CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Exploring the Impact of Soursop (*Annona muricata*) Leaves to Treat Breast Cancer: *In-silico* Approach

by

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

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I dedicate this thesis to my loving and supportive parents, brothers and husband who fully helped me in achieving my life goals.

CERTIFICATE OF APPROVAL

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Abstract

Breast cancer continues to be one of the most prevalent and life-threatening diseases affecting women globally. While current treatments such as chemotherapy, radiation, and targeted therapies have improved survival rates, they often come with serious side effects, high costs, and, in many cases, limited long-term efficacy due to drug resistance. These challenges have sparked growing interest in the use of natural products and traditional medicinal plants as potential alternatives or complementary therapies. One such plant is *Annona muricata*, commonly known as soursop or graviola, which has been used for centuries in traditional medicine across tropical regions to treat various ailments, including cancer. Its leaves, in particular, are known to contain powerful bioactive compounds like acetogenins, flavonoids, and alkaloids that have demonstrated cytotoxic effects in early laboratory studies. In this study, we explored the therapeutic potential of soursop leaf compounds against breast cancer using advanced *in-silico* techniques. Instead of starting in the lab, we began on the computer screening plant-derived compounds for their potential as drug-like molecules. We first identified several known phytochemicals from soursop leaves through scientific databases and literature. These compounds were then analyzed for drug-likeness and safety using tools like SwissADME, pkCSM, and ADMETlab 2.0, which helped predict how the compounds might behave in the human body in terms of absorption, metabolism, toxicity, and more. The most promising compounds were then virtually tested (through molecular docking) to see how well they could fit and bind with important breast cancer-related proteins such as BRCA1, HER2, and EGFR proteins that are often involved in the onset and progression of breast cancer. Software like AutoDock Vina CB Doock2, PyRx, and Discovery Studio Visualizer was used to simulate these interactions and examine how strongly the plant compounds could attach to the cancer-related targets. Compounds like quercetin, kaempferol, and annonacin showed strong and specific binding with key residues of the target proteins, suggesting a possible mechanism for anticancer activity. This research offers strong preliminary evidence that bioactive compounds from Annona muricata leaves may serve as potential therapeutic agents against breast cancer. While these findings

are based on computational models, they provide a valuable starting point for further experimental validation through laboratory testing and clinical trials. Our approach shows how the ancient wisdom of herbal medicine, when combined with modern computational tools, can open new doors in the fight against cancer. The results revealed that quercetin exhibited the most promising binding affinity, surpassing other phytochemicals, and demonstrated favorable pharmacokinetic properties comparable to the standard drug letrozole. Detailed docking analysis indicated stable interactions with key residues within the BRCA1 active site, suggesting its potential role in modulating BRCA1 function. Furthermore, ADMET predictions highlighted quercetin's acceptable absorption, distribution, and safety profile. Comparative analysis with letrozole validated the potential of quercetin as a lead compound for further development. The integration of pathway-based analysis supports the hypothesis that phytochemicals such as quercetin may exert anticancer activity by modulating these signaling cascades.

Keywords: *Annona muricata*, Phytochemicals, Breast cancer, Molecular docking, ADMET analysis, Protein-ligand interaction, Computational drug discovery.

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Abbreviations

ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
AI	Artificial Intelligence
AKT	Protein Kinase B
BC	Breast Cancer
BRCA1/BRCA2	Breast Cancer Gene 1 and Breast Cancer Gene 2 (a tumor suppressor gene)
CDK4/6	Cyclin-Dependent Kinase 4 and 6
DNA	Deoxyribonucleic Acid
EGFR	Epidermal Growth Factor Receptor
ER-α	Estrogen Receptor Alpha
FDA	Food and Drug Administration
FNA	Fine Needle Aspiration
HER2	Human Epidermal Growth Factor Receptor 2
HRT	Hormone Replacement Therapy
MOE	Molecular Operating Environment
MRI	Magnetic Resonance Imaging
PARP	Poly (ADP-ribose) Polymerase
PDB	Protein Data Bank
PI3K	Phosphoinositide 3-Kinase
ProTox-II	Prediction of Toxicity
SwissADME	Swiss Absorption, Distribution, Metabolism, and Excretion
TCGA	The Cancer Genome Atlas

WHO	World Health Organization
mTOR	Mechanistic Target of Rapamycin
pkCSM	Pharmacokinetics and Chemistry Structure Modeling

Chapter 1

Introduction

Cancer is a condition where cells start to grow uncontrollably and can spread to other parts of the body. Since the body is made up of trillions of cells, cancer can begin in almost any area. Normally, cells grow, divide, and replace old or damaged ones in a well-organized process. But sometimes this system breaks down, and damaged or abnormal cells begin to grow when they shouldn't. These unwanted cells can group together and form lumps of tissue called tumors. Tumors can be either benign, meaning they're not cancerous, or malignant, which means they can invade nearby tissues and spread further [1].

Breast cancer is a type of cancer that begins in the cells of the breast, most often in the milk-producing ducts or glands. It's one of the most common cancers around the world, affecting mostly women, though men can develop it too. Several factors can increase the risk, including age, family history, inherited genetic mutations, hormone levels, and lifestyle habits like drinking alcohol, lack of exercise, poor sleep patterns, and an unhealthy diet [2].

Common signs may include a lump or thickening in the breast sometimes painful, sometimes not changes in the shape or size of the breast, skin dimpling, unusual nipple discharge, redness, or a texture that looks like the skin of an orange. In many cases, though, symptoms don't show up until the cancer has already progressed, which makes regular screening important. Mammograms are especially

recommended for early detection, particularly for women over 40 or those with a family history of breast cancer [3].

In recent years, targeted medicines, which try to directly kill cancerous cells while inflicting minimum damage to normal cells, have improved therapeutic efficacy for select subtypes of BC. BC is widespread in Pakistan, with one out of every nine women developing it at some point in their lives. The age standardized incidence of BC in Pakistan is among the highest in Asia. In Pakistan, the prevalence of BC has increased considerably during the past two decades. BC frequency in Pakistan peaks between the ages of 45 and 59. Young women in Pakistan are frequently diagnosed with advanced BC, which might have a terrible prognosis. BC is the primary cause of cancer among Pakistani women [4].

Breast cancer is the most common cause of cancer and cancer-related deaths worldwide. Several factors can increase the risk of developing breast cancer, including older age, obesity, alcohol consumption, a family history of breast cancer, previous radiation exposure, reproductive history, tobacco use, and hormone therapy after menopause. Breast cancer is typically diagnosed through a combination of methods, starting with a physical exam and questions about symptoms. Doctors may then recommend imaging tests like mammograms, ultrasounds, or MRIs to get a clearer picture of the breast tissue. A mammogram is a specialized X-ray that provides detailed images of the breast, while an ultrasound uses sound waves to create images of the tissue. If necessary, a biopsy can be done to confirm the presence of cancer [5].

In order to obtain detailed photographs, the body is scanned with a magnet and computer. A biopsy is the removal of a tissue or fluid sample from the breast for microscopic examination. There are various types of biopsies, including fine-needle aspiration, core biopsy, and open biopsy. Following a diagnosis, more tests may be performed to establish the stage of the cancer, which determines how far it spreads [6].

Treatment regimens are created based on the findings of these tests, as well as the patient's needs and desires. Regular self-exams and mammograms can help

detect BC at an early stage, when it is more treatable. Symptoms may include breast dimpling, rash, or redness, as well as armpit discomfort or swelling. Breast pain or soreness that does not coincide with your monthly cycle. Doctors combine treatments to reduce the odds of cancer recurring.

These include surgical removal of the breast tumor, radiation therapy to lower the chance of recurrence in the breast and surrounding tissues, and drugs to kill cancer cells and prevent spread, such as hormone therapies, chemotherapy, or targeted biological therapies [7].

BC is the most common cancer in women globally, and *BRCA1* gene abnormalities are a major risk factor for its development. The *BRCA1* gene is essential for DNA damage repair, and mutations in this gene cause genetic instability, increasing the risk of developing BC. Patients with *BRCA1*-mutated BC frequently have aggressive tumor progression and few therapy options, making it critical to investigate innovative therapeutic agents.

sCurrently available treatments, such as PARP inhibitors (Olaparib, Niraparib, Talazoparib) and chemotherapy, have demonstrated efficacy but are associated with substantial toxicity, medication resistance, and unpleasant side effects. As a result, there is an urgent need for alternative, natural, and effective therapeutic agents that particularly target *BRCA1*-related BC while posing minimum risks [8].

Soursop leaves have been traditionally utilized for their therapeutic characteristics, and they include bioactive chemicals such as acetogenins, flavonoids, alkaloids, and phenolics, all of which have anticancer activity. However, their effectiveness against *BRCA1*-associated BC is mostly unknown. *In-silico* drug discovery approaches, such as molecular docking, molecular dynamics simulations, and ADMET analysis, provide a cost-effective and time-efficient way to screen and identify prospective drug candidates. The purpose of this work is to computationally analyze the binding potential and pharmacokinetic features of soursop leaf chemicals against *BRCA1* and other proteins involved in BC progression [9].

1.1 Problem Statement

BC remains a major global health challenge, requiring new therapeutic strategies for effective treatment. Traditional drug discovery is costly and time-intensive, but an *in-silico* approach can rapidly identify potential anti-cancer compounds. This study aims to analyze the bioactive compounds in soursop leaves using computational methods to predict their interactions with BC targets, offering a promising avenue for drug development.

1.2 Hypothesis

Bioactive compounds derived from soursop leaves, particularly acetogenins and flavonoids, can effectively bind to breast cancer-associated target proteins such as HER2, ER-α, and CDK4/6 with high affinity. These compounds are expected to exhibit favorable drug-likeness properties and acceptable ADMET profiles, making them suitable candidates for therapeutic development. It is further proposed that virtual screening and molecular docking will identify key soursop phytochemicals with binding energies comparable to or better than current standard treatments. Additionally, the compounds may modulate critical signaling pathways involved in breast cancer progression, such as the PI3K/AKT/mTOR pathway. Ultimately, the research anticipates that these natural compounds could be integrated into personalized medicine models, especially for patients with *BRCA1* mutations.

1.3 Gap Analysis

There is limited research that explores the specific interactions between bioactive compounds from soursop leaves and breast cancer-associated molecular targets using advanced *in-silico* models. Few studies have focused on the molecular docking of soursop compounds with key breast cancer proteins, limiting the understanding of their binding affinity and therapeutic potential. While ADMET analysis tools are crucial, there is a gap in research regarding the pharmacokinetics and toxicity

profiles of soursop compounds, which is essential for evaluating their drug-likeness and safety. Research seldom incorporates personalized medicine approaches by integrating patient-specific genomic and proteomic data, which could optimize treatment strategies for individuals with specific mutations like *BRCA1*.

1.4 Aim and Objectives

The aim of this study is to explore the therapeutic potential of bioactive compounds found in Annona muricata leaves against breast cancer, with a particular focus on *BRCA1*-associated pathways, using a comprehensive *in-silico* approach.

1.4.1 Research Objectives

- To explore and identify bioactive compounds present in Annona muricata leaves by conducting a thorough review of published research and chemical databases, focusing on their potential role in breast cancer treatment.

- To assess the effectiveness of these naturally occurring compounds against *BRCA1*-associated breast cancer using computer-based methods, including molecular docking, and ADMET analysis, to evaluate their interaction, stability, and safety as potential therapeutic agents.

1.4.2 Possible Outcomes

Novel bioactive chemicals from soursop leaves discovered to have a high binding affinity for *BRCA1* and associated proteins. Prediction of drug-like characteristics, stability, and ADMET profiles for soursop compounds. The identification of natural inhibitors for *BRCA1*- mutated BC could open up new options for medication development. Developing a computational paradigm for natural product-based medication development in *BRCA1*- related malignancies.

1.5 Significance of the Solution

The *in-silico* approach offers a fast, cost-effective, and precise method for identifying potential BC treatments from soursop leaves. By leveraging computational techniques, this study can predict the molecular interactions, toxicity, and pharmacokinetic properties of bioactive compounds, reducing the need for extensive laboratory experiments. If successful, the findings can contribute to the development of natural, plant-based cancer therapies, providing safer and more accessible treatment options for patients worldwide.

Chapter 2

Literature Review

2.1 Cancer

Cancer is a broad term that refers to a group of conditions in which cells in the body begin to grow uncontrollably, resulting in tumors or spreading to other parts of the body via the blood or lymphatic system. There are 100 different types of cancer, each classified according to the type of cell it begins in, such as lung cancer, breast cancer, skin cancer and colon cancer. Abnormal cell development can occur anywhere in the body, and the disease can be localized or metastasized, meaning it can spread to other organs or tissues. Cancer can be caused by genetic mutations, environmental factors including tobacco use, radiation exposure, or certain infections, as well as lifestyle decisions like nutrition, physical inactivity, and alcohol consumption [10].

Cancer is a broad group of diseases characterized by the uncontrolled growth and spread of abnormal cells that can originate in virtually any organ or tissue of the body. It begins when normal cells undergo genetic mutations that cause them to lose control over processes like cell division and programmed cell death. Unlike benign tumors, which remain localized and typically grow slowly, cancerous tumors have the capacity to invade surrounding tissues and penetrate into the bloodstream or lymphatic system. Once in circulation, cancer cells can establish secondary tumors in distant organs, a process known as metastasis, which is often

responsible for most cancer-related deaths. Cancers can originate from epithelial cells (carcinomas), connective tissues (sarcomas), blood-forming cells (leukemias), or immune system cells (lymphomas), among other types, and they can present with diverse biological behaviors and clinical outcomes [11].

