapies. In modern oncology, biomarkers are central to precision medicine and are increasingly integrated into routine clinical practice [32].

2.12 Therapeutic Approaches in Breast Cancer

2.12.1 Chemotherapy

Chemotherapy uses cytotoxic drugs to destroy dividing cancer cells. It is applied both early and advanced stages of breast cancer. Drugs may be given before surgery (neoadjuvant) to shrink tumors and after surgery (adjuvant) to prevent recurrence. Chemotherapy affects normal cells leading to side effects such as nausea, hair loss and fatigue. Combination therapies improve effectiveness and reduce resistance.

Response varies depending on cancer subtype. Despite challenges, chemotherapy remains a fundamental treatment option in breast cancer management. Newer

drug formulations and supportive care strategies are being developed to minimize toxicity and improve patient quality of life.

2.12.2 Hormonal Treatment

Hormonal therapy is used for hormone receptor positive breast cancers. Drugs such as tamoxifen block estrogen activity or reduce its production. This therapy slows tumor growth and lowers recurrence risk. It is widely used as adjuvant treatment and has fewer side effects compared to chemotherapy. Hormone therapy plays key role in long term BC management. Advances in selective estrogen receptor modulators and degraders are expanding treatment options.

2.12.3 Targeted Treatment

Targeted treatment focuses on specific molecular targets involved in the cancer growth. HER 2 targeted drugs such as trastuzumab are highly effective in HER2 positive cancers. These therapies are more affective and less toxic than the traditional chemotherapy. Targeted therapy is often combined with other treatments and has significantly improved survival outcomes. Research continues to identify new targets such as PI3K and CDK4/6 pathways, offering more personalized treatment strategies [29, 32].

2.12.4 Immune Based Therapy

Immune based therapy strengthens the body immune system to fight cancer. Immune checkpoint inhibitor are especially useful in aggressive cancers like triple negative breast cancer. This therapy helps overcome immune evasion by cancer cells. Ongoing research aims to improve its effectiveness and expand its use to other subtypes. Immunotherapy represents promising approach in the cancer treatment and is increasingly integrated into clinical trials [32].

2.13 Target Gene

BRCA1 is a tumor suppressor gene located on chromosome 17q21 that plays a vital role in protecting cells from cancer. It maintains DNA stability, regulates cell growth and division, and promotes cell death when repair is not possible. Under normal conditions, BRCA1 detects DNA breaks, recruits repair proteins, and pauses the cell cycle until the damage is corrected. If the damage cannot be repaired, BRCA1 triggers apoptosis, preventing unstable cells from multiplying. In this way, BRCA1 helps reduce the risk of tumor formation and supports the body's defense against genetic errors [33].

When BRCA1 functions normally, it repairs DNA damage caused by everyday cellular processes or environmental factors such as radiation, pollution, and chemicals. By keeping the genetic code accurate, BRCA1 prevents abnormal cell growth and lowers the risk of cancer. This protective role is especially important in tissues like the breast and ovaries, where cells divide rapidly. Proper functioning of BRCA1 therefore ensures long-term cell health and stability.

When BRCA1 is mutated, its protective functions are lost. The repair system becomes weak, DNA errors accumulate, and other important genes that control growth and cell death are affected. This instability leads to uncontrolled cell division and cancer. Mutations in BRCA1 are often hereditary, passed from parents to children, which explains the strong familial involvement in breast and ovarian cancers. Individuals who inherit a faulty BRCA1 gene are more likely to develop aggressive cancers, particularly triple-negative breast cancer, which is harder to treat. Men can also inherit BRCA1 mutations, increasing their risk of prostate and other cancers [34].

Environmental stress further worsens the impact of BRCA1 mutations. In healthy cells, BRCA1 repairs DNA damage caused by radiation or carcinogenic chemicals, but in mutated BRCA1 carriers, these stresses accelerate mutation accumulation. This combination of hereditary mutation and environmental exposure creates a high-risk environment for cancer development. Clinically, BRCA1 mutations are important because they make cancer cells vulnerable to targeted drugs such as

PARP inhibitors like Olaparib, which block alternative repair pathways and selectively kill tumor cells[35].

Normal BRCA1 protects DNA and prevents cancer, while mutated BRCA1 fails to repair damage, allowing mutations to build up and cause disease. Its role in hereditary transmission, environmental sensitivity, and therapeutic targeting makes BRCA1 one of the most critical genes in cancer biology and treatment.

2.14 Target Plant

A commonly selected medicinal plant is *Berberis lycium*, which is used in traditional herbal medicine. It is locally known as "Kashmal" or "Ishkeen" in Pakistan. This plant is a thorny evergreen shrub found in mountainous regions, especially in northern areas like Khyber Pakhtunkhwa and Azad Kashmir. It typically grows up to 2–3 meters tall and has yellow wood, small green leaves, and red or purple berries. The roots and bark are rich in important phytochemicals like berberine, which give it strong medicinal properties. Traditionally it is used for treating infections, diabetes, jaundice and eye diseases, and it is also being studied for the potential role in cancer treatment [36]. Taxonomic classification of *Berberis lycium* is shown in .

TABLE 2.1: Taxonomic classification of *Berberis lycium* Royle [36]

Taxonomic Rank	**Classification**
Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicotyledonae
Order	Ranunculales
Family	Berberidaceae
Genus	*Berberis*
Species	*Berberis lycium* Royle

Fig. 2.2 and Fig. 2.3 shows the flowers and leaves of *B. lycium*. *Berberis lycium* roots are woody and yellow inside due to the presence of the alkaloid berberine shown in Figure 2.5 . These roots have been used in traditional medicine for treating infections, inflammation, and other ailments [37].

The plant is native to mountain slopes in northwestern Indian Subcontinent, thriving in rocky and high-altitude regions [38].

FIGURE 2.2: Flowers of *B. lycium* [36].

FIGURE 2.3: Leaves of *B. lycium* [36] .

FIGURE 2.4: Fruits (berries) of *B. lycium* [36].

Berberis lycium, also known as Indian lycium, Indian barberry or boxthorn barberry, belongs to the family Berberidaceae [39]. It is a widespread species, and its fruit called kasmal is edible and consumed fresh, cooked or preserved [40].

FIGURE 2.5: Fruits (berries) of *B. lycium* [37].

The plant produces yellow flowers and small red to purple berries, which are incorporated into traditional foods. Medicinally, its roots and bark are highly valued for containing berberine, a compound with antimicrobial and also antioxidant properties. Traditionally, *Berberis lycium* has been use to treat jaundice, fever, diabetes, wounds and digestive disorders [41]. In addition, its berries are rich in

vitamins and minerals making them useful as a nutritional supplement in rural diets (Fig. 2.4).

Berberis lycium has been studied for the role in cancer treatment due to bioactive compounds, especially berberine. Research shows that berberine inhibits cancer cells growth and proliferation, while inducing apoptosis (programmed cell death). It reduces oxidative stress through antioxidant activity and prevents tumor progression with anti inflammatory effects.

Laboratory studies suggest potential benefits against breast, colon, and liver cancers. However, most findings are preclinical, so *Berberis lycium* should be considered a supportive herbal agent rather than a substitute for standard treatments like chemotherapy. More clinical studies needed to confirm its safety & effectiveness in humans [42].

2.15 Phytochemicals and Compounds in *Berberis lycium*

Berberis lycium is a medicinally important plant rich in bioactive phytochemicals, primarily extracted from its root bark, stem, and leaves using solvent based methods. The process involves drying and pulverizing plant material, followed by successive extraction with the solvents of increasing polarity such as petroleum ether, ethyl acetate and methanol or ethanol. This allows separation of non polar, semi polar, and polar compounds. Berberine, the principal isoquinoline alkaloid, is efficiently extracted using acidified alcohol due to its solubility in acidic conditions.

Related alkaloids such as palmatine, oxyberberine, and koumidine are isolated from alkaloid rich fractions using chromatographic techniques like high performance liquid chromatography and column chromatography.

Phenolic compounds including coumarins and stilbenes are extracted using semi polar solvents such as ethyl acetate or methanol. Modern techniques like ultrasound assisted and microwave assisted extraction enhance yield and stability.

It is important to note that favipiravir is a synthetic antiviral drug and not a natural phytochemical of *Berberis lycium*. Extraction efficiency depends on solvent polarity, compound chemistry, and purification methods. Recent studies also highlight antioxidant, antimicrobial, and anticancer properties of *Berberis lycium* phytochemicals, making them valuable for pharmacological applications.

Nanotechnology based delivery systems are being explored to improve bioavailability of these compounds. Overall, *Berberis lycium* remains a promising source of therapeutic agents with diverse biological activities [43].

2.16 Medicinal Properties

Berberis lycium Royle is a medicinal plant used in Pakistan and Himalayan regions. It contains the bioactive compounds like berberine, alkaloids, tannins and flavonoids that contribute to diverse pharmacological effects. Extracts of its root bark show strong antioxidant activity, reducing stress and protecting cells. It has antidiabetic properties by lowering blood glucose levels, and hepatoprotective effects that safeguard liver cells and improve liver function.

The plant demonstrates antimicrobial activity against bacteria such as *E. coli*, *Salmonella typhi*, and *Shigella dysenteriae*, supporting its traditional use in infections. It further shows anti inflammatory and antipyretic effects helping to reduce fever and inflammation.

Recent studies highlight its anticancer potential, with extracts showing cytotoxic effects against cancer cell lines, suggesting possible applications in breast cancer therapy.

Traditionally, it has also been used for wound healing, jaundice, diarrhea, cough, and skin diseases. Due to its rich phytochemical profile and multiple pharmacological actions, *Berberis lycium* is considered a promising natural source for developing herbal medicines and modern therapeutic agents Fig. 2.6 .

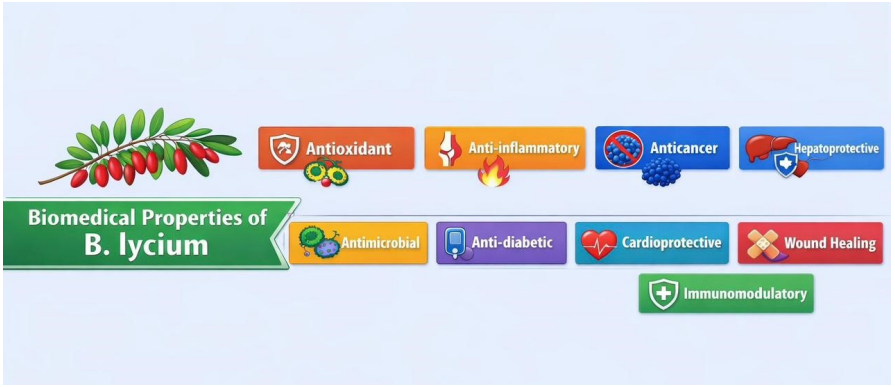


FIGURE 2.6: Flowchart representing biomedical properties of *B. lycium*[44].

2.17 History of Medicinal Plant Use

Medicinal plants used since ancient times for healing and maintaining health. Civilizations such as the Egyptians, Chinese, Greeks, and Indus Valley people relied on herbs for treatment. Systems like Ayurveda, Unani, and Traditional Chinese Medicine are based on plant remedies. Roots, leaves, bark, and flowers were used to prepare medicines, knowledge passed down through generations.

Many modern drugs originated from plants, such as aspirin from willow bark. Before modern medicine, plants were the primary source of healthcare. Historical records confirm their central role in both traditional and modern pharmacology [45].

2.18 Universal Applications of Medicinal Plants

Medicinal plants are used globally for preventing and treating diseases. They have applications in traditional medicine, pharmaceuticals, cosmetics, and nutraceuticals. Plant based compounds are developed into drugs for cancer, diabetes, infections, and cardiovascular diseases. Many people prefer herbal medicine for being natural and less toxic. They are also used in supplements to boost immunity and health. In rural areas, medicinal plants are often the first choice due to limited healthcare access. Global interest in herbal medicine is rising because of resistance

to synthetic drugs. Pharmaceutical industries extract active compounds for drug development, while scientific research validates their effectiveness. Thus, medicinal plants hold universal importance in healthcare worldwide [45, 46].

2.19 Therapeutic Herbs

Pakistan has rich biodiversity and a strong tradition of medicinal plant use. Many therapeutic herbs grow naturally in regions like Kashmir, Khyber Pakhtunkhwa, and Gilgit Baltistan. Common herbs include *Berberis lycium*, *Withania somnifera* (Ashwagandha), *Foeniculum vulgare* (Fennel), and *Nigella sativa* (Kalonji). These plants treat digestive problems, infections, diabetes, and inflammation. Pakistan's medicinal plants are also valuable for pharmaceutical research and drug development. Many have been scientifically studied for their bioactive compounds. Government and research institutions working to conserve these resources. Therapeutic herbs thus form an important part of Pakistan's healthcare system and cultural heritage [46, 47].

Chapter 3

Methodology

The methdology flowchart is shown in Figure 3.1

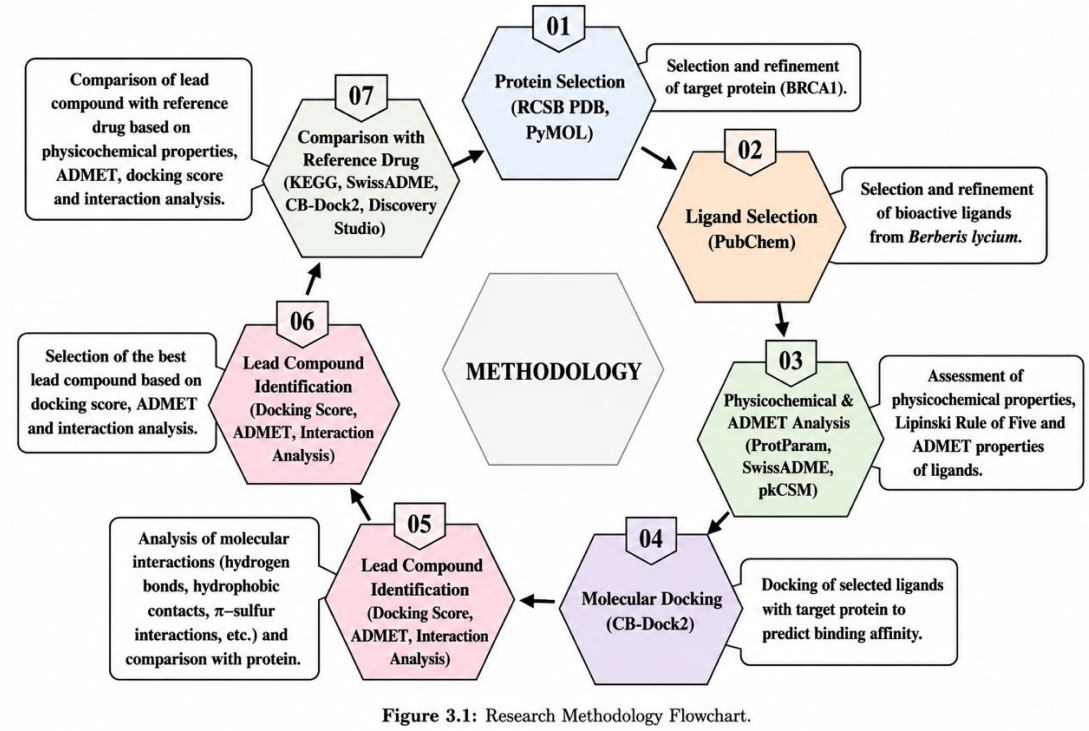


Figure 3.1: Research Methodology Flowchart.

FIGURE 3.1: Research Methodology Flowchart.

3.1 Protein Selection

The first step was to choose BRCA1 protein because it plays important role in repairing DNA and is strongly linked with breast cancer. The three dimensional structure of BRCA1 was taken from Protein Data Bank (PDB) which is a trusted online source for protein structures [48]. The model was selected based on good resolution and complete chain details so that the later docking study would be reliable.

3.2 Protein Refinement

After downloading, the protein structure was cleaned to make it ready for docking. This cleaning process involved removing water molecules, extra atoms, and incomplete residues that could disturb ligand binding. The structure was checked and edited using PyMOL which is common tool for protein visualization [49]. Refinement is necessary because raw protein structures often contain small errors or distortions from crystallography, and minimizing energy makes the protein more realistic for docking studies.

3.3 Ligand Selection

Bioactive compounds were chosen from *Berberis lycium*, a medicinal plant known for its anticancer activity. Important alkaloids such as Berberine, Oxyberberine, Palmatine, Koumidine, Stilbene and Favipiravir were identified as possible ligands. Their chemical structures were downloaded from PubChem, which is a free and reliable online database of chemical information [50]. The ligands were selected because of their reported biological activity and relevance to cancer treatment. Using PubChem ensures that the structures are accurate and standardized for computational studies.

3.4 Ligand Refinement

After downloading, the ligands were prepared for docking through a refinement process. The 3D structures from PubChem were retrieved. Energy minimization was then applied to reduce steric clashes and stabilize bond angles, making the ligands biologically realistic [51]. The structure was checked and edited using PyMOL which is common tool for protein visualization. This refinement step is important because raw ligand structures may contain irregularities that can affect docking accuracy. By optimizing the geometry, the ligands achieve stable conformations that better represent their behavior in biological systems.

3.5 Drug Likeness and Pharmacokinetic Evaluation

3.5.1 Physicochemical Properties

To check how the ligands behave, their basic chemical properties were calculated. These included molecular weight, hydrogen bond donors and acceptors, polar surface area (TPSA) and partition coefficient (LogP). The values were collected from PubChem and further analyzed using the SwissADME web server [50]. These properties help in predicting solubility, permeability, and stability, which are important for deciding if a compound can be developed as a drug.

3.5.2 Lipinski's Pharmacological Guideline

Ligands were then tested against Lipinski's Rule of 5, which is a guideline to see if a compound can be taken orally. According to the rule, a compound is likely to be orally active if:

i. Molecular mass below 500 Daltons.

ii. Hydrogen bond donors limited to five.

iii. Hydrogen bond acceptors not exceeding ten.

iv. Log P value is less than five.

These values were checked using SwissADME [52]. This step makes sure that the ligands are drug like and suitable for oral use.

3.5.3 ADMET Properties

Finally, the ligands were tested for ADMET properties like, absorption, distribution, metabolism, excretion and toxicity. Predictions were made using pkCSM online tool [53]. These tools give information about how well the compound is absorbed in the body, whether it can cross the blood brain barrier, how it is metabolized, how it is removed, and whether it has any toxic effects. This step helps in removing unsafe compounds and keeping only those with good pharmacokinetic profiles.

3.6 Molecular Docking

The prepared protein structure was taken in the PDB format from Protein Data Bank. The refined ligands were also converted into proper 3D formats. Both the protein and ligands were uploaded into the CB-Dock2, which is an online tool used for docking studies [54]. CB-Dock2 automatically finds possible binding pockets in protein and places the ligands inside them to predict how they interact.

The docking process then calculates binding affinity scores (Vina scores), cavity volume, and binding site coordinates. These results show how strongly each ligand binds to the protein and where the binding occurs. The docking step is very important because it gives a computer based prediction of protein–ligand interactions before any laboratory experiments are done.

The output of docking is a protein–ligand complex file in PDB format, which shows the 3D arrangement of ligand inside the protein's binding site. This file is then used in the next step, called interaction analysis.

3.7 Docking Interaction Analysis

After docking, the protein–ligand complexes were studied to see how ligands interact with the BRCA1 protein. The docking output files in PDB format were opened in Discovery Studio Visualizer, which provides both 2D and 3D interaction maps. This tool highlights important interactions such as hydrogen bonds, hydrophobic contacts and van der Waals forces between ligand and amino acid residues. The generated 2D interaction diagrams were saved as files so they could be used later for comparison, reporting, and documentation. These diagrams make it easy to identify which residues are directly involved in stabilizing ligand inside binding pocket.

This analysis step is important because it not only supports the docking scores but also gives a visual explanation of how the ligand fits into the protein. Saving the 2D files ensures that the results are properly recorded and can be referred to in future studies or publications [55].

3.8 Lead Compound Identification

If docking and interaction analysis completed, the ligands were compared to find most suitable candidate. The choice of the lead compound was based on three main factors: binding affinity scores, interaction strength, and ADMET properties. First, the docking results were checked to see which ligand had the lowest binding energy, showing stronger and more stable binding with the BRCA1 protein [56]. Next, the 2D interaction diagrams saved from Discovery Studio Visualizer were examined to confirm the presence of hydrogen bonds and hydrophobic interaction with amino acid residues [55].

Finally, the ADMET evaluation was reviewed to ensure that the ligand had good absorption, safe metabolism, proper distribution, and low toxicity. By combining these findings, the ligand with the best docking score, strong interactions, and favorable ADMET profile was selected as the lead compound. This compound was considered the most promising candidate for further drug development against BRCA1 related breast cancer, while other ligands with weaker binding or poor pharmacokinetic properties were kept only for comparison [53].

3.9 Drug Selection and Screening

The drug was chosen from the KEGG database, which provides information about approved and experimental compounds [56]. The selected drug was then used as a reference compound for comparison with the plant based ligands. Docking was performed to check how strongly the drug and ligands bind with the BRCA1 protein.

After docking, the complexes were studied through Discovery Studio Visualizer, where the binding site interactions were observed. The generated 2D interaction diagrams were saved to record hydrogen bonds and hydrophobic contacts. This allowed a clear comparison between the KEGG selected drug and the natural ligands, helping to identify which compound showed stronger and more stable binding [57] (Fig. 3.1).

Chapter 4

Results

4.1 Structural Analysis of BRCA1 Protein

We explored how natural compounds from the *Berberis lycium* may interact with the BRCA1 protein using computational methods. BRCA1 is an important protein that helps repair damaged DNA, controls the cell cycle and keeps cells functioning normally.

When this protein does not work properly, it can lead to serious diseases like breast and ovarian cancer. Because of this, BRCA1 is considered an important target for cancer research.

We selected different bioactive compounds from *Berberis lycium*, especially alkaloids such as berberine and palmatine, because they are known for their medicinal and anticancer properties. These compounds were tested to see how well they can bind with the BRCA1 protein.

The amino acid sequence of BRCA1 was taken from UniProt database (ID: P38398). After that, we analyzed its basic properties using the ExPASy ProtParam tool. These properties included molecular weight, isoelectric point (pI), stability index, aliphatic index and GRAVY value. Results showed that the protein is fairly stable and mostly hydrophilic, which is helpful for further structural studies.

To understand the 3D shape of the protein, we used homology modeling tools like SWISS-MODEL. We selected suitable template structures from the Protein Data Bank based on similarity. Special focus was given to important regions of the protein, such as the RING domain and BRCT domain, because they play key roles in DNA repair and are often affected in cancer.

After building the 3D model, we checked its quality to make sure the structure was reliable. Then, we performed docking to study how selected compounds bind with BRCA1. Docking was carried out using CB-Dock2. This method helped us find possible binding sites and measure how strongly each compound interacts with the protein.

We looked at binding energy, hydrogen bonds, and other interactions. Some compounds, especially berberine, showed strong binding with important amino acids in the protein.

This suggests that these natural compounds may help in improving or supporting the function of BRCA1. Overall, the results indicate that *Berberis lycium* could be a useful source of compounds for future cancer treatment research.

4.1.1 BRCA1 Protein Sequence Retrieval

The amino acid sequence of the BRCA1 protein was obtained from UniProtKB database using the accession number P38398. This database is widely used for reliable and well-annotated protein information.

BRCA1 is a large protein made up of 1,863 amino acids and is mainly found in the nucleus of the cell. It plays important role in DNA repair, maintaining genetic stability, and regulating cell growth.

The retrieved sequence was saved in FASTA format and used as the starting material for further analysis, including physicochemical characterization, structure prediction, and molecular docking. This step ensured that all subsequent computational work was based on accurate and standardized protein data.

4.1.2 BRCA1 Protein Sequence

The amino acid sequence of BRCA1 protein retrieved from UniProtKB (Acce–ssion ID: P38398) is shown below Figure 4.1.

```
>sp|P38398|BRCA1_HUMAN Breast cancer type 1 susceptibility protein
OS=Homo sapiens OX=9606 GN=BRCA1 PE=1 SV=2
MDLSALRVEEVQNVINAMQKILECPICLELIKEPVSTKCDHIFCKFCMLKLLNQKKGPSQ
CPLCKNDITKRSLQESTRFSQLVEELLKIICAFQLDTGLEYANSYNFAKKENNSPEHLKD
EVSIIQSMGYRNRAKRLLQSEPENPSLQETSLSVQLSNLGTVRTLRTKQRIQPQKTSVYI
ELGSDSSEDTVNKATYCSVGDQELLQITPQGTRDEISLDSAKKAACEFSETDVTNTEHHQ
PSNNDLNTTEKRAAERHPEKYQGSSVSNLHVEPCGTNTHASSLQHENSSLLLTKDRMNVE
KAEFCNKSKQPGLARSQHNRWAGSKETCNDRRTPSTEKKVDLNADPLCERKEWNKQKLPC
...
```

FIGURE 4.1: Retrieved amino acid sequence of the human BRCA1 protein (UniProt accession: P38398).

4.1.3 Physicochemical Properties of the BRCA1 Protein

Physicochemical properties of the BRCA1 protein were analyzed to understand its structural and functional characteristics. The amino acid sequence of BRCA1 (UniProt ID: P38398) was used as input in ExPASy ProtParam tool. It allows the calculation of various important properties based on the protein sequence.

Through this analysis, key parameters like molecular weight, isoelectric point (pI), instability index, aliphatic index and GRAVY value were obtained.

These properties provide useful insights into protein's stability, solubility and overall behavior in a biological system (Table 4.1).

The three dimensional structure of protein retreived from alpha fold in shown in Figure 4.2

The three-dimensional (3D) structure of the BRCA1 protein was predicted using the SWISS-MODEL server based on homology modeling. The model was selected because it demonstrated the best overall structural quality and reliability. The selected model was generated using the 6g2i.1.K template, representing the BRCT

TABLE 4.1: Predicted physicochemical characteristics of the BRCA1 protein obtained using the ExPASy ProtParam server.

No.	Properties	Results	Interpretation
1	Number of amino acids	1863	Indicates a large protein with multiple domains.
2	Molecular weight	207,785 Da	High molecular weight reflects complex biological role.
3	Theoretical isoelectric point (pI)	5.49	Protein is acidic in nature.
4	Total negatively charged residues (Asp + Glu)	212	Contributes to overall negative charge.
5	Total positively charged residues (Arg + Lys)	169	Lower than negative residues, confirming acidic protein nature.
6	Protein instability index	48.63	Value >40 indicates protein is unstable in vitro.
7	Aliphatic index	68.87	Suggests moderate thermal stability.
8	GRAVY (Hydropathy value)	-0.716	Negative value indicates protein is hydrophilic and soluble in aqueous environments.
9	Extinction coefficient	86,955	Useful for protein quantification in spectrophotometric assays.
10	Estimated half-life	30 hours (mammalian cells)	Indicates reasonable stability inside cells.

domain of the human BRCA1 protein. The model showed 100% sequence identity with the selected template and high template coverage for the modeled region.

The structural quality of the predicted model was evaluated using SWISS-MODEL validation tools. The model achieved a QMEANDisCo Global score of 0.77 ± 0.06, indicating good model reliability. The QMEAN Z-score was -1.46, suggesting that the predicted structure falls within the acceptable range when compared with experimentally determined protein structures.

Ramachandran plot analysis demonstrated that 96.70% of amino acid residues

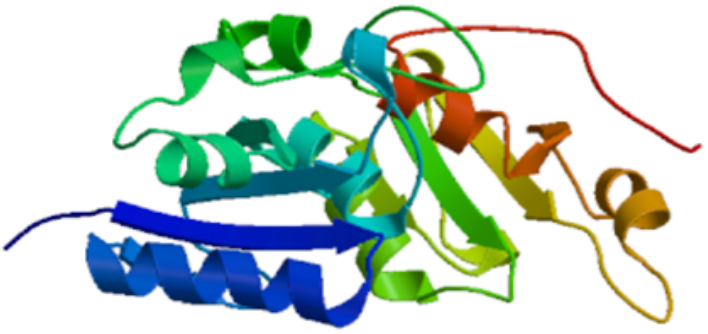


FIGURE 4.2: Three-dimensional (3D) structure of the BRCA1 protein predicted using the SWISS-MODEL server.

were located in the favored regions, while 0.00% of residues were present in outlier regions, confirming excellent stereochemical quality. Furthermore, the model exhibited a MolProbity score of 1.14 and a clash score of 1.75, indicating minimal steric clashes and good overall structural geometry. The model also contained 0 bad bonds (0/1761) and only 10 bad bond angles out of 2389, reflecting acceptable bond geometry. Based on these validation parameters, the predicted BRCA1 structure was considered reliable and was selected for subsequent molecular docking studies.

4.1.4 Functional Characteristics of BRCA1

BRCA1 is a tumor suppressor gene that helps to maintain genomic stability by repairing DNA damage, especially double strand breaks through homologous recombination. It controls cell cycle checkpoints to prevent damaged cells from dividing and also plays role in regulating gene expression that involved in DNA repair.

TABLE 4.2: Validation results of the selected BRCA1 BRCT domain model.

Parameter	**Value**
Modeling Server	SWISS-MODEL
Template	6g2i.1.K
Protein	BRCA1 BRCT domain
Sequence Identity	100%
QMEANDisCo Global	0.77 ± 0.06
QMEAN Z-score	-1.46
MolProbity Score	1.14
Clash Score	1.75
Ramachandran Favoured Residues	96.70%
Ramachandran Outliers	0.00%
Bad Bonds	0/1761
Bad Bond Angles	10/2389

BRCA1 has E3 ubiquitin ligase activity that helps regulate important cellular proteins. Overall, it protects cells from mutations and its dysfunction greatly increases the risk of breast and ovarian cancer.