Cancer often begins quietly, with abnormal cells developing in a localized area a stage known as carcinoma in situ. At this point, the cells are confined to their place of origin and have not yet invaded the surrounding tissues, making it an early and potentially treatable form of cancer. However, if left unchecked, these cells may progress to a more dangerous phase. As the tumor grows, it requires more oxygen and nutrients to survive. To meet this demand, it triggers a process called angiogenesis, which is the formation of new blood vessels. These newly formed vessels nourish the tumor and provide a route for cancer cells to move beyond their original site. Over time, some of these cells may break away, enter the bloodstream or lymphatic system, and travel to other parts of the body a process known as metastasis. This stage marks a turning point in cancer progression, as it allows the disease to spread to distant organs such as the lungs, liver, brain, or bones. Metastatic cancer is often harder to treat and is associated with more severe outcomes. Understanding these key stages starting from in situ development, through angiogenesis, to metastasis is vital for early detection, timely intervention, and improving survival rates [12].

Cancer is fundamentally a disease of uncontrolled cell division, often triggered by genetic mutations that disrupt normal cellular regulation. The process typically begins with increased mitosis and leads to tumor formation. Two major classes of genes are involved in the development of cancer: proto-oncogenes and tumor suppressor genes. Proto-oncogenes are normal genes that, when altered through mutations such as point mutations, amplifications, or translocations, become oncogenes that drive hyperactive growth and excessive protein stimulation. In contrast, tumor suppressor genes normally function to control cell division and ensure DNA integrity. When these genes undergo mutations that produce defective or missing proteins, their ability to regulate cell growth is lost, resulting in uncontrolled

cell proliferation. The combination of activated oncogenes and inactivated tumor suppressor genes creates an environment conducive to cancer development [13].

Cancer progresses through a series of defined stages, which help determine the severity of the disease and guide treatment decisions. The stages are typically classified from Stage 0 to Stage IV. Stage 0, also known as carcinoma in situ, refers to abnormal cells that are confined to their site of origin and have not yet invaded surrounding tissues. Stage I is considered an early stage, where the tumor is small and localized. In Stage II and III, the cancer grows larger and may spread to nearby lymph nodes or surrounding tissues, indicating increasing local advancement. Stage IV represents the most advanced form, where the cancer has metastasized to distant organs or body parts. The staging system plays a crucial role in prognosis, helping physicians determine the most appropriate course of therapy and assess the likely outcomes for the patient [14].

The progression of cancer usually occurs in a multistep process driven by accumulated genetic and epigenetic alterations that give cancer cells selective growth advantages. In its earliest stages, cancer may be confined to the tissue of origin, showing no signs of invasion. As it advances, cancer breaks through the basement membrane and invades neighboring tissues, eventually entering lymphatic and vascular channels to establish metastatic sites. Different cancers follow unique progression patterns and respond differently to therapeutic interventions, leading to the classification of cancers into various subtypes based on tissue of origin, molecular markers, and genetic profiles. For example, cancers can be divided into hormone receptor positive and negative, HER2-positive and –negative, or highly aggressive versus indolent subtypes. Understanding these cancer subtypes and their progression pathways is crucial for devising tailored treatments, predicting prognosis, and improving patient outcomes through early detection and precise therapeutic strategies [15].

Cancer grade refers to the description of a tumor based on how abnormal the cancer cells appear under a microscope and how quickly they are likely to grow and spread. Grading provides important information about the tumor's aggressiveness and potential behavior. Tumors are generally classified into Grade 1 to Grade 3

(or sometimes Grade 4). Grade 1 (low grade) tumors have cells that look more like normal cells and tend to grow slowly. Grade 2 (intermediate grade) tumors show moderately abnormal features and have a faster growth rate than Grade 1. Grade 3 (high grade) tumors contain cells that look very different from normal cells and are more likely to grow and spread aggressively. In some cancers, a Grade 4 is used to indicate highly undifferentiated or anaplastic tumors. Determining the cancer grade helps doctors estimate prognosis and choose the most effective treatment plan in conjunction with cancer staging [16].

Cancer prevalence is rising worldwide, driven by factors such as aging populations, lifestyle changes, and advances in detection and diagnosis. According to the World Health Organization (WHO), cancer is today one of the top causes of death worldwide, accounting for roughly one in every six deaths. Furthermore, modern lifestyle variables such as poor diets, a lack of physical activity, and increased tobacco and alcohol consumption have all contributed to the rising incidence of many malignancies. The most prevalent kinds of cancer in high-income countries are breast, prostate, lung, and colorectal cancers, but low- and middle-income countries frequently have a higher prevalence of liver and cervical cancers [17].

Cancer is the largest cause of morbidity and mortality globally, with an estimated 19.3 million new cases and 10 million cancer-related deaths expected in 2020. The most common types of cancer differ by area, with lung cancer being the most prevalent in both men and women worldwide, followed by BC in women and prostate cancer in men. Despite the worrisome statistics, breakthroughs in early identification, treatment, and prevention have resulted in higher survival rates for many cancers. Early detection technologies, such as mammography for BC and colonoscopies for colorectal cancer, have helped to discover malignancies at an earlier stage, resulting in more effective treatment options [17].

The global prevalence of cancer also demonstrates the enormous disparity in cancer outcomes among countries. High-income countries often have access to advanced healthcare systems, early diagnostic tools, and cutting-edge therapies, resulting in greater survival rates. In contrast, low- and middle-income countries confront obstacles such as limited access to healthcare, a lack of screening programs, and

a scarcity of specialized treatment choices, all of which contribute to inferior cancer outcomes. This disparity highlights the importance of increasing investment in healthcare infrastructure and cancer research to ensure that effective preventive, early diagnosis, and treatment techniques are available to all populations, regardless of socioeconomic position [18].

2.2 Breast Cancer

BC is one of the most common and serious health concerns worldwide, particularly among women. It is a kind of cancer that starts in the breast tissue, usually in the milk- producing glands called lobules or the ducts that transport milk to the nipple. Although men can develop BC, women account for the great majority of instances. Significant progress has been made in our understanding of BC over the years, but the illness remains a serious challenge in terms of prevention, detection, and therapy. BC develops due to a variety of variables, including genetic susceptibility, hormonal effects, and lifestyle decisions. Individuals with mutations in specific genes, such as *BRCA1* and *BRCA2*, are more likely to develop the condition, therefore family history is important [19].

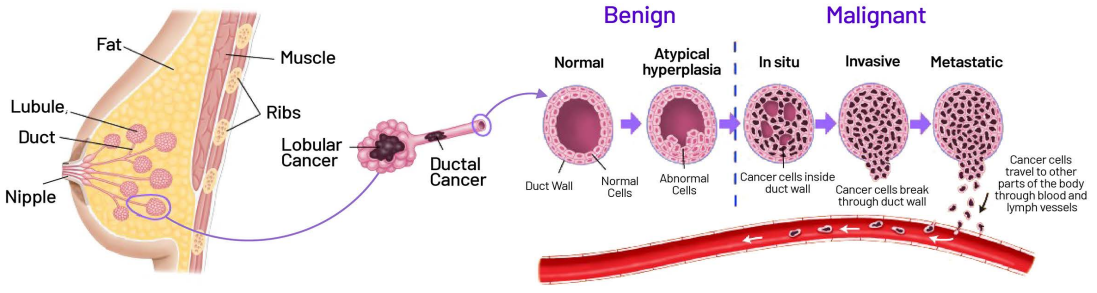

FIGURE 2.1: Progression of Breast Cancer. Anatomical structure of the breast showing lobules, ducts, fat, muscle, and ribs key areas where cancer may originate [20].

The image illustrates the development and progression of breast cancer, starting from normal breast anatomy to the advanced stages of malignancy. It shows two primary origins of breast cancer: lobular and ductal, with the latter being

more common. The process begins in the ducts or lobules of the breast, where normal cells may undergo changes leading to atypical hyperplasia an early benign condition characterized by abnormal cell growth. If this progresses, cancer cells can remain within the duct wall in what is known as ductal carcinoma in situ (DCIS), still considered non-invasive. As the disease advances, cancer cells break through the duct wall, becoming invasive and capable of spreading to surrounding breast tissue. In the final stage, metastatic cancer occurs when these malignant cells enter blood or lymphatic vessels, traveling to distant organs. The image clearly differentiates benign stages from malignant ones and highlights the critical transition from localized to systemic disease [20].

The actual origin of BC is unknown, but experts have found numerous major risk factors. The most major factor is age, with the risk of having BC increasing as women become older, particularly after the age of 50. Hormonal factors also play a crucial role in risk; women who have had prolonged estrogen exposure, such as those who begin menstruation early, suffer late menopause, or use hormone replacement therapy, may be at a higher risk. Furthermore, lifestyle variables such as alcohol consumption, poor diet, physical inactivity, and obesity have all been related to a higher risk of BC. Genetic abnormalities in the *BRCA1* and *BRCA2* genes can significantly raise the risk, with women inheriting these mutations having an up to 80% lifetime risk of acquiring the disease [21].

Early identification of BC is critical for increasing the odds of successful therapy. Mammography, a form of X-ray used to evaluate the breast, is the most prevalent screening method for abnormalities. It can detect cancerous cells before any symptoms appear, making it an effective tool in the fight against BC. Breast ultrasounds and MRIs are also used as screening tools, particularly in women with dense breast tissue or higher risk factors. In addition to screening, self-examination, in which people check their own breasts for lumps or other changes, can be an effective strategy to detect abnormalities early, albeit it should be done in conjunction with professional screening [22].

Once identified, BC treatment is determined by the cancer's type, stage, and location, as well as the patient's overall condition. The primary treatment choices are

surgery, chemotherapy, radiation therapy, hormone therapy, and targeted therapy. Surgery is frequently the initial line of treatment, and it may include removing the tumor or, in more advanced cases, a mastectomy, which removes the entire breast. Chemotherapy, which employs medications to destroy cancer cells, is frequently used in conjunction with surgery, particularly when the cancer has gone beyond the breast. Radiation therapy may also be used to target. Hormone therapy for hormone-receptor-positive tumors works by inhibiting the hormones that promote cancer growth. Targeted treatments, which attack specific molecular targets involved in cancer cell proliferation, have also been increasingly effective, providing patients with more individualized therapy options [23].

The Substantial breakthroughs in therapy and early detection, the prognosis for BC differs according to the stage at which it is found. If BC is detected early, the five- year survival rate for localized BC is approximately 99%. However, if the cancer has progressed to other places of the body, the survival rate drops. Late-stage BC, commonly known as metastatic BC, is more difficult to treat and requires rigorous therapy. BC research is constantly evolving, with a focus on improving diagnostic techniques, better understanding the disease's genetic roots, and generating more effective and customized treatments. New medicines are being developed to better target cancer cells while causing minimal damage to healthy tissue [24].

2.3 Risk factors

BC is impacted by a variety of genetic, environmental, and lifestyle factors. While the specific etiology of BC is unknown, various risk factors have been found that enhance the likelihood of developing the disease. Understanding these risk factors is essential for early detection, prevention, and effective disease management. One of the most important risk factors for breast cancer is female gender, as women are far more likely than men to develop the disease. Another crucial factor is age; as women age, their risk of having BC increases, with the majority of cases occurring after the age of 50. The aging process is known to cause changes in

breast tissue and hormone levels, raising the risk of abnormal cell growth. A family history of BC can considerably raise an individual's risk, especially if a close member (mother, sister, or daughter) gets the disease. Inherited genetic mutations, particularly those in the *BRCA1* and *BRCA2* genes, have been associated to an increased risk of BC. These mutations can be handed down from one generation to the next, significantly increasing the risk of getting breast and ovarian cancer. Other gene mutations, such as those affecting TP53 or PTEN, can raise cancer risk but are less prevalent. [25]

Hormonal exposure has an important role in the development of BC. Women who have early menstruation (before age 12) or late menopause (beyond age 55) have a higher lifetime estrogen exposure, which is likely to increase the chance of developing BC. Furthermore, women who utilize hormone replacement therapy (HRT) to treat menopausal symptoms, particularly combination estrogen and progesterone therapy, may be at an elevated risk. Pregnancy history is also important, with women who have their first kid later in life or who do not have children having a higher risk than those who have children at a younger age [26].

Lifestyle decisions are another important factor influencing BC risk. Alcohol use is a well-established risk factor, with research indicating that the more alcohol a woman consumes, the greater her risk of developing BC. Obesity, particularly after menopause, has been related to an increased risk because fat cells release more estrogen. A lack of physical activity and a bad diet may also contribute to an increased risk, as regular exercise and a balanced diet have been shown to lessen the likelihood of acquiring several types of cancer, including BC.

Exposure to ionizing radiation, particularly in childhood, is a well-known risk factor for BC. Women who got chest radiation therapy as children for illnesses such as Hodgkin lymphoma are more likely to acquire BC later in life. Furthermore, radiation exposure from certain medical imaging examinations, while usually lower than therapeutic dosages, may contribute to a slight increase in risk. Women who have thick breast tissue are more likely to get BC. Dense breasts include less fat and more glandular tissue, making mammograms less effective for cancer detection.

High breast density may also indicate a higher risk of aberrant cell development, which could lead to cancer [27].

While some risk factors like age and genes cannot be changed, leading a good lifestyle will greatly lower the likelihood of BC. Regular exercise, limited alcohol use, maintaining a healthy weight, and avoiding hormone replacement medication as much as possible are all beneficial risk-reduction strategies. Breastfeeding may also provide protective benefits, since it has been proven to marginally reduce the incidence of BC, especially in women who breastfeed for an extended period of time [28].