4.1.4.1 BRCA1 Domains

BRCA1 is a key tumor suppressor protein that plays an essential role in repairing DNA, regulating the cell cycle and preserving genomic stability.

It contains several important functional regions, including the RING domain at the N-terminal end, which provides E3 ubiquitin ligase activity and supports interactions with proteins such as BARD1, and the BRCT domains at the C-terminal end, which bind phosphorylated proteins and are crucial for DNA damage signaling.

In addition, the central region includes coiled-coil domains that enable interaction with proteins like PALB2, connecting BRCA1 to homologous recombination repair mechanisms.

Together, these domains coordinate the detection of DNA damage and the recruitment of repair systems, helping to prevent mutations that may result in cancers, especially breast and ovarian cancer.

FIGURE 4.3: BC type 1 susceptibility protein's sequence alignment , structure features and binding sites.

4.1.5 3D Structure Prediction of BRCA1

After getting the FASTA sequence of BRCA1, predicted its three dimensional structure using tools like SWISS-MODEL.

TABLE 4.3: Functional features of the BRCA1 protein from ProtParam.

Region	**Residues**	**Role**	**Key Interactions**
BRCT 1	1642–1736	Phosphoprotein binding domain; recognizes phosphorylated partners in DNA repair.	Interacts with CtIP, Abraxas, BRIP1, and other DNA damage response proteins.
BRCT 2	1756–1855	Works with BRCT 1 and stabilizes binding to phosphorylated proteins.	Same partners as BRCT 1; essential for checkpoint signaling and repair complex assembly.
RING-type domain	24–65	Provides E3 ubiquitin ligase activity and stabilizes BRCA1.	Strongly interacts with BARD1 to form the BRCA1–BARD1 heterodimer.
PALB2 interaction site	1397–1424	Connects BRCA1 to PALB2, enabling BRCA2 recruitment for homologous recombination.	Forms the BRCA1–PALB2–BRCA2 complex; essential for RAD51 loading.
Turn motif	5–7	Provides structural flexibility and proper folding of the RING domain.	Helps orient zinc-binding residues in the RING domain.
Basic/Acidic residues	327–338	Forms a charged patch important for DNA/protein binding.	May interact with nucleic acids or regulatory proteins.
ComBias region	1445–1470	Low-complexity region; mediates flexible or transient interactions.	Could bind repair proteins or regulatory factors in a dynamic manner.

4.1.5.1 SWISS-MODEL

It uses homology modeling, which means it builds the BRCA1 structure based on similarity to proteins whose structures are already solved. If a BRCA1 domain

has a close match in the database, SWISS-MODEL can create a reliable model.

For example, the BRCT domains of BRCA1 have good template coverage, so SWISS-MODEL can generate accurate models of these regions.

4.2 Phytochemical Analysis of *Berberis lycium*

4.2.1 Compound Selection

In our literature based compound selection, we included berberine, oxyberberine, palmatine, koumidine, stilbene, and favipiravir. Most of these compounds are naturally present in *Berberis lycium* (Indian barberry) and related medicinal plants, which are traditionally used for their therapeutic properties.

The basis of their selection was the scientific evidence reporting strong bioactivity, particularly anticancer, antioxidant and antiviral effects.

Since *Berberis lycium* is rich in isoquinoline alkaloids like berberine, oxyberberine, palmatine, and koumidine, it served as the primary plant source in our study.

Coumarin and stilbene derivatives are also well known plant compounds with medicinal relevance, while favipiravir was included as a synthetic drug due to its pharmacological importance.

Altogether, these compounds were chosen based on published evidence of bioactivity with *Berberis lycium* serving as the primary natural source for several of these alkaloids, making it a key plant in our study for docking and interaction analysis with BRCA1 (Table 4.4).

4.2.2 Compounds with IUPAC Names and 2D structures

The selected compounds and their 2d structure and IUPAC names are shown in (Tables 4.5 and 4.6).

TABLE 4.4: Selected ligands and their types retrieved from *Berberis lycium*.

Sr. No.	Ligand Name	PubChem CID	Class	Structure Type
1	Berberine	2353	Alkaloid	Heteropentacyclic
2	Oxyberberine	11066	Alkaloid	Oxidized polycyclic
3	Palmatine	19009	Alkaloid	Heterotetracyclic
4	Koumidine	44584550	Alkaloid	Polycyclic indole
5	Stilbene	5280794	Aromatic hydrocarbons	Linear diaryl ethene
6	Favipiravir	492405	Pyrazines (Antiviral drug)	Heterocyclic

4.3 Compound Structure Retrieval (PubChem)

For this study, the required 2D and 3D compound structures were obtained using PubChem. The compounds were saved in suitable formats such as SDF, MOL, and PDB to ensure compatibility with docking software.

Preparing these structures was a critical step, as correct molecular geometry and proper formatting are essential for reliable docking simulations, enabling precise evaluation of ligand–protein interactions with BRCA1 and supporting the identification of potential drug candidates (Tables 4.5 and 4.6).

4.4 Lipinski Rule of 5

Based on the Lipinski Rule of 5, a compound is generally expected to have good oral bioavailability if it satisfies most of the following conditions:

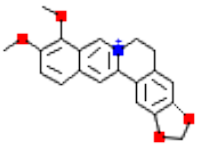
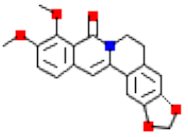
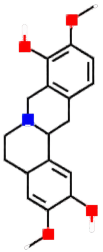
i. Molecular weight should not exceed 500 daltons.

ii. LogP (octanol–water partition coefficient) should remain at 5 or below.

iii. Hydrogen bond donors should be limited to 5 or fewer.

iv. Hydrogen bond acceptors should be capped at 10 or fewer.

TABLE 4.5: Compounds, IUPAC names, molecular formulae, molecular weights and 2D structures (Part I).

Compound	**IUPAC**	**MF**	**MW**	**2D Structure**
Berberine	16,17-dimethoxy-5,7-dioxa-13-azoniapentacyclo[11.8.0–.$0^{2,10}$.$0^{4,8}$.$0^{15,20}$]henicosa-1(13),2,4(8),9,14,16,18,20-octaene	$C_{20}H_{18}NO_4^+$	336.4	
Oxyberberine	16,17-dimethoxy-5,7-dioxa-13-azapentacyclo[11.8.0–.$0^{2,10}$.$0^{4,8}$.$0^{15,20}$] henicosa-1(21),2,4(8),9,15(20),16,18-heptaen-14-one	$C_{20}H_{17}NO_5$	351.4	
Palmatine	2,3,9,10-tetramethoxy-5,6-dihydroisoquinolino[2,1-b]isoquinolin-7-ium	$C_{21}H_{22}NO_4^+$	352.4	

v. A compound is considered likely to be orally active if it does not violate more than one of these guidelines.

Our compounds follow the Lipinski Rule of Five, meaning they have suitable molecular weight, LogP, hydrogen bond donors and hydrogen bond acceptors within the required limits. Since they do not violate more than one of these guidelines, they are considered likely to have good oral bioavailability and drug-like properties (Table 4.7).

TABLE 4.6: Compounds, IUPAC names, molecular formulae, molecular weights and 2D structures (Part II).

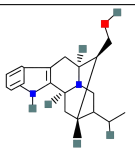
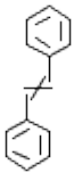
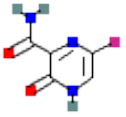
Compound	**IUPAC**	**MF**	**MW**	**2D Structure**
Koumidine	[(1S,12S,13S,14R,15Z)-15-ethylidene-3,17-diazapentacyclo[12.3.1.$0^{2,10}$.$0^{4,9}$.$0^{12,17}$]octadeca-2(10),4,6,8-tetraen-13-yl]methanol	$C_{19}H_{22}N_2O$	294.4	
Stilbene	Stilbene	$C_{14}H_{12}$	180.24	
Favipiravir	5-fluoro-2-oxo-1H-pyrazine-3-carboxamide	$C_5H_4FN_3O_2$	157.1	

TABLE 4.7: Physicochemical properties of *Berberis lycium* ligands.

Category	**Parameter**	**Berberine**	**Oxyberberine**	**Palmatine**	**Koumidine**	**Stilbene**	**Favipiravir**
Physicochemical Properties	Formula	$C_{20}H_{18}NO_4^+$	$C_{20}H_{17}NO_5$	$C_{21}H_{22}NO_4^+$	$C_{19}H_{22}N_2O$	$C_{14}H_{12}$	$C_5H_4FN_3O_2$
Physicochemical Properties	Molecular Weight	336.36 g/mol	351.35 g/mol	352.40 g/mol	294.39 g/mol	180.25 g/mol	157.10 g/mol
Physicochemical Properties	No. of H-bond Acceptors	4	5	4	2	0	4
Physicochemical Properties	No. of H-bond Donors	0	0	0	2	0	2
Lipophilicity	Log $P_{o/w}$	2.53	3.04	2.64	2.55	4.10	-0.27
Water Solubility	Log S (ESOL)	-4.55	-4.19	-4.58	-3.22	-4.49	-0.80
Pharmacokinetics	GI Absorption	High	High	High	High	Low	High
Drug-likeness	Lipinski Rule	Yes	Yes	Yes	Yes	Yes	Yes

4.5 ADMET Profile and Drug-Likeness

For ADMET evaluation, we collected the SMILES strings of our selected compounds directly from the PubChem database. These structures were then processed through the pkCSM platform, which generated predictions for absorption, distribution, metabolism, excretion, and toxicity.

4.5.1 Berberine

Berberine is a plant-derived alkaloid commonly found in barberry, goldenseal, and Oregon grape, recognized for its bright yellow color and traditional medicinal use. It shows promising antimicrobial, antioxidant, and metabolic effects, making it useful in managing conditions like diabetes, obesity, and high cholesterol. Although widely available as a dietary supplement, it is not officially approved as a prescription drug in most countries, but research continues to highlight its potential for cardiovascular and metabolic health.

4.5.1.1 Absorption Profile

Berberine has low water solubility with a predicted value of -3.973, which makes it harder to dissolve. Even so, it shows good movement across intestinal cells with a Caco-2 value of 1.734 and a very high human intestinal absorption rate of 97.147%, meaning it can be absorbed well once it is dissolved. Its skin permeability is low at -2.576, so it is not effective for absorption through the skin.

Berberine is also a substrate of P-glycoprotein, which reduces how much of it is absorbed, and while it does not block P-gp I, it does block P-gp II, which may affect how it interacts with other medicines. Overall, berberine's absorption is limited by solubility and efflux transporters, but it still has strong natural potential for intestinal uptake as shown in Table 4.8.

TABLE 4.8: Absorption profile of Berberine.

Property	**Value**
Aqueous solubility	-3.973
Caco-2 cell permeability	1.734
Human intestinal absorption (%)	97.147
Dermal permeability	-2.576
P-glycoprotein substrate status	Yes
P-glycoprotein I inhibition	No
P-glycoprotein II inhibition	Yes

4.5.1.2 Distribution Profile

The distribution profile of berberine shows a steady-state volume of distribution (VDss) of 0.58 in humans, which reflects moderate spread into tissues. Its fraction unbound value of 0.262 means that about a quarter of the compound remains free in plasma and available for activity.

The predicted blood–brain barrier permeability of 0.198 indicates limited ability to cross into the brain, while the CNS permeability score of -1.543 further confirms restricted penetration into the central nervous system.

Overall, berberine distributes mainly to peripheral organs such as the liver and kidneys, with only modest access to the brain (Table 4.9).

TABLE 4.9: Distribution profile of Berberine.

Property	**Value**
Volume of distribution (human)	0.58
Fraction unbound (human plasma)	0.262
Blood–brain barrier permeability	0.198
Central nervous system permeability	-1.543

4.5.1.3 Metabolism Profile

Berberine's metabolism shows that it is not processed by CYP2D6 but is broken down by CYP3A4. It can block several liver enzymes, including CYP1A2, CYP2D6, and CYP3A4, which means it may interfere with how other medicines are metabolised.

However, it does not affect CYP-2C19 or CYP-2C9. Berberine mainly relies on CYP3A4 for its breakdown and has the ability to inhibit some key enzymes, which could lead to drug interactions (Table 4.10).

TABLE 4.10: Metabolism profile of Berberine.

Property	**Value**
CYP-2D6 substrate	No
CYP-3A4 substrate	Yes
CYP-1A2 inhibitor	Yes
CYP-2C19 inhibitor	No
CYP-2C9 inhibitor	No
CYP-2D6 inhibitor	Yes
CYP-3A4 inhibitor	Yes

4.5.1.4 Excretion Profile

Berberine's excretion profile shows a total clearance of 1.27, meaning it is eliminated from the body at a moderate speed.

It is not a substrate of the renal OCT2 transporter, so this pathway does not contribute to its removal through the kidneys.

Berberine is cleared fairly quickly, mainly through bile and urine, rather than relying on OCT2-mediated kidney transport (Table 4.11).

TABLE 4.11: Excretion profile of Berberine.

Property	**Value**
Drug clearance	1.27
Renal OCT2 transporter	No

4.5.1.5 Toxicity Profile

Berberine's toxicity profile shows it is AMES toxic, meaning it has mutagenic potential, and its maximum tolerated dose in humans is quite low at 0.144. It does not affect heart rhythm since it does not inhibit hERG I or hERG II channels.

In rats, acute oral toxicity (LD_{50}) is 2.571 and chronic toxicity (LOAEL) is 1.89, pointing to possible long-term effects at higher doses.

It is linked to liver toxicity but does not cause skin sensitisation. Other models suggest moderate toxicity in *Tetrahymena pyriformis* and low toxicity in minnows. In short, berberine carries risks of genetic and liver toxicity but is less harmful for the heart and skin (Table 4.12).

TABLE 4.12: Toxicity profile of Berberine.

Property	**Value**
AMES assessment	Yes
Maximum tolerated dose (human)	0.144
hERG I inhibitor	No
hERG II inhibitor	No
Oral acute toxicity in rats (LD_{50})	2.571
Oral chronic toxicity in rats (LOAEL)	1.89
Hepatotoxicity	Yes
Skin sensitisation	No
Tetrahymena pyriformis toxicity	0.354
Minnow aquatic toxicity	-0.277

4.5.2 Oxyberberine

Oxyberberine is a natural compound made when berberine goes through oxidation, and it is found in plants like *Arcangelisia gusanlung* and *Thalictrum foliolosum*. It has a slightly different structure than berberine, which gives it stronger antioxidant effects and makes it very good at fighting harmful free radicals in the body.

Even though it does not dissolve well in water, researchers see it as a promising candidate for improving metabolic health and heart health.

4.5.2.1 Absorption Profile

Oxyberberine has poor water solubility with a value of -4.218, making it harder to dissolve, but it shows moderate permeability across intestinal cells (1.077) and excellent intestinal absorption at 100%. Its skin permeability is low (-2.623), so it is not suitable for absorption through the skin.