2.4 Epidemiology of BC

BC is one of the most frequent malignancies in the world, accounting for the majority of cancer-related fatalities, particularly among women. BC epidemiology is the study of the disease's prevalence, determinants, and trends in different populations, with a particular emphasis on how risk factors, incidence, mortality, and survival rates fluctuate by geographic location, age, gender, and other factors.

Breast cancer is the most often diagnosed disease in the world, with an estimated 19.3 million new cases and 10 million deaths by 2020. It accounts for roughly 24% of all cancer cases and 15% of cancer-related deaths in women. BC is more common in high-income nations, such as the United States, Canada, and Western Europe, due to increased screening, early identification, and advanced healthcare systems. However, BC rates are increasing in many low- and middle-income nations as a result of urbanization, lifestyle changes, and advances in healthcare that enable better diagnosis [29, 30].

In these high-income countries, incidence rates are higher as a result of increased awareness, more widespread screening programs, and earlier detection tools like as mammography. For example, in North America and Europe, the age-standardized incidence rate can approach 90- 120 per 100,000 women each year. Lower rates are observed in sub-Saharan Africa and portions of Asia, where access to healthcare,

illness knowledge, and screening procedures are frequently inadequate. However, as living standards rise and urbanization expands in these areas, the incidence of BC gradually increases [31].

According to epidemiological research, the incidence of BC varies with age, ethnicity, and reproductive history. The condition primarily affects women, with less than 1% of instances involving men. The risk of BC rises dramatically with age, particularly after the age of 50, due to hormonal changes and increased cumulative estrogen exposure. Family history is also important; women with close relatives who have had BC are at a higher risk, especially if the relative was diagnosed at a young age. Inherited genetic abnormalities, particularly those in the *BRCA1* and *BRCA2* genes, are also linked to an increased chance of developing BC.

Reproductive characteristics such as age at first menstruation, age at first childbirth, and nursing history all have an impact on BC risk. Women who have early menarche (before the age of 12) and late menopause (beyond the age of 55) have a higher lifetime estrogen exposure, increasing the risk. Women who have children at a younger age or who breastfeed for a longer amount of time are less likely to be at risk. Additionally, hormonal replacement treatment use has been related to an increased risk of BC, particularly when used for an extended period of time [32].

BC mortality has been consistently dropping in many high-income nations, owing mostly to breakthroughs in early identification, improved treatment approaches, and more access to healthcare. In the United States, for example, the five-year survival rate for women diagnosed with BC is around 90%, with early-stage tumors having survival rates of nearly 99%. However, the prognosis remains significantly worse in low- and middle-income nations, where late-stage diagnosis, limited access to healthcare, and a lack of treatment alternatives all contribute to reduced survival rates. BC survival rates in some regions might be as low as 50

Metastatic BC, in which the disease has spread to other parts of the body, is far more difficult to treat and continues to pose a significant challenge in increasing survival rates. While targeted medicines, chemotherapy, and immunotherapy

have improved the prognosis for many women, metastatic cancer frequently necessitates long-term maintenance rather than a cure. Furthermore, the global burden of BC is increasing, driven by population aging and lifestyle variables such as increased alcohol intake, obesity, and sedentary behavior, all of which raise the risk of acquiring the illness [31].

Epidemiological studies have found considerable regional and socioeconomic variations in BC outcomes. Because high-income countries have access to early screening and improved therapies, BC is frequently discovered at an earlier stage, resulting in higher survival rates. In low-income nations, where screening and treatment resources are limited, women are frequently detected at a later stage, resulting in less favorable outcomes. Furthermore, socioeconomic status has a role; women in affluent communities have greater access to healthcare facilities, including preventive treatments such as mammography, which lowers their chance of late-stage diagnosis. On the other hand, women in lower socioeconomic categories may face challenges to early detection, such as a lack of education, limited access to healthcare, and financial restraints.

The global epidemiology of BC is changing. While incidence rates remain high in rich countries, the number of cases in emerging countries has increased significantly due to urbanization, changing lifestyles, and an aging population. To reduce the disease's impact in these regions, efforts must be made to promote early detection, provide access to treatment, and raise awareness about BC. Genetic and environmental research, as well as advances in precision medicine, have the potential to lead to more effective therapies and individualized prevention efforts [32].

2.5 Diagnosis of BC

Breast cancer is usually diagnosed through a step-by-step process that starts with reviewing a patient's medical history and doing a physical exam. This is often followed by imaging tests, such as mammograms or ultrasounds. If there's anything suspicious, a biopsy may be done to check for the presence of cancerous cells and confirm the diagnosis. The healthcare professional will inquire about the patient's

personal and family medical history, including any prior breast concerns, a family history of BC, hormonal variables, and lifestyle choices. The doctor will examine your breasts for any abnormalities, such as lumps, changes in skin texture, or unusual discharge from the nipples. This is frequently the initial step in detecting probable BC [33].

Imaging is critical in the diagnosis of BC because it identifies anomalies in the breast that a physical exam may not reveal. Mammography is the most often used BC screening method. It entails taking low-dose X-rays to obtain detailed images of breast tissue. Mammograms can reveal cancers that are too tiny to feel during a physical examination. Women who have thick breast tissue may require extra imaging tests. Ultrasound is a test that employs sound waves to produce images of the breast tissue. It is commonly used to distinguish between solid tumors (which may be malignant) and Fluid- filled cysts. Ultrasound can also be used in conjunction with mammography to further investigate problematic spots.

Magnetic resonance Imaging MRI scans employ magnets and radio waves to provide detailed images of breast tissue. MRIs are commonly utilized for high-risk patients or those with thick breast tissue. It can also assist determine the size of the malignancy in the breast and adjacent tissues. Breast Tomosynthesis (3D Mammography) is an enhanced mammography technique that produces three-dimensional images of the breast. It can help detect cancers and prevent false positives, particularly in women with thick breasts [34].

If an imaging test detects an abnormality, a biopsy may be required to determine whether it is malignant. Fine Needle Aspiration involves using a fine, hollow needle to extract a small sample of tissue from the questionable location. FNA is commonly utilized for cysts or easily accessible lumps. Core Needle Biopsy: A bigger needle is used to extract a sample of tissue from the breast. It is more typically employed when a tumor is suspected because it yields more tissue for investigation. Surgical biopsy is utilized in some circumstances when a needle biopsy is not possible or does not give enough tissue. This entails removing a portion or all of the suspicious lump for examination. Sentinel lymph node biopsy. If the breast tissue biopsy confirms malignancy, a sentinel lymph node biopsy may

be performed to determine whether the disease has progressed to the lymph nodes [35].

After the biopsy sample is collected, a pathologist examines the tissue under a microscope to see if malignancy is present. The findings of this test can assist define the type of BC (e.g., invasive ductal carcinoma, invasive lobular carcinoma) and whether it is hormone receptor-positive, HER2-positive, or triple-negative. This information is crucial for planning treatment. Hormone Receptor Tests determine whether BC cells contain receptors for estrogen or progesterone. Hormone treatments are commonly used to treat cancers that express these receptors. The HER2 gene creates a protein that stimulates cancer cell proliferation. In some malignancies, HER2 is overexpressed, resulting in more aggressive illness. HER2-positive BCs are treated with targeted treatments. *BRCA1* and *BRCA2* gene tests can help establish whether an inherited mutation raises the risk of BC. These tests are especially relevant for women who have a family history of the condition [36].

After BC is discovered, more tests may be performed to assess the cancer's stage, which defines how far it has spread. CT or PET scans may be used to detect the spread of cancer to other parts of the body (for example, bones, liver, or lungs). If symptoms indicate that cancer has progressed to the bones, a bone scan may be performed to detect metastases. Following diagnosis and staging, a multidisciplinary team of experts, including oncologists, surgeons, and radiologists, will examine the case and offer a specific treatment plan depending on the type and stage of the cancer, as well as the patient's overall health and preferences [37].

2.6 Screening of BC

BC screening is an important step in early detection, as it can help save lives by detecting cancer before symptoms arise. BC screening is a series of tests and examinations designed to detect signals of BC in patients who do not have symptoms. The primary screening approach is mammography, an X-ray of the breast that can detect cancers or abnormalities in the tissue before they become visible or cause symptoms [38].

Many health groups, like the American Cancer Society and the US Preventive Services Task Force (USPSTF), recommend that women aged 40 to 50 begin receiving annual or biennial mammograms. Women aged 50 to 74 should receive mammograms every two years. Women with a family history of BC, genetic abnormalities (such as *BRCA1* or *BRCA2*), or other risk factors may begin screenings sooner or have more regular testing, including MRI (Magnetic Resonance Imaging). Screening provides for the early discovery of cancer, boosting the chances of successful treatment and survival [39].

Regular screening can help reduce BC mortality by finding cancers before they spread. Screening may falsely show the presence of cancer, resulting in unneeded biopsies, worry, and more procedures. Screening may miss some tumors, particularly in thick breasts, creating a false sense of security. Some tumors found through screening may be slow- growing and non-life-threatening, resulting in unneeded treatment [40].

2.7 Key Signaling Pathways in Breast Cancer Targeted by Natural Compounds

Breast cancer is a complex and diverse condition that involves the disruption of several molecular pathways responsible for regulating cell growth, survival, programmed cell death, and the spread of cancer cells. In recent years, there has been growing scientific interest in plant-based compounds as potential therapeutic agents. Among these, *Annona muricata* (commonly known as soursop) has attracted attention for its bioactive components. The leaves of this plant are rich in acetogenins and flavonoids, which have demonstrated promising effects in both computational and laboratory studies, particularly in influencing critical signaling pathways linked to cancer progression and suppression.

2.7.1 PI3K/AKT/mTOR Pathway

The phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway is a central regulator of cell growth, proliferation, metabolism, and survival. Dysregulation of this pathway is a common feature in breast cancer, particularly in aggressive subtypes such as HER2-positive and triple-negative breast cancers, where mutations or amplifications in the *PIK3CA* gene—the gene encoding the catalytic subunit of *PI3K*—are often observed. Aberrant activation of PI3K/AKT/mTOR drives tumor development by suppressing programmed cell death and enhancing protein synthesis, thereby favoring uncontrolled growth. Natural compounds have shown promise in targeting this pathway. For instance, acetogenins derived from Annona muricata are reported to inhibit mitochondrial Complex I, reducing cellular ATP levels and consequently downregulating mTOR activity. Similarly, flavonoids like quercetin and kaempferol exert inhibitory effects by blocking AKT phosphorylation and suppressing downstream mTOR signaling, making them potential candidates for complementary breast cancer therapy [41].

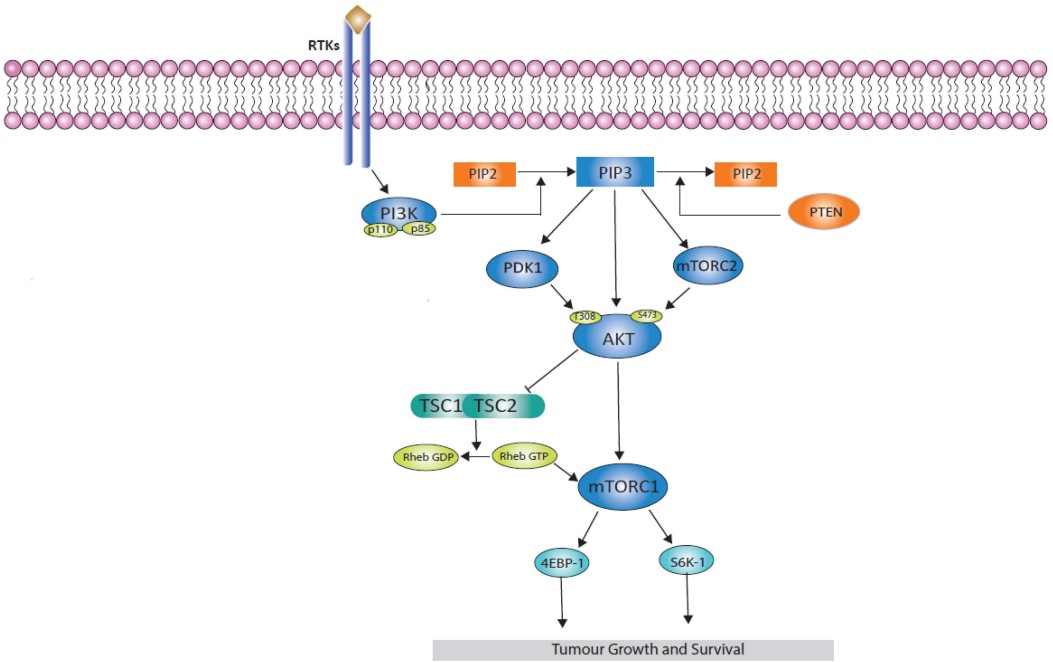

FIGURE 2.2: mTOR Signaling Pathway: Regulation of Cell Growth, Metabolism, and Survival [42]

This figure illustrates the mTOR signaling pathway, a fundamental cellular network that governs growth, metabolism, and survival. The cascade is initiated at the cell membrane, where growth factors such as IGF1 bind to IGF1R, and epidermal growth factor receptors (EGFR/HER2/HER3) are activated. These receptor tyrosine kinases (RTKs), along with insulin receptor substrate (IRS) proteins, stimulate PI3K activity. Once activated, PI3K catalyzes the conversion of PIP2 into PIP3, which in turn recruits AKT to the membrane for activation through phosphorylation by PDK1. Activated AKT phosphorylates and suppresses the TSC1/2 complex, a negative regulator of the small GTPase Rheb. When TSC1/2 inhibition occurs, Rheb remains in its active, GTP-bound state, which directly stimulates mTOR complexes. Conversely, the tumor suppressor PTEN counterbalances PI3K by converting PIP3 back to PIP2, thereby acting as a critical brake on the pathway.

The mTOR protein functions within two structurally distinct complexes: mTORC1 and mTORC2. mTORC1, composed of mTOR, Raptor, PRAS40, DEPTOR, and mLST8, plays a pivotal role in protein synthesis, cell growth, and metabolic regulation by activating effectors such as S6K1 and inhibiting 4EBP1. It also enhances angiogenesis through HIF-1 activation and promotes glucose uptake via regulation of transporters like GLUT1. In contrast, mTORC2, which consists of mTOR, Rictor, DEPTOR, and mLST8, primarily supports cell survival and cytoskeletal organization by facilitating AKT phosphorylation. Together, these complexes act as a central hub for integrating extracellular growth signals with intracellular metabolic demands, highlighting mTOR as a key therapeutic target in breast cancer and metabolic disorders [43].

2.7.2 Estrogen Receptor (ER) Signaling Pathway

Estrogen receptor-alpha is a key driver of luminal-type breast cancers. Upon estrogen binding, ER-α dimerizes and translocates to the nucleus, where it regulates the expression of genes involved in proliferation and survival. About 70% of breast cancers are ER-positive and depend on estrogen signaling for growth. Flavonoids

have structural similarity to estrogens and can act as selective estrogen receptor modulators. In silico docking studies indicate strong binding affinity of certain flavonoids to ER-α, potentially blocking its activation.

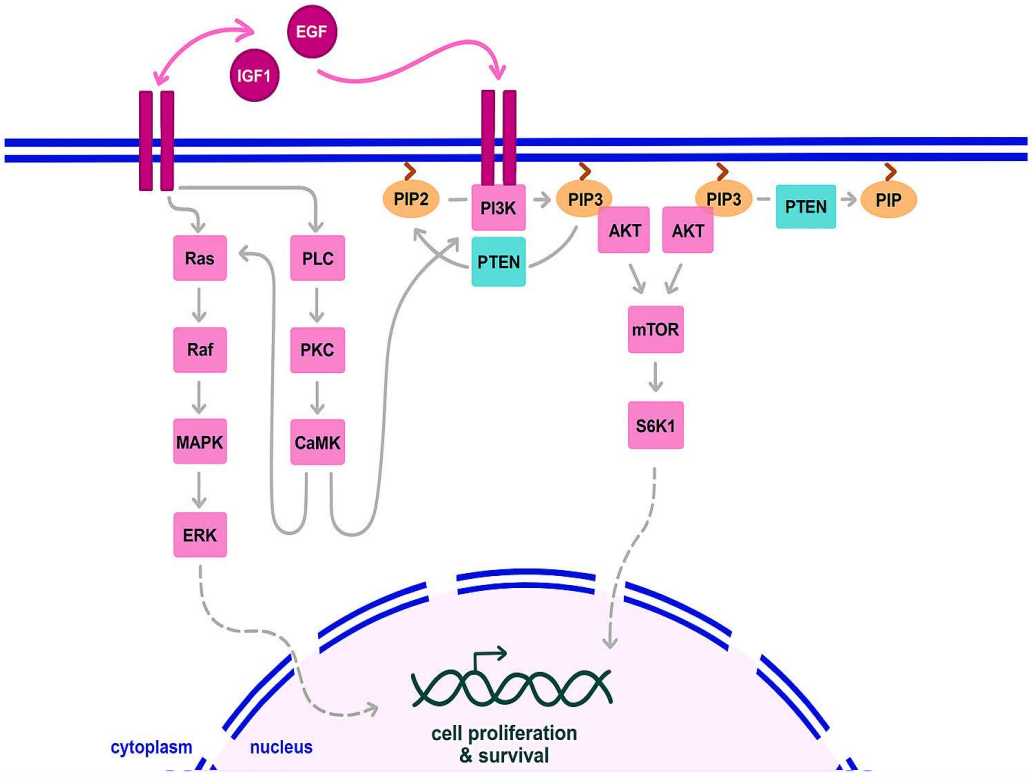

FIGURE 2.3: Intracellular signaling events triggered by epidermal growth factor (EGF) and insulin-like growth factor1 (IGF-1) [44].

This figure illustrates the major intracellular signaling pathways activated by growth factors such as epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF-1). Binding of these ligands to their respective receptors initiates several downstream cascades that regulate fundamental cellular processes. One of the key pathways highlighted is the Ras/Raf/MEK/ERK signaling cascade, which transmits extracellular cues to the nucleus and modulates gene expression programs that drive cell proliferation and survival. In parallel, other signaling routes including PLC/PKC and CaMK are activated, contributing to cellular growth, differentiation, and metabolic adaptation.

On the right side of the figure, the PI3K/AKT/mTOR pathway is depicted as another central regulator of cellular homeostasis. Activation of PI3K leads to the phosphorylation of PIP2 into PIP3, which subsequently recruits and activates

AKT. Once activated, AKT stimulates downstream effectors such as mTOR and S6K1, enhancing protein synthesis, metabolism, and cell survival. The tumor suppressor PTEN serves as a negative regulator of this cascade by dephosphorylating PIP3 back to PIP2, thus preventing excessive AKT signaling. Ultimately, both the Ras/MAPK and PI3K/AKT/mTOR pathways converge at the nuclear level, where they orchestrate transcriptional programs that promote growth and survival. Dysregulation of these interconnected pathways is strongly linked to uncontrolled proliferation and cancer progression [45].

2.7.3 HER2/EGFR Signaling Pathway

HER2 (human epidermal growth factor receptor 2) belongs to the ERBB family of receptor tyrosine kinases and plays a pivotal role in cellular signaling. When overexpressed, HER2 undergoes constitutive dimerization, leading to persistent activation of downstream signaling cascades, most notably the PI3K/AKT and MAPK pathways. This aberrant signaling drives uncontrolled cell proliferation and survival, contributing to the development of aggressive breast cancer subtypes that are often linked with poor clinical outcomes. Recent molecular docking studies have indicated that acetogenins, bioactive compounds derived from Annona muricata, are capable of binding to the HER2 kinase domain. Such interactions suggest their potential utility as natural inhibitors of HER2 activity, offering a promising alternative or complementary approach in the management of HER2-positive breast cancers.

This figure highlights the major signaling cascades activated by the Epidermal Growth Factor Receptor (EGFR). Upon ligand binding by molecules such as EGF, TGF- , amphiregulin, or epiregulin the receptor undergoes dimerization and subsequent activation of its intrinsic tyrosine kinase (TK) domains through autophosphorylation. These phosphorylated residues then serve as initiation points for several downstream signaling pathways, including MAPK, STAT, and PI3K/AKT.

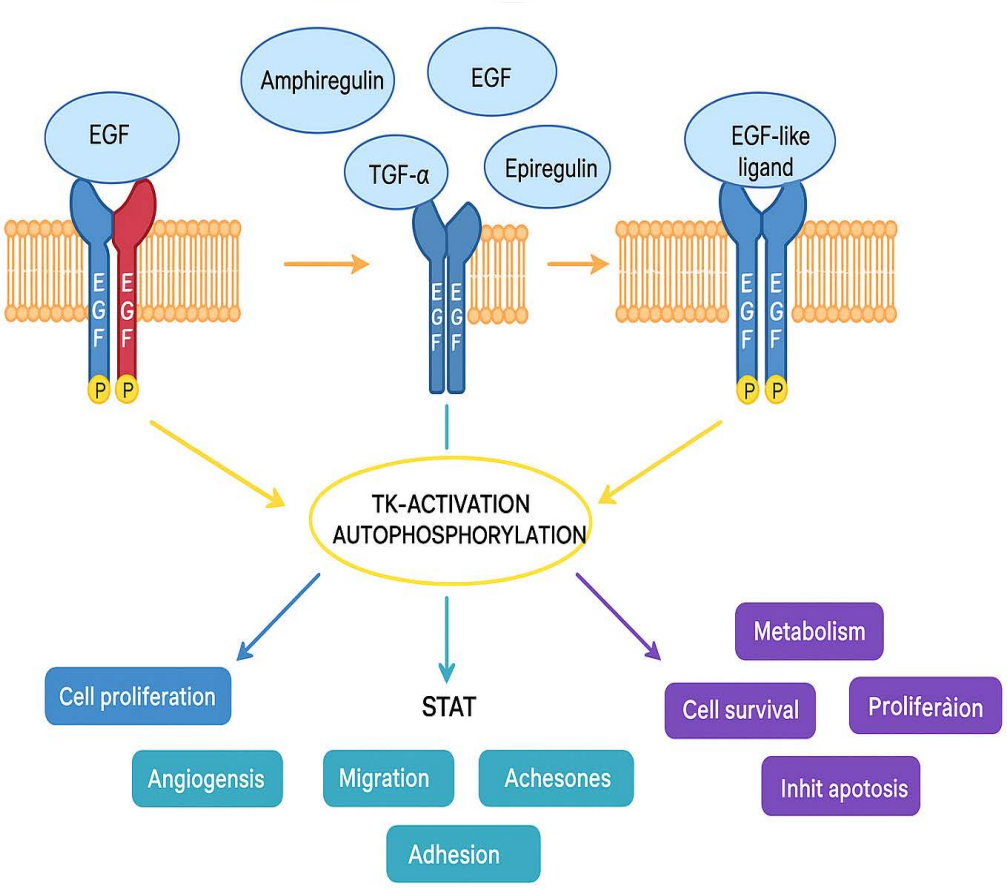

FIGURE 2.4: Signaling cascades initiated upon epidermal growth factor receptor (EGFR) activation [45]

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Collectively, these cascades regulate essential cellular processes such as proliferation, migration, adhesion, angiogenesis, survival, and suppression of apoptosis, thereby maintaining cellular growth and homeostasis.

In the context of breast cancer, dysregulated EGFR activity is a critical driver of tumor initiation and progression. Overexpression or mutations in EGFR can result in constitutive signaling, leading to excessive cell proliferation, sustained angiogenesis, and resistance to programmed cell death. Among these pathways, persistent activation of PI3K/AKT signaling is particularly associated with therapeutic resistance and unfavorable clinical outcomes. Understanding this schematic is therefore important, as it provides the molecular rationale for the use of targeted therapies such as tyrosine kinase inhibitors and monoclonal antibodies that are designed to block aberrant EGFR signaling and improve treatment strategies in breast cancer management [46].

2.7.4 CDK4/6–Rb Pathway

Cyclin-dependent kinases 4 and 6 (CDK4/6) are key regulators of cell cycle progression. In association with cyclin D, these kinases phosphorylate the retinoblastoma (Rb) protein, thereby releasing E2F transcription factors and allowing the transition from the G1 to S phase. In hormone receptor-positive (HR+) breast cancers, CDK4/6 activity is often hyperactivated, resulting in deregulated cell cycle progression and uncontrolled proliferation. Targeting this pathway has become a therapeutic focus, with CDK4/6 inhibitors such as Palbociclib demonstrating clinical efficacy. Interestingly, several plant-derived compounds, including flavonoids and acetogenins, have exhibited potential CDK4/6 inhibitory activity in silico. These findings suggest their ability to induce G1 cell cycle arrest, mimicking the mechanism of action of clinically approved CDK4/6 inhibitors and highlighting their promise as natural therapeutic candidates in breast cancer management.

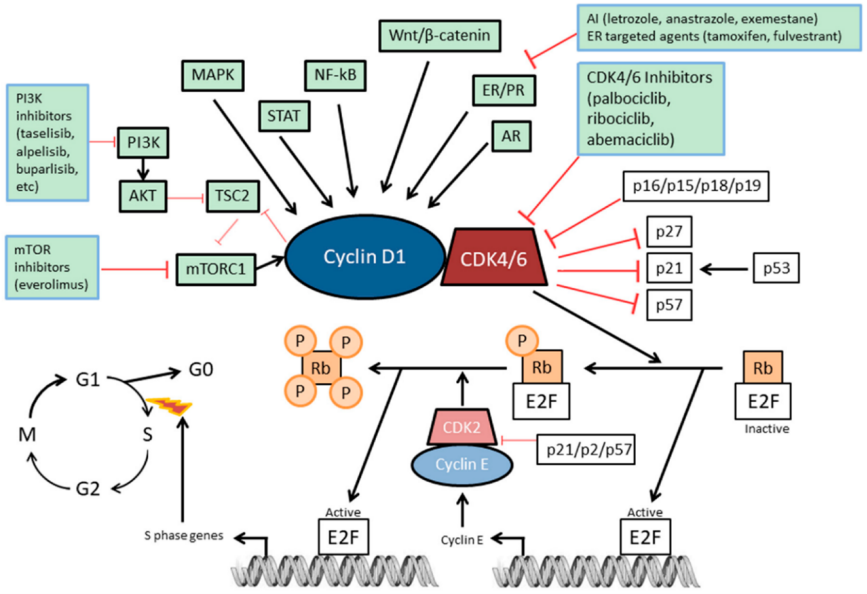


FIGURE 2.5: Cyclin D1–CDK4/6–Rb signaling axis, a central regulator of cell cycle progression in breast cancer [47].