Unlike berberine, it is not a P-glycoprotein substrate, which helps avoid efflux problems, but it does inhibit both P-gp I and P-gp II, meaning it could interact with other drugs. In short, oxyberberine absorbs well in the intestine but struggles with solubility (Table 4.13).

TABLE 4.13: Absorption profile of Oxyberberine.

Model	**Outcome Value**
Aqueous solubility	-4.218
Caco-2 cell permeability	1.077
Human intestinal absorption (%)	100
Dermal permeability	-2.623
P-glycoprotein substrate status	No
P-glycoprotein I inhibition	Yes
P-glycoprotein II inhibition	Yes

4.5.2.2 Distribution Profile

Oxyberberine's distribution shows a very low VDss of −0.025, meaning it does not spread much into tissues. Its fraction unbound is 0.168, so only a small amount stays free in plasma. The blood–brain barrier permeability is −0.07 and central nervous system permeability is −2.116, both indicating very limited entry into the brain. Oxyberberine mostly stays in circulation with little distribution to the central nervous system (Table 4.14).

TABLE 4.14: Distribution profile of Oxyberberine.

Model	**Outcome Value**
Volume of distribution (human)	-0.025
Fraction unbound (human plasma)	0.168
Blood–brain barrier permeability	-0.07
Central nervous system permeability	-2.116

4.5.2.3 Metabolism Profile

Oxyberberine's metabolism shows it is not processed by CYP2D6 but is broken down by CYP3A4. It can block several enzymes, including CYP1A2, CYP2C19, CYP2C9, and CYP3A4, which means it may interfere with the breakdown of other medicines.

However, it does not inhibit CYP2D6. Oxyberberine mainly depends on CYP3A4 for metabolism and has the potential to interact with drugs by inhibiting multiple liver enzymes (Table 4.15).

4.5.2.4 Excretion Profile

Oxyberberine's excretion profile indicates a low total clearance of 0.121, meaning it is removed from the body slowly. Unlike berberine, it is a substrate of the renal OCT2 transporter, showing that kidney transport contributes to its elimination. In short, oxyberberine clears at a slower rate and relies on OCT2 for excretion (Table 4.16).

TABLE 4.15: Metabolism profile of Oxyberberine.

Model	**Outcome Value**
CYP-2D6 substrate	No
CYP-3A4 substrate	Yes
CYP-1A2 inhibitor	Yes
CYP-2C19 inhibitor	Yes
CYP-2C9 inhibitor	Yes
CYP-2D6 inhibitor	No
CYP-3A4 inhibitor	Yes

TABLE 4.16: Excretion profile of Oxyberberine.

Property	**Value**
Drug clearance	0.121
Renal OCT2 transporter	Yes

4.5.2.5 Toxicity Profile

Oxyberberine's toxicity profile shows it is not mutagenic (no AMES toxicity) and does not affect heart rhythm since it does not inhibit hERG I or hERG II channels. Its maximum tolerated dose in humans is very low (-0.234). In rats, acute toxicity (LD_{50}) is 2.394 and chronic toxicity (LOAEL) is 2.226, pointing to possible long-term effects. It is linked to liver toxicity but not skin sensitisation, with moderate toxicity in *Tetrahymena pyriformis* and mild toxicity in minnows. In short, oxyberberine mainly poses liver risks but is safer for the heart, skin, and genetics (Table 4.17).

4.5.3 Palmatine

Palmatine is a natural isoquinoline alkaloid belonging to the protoberberine family. It is structurally related to compounds like berberine and oxyberberine, sharing similar chemical features and biological activities. Palmatine is recognized for its bright yellow color and has been studied for its antimicrobial, anti-inflammatory, and antioxidant effects, making it an important member of the protoberberine group with potential medicinal applications.

TABLE 4.17: Toxicity profile of Oxyberberine.

Property	**Value**
AMES assessment	No
Maximum tolerated dose (human)	-0.234
hERG I inhibitor	No
hERG II inhibitor	No
Oral acute toxicity in rats (LD_{50})	2.394
Oral chronic toxicity in rats (LOAEL)	2.226
Hepatotoxicity	Yes
Skin sensitisation	No
Tetrahymena pyriformis toxicity	0.414
Minnow aquatic toxicity	0.165

4.5.3.1 Absorption Profile

Palmatine's absorption profile shows poor water solubility (−4.194) but still achieves moderate Caco-2 permeability (0.863) and high intestinal absorption 97.084%. Its skin permeability is low (−2.554), limiting absorption through the skin. Palmatine is both a substrate and inhibitor of P-glycoprotein I and II, suggesting possible drug interactions.

It absorbs well in the intestine despite low solubility and interacts with P-glycoprotein transporters [Table 4.18].

TABLE 4.18: Absorption profile of Palmatine.

Property	**Value**
Aqueous solubility	-4.194
Caco-2 permeability	0.863
Human intestinal absorption (%)	97.084
Skin permeability	-2.554
P-glycoprotein substrate	Yes
P-glycoprotein I inhibitor	Yes
P-glycoprotein II inhibitor	Yes

4.5.3.2 Distribution Profile

Palmatine's distribution profile indicates a moderate VDss of 0.641, showing it spreads somewhat into tissues. Its fraction unbound is 0.245, meaning about a quarter remains free in plasma.

With blood–brain barrier permeability at −0.112 and central nervous system permeability at −1.535, it has limited ability to cross into the brain. Palmatine distributes moderately in the body but shows poor penetration into the central nervous system[Table 4.19].

TABLE 4.19: Distribution profile of Palmatine.

Property	**Value**
Volume of distribution (VDss, human)	0.641
Fraction unbound (human plasma)	0.245
Blood-brain barrier permeability	-0.112
Central nervous system permeability	-1.535

4.5.3.3 Metabolism Profile

Palmatine's metabolism profile reveals that it is processed by CYP3A4 but not by CYP2D6. It shows inhibitory effects on CYP1A2 and CYP2D6, which could influence the breakdown of certain drugs, while having no impact on CYP2C19, CYP2C9, or CYP3A4. Overall, palmatine depends on CYP3A4 for metabolis[Table 4.20].

TABLE 4.20: Metabolism profile of Palmatine.

Property	**Value**
CYP2D6 substrate	No
CYP3A4 substrate	Yes
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	No

4.5.3.4 Excretion Profile

Palmatine's excretion profile indicates a total clearance of 1.246, showing it is eliminated from the body at a relatively faster rate. It is not a substrate of the renal OCT2 transporter, meaning kidney transport does not significantly contribute to its removal. Palmatine is cleared more efficiently without reliance on OCT2 pathways [Table 4.21].

TABLE 4.21: Excretion profile of Palmatine.

Property	**Value**
Drug clearance	1.246
Renal OCT2 transporter	No

4.5.3.5 Toxicity Profile

Palmatine's toxicity profile shows mutagenic potential (AMES positive) and a low maximum tolerated dose (0.231). It does not block hERG I but does inhibit hERG II, suggesting some cardiac risk.

Rat studies report acute toxicity (LD_{50} 2.551) and chronic toxicity (LOAEL 1.536). It is hepatotoxic but not a skin sensitiser, with moderate *Tetrahymena pyriformis* toxicity (0.388) and low minnow aquatic toxicity (-0.313). Overall, palmatine poses risks for genetic damage, liver effects, and certain heart concerns, while being less harmful to the skin and aquatic species [Table 4.22].

4.5.4 KOUMIDINE

Koumidine is a natural alkaloid from the sarpagan family, related to compounds like koumine. It has drawn interest in research because of its possible effects on inflammation and pain regulation. Due to its connection with other active alkaloids, koumidine is considered a promising subject for further studies in neurological and therapeutic applications.

TABLE 4.22: Toxicity profile of Palmatine.

Property	**Value**
AMES assessment	Yes
Maximum tolerated dose (human)	0.231
hERG I inhibitor	No
hERG II inhibitor	Yes
Oral acute toxicity in rats (LD_{50})	2.551
Oral chronic toxicity in rats (LOAEL)	1.536
Hepatotoxicity	Yes
Skin sensitisation	No
Tetrahymena pyriformis toxicity	0.388
Minnow aquatic toxicity	-0.313

4.5.4.1 Absorption Profile

Koumidine's absorption profile highlights poor water solubility (−3.913), yet it shows fairly strong Caco-2 permeability (1.495) and high intestinal uptake 95.26%. Its skin permeability is low (−2.941), limiting dermal absorption.

It acts as a P-glycoprotein substrate but does not inhibit P-gp I or II. Altogether, Koumidine is efficiently absorbed in the intestine despite low solubility, with minimal skin absorption and no inhibitory effects on P–gp transport [Table 4.23].

TABLE 4.23: Absorption profile of Koumidine.

Property	**Value**
Aqueous solubility	-3.913
Caco-2 cell permeability	1.495
Human intestinal absorption	95.26
Dermal permeability	-2.941
P-glycoprotein substrate status	Yes
P-glycoprotein I inhibition	No
P-glycoprotein II inhibition	No

4.5.4.2 Distribution Profile

Koumidine's distribution profile indicates a volume of distribution of 1.694, suggesting it disperses well into body tissues. Around 29.7% remains unbound in plasma, allowing moderate circulation.

With BBB permeability at 0.241, it shows some ability to cross the blood–brain barrier, though the CNS permeability value of −1.753 reflects limited entry into the central nervous system. In essence, Koumidine spreads broadly in the body but has restricted brain penetration [Table 4.24].

TABLE 4.24: Distribution profile of Koumidine.

Property	**Value**
Volume of distribution (human)	1.694
Fraction unbound (human plasma)	0.297
Blood–brain barrier permeability	0.241
Central nervous system permeability	-1.753

4.5.4.3 Metabolism Profile

Koumidine's metabolism profile shows that it is processed by both CYP2D6 and CYP3A4. It inhibits CYP1A2 and CYP2D6, which may interfere with the metabolism of certain drugs, while it does not affect CYP-2C19, CYP-2C9 or CYP3A4. Overall, Koumidine depends on CYP2D6 and CYP3A4 for metabolism and selectively blocks CYP1A2 and CYP2D6 activity [Table 4.25].

TABLE 4.25: Metabolism profile of Koumidine.

Property	**Value**
CYP-2D6 substrate	Yes
CYP-3A4 substrate	Yes
CYP-1A2 inhibitor	Yes
CYP-2C19 inhibitor	No
CYP-2C9 inhibitor	No
CYP-2D6 inhibitor	Yes
CYP-3A4 inhibitor	No

4.5.4.4 Excretion Profile

Koumidine's excretion profile shows a total clearance value of 1.143, reflecting a moderate elimination rate. It is also a substrate of the renal OCT2 transporter, meaning kidney transport mechanisms contribute to its removal. Koumidine is cleared at a steady pace with OCT2 involvement in its excretion [Table 4.26].

TABLE 4.26: Excretion profile of Koumidine.

Property	**Value**
Drug clearance	1.143
Renal OCT2 transporter	Yes

4.5.4.5 Toxicity Profile

Koumidine's toxicity profile suggests mutagenic activity (AMES positive) and low maximum tolerated dose in humans (−0.666). It does not affect hERG I but does inhibit hERG II, pointing to potential cardiac concerns. In rats, acute oral toxicity (LD_{50}) is 2.888, while chronic toxicity (LOAEL) is 2.074.

The compound shows liver toxicity but no skin sensitisation. Additional models indicate moderate *Tetrahymena pyriformis* toxicity (0.438) and mild minnow toxicity (0.182). Overall, Koumidine carries risks related to genetic damage, liver effects, and some heart issues, with limited impact on skin and aquatic organisms [Table 4.27].

4.5.5 STILBENE

Stilbene is an aromatic hydrocarbon made up of two benzene rings joined by a double bond and it acts as the base structure for many natural and synthetic compounds. Derivatives of stilbene, such as resveratrol, are known for their antioxidant, anti-inflammatory and anticancer properties. Because of these biological activities, stilbene and its related compounds are considered important in medicinal chemistry and natural product research.

TABLE 4.27: Toxicity profile of Koumidine.

Property	**Value**
AMES assessment	Yes
Maximum tolerated dose (human)	-0.666
hERG I inhibitor	No
hERG II inhibitor	Yes
Oral acute toxicity in rats (LD_{50})	2.888
Oral chronic toxicity in rats (LOAEL)	2.074
Hepatotoxicity	Yes
Skin sensitisation	No
Tetrahymena pyriformis toxicity	0.438
Minnow aquatic toxicity	0.182

4.5.5.1 Absorption Profile

Stilbene's absorption profile reveals very poor water solubility (−4.939), yet it maintains good Caco-2 permeability (1.579) and high intestinal absorption value (94.791%). Its skin permeability is low (−1.886), limiting dermal uptake.

Unlike Koumidine, it is neither a substrate nor an inhibitor of P-glycoprotein. Stilbene is well absorbed in the intestine despite low solubility, with minimal skin absorption and no P-gp interaction [Table 4.28].

TABLE 4.28: Absorption profile of Stilbene.

Property	**Value**
Aqueous solubility	-4.939
Caco-2 cell permeability	1.579
Human intestinal absorption	94.791
Dermal permeability	-1.886
P-glycoprotein substrate status	No
P-glycoprotein I inhibition	No
P-glycoprotein II inhibition	No

4.5.5.2 Distribution Profile

Stilbene's distribution profile shows a VDss of 0.73, suggesting moderate tissue spread. Its fraction unbound is extremely low (0.001), meaning it binds almost entirely to plasma proteins.

With BBB permeability at 0.608, it can cross the blood–brain barrier, but the CNS permeability value (−1.174) indicates limited penetration into the central nervous system. It distributes moderately in tissues, binds strongly in plasma, and has restricted brain access [Table 4.29].

TABLE 4.29: Distribution profile of Stilbene.

Property	**Value**
Volume of distribution (human)	0.73
Fraction unbound (human plasma)	0.001
Blood–brain barrier permeability	0.608
Central nervous system permeability	-1.174

4.5.5.3 Metabolism Profile

Stilbene's metabolism profile indicates that it is not metabolized by CYP2D6 but does undergo metabolism via CYP3A4. It inhibits CYP1A2, CYP2C19, and CYP2C9, which could interfere with the breakdown of drugs processed by these enzymes. However, it does not inhibit CYP2D6 or CYP3A4. It depends on CYP3A4 for metabolism while selectively blocking CYP1A2, CYP2C19, and CYP2C9 activity [Table 4.30].

4.5.5.4 Excretion Profile

Stilbene's excretion profile indicates a total clearance of 0.166, pointing to a relatively slow elimination rate. It is not recognized as a substrate of the renal OCT2 transporter, suggesting that kidney transport pathways are not significantly involved in its removal. Stilbene is cleared at a modest pace without OCT2 participation [Table 4.31].

TABLE 4.30: Metabolism profile of Stilbene.

Property	**Value**
CYP-2D6 substrate	No
CYP-3A4 substrate	Yes
CYP-1A2 inhibitor	Yes
CYP-2C19 inhibitor	Yes
CYP-2C9 inhibitor	Yes
CYP-2D6 inhibitor	No
CYP-3A4 inhibitor	No

TABLE 4.31: Excretion profile of Stilbene.