In most human cancers, the Cyclin D1–CDK4/6–Rb signaling axis is frequently disrupted through a variety of mechanisms. These include loss of Rb function, amplification or overexpression of Cyclin D1, mutations in CDK4 that confer resistance to inhibitors, or deletion of tumor suppressors such as p16^INK4a. Such alterations enable cancer cells to bypass normal checkpoints, resulting in

uncontrolled proliferation. Under physiological conditions, external stimuli activate signaling networks including PI3K/AKT/mTOR, MAPK, STAT, and Wnt/β-catenin, which collectively enhance Cyclin D1 expression and stability. Cyclin D1 subsequently binds to CDK4/6, initiating phosphorylation of the Rb protein. This phosphorylation event releases E2F transcription factors, which in turn activate Cyclin E–CDK2 complexes, promote further Rb hyperphosphorylation, and stimulate the transcription of S-phase genes, ultimately driving the G1–S phase transition and cancer cell growth.

Therapeutically, CDK4/6 inhibitors function by blocking this G1-to-S progression, restoring Rb activity through dephosphorylation, and halting uncontrolled cell division. Endogenous inhibitors such as the INK4 and CIP/KIP protein families normally regulate CDK activity, but their functions are frequently impaired in cancer cells. The development of selective CDK4/6 inhibitors including Palbociclib, Ribociclib, and Abemaciclib represents a major advance in breast cancer therapy, particularly in hormone receptor–positive tumors where Cyclin D1 is commonly overexpressed. These agents effectively suppress tumor growth by preventing Rb phosphorylation, though their efficacy requires intact Rb function. Importantly, while loss of p16^INK4a or Rb independently contributes to tumorigenesis, the predictive value of Rb status for determining CDK4/6 inhibitor responsiveness in clinical settings remains uncertain. This underscores the need for improved biomarkers to guide patient selection and optimize therapeutic outcomes [48].

2.7.5 BRCA1/BRCA2 and DNA Damage Repair Pathways

BRCA1 and BRCA2 function as key tumor suppressor genes that play essential roles in homologous recombination (HR)-mediated DNA repair. Mutations in either gene compromise the efficiency of this repair mechanism, resulting in genomic instability and an elevated risk of developing cancer. Germline mutations in BRCA1 are particularly linked to triple-negative breast cancer (TNBC), a highly aggressive subtype with limited targeted treatment options. Although most therapeutic strategies do not directly inhibit BRCA proteins, agents that either induce

DNA damage or block alternative repair mechanisms can exploit the vulnerability of BRCA-mutant cells. A notable example is the use of poly (ADP-ribose) polymerase (PARP) inhibitors, which interfere with base excision repair, creating a condition of synthetic lethality that selectively eliminates BRCA-deficient tumor cells while sparing normal cells.

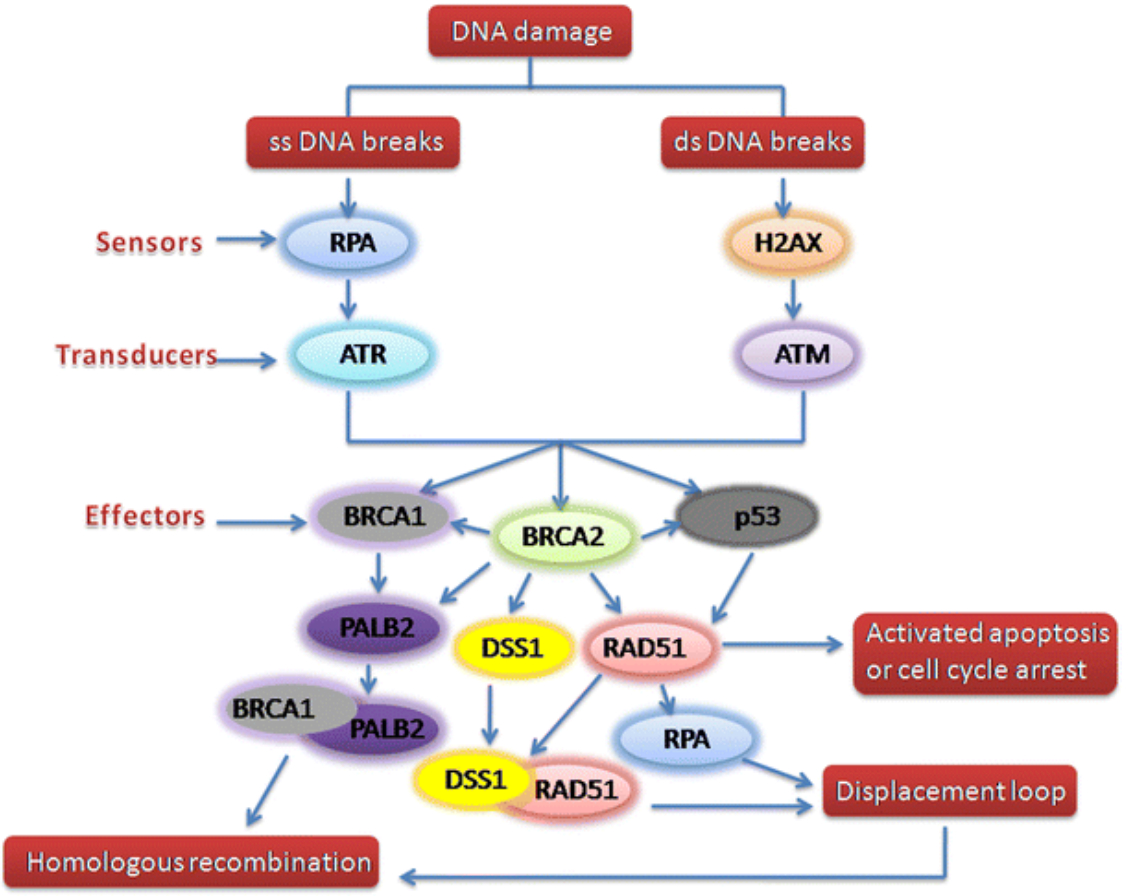


FIGURE 2.6: Role of BRCA2 in the repair of DNA double-strand breaks through homologous recombination (HR) [49].

BRCA2 serves as a central mediator of homologous recombination (HR), the most precise mechanism for repairing DNA double-strand breaks (DSBs). Following DNA damage, single-stranded breaks are detected by replication protein A (RPA), which activates the ATR kinase, whereas DSBs are recognized through phosphorylation of H2AX, leading to ATM activation. These kinases subsequently trigger downstream repair responses by activating BRCA1, BRCA2, and p53. BRCA2 interacts with RAD51 and DSS1 to form a repair complex that facilitates displacement loop (D-loop) formation, a critical step in strand invasion during HR. BRCA1 contributes earlier in the process by sensing DNA damage, initiating end

resection, and recruiting repair factors, while also coordinating with PALB2 to support BRCA2 function.

The functional cooperation between BRCA1 and BRCA2 is essential for safeguarding genomic integrity. BRCA1 primarily governs DNA damage recognition, signaling, and end resection, whereas BRCA2 directly mediates RAD51 filament formation, enabling accurate strand pairing and recombination. PALB2 acts as a molecular bridge, stabilizing BRCA1–BRCA2 interactions and ensuring efficient nuclear localization of the repair machinery. In parallel, p53 modulates cellular outcomes by interacting with RAD51 to promote cell cycle arrest or apoptosis when DNA repair is insufficient. Mutations in BRCA1 or BRCA2 compromise these repair processes, resulting in genomic instability, tumor development, and elevated susceptibility to breast and ovarian cancers. Importantly, such defects also carry therapeutic implications, as BRCA-mutant tumors exhibit heightened sensitivity to PARP inhibitors and DNA-damaging agents through synthetic lethality [50].

2.8 Selected Gene

The *BRCA1* gene, located on chromosome 17, encodes a protein that is essential for preserving genomic integrity. It is essential for homologous recombination, DNA repair, and proper cell division. *BRCA1* also interacts with other proteins to control the cell cycle, apoptosis, and transcription. Mutations in *BRCA1* disturb these mechanisms, resulting in genetic instability and increased cancer risk. More than 1,000 *BRCA1* mutations have been found, with different impacts on protein function. Some mutations truncate the protein, while others affect its ability to bind DNA or interact with other proteins.

Individuals with hereditary *BRCA1* mutations had significantly higher lifetime risks for breast (45-72%), ovarian (39-46%), and other malignancies. *BRCA1* mutations can be identified by family history and genetic testing. Carriers may

choose enhanced screening (MRI, mammography), preventive measures (mastectomy, salpingo- oophorectomy), or targeted therapy. The research focuses on enhancing risk assessment, creating effective prevention techniques, and optimizing treatment alternatives. PARP drugs, for example, have demonstrated potential in the treatment of *BRCA1*- related malignancies. Individuals with *BRCA1* mutations benefit from genetic counseling and testing, which allows them to make informed decisions and receive individualized care. Regular observation and preemptive actions can considerably improve the outcomes for people at risk [51].

BRCA1 is a large, multifunctional gene that encodes a protein consisting of 1,863 amino acids and contains several functional domains essential for its tumor - suppressive activities. These domains include the N-terminal RING finger domain, which mediates protein-protein interactions and ubiquitin ligase activity, and two C-terminal BRCT (*BRCA1* C-Terminus) domains, which facilitate the recognition of phosphorylated proteins involved in the DNA damage response. The RING domain enables *BRCA1* to form a heterodimer with its partner BARD1, a critical interaction for its role in ubiquitination and regulation of the DNA repair machinery. The BRCT domains serve as a scaffold for repair protein assembly, ensuring accurate repair of DNA double-strand breaks through homologous recombination. Beyond DNA repair, *BRCA1* is also involved in regulating transcription and chromatin structure, participating in centrosome duplication and cell cycle checkpoint activation, further underlining its importance in genomic integrity [52].

Inherited mutations in *BRCA1* often produce truncated or nonfunctional protein, severely compromising its repair capacity and cellular control mechanisms. These mutations show a strong autosomal-dominant inheritance pattern, and carriers can have up to a 60–80% lifetime risk of breast cancer and a 35–50% lifetime risk of ovarian cancer. The tumors arising in *BRCA1* mutation carriers often display distinctive histopathological features such as a high-grade invasive ductal phenotype and are more commonly triple-negative (lacking estrogen, progesterone, and HER2 receptors), which correlates with a more aggressive clinical course and limited targeted treatment options. Research into *BRCA1* has also spurred advances in personalized medicine; for example, the use of PARP inhibitors exploits the

concept of synthetic lethality to selectively kill cancer cells deficient in *BRCA1*-mediated DNA repair. Furthermore, ongoing studies into *BRCA1*'s regulation, interactions with other DNA repair proteins like RAD51 and *BRCA2*, and its role in maintaining genomic stability continue to inform new strategies for prevention, diagnosis, and treatment of hereditary cancers [53].

2.9 Selected Plant

Soursop (*Annona muricata*):

Scientific classification

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Magnoliids
Order:	Magnoliales
Family:	Annonaceae
Genus:	Annona
Species:	*muricata*

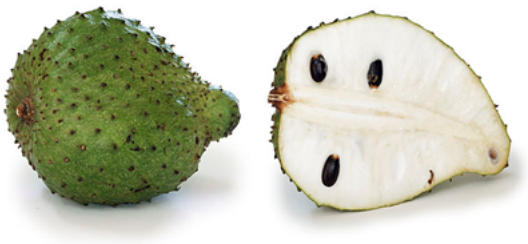


FIGURE 2.7: Soursop (*Annona muricata*) [54]

FIGURE 2.8: *A. muricata* leaves [54]

Soursop (also known as graviola, guyabano, or guanábana in Latin America) is the fruit of *Annona muricata*, an evergreen tree with broadleaf leaves and flowers shown in figure 2.2. It is widely dispersed throughout the Americas and the Caribbean's tropical regions. Soursop, a tropical fruit- bearing plant, has received interest for its possible therapeutic benefits, particularly in cancer treatment. It thrives best in warm, humid climates and is not indigenous to Pakistan.

However, soursop is not commonly grown in Pakistan because the climate in most parts of the country (especially in Punjab and Khyber Pakhtunkhwa) is not ideally tropical or humid enough for its commercial cultivation. That said, with proper greenhouse or controlled-environment farming, it is possible to grow soursop on a small scale in warmer southern parts of Pakistan, especially in Sindh or coastal areas like Karachi. Occasionally, you might find its fruits or leaves sold as imports in large urban markets or online organic/herbal product sellers, but it is not a commonly cultivated fruit tree like mango or guava [55].

Soursop, which is endemic to tropical regions of the Americas, is well-known for its tasty fruit and traditional medicinal uses. Several portions of the plant, including the leaves, bark, and seeds, contain bioactive chemicals known as acetogenins, alkaloids, and phenolics, which have been researched for their medicinal potential. It belongs to the Annonaceae family and is in the same genus as cherimoya [56].

Soursop is found in many places around the world and thrives best in warm, humid climates. It doesn't do well in the cold temperatures below 5^oC (41^oF) can

damage its leaves and branches, and anything below 3°C (37°F) might actually kill the plant. When the fruit dries out, it's no longer good for making concentrated products. Fresh soursop has a tropical, inviting aroma similar to pineapple. Its flavor is a unique mix of strawberries and apple, with a tangy citrus twist, all wrapped up in a creamy, banana-like texture [57].

Soursop, also known as graviola, is often mentioned as a natural remedy for cancer, even though there's no strong scientific evidence to support its effectiveness against cancer or other serious diseases. The fruit, seeds, and leaves of the plant contain a compound called anonacin. Its leaves also have annonamine an alkaloid similar to aporphine, featuring a quaternary ammonium group and a chemical called lichexanthone, which belongs to the xanthone family. Traditionally, soursop has been used in folk medicine to help with fevers, infections, parasites, inflammation, diabetes, high blood pressure, and even as a preventive approach or complementary aid in cancer care [58, 59].

Soursop-derived compounds were docked to the *BRCA1* protein to determine their binding affinity. Annonacin and bullatacin exhibited substantial interactions, indicating that they could inhibit *BRCA1* mutations. Computational simulations revealed that soursop chemicals stabilize *BRCA1* protein connections, lowering the cancer-promoting effects. SwissADME and pkCSM were used to screen soursop compounds for pharmacokinetic properties. Many compounds adhered to Lipinski's Rule of Five, showing favorable drug-like characteristics [60].

Soursop leaves have been gaining attention lately because they're packed with natural compounds that could support our health in a variety of ways. Researchers have found that these leaves contain unique substances like acetogenins, flavonoids, alkaloids, and tannins, which give them their powerful medicinal qualities. Acetogenins, in particular, have been studied for their ability to target cancer cells, especially in breast, liver, and colon cancers, by triggering cell death while leaving healthy cells unharmed. This is why soursop leaves are often talked about as a promising natural option in cancer research [61].

Beyond their anticancer potential, people have been using soursop leaves for a long time in traditional medicine to help with infections and inflammation. Recent studies back this up, showing that extracts from these leaves can fight bacteria, fungi, and even drug-resistant infections. They also seem to have strong anti-inflammatory effects, which could make them useful in treating everyday conditions like arthritis or gut inflammation. Some newer research even suggests they might help regulate blood sugar, protect the liver, and support nerve health. Still, most of this evidence comes from lab tests and early-stage research, so more clinical studies are needed before we fully understand the safest and most effective ways to use soursop leaves. But overall, these findings make soursop leaves an exciting topic in natural medicine and highlight their potential as a supportive therapy for various health conditions [62].

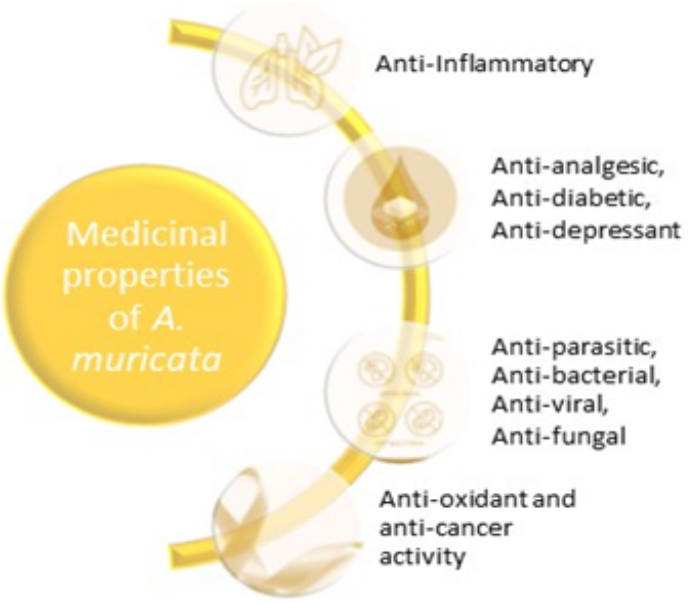


FIGURE 2.9: Flowchart representing the biomedical properties of *A. muricata* [63]

Chapter 3

Methodology

3.1 Proposed Diagram

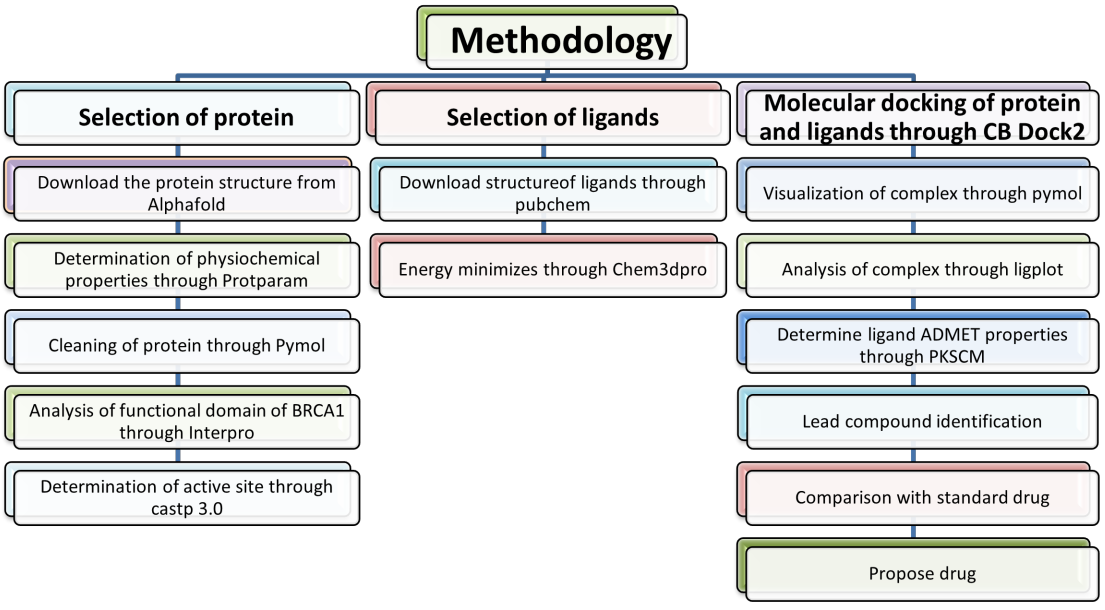


FIGURE 3.1: Detail outlines of my research methodology.

3.2 Data Collection and Preprocessing

The first step was collecting relevant data, including phytochemical compounds from soursop leaves and BC-associated molecular targets.

3.2.1 Phytochemical Data Collection

The bioactive compounds present in soursop leaves was retrieved from various databases, including: Dr. Duke's Phytochemical and Ethnobotanical Database (for plant- derived bioactive compounds). PubChem, ChEMBL, and ZINC databases (for chemical structures and molecular properties). Traditional medicine literature and scientific journals [64].

The key bioactive compounds include: Acetogenins (e.g., Annomuricin, Bullatacin, Squamocin) Potent inhibitors of cancer cell respiration, Flavonoids (e.g., Quercetin, Kaempferol, Luteolin) Known for their antioxidant and anti - inflammatory properties, Alkaloids (e.g., Coreximine, Reticuline) Exhibit cytotoxic activity, Phenolic Compounds (e.g., Gallic acid, Caffeic acid) Induce apoptosis in cancer cells [65].

3.2.2 BC Target Identification

To evaluate the anticancer potential of soursop-derived compounds, BC-related target proteins was selected from various sources: The Cancer Genome Atlas (TCGA) Provides genomic and transcriptomic profiles of BC. UniProt and Protein Data Bank Contain 3D structures of BC-associated proteins. KEGG Pathway Database helps to identify molecular pathways involved in BC progression. Key target proteins include: Estrogen Receptor (ER-) (PDB ID: 3ERT) overexpressed in ER-positive BC. HER2 (PDB ID: 3PP0) drives HER2-positive BC progression. EGFR (PDB ID: 1M17) plays a role in tumor proliferation. Cyclin-dependent kinases (CDK4/6) (PDB ID: 2W9Z) regulate cell cycle progression. PI3K/AKT/mTOR pathway proteins frequently mutated in BC [66].

3.3 Molecular Docking Studies

Molecular docking was used to predict the binding affinity and interactions between soursop bioactive compounds and BC target proteins.

3.3.1 Ligand Preparation

The 2D and 3D structures of selected bioactive compounds was downloaded from PubChem, ChEMBL, and ZINC databases. The compounds are energy- minimized using ChemSketch, Open Babel, and Avogadro to remove steric clashes and optimize geometry. Molecular properties (e.g., logP, molecular weight, hydro- gen bond donors/acceptors) was analyzed using SwissADME [67].

3.3.2 Receptor Preparation

BC target proteins are retrieved from the Protein Data Bank (PDB). Water molecules and non-essential ligands are removed using PyMOL and Chimera. Energy minimization is performed using AutoDockTools to optimize the protein structure [68].

3.3.3 Molecular Docking Procedure

Docking Software Used: AutoDock Vina (for flexible ligand docking), Schrödinger Glide (for high-precision docking), Molecular Operating Environment (MOE) (for detailed interaction analysis). CB-Dock2 is an advanced molecular docking tool designed for automated and user-friendly protein–ligand docking. It builds upon the original CB-Dock by offering improved accuracy and efficiency in predicting binding poses and affinities. One of its key features is its ability to automatically identify potential binding cavities in the target protein structure using an efficient cavity detection algorithm. Once cavities are detected, the tool performs docking simulations using the AutoDock Vina engine, allowing users to upload either single

ligands or small ligand libraries. CB-Dock2 supports flexible file input for- mats, generates visual docking results, and provides scoring functions that help in assessing ligand binding strength. Its web-based interface makes it accessible for both beginners and experienced researchers, playing a valuable role in virtual screening, drug discovery, and structure-based design studies. The docking results was evaluated based on: Binding energy (ΔG, kcal/mol) lower values indicate stronger binding. Hydrogen bonding, hydrophobic interactions, and - stacking determine binding stability [69].

3.4 ADMET and Toxicity Prediction

To evaluate the potential of the bioactive compounds derived from soursop (*Annona muricata*) leaves as safe and effective drug candidates, an *in-silico* ADMET analysis is performed using established computational tools. This approach allows the early assessment of the Absorption, Distribution, Metabolism, Excretion, and Toxicity profiles of these compounds before moving to experimental validation.

3.4.1 Pharmacokinetic Properties Prediction

The pharmacokinetic properties of the selected compounds are predicted using SwissADME and pkCSM platforms. These tools help estimate key parameters that influence oral bioavailability and overall drug-likeness. Lipinski's Rule of Five is applied to determine whether the compounds possess physicochemical properties suitable for oral administration, such as molecular weight, hydrogen bond donor and acceptor counts, and lipophilicity. Blood–brain barrier (BBB) permeability is also predicted to assess the potential of the compounds to cross into the central nervous system, which is especially important when evaluating off-target effects or CNS-related therapies. Gastrointestinal (GI) absorption is estimated to understand whether the compounds would likely be well-absorbed when administered orally. Metabolic interactions with Cytochrome P450 enzymes (CYP450s) are analyzed to identify the possibility of rapid metabolism or potential drug–drug

interactions, as these enzymes play a central role in the biotransformation of most pharmaceutical agents.

3.4.2 Toxicity Screening

In addition to the pharmacokinetics, the safety profile of these compounds is investigated using ProTox-II and Toxtree, which predict potential adverse effects based on chemical structure. ProTox-II estimates key toxicity endpoints, including hepatotoxicity (the risk of liver damage), carcinogenicity (the potential to cause cancer), and mutagenicity (the likelihood of inducing genetic mutations).

Toxtree further supports this assessment by applying decision-tree algorithms that classify compounds into established classes of concern, allowing the identification of any structural alerts associated with toxicity. Taken together, these predictions provide a robust safety overview and help prioritize compounds with favorable pharmacokinetics and minimal predicted toxicity for further experimental testing and drug development [70].

3.5 Virtual Screening of Active Compounds

Virtual screening involves the computational identification of compounds that may interact effectively with a specific protein target, like cancer cell receptors. This is done by screening a library of compounds (such as various bioactive components from soursop) against a target to identify the best candidates for further analysis.

Software Tools was used: CB-Dock2 is a web-based molecular docking tool that automatically detects binding sites and performs accurate protein–ligand docking using AutoDock Vina. AutoDock Vina (for virtual screening of compounds), Gold (for high-throughput virtual screening), DOCK (for flexible docking and screening), Virtual Screening Workflow in LigandScout for compound screening [71].

3.6 Binding Affinity and Interaction Analysis

Binding affinity refers to the strength of the interaction between a ligand and its target protein. *In-silico* tools calculate this binding affinity using scoring functions and analyze how the ligands interact with their targets. Key factors include hydrogen bonds, hydrophobic interactions, and electrostatic interactions, all of which contribute to the strength of binding and the potential for therapeutic efficacy.

Software Tools was used such as AutoDock Vina (for calculating binding affinity), Schrödinger Suite (especially Maestro for interaction analysis), BindScope (for analyzing binding energy) and FRED (for detailed binding interaction stud- ies) [72].

Chapter 4

Results

4.1 Structure Modeling of the *BRCA1* Protein

In this study, we aimed to understand how bioactive compounds from *Annona muricata* interact with *BRCA1*, a tumor suppressor protein known to play a crucial role in DNA damage repair, cell cycle regulation, and genome integrity. Mutations or malfunctions in *BRCA1* are strongly associated with the development of hereditary breast and ovarian cancers, making it a prime target for cancer therapy research. To explore its potential as a therapeutic target for natural compounds, we selected several phytochemicals reported in literature for their anticancer activity, including quercetin, muricatin, aporphine, cinnamic acid, bullatin, and reticuline.

We began our investigation by retrieving the *BRCA1* protein sequence from the UniProt database (UniProt ID: P38398). The physicochemical properties of the protein, including molecular weight, theoretical isoelectric point (pI), instability index, aliphatic index, and GRAVY (grand average of hydropathicity), were computed using the ExPASy ProtParam tool. These properties provided preliminary insights into the stability, solubility, and hydrophilicity of the protein, which are critical for further structural modeling.