Property	**Value**
Drug clearance	0.166
Renal OCT2 transporter	No

4.5.5.5 Toxicity Profile

Stilbene's toxicity profile indicates it is not mutagenic (AMES negative) and has a relatively higher maximum tolerated dose in humans (0.947). It does not inhibit hERG I or hERG II, suggesting little risk of cardiac effects. In rats, both acute oral toxicity (LD_{50}) and chronic toxicity (LOAEL) are measured at 1.916. The compound is free from hepatotoxicity but does cause skin sensitisation.

Additional models show notable *Tetrahymena pyriformis* toxicity (1.501) and moderate minnow toxicity (0.62). Overall, it appears safe in terms of genetic and liver effects, though it may trigger skin reactions and display some toxicity in aquatic organisms [Table 4.32].

4.5.6 Favipiravir

Favipiravir is an antiviral drug first developed in Japan to treat influenza strains that resist regular medicines. It works by stopping the enzyme RNA-dependent RNA polymerase, which viruses need to multiply, and has shown effectiveness against several RNA viruses.

TABLE 4.32: Toxicity profile of Stilbene.

Property	**Value**
AMES assessment	No
Maximum tolerated dose (human)	0.947
hERG I inhibitor	No
hERG II inhibitor	No
Oral acute toxicity in rats (LD_{50})	1.916
Oral chronic toxicity in rats (LOAEL)	1.916
Hepatotoxicity	No
Skin sensitisation	Yes
Tetrahymena pyriformis toxicity	1.501
Minnow aquatic toxicity	0.62

Besides influenza, it has also been tested for illnesses like Ebola and COVID-19. Although usually well tolerated, it can cause side effects such as changes in liver function and is unsafe during pregnancy. At present, Favipiravir is officially approved mainly in Japan, while in other countries it has been used only under emergency or research settings.

4.5.6.1 Absorption Profile

Favipiravir shows moderate solubility (−2.121) and has low Caco-2 permeability (0.623), yet achieves strong intestinal absorption (91.69%). Skin absorption is negligible (−3.28), and it has no interaction with P-glycoprotein. Favipiravir is well absorbed in the gut, poorly soluble, minimally absorbed through skin, and free from P-gp effects [Table 4.33].

TABLE 4.33: Absorption profile of Favipiravir.

Property	**Value**
Aqueous solubility	-2.121
Caco-2 cell permeability	0.623
Human intestinal absorption	91.69
Dermal permeability	-3.28
P-glycoprotein substrate status	No
P-glycoprotein I inhibition	No
P-glycoprotein II inhibition	No

4.5.6.2 Distribution Profile

Favipiravir's distribution profile reveals a Volume of distribution of −0.218, indicating limited tissue spread, while its fraction unbound is relatively high (0.783), meaning much of the drug remains free in plasma rather than bound to proteins.

The BBB permeability value (−0.127) suggests poor ability to cross the blood–brain barrier, and the CNS permeability (−3.081) further confirms very restricted entry into the CNS.

Favipiravir shows weak tissue distribution, remains largely unbound in plasma, and has minimal penetration into the brain and CNS [Table 4.34].

TABLE 4.34: Distribution profile of Favipiravir.

Property	**Value**
Volume of distribution (human)	-0.218
Fraction unbound (human plasma)	0.783
Blood–brain barrier permeability	-0.127
Central nervous system permeability	-3.081

4.5.6.3 Metabolism Profile

Favipiravir's metabolism profile indicates that it is neither a substrate nor an inhibitor of the key CYP enzymes tested, including CYP2D6, CYP3A4, CYP1A2, CYP2C19, and CYP2C9.

This means the drug is unlikely to be metabolized through these pathways or interfere with the metabolism of other compounds that rely on them.

Also, it shows minimal involvement with CYP enzymes, lowering the likelihood of drug–drug metabolic interactions [Table 4.35].

TABLE 4.35: Metabolism profile of Favipiravir.

Property	**Value**
CYP-2D6 substrate	No
CYP-3A4 substrate	No
CYP-1A2 inhibitor	No
CYP-2C19 inhibitor	No
CYP-2C9 inhibitor	No
CYP-2D6 inhibitor	No
CYP-3A4 inhibitor	No

4.5.6.4 Excretion Profile

Favipiravir's excretion profile indicates a total clearance of 0.518, reflecting a moderate elimination rate. It is not a substrate of the renal OCT2 transporter, suggesting that kidney transport pathways are not significantly involved in its removal. In short, Favipiravir clears at a moderate pace without OCT2 participation [Table 4.36].

TABLE 4.36: Excretion profile of Favipiravir.

Property	**Value**
Drug clearance	0.518
Renal OCT2 transporter	No

4.5.6.5 Toxicity Profile

Favipiravir's toxicity profile indicates it is not mutagenic (AMES negative) and has a relatively high maximum tolerated dose in humans (1.291). It does not act as an inhibitor of hERG I or hERG II, suggesting minimal cardiac risk. In rats, acute oral toxicity (LD_{50}) is 1.941 and chronic toxicity (LOAEL) is 2.023.

The drug is not hepatotoxic and does not cause skin sensitisation. Toxicity models show very low *Tetrahymena pyriformis* toxicity (0.099) but higher minnow toxicity (3.407).

Favipiravir appears safe with respect to genetic, liver, and skin effects, though it demonstrates notable toxicity in aquatic species [Table 4.37].

TABLE 4.37: Toxicity profile of Favipiravir.

Property	**Value**
AMES assessment	No
Maximum tolerated dose (human)	1.291
hERG I inhibitor	No
hERG II inhibitor	No
Oral acute toxicity in rats (LD_{50})	1.941
Oral chronic toxicity in rats (LOAEL)	2.023
Hepatotoxicity	No
Skin sensitisation	No
Tetrahymena pyriformis toxicity	0.099
Minnow aquatic toxicity	3.407

4.6 Molecular Docking Analysis

4.6.1 Docking Tool (CB-Dock2)

CB-Dock2 is a web-based docking tool that automatically detects potential binding pockets on the protein surface and performs docking simulations with user-provided ligands.

It offers an integrated workflow where pocket prediction and binding affinity evaluation are carried out together, making the process efficient and reliable. When

applied to the BRCA1 protein structure, CB-Dock2 identified suitable binding sites and generated docking scores, which provided valuable insights into ligand–protein interactions and supported the recognition of promising druggable regions for therapeutic research.

4.6.2 Docking Results of Compounds

The docking table presents the interaction outcomes for each compound tested with the protein. It outlines values such as docking score, cavity volume, and docking structure, which together indicate how well a ligand fits into the binding pocket and the strength of its binding. In essence, it provides a clear comparison of compounds, highlighting those with stronger affinities and better suitability for potential drug development.

In CB-Dock2, docking simulations were conducted for six compounds: Berberine, Oxyberberine, Palmatine, Koumidine, Stilbene, and Favipiravir. The software automatically identified binding cavities in the BRCA1 protein and calculated their cavity volumes.

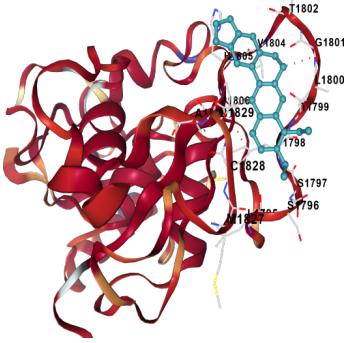
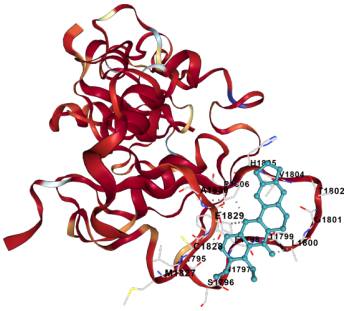
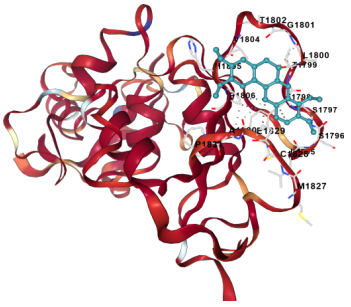
Each ligand was then docked into the predicted pockets to evaluate how well it fit within the available binding space and to analyze its interaction potential.

A key aspect of the docking results is the Vina score, which represents binding affinity. In this scoring system, the more negative the value, the stronger the binding interaction.

This means ligands with lower (more negative) scores are more likely to form stable complexes with the protein. By combining this information with cavity volume data, we were able to assess the suitability of each compound for binding and identify those with more favorable interaction tendencies.

This structured approach ensured that all six ligands were assessed under consistent conditions, offering a clear overview of their binding behavior.

TABLE 4.38: Docking score of compounds using CB-Dock2 (Berberine to Palmatine).

Ligand	**Vina Score (kcal/mol)**	**Cavity Volume ($Å^3$)**	**Docking Results**
Berberine	-7.0	79	
Oxyberberine	-7.0	79	
Palmatine	-6.6	79	

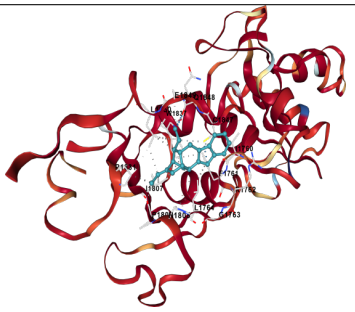
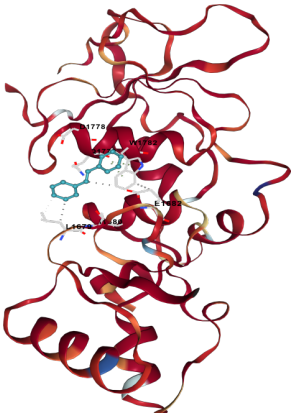
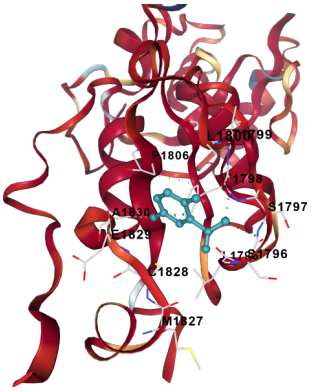
The results emphasize the importance of docking scores and cavity volumes in predicting ligand–protein compatibility, laying the groundwork for further exploration in therapeutic research [Table 4.39].

4.7 Analysis and Visualization of Protein–Lig–and Interactions

After docking the CB-Dock2 ligand with the protein, the resulting protein–ligand complex was saved in PDB format and then analysed using Discovery Studio

Visualizer.

TABLE 4.39: Docking score of compounds using CB-Dock2 (Koumidine to Favipiravir).

Ligand	**Vina Score (kcal/mol)**	**Cavity Volume ($Å^3$)**	**Docking Results**
Koumidine	-6.4	225	
Stilbene	-5.4	94	
Favipiravir	-4.4	79	

This software allowed us to examine binding interactions in detail, including hydrogen bonds, hydrophobic contacts, and the overall orientation of the ligand inside the active site.

Through 3D visualization, we could clearly see how the ligand fits into the binding pocket and which amino acid residues contribute to stabilizing the interaction.

Discovery Studio Visualizer is a specialized tool for molecular modeling and interaction analysis. It provides both 3D and 2D representations, making docking results easier to interpret and present. In our study, we generated a 2D interaction diagram that highlighted hydrogen bonds, van der Waals forces, and other non-bonded contacts between the ligand and protein residues. This diagram was downloaded to document the findings, offering clear graphical evidence of the binding mechanism and supporting the docking study with visual data.

4.7.1 Interaction Analysis of Berberine

This illustration highlights how a ligand achieves stability within a protein binding pocket through a network of non-covalent forces. The compound at the center, characterized by aromatic rings and heteroatoms, is surrounded by markers that denote different interaction types. Yellow dashed lines correspond to Pi–Sulfur contacts, pink lines represent Alkyl and Pi–Alkyl associations, while green shading indicates van der Waals forces.

Collectively, these interactions establish a robust binding framework, underscoring the ligand's potential as a lead candidate in molecular docking and drug discovery studies [Fig. 4.4].

4.7.2 Interaction Analysis of Oxyberberine

This figure depicts how a ligand maintains stability inside a protein binding pocket through a variety of non-covalent forces. The compound at the center, composed of aromatic rings and functional groups, is surrounded by markers that highlight its engagement with the binding environment.

The interactions include van der Waals forces, carbon–hydrogen bonding, alkyl contacts, Pi–Alkyl associations, and Pi–Sulfur contacts. Together, these forces establish a strong and diverse binding profile, reinforcing the ligand's potential relevance in molecular docking studies and drug discovery applications [Fig. 4.5].

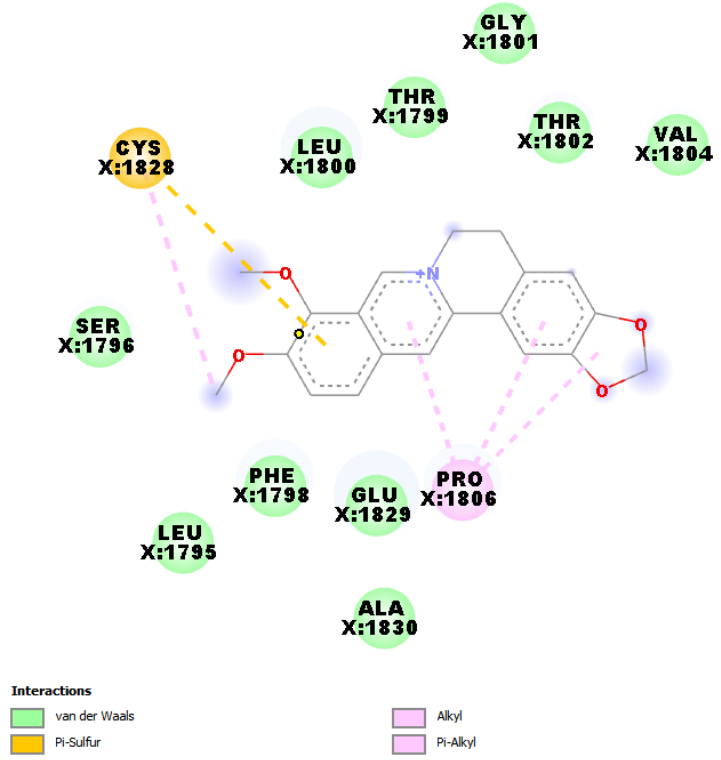


FIGURE 4.4: Interaction of Berberine with the BRCA1 protein generated using Discovery Studio Visualizer.