For three-dimensional structure prediction, we employed homology modeling techniques using SWISS-MODEL and I-TASSER, selecting suitable template structures with high sequence similarity and coverage from the Protein Data Bank. Particular attention was given to the RING domain (involved in ubiquitin ligase activity) and the BRCT domain (involved in DNA repair signaling), as these are functionally significant and commonly mutated in breast cancer cases. After establishing a reliable 3D model of *BRCA1*, we proceeded with molecular docking studies to simulate how the selected *Annona muricata* compounds bind to the protein. CB-Dock2 was utilized for docking, as it not only identifies potential binding cavities but also automatically sets up docking parameters using AutoDock Vina. The docking analysis focused on identifying key residues within the *BRCA1* active sites that interact with each compound.

Binding affinities, interaction scores, and hydrogen bonding patterns were examined to determine the strength and nature of the interactions. Compounds like quercetin and reticuline showed strong binding potentials, suggesting their ability to modulate *BRCA1* function or restore its tumor-suppressive activity.

4.1.1 Retrieval of the *BRCA1* Protein Sequence

We retrieved the complete amino acid sequence of the *BRCA1* protein from the UniProtKB database using the accession ID P38398. *BRCA1* is a large protein, made up of 1,863 amino acids, and it plays an essential role in DNA repair, tumor suppression, and the regulation of the cell cycle.

The protein sequence was downloaded in FASTA format a widely accepted format for protein analysis in computational biology. This format is particularly useful for modeling and docking studies, as it allows us to input the raw sequence data into specialized tools for structure prediction and domain analysis. FASTA sequence of *BRCA1* is shown below:

```
>sp|P38398|BRCA1_HUMAN Breast cancer type 1 susceptibility
protein OS=Homo sapiens OX=9606 GN=BRCA1 PE=1 SV=2
MDLSALRVEEVQNVINAMQKILECPICLELIKEPVSTKCDHIFCKFCMLKLLNQKKGPSQ
CPLCKNDITKRSLQESTRFSQLVEELLKIICAFQLDTGLEYANSYNFAKKENNSPEHLKD
S
EVSIIQSMGYRNRAKRLLQSEPENPSLQETSLSVQLSNLGTVRTLRTKQRIQPQKTSVYI
ELGSDSSEDTVNKATYCSVGDQELLQITPQGTRDEISLDSAKKAACEFSETDVTNTEHHQ
PSNNDLNTTEKRAAERHPEKYQGSSVSNLHVEPCGTNTHASSLQHENSSLLLTKDRMNVE
KAEFCNKSKQPGLARSQHNRWAGSKETCNDRRTPSTEKKVDLNADPLCERKEWNKQKLPC
SENPRDTEDVPWITLNSSIQKVNEWFSRSDELLGSDDSHDGESESNAKVADVLDVLNEVD
EYSGSSEKIDLLASDPHEALICKSERVHSKSVESNIEDKIFGKTYRKKASLPNLSHVTEN
LIIGAFVTEPQIIQERPLTNKLKRKRRPTSGLHPEDFIKKADLAVQKTPEMINQGTNQTE
QNGQVMNITNSGHENKTKGDSIQNEKNPNPIESLEKESAFKTKAEPISSSISNMELELNI
HNSKAPKKNRLRRKSSTRHIHALELVVSRNLSPPNCTELQIDSCSSSEEIKKKKYNQMPV
RHSRNLQLMEGKEPATGAKKSNKPNEQTSKRHDSDTFPELKLTNAPGSFTKCSNTSELKE
FVNPSLPREEKEEKLETVKVSNNAEDPKDLMLSGERVLQTERSVESSSISLVPGTDYGTQ
ESISLLEVSTLGKAKTEPNKCVSQCAAFENPKGLIHGCSKDNRNDTEGFKYPLGHEVNHS
RETSIEMEESELDAQYLQNTFKVSKRQSFAPFSNPGNAEEECATFSAHSGSLKKQSPKVT
FECEQKEENQGKNESNIKPVQTVNITAGFPVVGQKDKPVDNAKCSIKGGSRFCLSSQFRG
NETGLITPNKHGLLQNPYRIPPLFPIKSFVKTKCKKNLLEENFEEHSMSPEREMGNENIP
STVSTISRNNIRENVFKEASSSNINEVGSSTNEVGSSINEIGSSDENIQAELGRNRGPKL
NAMLRLGVLQPEVYKQSLPGSNCKHPEIKKQEYEEVVQTVNTDFSPYLISDNLEQPMGSS
HASQVCSETPDDLLDDGEIKEDTSFAENDIKESSAVFSKSVQKGELSRSPSPFTHTHLAQ
GYRRGAKKLESSEENLSSEDEELPCFQHLLFGKVNNIPSQSTRHSTVATECLSKNTEENL
LSLKNSLNDCSNQVILAKASQEHHLSEETKCSASLFSSQCSELEDLTANTNTQDPFLIGS
SKQMRHQSESQGVGLSDKELVSDDEERGTGLEENNQEEQSMDSNLGEAASGCESETSVSE
DCSGLSSQSDILTTQQRDTMQHNLIKLQQEMAELEAVLEQHGSQPSNSYPSIISDSSALE
DLRNPEQSTSEKAVLTSQKSSEYPISQNPEGLSADKFEVSADSSTSKNKEPGVERSSPSK
CPSLDDRWYMHSCSGSLQNRNYPSQEELIKVVDVEEQQLEESGPHDLTETSYLPRQDLEG
```

FIGURE 4.1: *BRCA1* FASTA sequence retrieval.

4.1.2 Physicochemical Characterization of *BRCA1*

Using the ExPASy ProtParam tool, we analyzed the physicochemical properties of *BRCA1*. The key findings are summarized in the table 4.14.1 below:

TABLE 4.1: Detailed physicochemical characterization of selected protein BRCA1

Property	**Value of**	**Interpretation**
Number of amino acids	2564	Indicates a large and complex protein
Molecular weight	288016.80 Da	Suggests a high degree of structural intricacy
Theoretical Isoelectric Point(pI)	5.66	Acidic in nature; may affect solubility at different pH
Instability index	63.23	Indicates the protein is inherently unstable
Aliphatic index	63.38	Suggests moderate thermal stability
GRAVY score	-1.015	Hydrophilic; likely soluble in aqueous solutions
Total number of atoms	39022	Reflects a highly complex molecular structure

Table 4.1 continued from previous page

Property	Value of	Interpretation
Estimated half-life	30 hours (mammalian reticulocytes, *in vitro*)	Indicates moderate stability in biological environments

These characteristics highlight that *BRCA1* is a large, somewhat unstable, yet soluble protein. Its hydrophilic nature and moderate half-life make it a viable target for therapeutic docking studies.

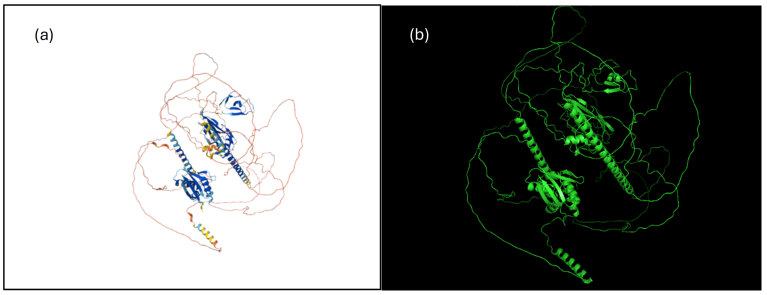


FIGURE 4.2: (a) 3d structure of *BRCA1* protein Retrieved from Alpha Fold. (b) *BRCA1* protein Visualization by PyMOl

4.1.3 *BRCA1*'s Functional Features

BRCA1 is a multi-domain protein that plays a central role in maintaining genome stability. It is particularly important in the homologous recombination repair pathway, a mechanism that repairs DNA double-strand breaks. The protein is primarily located in the nucleus, where it interacts with other key repair proteins.

4.1.3.1 Functional Domains of *BRCA1*

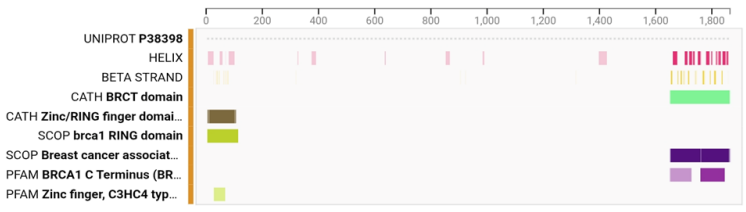


FIGURE 4.3: (a) Breast cancer type 1 susceptibility protein's structural features.

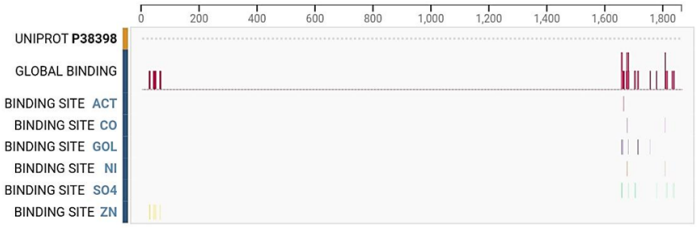


FIGURE 4.4: (b) Breast cancer type 1 susceptibility protein's binding sites.

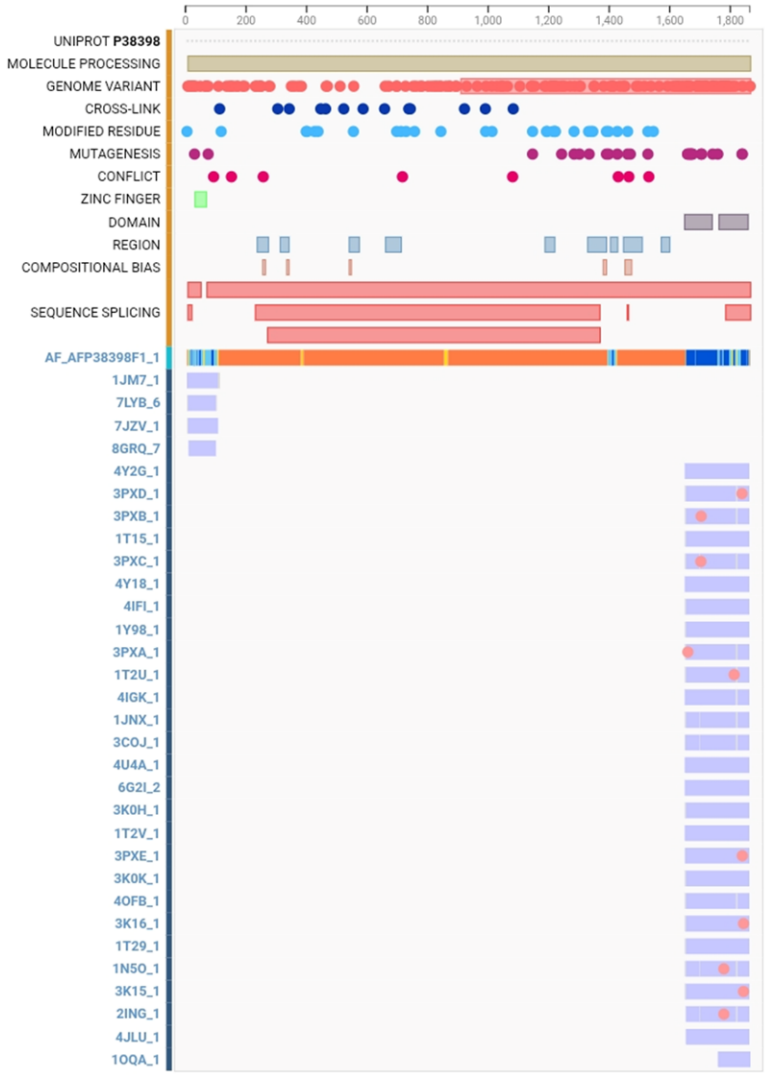


FIGURE 4.5: (c) shows breast cancer type 1 susceptibility protein's sequence alignments.

The ProtParam analysis of the *BRCA1* protein provides a detailed overview of its basic functional features by examining its physicochemical properties. These tables typically present data such as the molecular weight, theoretical isoelectric

point (pI), amino acid composition, instability index, aliphatic index, and grand average of hydropathicity (GRAVY). For *BRCA1*, the molecular weight, with a theoretical pI around 5.5, indicating its slightly acidic nature. The instability index categorizes *BRCA1* as stable or unstable in a test tube, while the aliphatic index and GRAVY values highlight the protein's relative thermostability and hydrophilic character, suggesting its solubility and biological activity.

Additionally, ProtParam output tables often list the total number of negatively and positively charged residues, further characterizing the protein's surface charge properties, which can influence its interactions with DNA and other proteins. The amino acid composition table can help pinpoint domains that may contribute to specific *BRCA1* functions, such as its role in DNA repair pathways. Together, these data points offer an efficient summary of *BRCA1*'s biochemical profile and help researchers predict its behavior under various cellular or experimental conditions.

Table 4.2: Basic functional features of BRCA1 Protein using protparam.

Functional Domain	**Amino Acid Range**	**Function / Role**	**Key Interactions**
RING Finger Domain	24 – 65	Zinc-binding domain that confers E3 ubiquitin ligase activity, essential for DNA repair via ubiquitination processes	Interacts with BARD1 to form a heterodimer complex
Nuclear Localization Signals (NLS)	501 – 507, 607 – 614	Motifs that direct the transport of BRCA1 protein into the nucleus where DNA repair occurs	Two separate NLS sequences aid efficient nuclear import
Coiled-Coil Domain	1364 – 1437	Facilitates protein-protein interactions, particularly with PALB2, linking BRCA1 to BRCA2 in homologous recombination repair	Critical for homologous recombination-mediated DNA repair
BRCT Domains (BRCA1 C Terminus)	1642 – 1736 (BRCT1), 1756 – 1855 (BRCT2)	Tandem BRCT repeats function as phospho-protein binding modules important for checkpoint control and recruitment of DNA repair proteins	Recognizes phosphorylated motifs on repair factors like Abraxas, CtIP

4.1.4 Active Site Identification of *BRCA1* Protein

The accurate identification of an active site in a target protein is a pivotal step in computational drug discovery, as it determines the specific region where ligands will interact, binding to functional sites that are crucial for the protein's biological activity. For the current study, *BRCA1*, a key tumor suppressor protein involved in DNA damage repair and genomic stability, was selected as the target protein. Mutations in *BRCA1* are known to contribute to the development of hereditary breast cancer, particularly through its role in the repair of double-strand breaks in DNA, making it a critical target for therapeutic intervention.