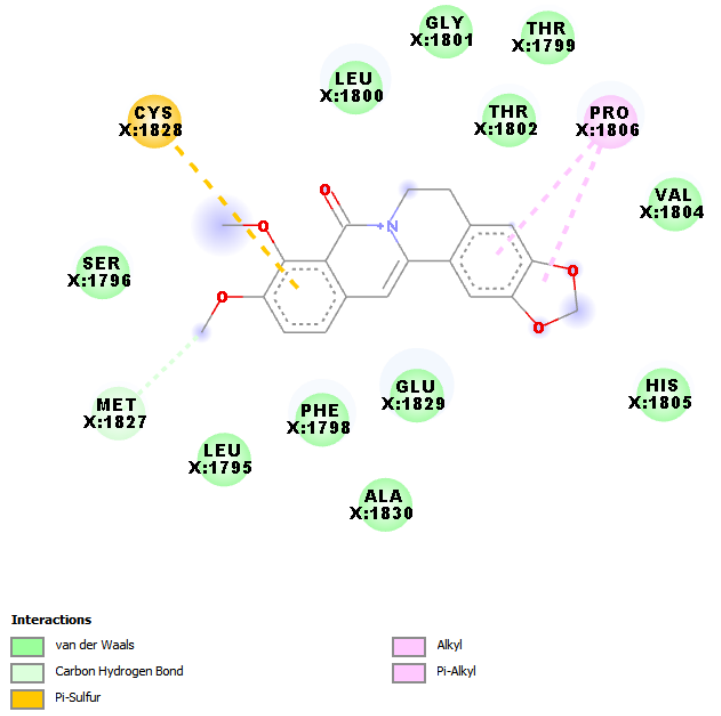


FIGURE 4.5: Interaction of Oxyberberine with the BRCA1 protein generated using Discovery Studio Visualizer.

4.7.3 Interaction Analysis of Palmatine

This figure illustrates how a ligand functions within a protein binding pocket by forming a network of stabilizing non-covalent interactions.

The central molecule, composed of fused aromatic rings with oxygen and nitrogen atoms, engages its surroundings through van der Waals forces, hydrogen bonding, alkyl associations, Pi–Alkyl contacts, and Pi–Sulfur interactions.

These forces not only secure the ligand's position but also contribute to binding specificity and modulation of protein activity [Fig. 4.6].

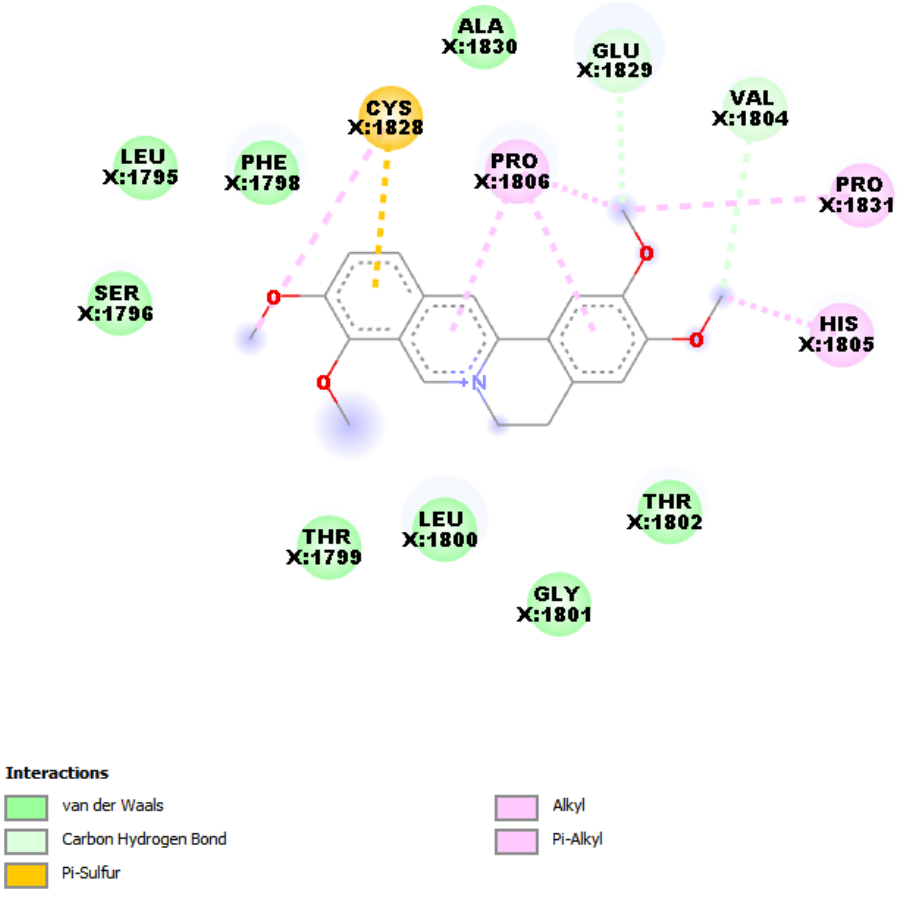


FIGURE 4.6: Interaction of Palmatine with the BRCA1 protein generated using Discovery Studio Visualizer.

4.7.4 Interaction Analysis of Koumidine

This figure portrays the functional role of a ligand within a protein binding site, emphasizing how it maintains stability through a combination of non-covalent forces. The heterocyclic compound at the center interacts with its surroundings via van der Waals contacts, hydrogen bonds, and alkyl or Pi–Alkyl associations.

These interactions not only secure the ligand's position but also enhance binding specificity and influence protein activity. Altogether, this interaction profile highlights the compound's relevance in molecular docking research and its potential as a candidate for drug discovery [Fig. 4.7].

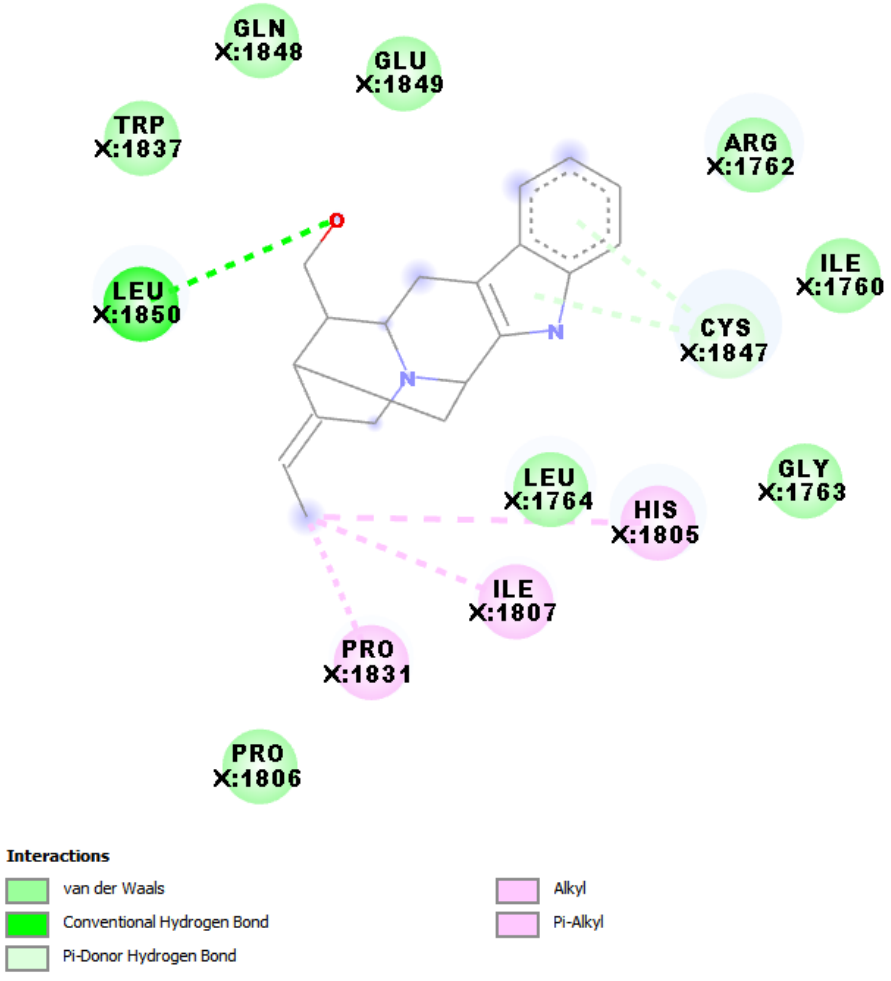


FIGURE 4.7: Interaction of Koumidine with the BRCA1 protein generated using Discovery Studio Visualizer.

4.7.5 Interaction Analysis of Stilbene

This figure depicts how an aromatic ligand operates within a protein binding site by establishing several stabilizing non-covalent interactions. The central structure, made up of two fused benzene rings, engages with its environment through van der Waals forces, Pi-donor hydrogen bonding, Pi–Pi stacking, and Pi–Alkyl contacts.

These interactions not only anchor the ligand firmly in place but also enhance its binding selectivity and influence the protein's functional behavior [Fig. 4.8].

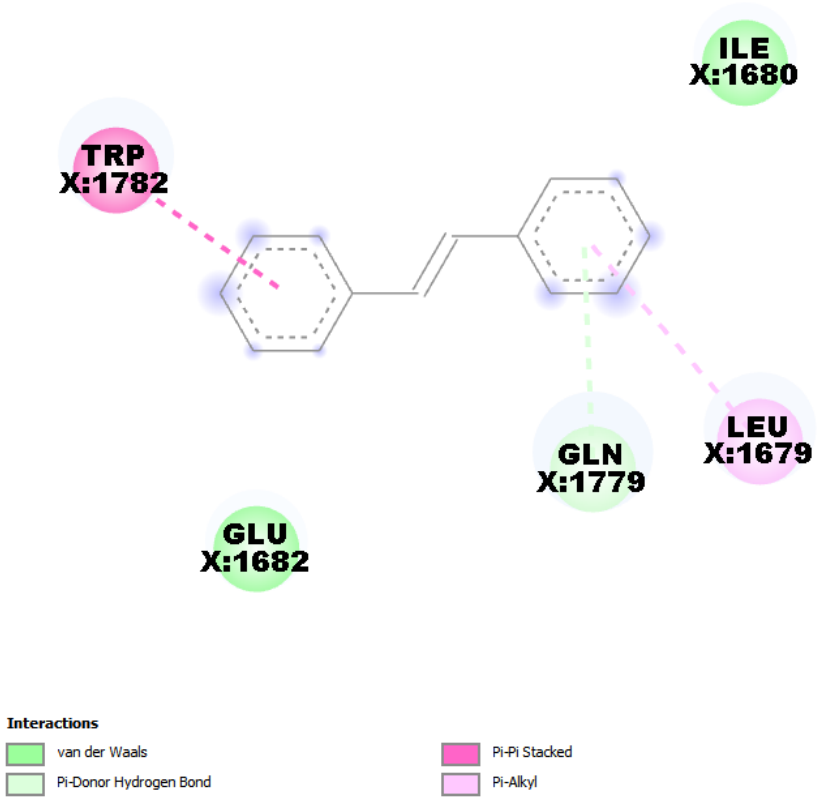


FIGURE 4.8: Interaction of Stilbene with the BRCA1 protein generated using Discovery Studio Visualizer.

4.7.6 Interaction Analysis of Favipiravir

The interaction diagram illustrates that the ligand establishes one hydrogen bond with the protein, while residues such as Leu, Pro, Ala, Phe, and Cys contribute

to multiple hydrophobic and van der Waals interactions.

This combination of a single hydrogen bond for specificity and several hydrophobic contacts for stability ensures that the ligand is securely positioned within the binding pocket, highlighting the balance of forces that drive effective protein–ligand binding [Fig. 4.9].

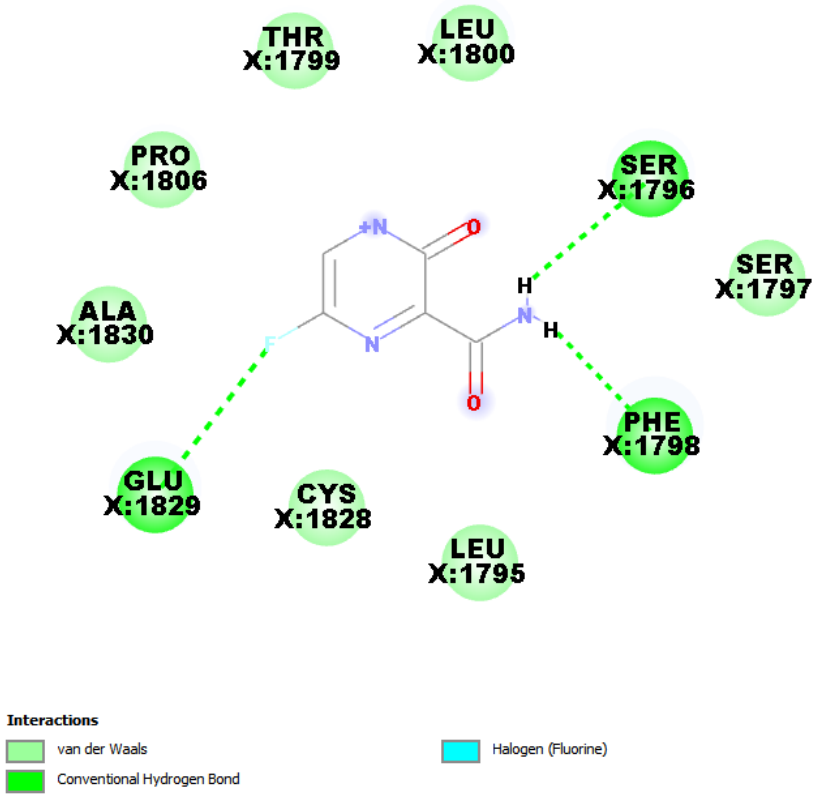


FIGURE 4.9: Interaction of Favipiravir with the BRCA1 protein generated using Discovery Studio Visualizer.

4.8 Selection of Potential Lead Compound

Berberine is acknowledged as the lead compound among *Berberis lycium* phytochemicals tested against BRCA1, standing out as the most promising candidate. A lead compound is defined as one that demonstrates superior binding affinity, adherence to Lipinski's Rule of Five, favorable ADMET properties, and validated biological activity. Berberine meets all these requirements, making it the central

focus for further drug development. Berberine proves to be the most effective lead compound, showing the strongest binding affinity (Vina score –7.0) and stable fit within a cavity volume of 79 Å^3. Its interaction profile indicates reliable stabilization and high specificity, making it the most promising candidate for drug design.

Structurally, berberine is an isoquinoline alkaloid characterized by a planar aromatic framework and nitrogen atoms that facilitate hydrogen bonding and hydrophobic interactions. This molecular architecture allows berberine to fit effectively into protein binding pockets, particularly BRCA1, and form stable complexes. Its chemical nature is a key factor behind its consistent docking performance.

From a drug-likeness standpoint, berberine fully complies with Lipinski's Rule of Five. Its molecular weight is under 500 Da, LogP value lies within the acceptable range, and the number of hydrogen bond donors and acceptors falls within permissible limits. These features confirm berberine's potential for oral bioavailability and pharmacological suitability, which are essential for lead compound designation.

ADMET profiling further supports berberine's candidacy. It demonstrates good intestinal absorption, moderate distribution, and metabolic stability. Toxicity predictions suggest low mutagenic and carcinogenic risks, indicating that berberine is safe for therapeutic use. Despite these strengths, one limitation is its low oral bioavailability due to poor absorption and rapid metabolism.

Comparative analysis shows that Oxyberberine, with the same docking score and cavity size, demonstrates similar potential but lacks the extensive biological validation that supports Berberine's role. Palmatine, although structurally related, shows slightly weaker binding (–6.6) in the same cavity, reflecting reduced stability compared to Berberine. Meanwhile, Koumidine binds in a larger cavity (225 Å^3) but with lower affinity (–6.4), suggesting less effective stabilization despite occupying more space. Stilbene (–5.4) and Favipiravir (–4.4) display the weakest docking scores, indicating poor binding strength and limited relevance as candidates in

this context. Oxyberberine and Palmatine act as secondary hits, whereas Stilbene and Favipiravir show limited relevance. This comparison underscores Berberine's superiority and justifies its selection as the lead compound. The results confirm Berberine's superior binding strength and pharmacological promise, positioning it as the most reliable lead compound, while Oxyberberine may serve as a secondary option for further exploration, and the remaining compounds show progressively weaker potential.

Experimental validation adds further credibility to berberine's role. Wet-lab studies confirm its anticancer, antioxidant, and antimicrobial properties. Berberine induces apoptosis, inhibits tumor growth, and modulates signaling pathways, all of which are highly relevant to breast cancer research. Another dimension often overlooked is berberine's interaction with gut microbiota, which indirectly influences glucose metabolism, lipid regulation, and immune responses. This systemic effect adds a modern pharmacological perspective to its traditional use.

4.9 Drug Identification

For drug identification, we relied on the KEGG database, which provides a systematic link between compounds, their targets, and biological pathways. Through KEGG's drug entries and pathway mapping, our selected ligands were connected to the BRCA1 pathway, allowing us to validate docking outcomes. This process confirmed Berberine as the lead compound, since its docking strength was supported not only by computational scores but also by pathway-level evidence, making the identification more reliable and biologically relevant.

4.9.1 Olaparib

Olaparib is a PARP inhibitor prescribed for cancers linked to BRCA1 mutation, including ovarian, breast, pancreatic, and prostate cancers. It works by preventing DNA repair in tumor cells, which leads to selective cancer cell death. Clinically,

it is given orally, often as maintenance therapy after chemotherapy, to extend remission and improve patient survival outcomes. The 3D structure of Olaparib was downloaded from PubChem [50] [Fig. 4.10].

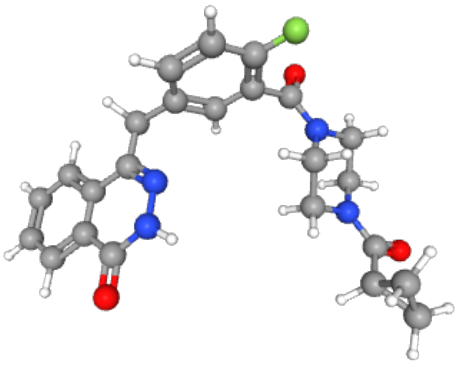


FIGURE 4.10: 3D structure of Olaparib downloaded from PubChem.

4.9.2 Chemical and Structural Data

TABLE 4.40: Chemical and structural information of Olaparib retrieved from PubChem.

Sr. No.	Property	Value
1	Drug	Olaparib
2	Compound CID	23725625
3	Molecular Formula	$C_{24}H_{23}FN_4O_3$
4	Molecular Weight	434.5 g/mol

4.9.3 Physicochemical Comparison of Olaparib and Berberine

Information related to the drug was retrieved through pkCSM.

TABLE 4.41: Physicochemical properties of Olaparib compared with Berberine.

Sr. No.	Property	Olaparib Value	Berberine Value
1	Molecular weight	434.46 g/mol	336.367
2	LogP value	2.3474	3.0963
3	H-bond donors	1	0
4	H-bond acceptors	4	4
5	Rotatable bonds	4	2
6	Surface area	183.385	144.867

4.9.4 Olaparib as Standard Comparator

For BRCA1 targeting, Olaparib was selected as the reference drug because it is an FDA-approved PARP inhibitor that directly exploits BRCA1 mutations through the principle of synthetic lethality. By blocking PARP-mediated DNA repair, Olaparib causes BRCA1-deficient cancer cells to accumulate unrepaired DNA damage, which ultimately triggers apoptosis.

This mechanism makes Olaparib the most scientifically relevant benchmark drug for evaluating the anticancer potential of natural phytochemicals such as berberine. Its established clinical application in breast, ovarian, pancreatic, and prostate cancers associated with BRCA mutations provides strong validation, ensuring that the comparison between berberine and Olaparib is meaningful and aligned with current therapeutic strategies [57].

4.9.5 Mode of Action

i. Olaparib functions as a PARP inhibitor, blocking the enzyme poly(ADP-ribose) polymerase that normally repairs single-strand DNA breaks.

ii. Inhibition of PARP causes DNA damage to accumulate, which then progresses into double-strand breaks during DNA replication.

iii. In healthy cells, BRCA1/2 proteins repair these double-strand breaks via homologous recombination, but this pathway is defective in BRCA-mutated cancer cells.

iv. The combined loss of PARP-mediated repair and BRCA1/2 function results in synthetic lethality, selectively killing cancer cells while sparing normal cells [58].

4.10 Comparison of ADMET Properties

4.10.1 Absorption Profile Comparison

The absorption comparison indicates that Berberine demonstrates stronger uptake potential than Olaparib, particularly in terms of intestinal absorption and permeability, which points to improved oral bioavailability. Both compounds show similar solubility and skin permeability, suggesting comparable diffusion behavior.

Importantly, they share the same profile as P-glycoprotein substrates, meaning efflux transport could limit their intracellular concentration, and both act as P-gp II inhibitors, which may influence drug–drug interactions.

Overall, Berberine presents slightly more favorable absorption characteristics, reinforcing its promise as a natural lead compound when assessed against Olaparib as the standard comparator [Table 4.42].

4.10.2 Distribution Profile Comparison

Berberine demonstrates broader systemic reach compared to Olaparib, with a larger volume of distribution and a greater proportion of unbound drug available in plasma, which enhances its potential biological activity.

TABLE 4.42: Absorption profile comparison of Olaparib and Berberine.

Model	**Outcome Value of Olaparib**	**Outcome Value of Berberine**
Aqueous solubility	-4.014	-3.973
Caco-2 cell permeability	1.08	1.734
Human intestinal absorption	91.926	97.147
Dermal permeability	-2.736	-2.576
P-glycoprotein substrate status	Yes	Yes
P-glycoprotein I inhibition	No	No
P-glycoprotein II inhibition	Yes	Yes

Unlike Olaparib, Berberine shows more favorable penetration across the blood–brain barrier and into the central nervous system, suggesting possible neurological accessibility. In contrast, Olaparib remains more restricted in circulation and CNS entry, reflecting its focused pharmacological design.

Overall, Berberine's distribution characteristics point toward wider tissue availability, strengthening its role as a natural lead compound when assessed against Olaparib as the standard comparator [Table **??**].

TABLE 4.43: Distribution profile comparison of Olaparib and Berberine.

Model	**Outcome Value of Olaparib**	**Outcome Value of Berberine**
Volume of distribution in human	0.215	0.58
Fraction unbound (human plasma)	0.001	0.262
Blood brain barrier permeability	-0.85	0.198
Central nervous system permeability	-2.655	-1.543

4.10.3 Metabolism Profile Comparison

Berberine and Olaparib share some common traits but differ in their enzyme interactions. Both act as CYP3A4 substrates and inhibitors, meaning they are metabolized through this pathway and can influence drug–drug interactions.

Neither compound is a substrate for CYP2D6, yet Berberine uniquely inhibits CYP1A2 and CYP2D6, while Olaparib inhibits CYP2C19 and CYP2C9 instead.

These differences highlight that Berberine may affect metabolic clearance through a broader enzyme spectrum, whereas Olaparib shows a more selective inhibition profile aligned with its clinical design.

Overall, Berberine's wider inhibitory activity suggests potential variability in metabolism compared to Olaparib as the standard comparator [Table 4.44].

TABLE 4.44: Metabolism profile comparison of Olaparib and Berberine.

Model	**Outcome Value of Olaparib**	**Outcome Value of Berberine**
CYP-2D6 substrate	No	No
CYP-3A4 substrate	Yes	Yes
CYP-1A2 inhibitor	No	Yes
CYP-2C19 inhibitor	Yes	No
CYP-2C9 inhibitor	Yes	No
CYP-2D6 inhibitor	No	Yes
CYP-3A4 inhibitor	Yes	Yes

4.10.4 Excretion Profile Comparison

The excretion profile shows that Berberine clears from the body more efficiently than Olaparib, reflecting faster elimination and potentially shorter systemic retention. Both compounds are not substrates of the renal OCT2 transporter, indicating they follow similar kidney excretion routes.

Berberine demonstrates stronger clearance capacity, while Olaparib maintains a slower elimination pattern consistent with its targeted pharmacological role [Table 4.45].

TABLE 4.45: Excretion profile comparison of Olaparib and Berberine.

Model	**Outcome Value of Olaparib**	**Outcome Value of Berberine**
Drug Clearance	0.569	1.27
Renal OCT2 transporter	No	No

4.10.5 Toxicity Profile Comparison

This profile shows that Berberine and Olaparib share some overlapping risks but differ in important ways. Berberine is predicted to be mutagenic in the AMES test, while Olaparib is not, and both display potential hepatotoxicity. Neither compound causes skin sensitisation, and their acute and chronic oral toxicity levels in rats are comparable.

A key distinction is that Olaparib acts as a hERG II inhibitor, which may raise cardiac safety concerns, whereas Berberine does not. Differences also appear in aquatic toxicity models, where Berberine shows higher sensitivity in certain species. Overall, Berberine carries mutagenic concerns, while Olaparib presents more cardiac-related risks, reflecting distinct safety considerations for each compound [Table 4.46].

TABLE 4.46: Toxicity profile comparison of Olaparib and Berberine.

Model	**Outcome Value of Olaparib**	**Outcome Value of Berberine**
AMES assessment	No	Yes
Max. Tolerated dose in human	0.204	0.144
hERG inhibitor type I	No	No
hERG inhibitor type II	Yes	No
Oral Acute Toxicity in rats (LD50)	2.623	2.571
Oral Chronic Toxicity in rats (LOAEL)	1.799	1.89
Liver toxicity	Yes	Yes
Dermal Sensitisation	No	No
T-Pyriformis toxicity	0.298	0.354
Minnow aquatic toxicity	1.967	-0.277

4.11 Docking Score Comparison

In the docking study, both the lead compound Berberine and the reference drug Olaparib were docked against target protein BRCA1 to assess their binding affinities and interaction profiles [Table 4.47].

Olaparib achieved a stronger binding affinity with a Vina score of –7.9, occupying a larger cavity volume of 94 $Å^3$ at coordinates (28, 59, 46) with a docking box size of 23 × 23 × 23. This reflects its stable and precise fit within the BRCA1 binding pocket.

In contrast, Berberine showed a slightly lower affinity (–7.0) and was accommodated in a smaller cavity volume of 79 $Å^3$ at coordinates (10, 80, 36) with a docking box size of 22 × 22 × 22. These outcomes indicate that Olaparib binds more tightly and stabilizes better, while Berberine still demonstrates competitive docking behavior, supporting its potential as a natural lead compound for further exploration.

TABLE 4.47: Docking score comparison of Olaparib and Berberine.

Ligand	**Vina Score**	**Cavity Volume**	**Center (x,y,z)**	**Docking Size (x,y,z)**
Olaparib	-7.9	94	28, 59, 46	23,23,23
Berberine	-7.0	79	10,80,36	22,22,22

4.12 Molecular Interaction Analysis of Olaparib

This diagram illustrates the binding mechanism of the ligand with BRCA1 in a simplified way. The ligand is stabilized inside the protein pocket through a combination of hydrogen bonding, hydrophobic contacts, and electrostatic forces.

These interactions collectively enhance the ligand's binding affinity and ensure a reliable docking pose. It demonstrates how a balanced network of molecular forces

contributes to strong and stable ligand–protein binding, making it significant for drug design and therapeutic applications.

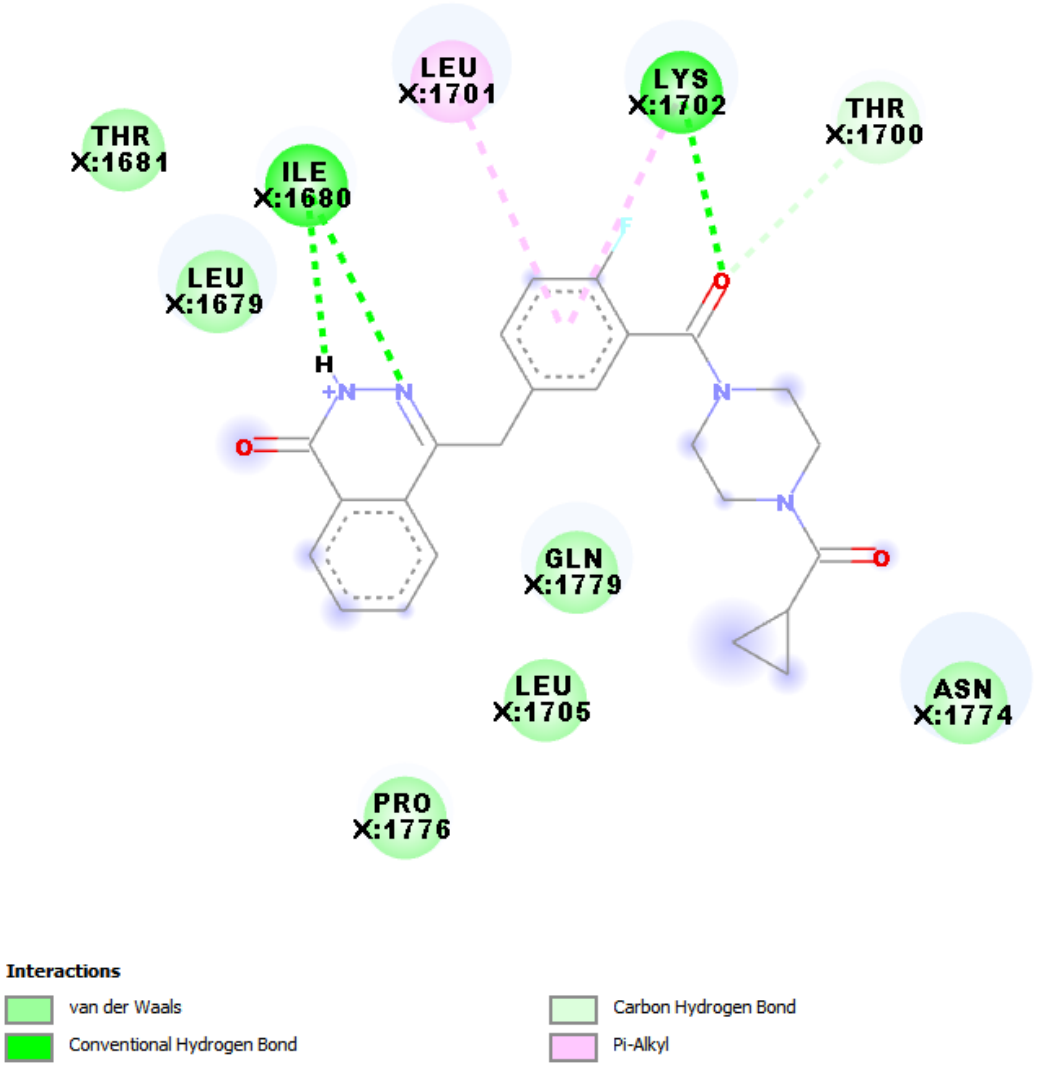


FIGURE 4.11: Interaction analysis of Olaparib with BRCA1.

Berberine, on the other hand, demonstrates a moderate affinity, forming fewer hydrogen bonds but maintaining stability through hydrophobic and Pi–alkyl interactions.

Berberine's consistent binding behavior highlights its potential as a natural lead compound, offering therapeutic promise despite its slightly weaker binding strength [Fig. 4.11].

4.12.1 Comparison between Olaparib and Berberine

TABLE 4.48: Interaction analysis comparison between drug and lead compound.

Feature	**Olaparib Interaction Analysis**	**Berberine Interaction Analysis**
Profile	Stronger affinity due to diverse hydrogen bonding and electrostatic interactions	Stable binding with fewer hydrogen bonds, relying more on hydrophobic and Pi–donor contacts
Binding Affinity (Vina Score)	-7.9 kcal/mol $\rightarrow$ stronger binding	-7.0 kcal/mol $\rightarrow$ moderate binding
Hydrogen Bonds	3 conventional + 1 carbon hydrogen bond (total 4)	2 conventional hydrogen bonds (total 2)
Interaction Types	Conventional hydrogen bonds, carbon hydrogen bonds, Pi–cation, Pi–alkyl, van der Waals	Conventional hydrogen bonds, Pi–donor hydrogen bonds, alkyl/Pi–alkyl, van der Waals

Chapter 5

Discussions

Breast cancer is the most frequently diagnosed malignancy in women worldwide and remains a leading cause of cancer related deaths. It develops when abnormal cells in the breast, most commonly within the ducts or lobules, proliferate uncontrollably, forming tumors that may infiltrate nearby tissues or metastasize to distant organs. The disease is influenced by several risk factors, including age, hormonal influences, lifestyle choices, and genetic predisposition, with inherited mutations in the BRCA1 gene being among the most critical determinants [59].

Advances in research have significantly improved both diagnosis and treatment. Early detection through mammography and genetic screening has proven vital in lowering mortality rates by enabling timely intervention [60]. Conventional therapies such as surgery, chemotherapy and radiation remain central to management, but modern approaches now include targeted treatments. Hormone therapies like tamoxifen and aromatase inhibitors are effective in estrogen receptor positive cancers, while HER2 positive tumors respond to monoclonal antibodies such as trastuzumab [61].

Recent investigations also highlight the potential of immunotherapy and plant derived compounds in breast cancer management. Natural agents such as berberine from *Berberis lycium* have demonstrated cytotoxicity, apoptosis induction, and multi pathway activity against breast cancer cells. Integrating these phytochemicals with modern targeted therapies offers a promising avenue for affordable and

accessible treatment, particularly in regions where synthetic drugs remain limited [62].

The BRCA1 gene is fundamental for maintaining genomic integrity by repairing DNA damage through homologous recombination. Mutations in BRCA1 impair this repair mechanism, resulting in uncontrolled cell division and a heightened risk of breast and ovarian cancers. Consequently, BRCA1 has become a major focus in cancer biology and drug discovery [63]. Synthetic drugs, particularly PARP inhibitors, exploit this weakness through synthetic lethality. Among them, Olaparib, an FDA approved PARP inhibitor, stands out as the most successful example. It demonstrates strong binding affinity and clinical efficacy by blocking DNA repair pathways. Its proven effectiveness in breast, ovarian, pancreatic, and prostate cancers has established it as a benchmark drug [64]. However, the high cost, adverse effects, and limited accessibility of Olaparib in developing regions highlight the urgent need for safer, affordable, and more widely available alternatives.

Berberis lycium, a medicinal shrub native to South Asia, emerges as a promising candidate. Traditionally, it has been used to treat diabetes, liver ailments, eye infections, skin conditions, and gastrointestinal disorders, reflecting its broad pharmacological potential. Plant contains isoquinoline alkaloids such as berberine, palmatine, koumidine, oxyberberine, and stilbene, which have been reported to possess antimicrobial, anti-inflammatory, antioxidant and anticancer properties [65].

Modern research has begun to validate these traditional claims. For example, a Springer study (2026) demonstrated that a chitosan-based nano formulation of berberine from *Berberis lycium* produced sustained cytotoxic effects against MCF-7 breast cancer cells, confirming its anticancer potential. Similarly, a PLOS One study (2023) highlighted berberine's neuroprotective role in Alzheimer's disease models, supported by docking with β-secretase, thereby proving its versatility in targeting multiple biological pathways.

The docking analysis offered valuable insights into how Olaparib and Berberine interact with BRCA1 at the molecular level. Olaparib displayed a strong binding

TABLE 5.1: Comparison of the mode of action between Olaparib and Berberine.

Compound	Target	Mode of Action	Cancer Relevance
Olaparib	PARP1/2 enzymes	Blocks DNA single-strand break repair; traps PARP on DNA, forming toxic complexes; causes synthetic lethality in BRCA1/2-mutated cells.	Used in breast, ovarian, pancreatic, and prostate cancers, especially in patients with BRCA mutations.
Berberine	PI3K/Akt/mTOR pathway, caspases, MMPs	Induces apoptosis through caspase activation; arrests cell cycle progression; suppresses metastasis by reducing MMP expression and increasing E-cadherin; exhibits antioxidant and anti-inflammatory activities.	Demonstrates anticancer activity against breast, lung, liver, and colorectal cancers and is considered a promising natural therapeutic candidate.

affinity, forming multiple hydrogen bonds and hydrophobic contacts that firmly anchor it within the active site. This interaction explains its established role as a PARP inhibitor, effectively blocking DNA repair and triggering synthetic lethality in BRCA1-mutated cells. In contrast, Berberine showed competitive, though slightly weaker, binding affinity. Its stabilization within the BRCA1 pocket was achieved mainly through hydrophobic and Pi–Sulfur interactions. These results indicate that Berberine can partially replicate Olaparib's binding behavior, thereby disrupting DNA repair pathways. When linked to mechanism, the evidence suggests that while Olaparib acts as a precise PARP inhibitor, Berberine exerts broader effects by not only binding BRCA1 but also influencing apoptosis, cell cycle regulation, and metastasis suppression. Thus, docking outcomes provide a mechanistic rationale for Berberine's anticancer activity and support the hypothesis that natural compounds can achieve molecular interactions similar to synthetic drugs, reinforcing their potential role in modern oncology[66].

The ADMET predictions further reinforced these observations. Berberine and related alkaloids displayed favorable absorption and distribution properties, along with acceptable toxicity levels. Compliance with Lipinski's Rule of Five confirmed

their drug likeness, ensuring suitability for oral administration [67]. These results suggest that *Berberis lycium* compounds are not only effective but also safe enough to be considered for drug development. Together, docking and ADMET profiling provide a strong computational foundation for identifying natural anticancer agents, reducing the need for early laboratory experiments and saving both time and resources.

Beyond oncology, *Berberis lycium* has demonstrated potential in treating metabolic disorders such as diabetes, where berberine improves insulin sensitivity and lowers blood glucose levels. It has also been reported to have hepatoprotective effects, supporting liver function and reducing oxidative stress. Its antimicrobial activity against bacteria and fungi makes it useful in treating infections, while its anti-inflammatory properties contribute to wound healing and skin health [68]. These diverse pharmacological actions highlight the plant's therapeutic versatility, making it an attractive candidate for integrated drug discovery.

Several previous studies further support the anticancer relevance of berberine and *Berberis lycium*. A Frontiers in Pharmacology (2022) article reported that berberine induced apoptosis in breast cancer cells by activating caspase pathways and suppressing PI3K/Akt signaling, confirming its ability to interfere with cancer cell survival mechanisms. Another Journal of Ethnopharmacology (2021) study showed that berberine reduced tumor growth in xenograft models, highlighting its translational potential from computational docking to animal studies [69]. These findings strengthen the argument that berberine is not only computationally promising but also biologically active in cancer therapy.

Additionally, a Molecules (2020) study demonstrated that berberine exhibited strong binding affinity to DNA and topoisomerase enzymes, suggesting multiple mechanisms of anticancer action. This aligns with the docking results against BRCA1, showing that berberine can interact with diverse molecular targets [70]. Such versatility is valuable because cancer is a multi-pathway disease, and compounds capable of acting on several targets may provide broader therapeutic benefits.

Another relevant study in Biomedicine & Pharmacotherapy (2022) reported that berberine enhanced the sensitivity of cancer cells to chemotherapy drugs, reducing resistance and improving treatment outcomes. This highlights a critical gap in current research: the potential synergistic effects of combining berberine with synthetic drugs like Olaparib [71]. Globally, similar approaches have been applied to other medicinal plants. For example, *Lycium barbarum* has demonstrated anticancer effects through docking and network pharmacology, confirming that traditional herbs can bind strongly to cancer targets. This global trend validates the approach of combining computational docking, ADMET profiling, and traditional knowledge to identify affordable anticancer agents [72]. By situating *Berberis lycium* within this broader research landscape, the study demonstrates that traditional medicinal plants can play a significant role in modern oncology.

From the integration of these studies, several gaps become evident. First, most current work, including this study, relies heavily on computational docking and ADMET predictions. Wet lab validation through cell culture, animal models, and clinical trials is urgently required to confirm therapeutic potential. Second, only a limited number of alkaloids from *Berberis lycium* have been tested. Broader screening could reveal stronger candidates with improved binding affinities.

Third, the synergistic potential of combining berberine with synthetic drugs like Olaparib remains unexplored. Such combinations could reduce side effects, overcome drug resistance, and provide innovative treatment strategies.

Fourth, molecular dynamics simulations are missing. Docking provides static predictions, but dynamic simulations would confirm the stability of ligand–protein complexes over time. Fifth, pathway analysis is needed to understand how phytochemicals influence downstream signaling in cancer progression. Docking shows binding affinity but does not explain biological outcomes. Sixth, even if natural compounds prove effective, clinical accessibility remains a challenge. Standardized formulations, dosage optimization, and regulatory approval are necessary for translation into practice. Finally, comparative plant studies are lacking [73]. While *Lycium barbarum* and other herbs have been studied, direct comparisons between

Berberis lycium and other medicinal plants in cancer therapy are limited, leaving a gap in understanding relative efficacy.

While Olaparib remains the benchmark drug for BRCA1 related cancers, *Berberis lycium* and its alkaloids, particularly berberine, demonstrate competitive binding, favorable ADMET profiles, and proven cytotoxicity in breast cancer cells. Integrating computational predictions with wet lab validation will bridge gap between traditional medicine and modern oncology, offering affordable and accessible cancer therapies for regions where synthetic drugs are limited [74]. This research not only highlights the potential of *Berberis lycium* but also underscores the importance of combining cultural knowledge with advanced computational methods to develop innovative anticancer strategies.

Chapter 6

Conclusion and Future Recommendations

6.1 Conclusion

This research confirms that BRCA1 is a crucial therapeutic target in metastatic breast cancer, and both synthetic and natural compounds can interact with it effectively. The primary outcome is that Olaparib exhibited the strongest stabilization and binding affinity, forming multiple hydrogen bonds and hydrophobic contacts within the BRCA1 active site. This validates its established role as a clinically approved PARP inhibitor for BRCA1-mutated cancers. In comparison, bioactive alkaloids from *Berberis lycium* including Berberine, Palmatine, Koumidine, Oxyberberine, Favipiravir and Stilbene derivatives achieved competitive docking scores, with Berberine emerging as the most promising candidate. Berberine stabilized mainly through hydrophobic and Pi–Sulfur interactions, while Palmatine and Oxyberberine also demonstrated notable binding affinities, suggesting that these natural compounds can mimic aspects of Olaparib's binding behavior and interfere with DNA repair pathways.

The ADMET profiling further strengthened these findings, confirming that Berberine, Palmatine, and Oxyberberine possess favorable absorption, distribution, and

acceptable toxicity levels. Their compliance with Lipinski's Rule of Five validated their drug-likeness and suitability for oral administration. Collectively, docking and ADMET analyses highlight the core contribution of this study: *Berberis lycium* phytochemicals not only show potential as BRCA1 inhibitors but also exhibit broader multi-target effects such as apoptosis induction, cell cycle arrest and suppression of metastasis.

The integration of computational docking with ADMET predictions underscores the importance of *in silico* approaches in early drug discovery, particularly in regions where access to expensive therapies is limited. By evaluating natural compounds alongside established drugs, new treatment strategies can be identified that may reduce side effects and improve patient outcomes. Nevertheless, the study emphasizes the need for wet-lab experiments, molecular dynamics simulations, pathway analysis and clinical trials to validate these computational predictions and translate them into clinical practice.

Importantly, the affordability and accessibility of plant-based medicines such as *Berberis lycium* make them especially relevant for countries where modern drugs are either unavailable or prohibitively costly. In this way, *Berberis lycium* emerges not only as a promising anticancer agent but also as a bridge between traditional medicine and modern oncology, offering hope for more effective, affordable, and accessible cancer therapies worldwide.

6.2 Future Recommendations

First the results from computational studies should be tested in the laboratory. This means starting with cell culture experiments to check how *Berberis lycium* compounds affect cancer cells, then moving to animal studies to see their safety and effectiveness in living systems. In the long run, clinical trials will be needed to confirm whether these compounds can be used safely and effectively in humans. At the same time, more phytochemicals from *Berberis lycium* should be screened,

because other compounds may show even stronger binding and better anticancer activity than berberine.

The future work should look at combining natural compounds with synthetic drugs like Olaparib. This type of combination could help reduce side effects, fight drug resistance, and improve treatment results. On the computational side, molecular dynamics simulations should be used to study how stable the compound–protein complexes remain over time. In addition, pathway analysis and network pharmacology can explain how these compounds affect cancer-related biological processes, giving a clearer picture of their role in therapy.

For these findings to be useful in real life, researchers must focus on dosage optimization, standardized formulations, and affordability. Comparative studies with other medicinal plants such as *Lycium barbarum* can help identify which natural agents are most effective. Importantly, efforts should be made to make these therapies accessible in resource-limited regions, where expensive synthetic drugs are often unavailable. By combining computational predictions, laboratory testing, and clinical translation, *Berberis lycium* can become a practical and affordable option in modern cancer treatment.

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