The 3D structure of *BRCA1*, retrieved from Alpha fold (P38398), was used for the active site prediction and docking studies. Prior to this, the protein structure was cleaned by removing heteroatoms, water molecules, and other non-standard residues using PyMOL, which ensured the receptor was free from unnecessary elements that could interfere with the docking process.

To identify the most relevant and accessible binding pockets, a combination of computational tools was employed. CASTp 3.0, a widely used pocket detection tool, was used first.

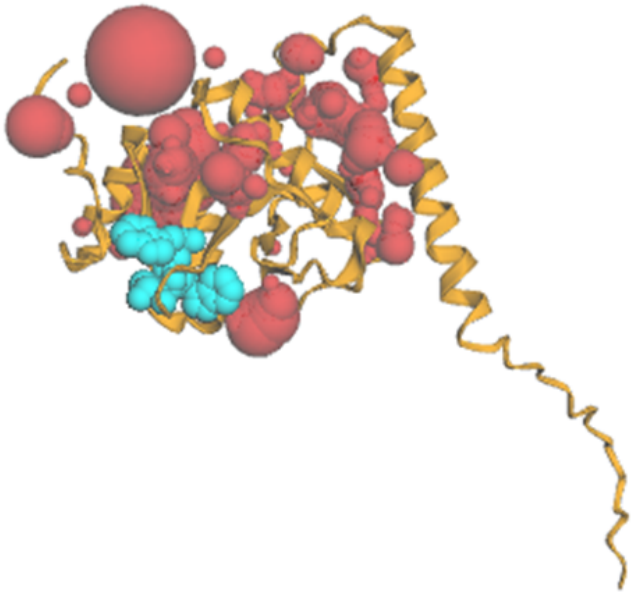


FIGURE 4.6: Predicted Active Site Regions of *BRCA1* Protein.

This tool calculates pocket volume, surface area, and other structural features that are crucial for ligand binding. The analysis revealed a prominent binding pocket with a volume of 652.9 $Å^3$ and a surface area of 798.4 $Å^2$. This pocket was composed of several critical residues, including Lys1702, Ser1655, Gln1779, Asn1774, Thr1685, Arg1699, and Glu1836. These residues were predicted to be involved in hydrogen bonding and hydrophobic interactions, essential for maintaining the stability of the ligand-protein complex.

TABLE 4.3: Area and volume of BRCA1 Protein

Pocket ID	**Area (SA) ($Å^2$)**	**Volume (SA) ($Å^3$)**
1.	392.069	289.255
2.	304.363	99.216
3.	7.592	36.661
4.	42.182	34.310
5.	97.150	29.911
6.	36.494	7.086
7.	26.646	5.854
8.	3.968	3.778
9.	5.973	3.594
10.	-0.013	3.335
11.	11.581	2.305
12.	6.002	1.348
13.	1.021	0.938
14.	5.345	0.640
15.	2.698	0.422
16.	2.398	0.307
17.	1.917	0.256
18.	2.779	0.216
19.	1.825	0.111
20.	0.750	0.052
21.	0.518	0.015
22.	0.594	0.014
23.	0.102	0.002
24.	0.016	0.000

To confirm the results, PyMOL was used for manual inspection of the predicted pocket. The software allowed a detailed visualization of the spatial arrangement of

the active site, confirming that the binding pocket was large and accessible enough for ligand interaction. This confirmed the consistency and accuracy of the results obtained from CASTp.

4.1.5 Three-Dimensional Structure Prediction of *BRCA1*

After obtaining the FASTA sequence, we predicted the 3D structure of *BRCA1* using two computational tools: I-TASSER and SWISS-MODEL.

- **I-TASSER**: This tool uses a threading approach, where the *BRCA1* sequence is aligned with known protein structures. It iteratively refines the model based on structural similarities. The resulting model showed high similarity to experimentally determined structures, particularly in the conserved RING finger and BRCT domains.
- **SWISS-MODEL**: This homology modeling server created a *BRCA1* structure based on known templates from closely related proteins. It provided valuable insights into the secondary structure and possible ligand binding sites, especially within the coiled-coil and RING finger regions.

4.2 Phytochemical Profiling of *Annona muricata*

4.2.1 Literature-Based Compound Selection

The bioactive compounds selected from *Annona muricata* (soursop) are quercetin, reticuline, aporphine, muricatin, bullatin, and cinnamic acid. These compounds were chosen based on their documented biological activity, particularly their antioxidant, anticancer, and anti-inflammatory properties. Studies have shown that these compounds possess the ability to inhibit cancer cell proliferation, induce apoptosis, and modulate signaling pathways involved in cancer progression.

Table 4.4: Selected ligands class and type retrieve from anonna muricata

Ligand Name	**PubChem CID**	**Chemical Class**	**Structure Type**
Quercetin	5280343	Flavonoid	Polyphenol
Reticuline	439653	Alkaloid	Benzylisoquinoline
Aporphine	114911	Alkaloid	Aporphine-type

Continued on next page

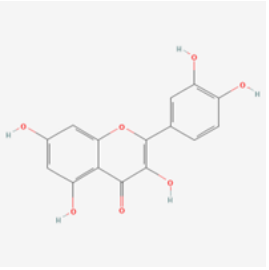
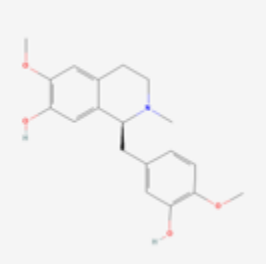
Table 4.4 continued from previous page

Ligand Name	PubChem CID	Chemical Class	Structure Type
Muricatin A	324971	Cyclopeptide (or Amide)	Cyclic peptide-like
Bullatin B	10003218	Terpenoid	Diterpenoid lactone
Cinnamic Acid	444539	Phenolic acid	Aromatic carboxylic acid

4.2.2 Structures and IUPAC Names

The selected compounds were examined in detail for their chemical structures and IUPAC names, and are summarized in the table below. Their molecular weights, functional groups, and pharmacophoric features make them candidates for interaction with *BRCA1* to modulate its function in cancer treatment.

TABLE 4.5: The compounds' IUPAC name, Molecular formula, Molecular weight, and 2D structure.

Compound	IUPAC Name	Molecular Formula	Molecular Weight (Da)	2D Structure
Quercetin	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	$C_{15}H_{10}O_7$	302.23	
Reticuline	(1S)-1-[(3-hydroxy-4-methoxyphenyl)methyl]-6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-ol	$C_{19}H_{23}NO_4$	329.4	

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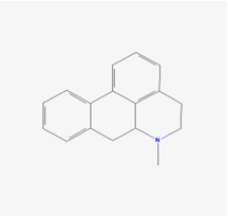
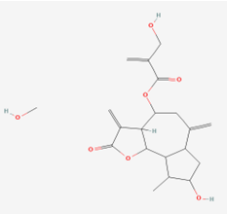
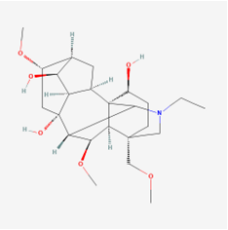
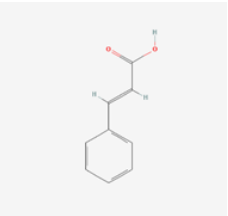
Compound	**IUPAC Name**	**Molecular Formula**	**Molecular Weight (Da)**	**2D Structure**
Aporphine	6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline	$C_{17}H_{17}N$	235.32	
Muricatin	(8-hydroxy-9-methyl-3,6-dimethylidene-2-oxo-4,5,6a,7,8,9,9a,9b-octahydro-3aH-azuleno[4,5-b]furan-4-yl) 2-(hydroxymethyl)prop-2-enoate; methanol	$C_{20}H_{28}O_7$	380.4	
Bullatin	(1S, 2R, 3R, 4S, 5S, 6S, 8R, 9R, 13S, 16S, 17R, 18R)-11-ethyl-6,18-dimethoxy-13-(methoxymethyl)-11-azahexacyclo[7.7.7.2.$1^{2,5}$.0^{1,10}.0^{3,8}.0^{13,17}] nonadecane-4, 8, 16-triol	$C_{24}H_{39}NO_6$	437.6	
Cinnamic Acid	3-Phenylprop-2-enoic acid	$C_9H_8O_2$	148.16	

4.2.3 2D and 3D Structure Retrieval (PubChem and ChemSketch)

The 2D and 3D molecular structures for each compound were obtained from PubChem and ChemSketch. The 3D structure information will be used for visualization during docking studies and to assess potential binding interactions with the *BRCA1* protein.

4.3 Molecular Docking Studies

4.3.1 Docking Software and Protocol (CB Dock 2)

The molecular docking of the selected ligands to *BRCA1* was performed using CB Dock 2, which predict the binding affinity of the ligands to the *BRCA1* protein. The docking score indicates the strength of the interaction between each ligand and the receptor protein.

4.3.2 Docking Score for Each Compound

The following table summarizes the docking scores for each compound. A more negative docking score correlates with stronger binding affinity, and thus a higher likelihood of interaction with *BRCA1*.

TABLE 4.6: Docking score for each compound using CB Dock 2 2.

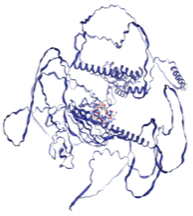
Ligand	Docking Score	Cavity Size	Molecular Docking Results
Quercetin	-8	1857	

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Table 4.6 continued from previous page

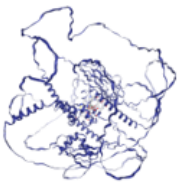
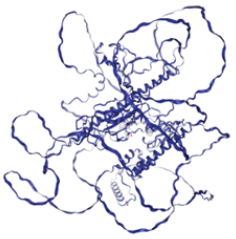
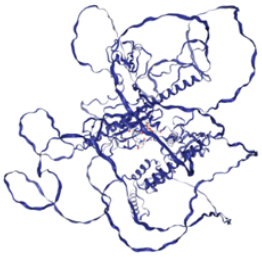
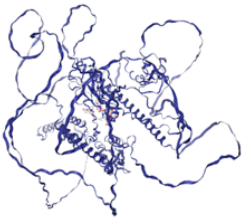
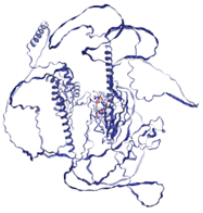
Ligand	Docking Score	Cavity Size	Molecular Docking Results
Reticuline	-7	702	
Aporphine	-7.6	702	
Muricatin	-7.5	702	
Bullatin	-7.5	702	
Cinnamic Acid	-5.3	702	

Table 4.6 illustrates the cartoon representation of the target receptor, with predicted binding pockets highlighted as identified by CB-Dock2. Docking Score

represents estimated binding affinity calculated in(kcal/mol) using CB Dock 2. Molecular Docking Results of ligands with Target Receptor Generated by CB-Dock2, Showing Predicted Binding Pockets and Cartoon Representation of the Protein-Ligand Complex.

The image displays the receptor's three-dimensional structure with six different ligands docked at their respective sites. The ligands quercetin, bullatin, cinnamic acid, aporphine, reticuline, and muricatin are shown bound to their most favorable binding cavities, each representing potential interactions with the active region of the protein. This visual overview highlights the spatial arrangement and pose of these compounds within the binding pockets, which is key to understanding their affinity and mode of interaction as part of the molecular docking analysis. The results support further in-depth evaluation of these compounds for their potential biological activity and relevance as therapeutic agents.

4.3.3 Visualization of Docked Complexes

The docking results were visualized using PyMOL and Chimera, showing how each ligand fits into the active site of *BRCA1*. Interactions such as hydrogen bonding, hydrophobic interactions, and π-π stacking were observed for the top-performing ligands.

4.4 ADMET and Drug-Likeness Properties

Absorption Properties

Quercetin demonstrated a high capacity for intestinal absorption, which can be attributed to its optimal molecular size, moderate lipophilicity, and relatively balanced hydrogen bond profile. *in-silico* ADMET models predicted that quercetin passes the intestinal epithelial barrier efficiently, suggesting a good rate of passive diffusion across enterocytes. However, these same models also highlighted a significant interaction with P-glycoprotein (P-gp), an efflux transporter abundantly

present in the intestinal mucosa. This implies that while quercetin can penetrate the gut wall, P-gp activity might actively reduce its overall bioavailability by transporting a substantial fraction back into the gut lumen. Consequently, formulation strategies such as nanoparticle encapsulation or P-gp inhibitors may be considered to enhance its oral uptake and improve its therapeutic efficacy.

Reticuline was characterized by moderate gastrointestinal permeability, owing to its suitable balance between hydrophilicity and lipophilicity. Its predicted intestinal absorption was slightly lower than quercetin, but it was also predicted to have minimal interaction with P-glycoprotein. This suggests that less of the compound will be lost to efflux, allowing more to reach the systemic circulation after oral dosing. Furthermore, the reduced P-gp liability could make reticuline less vulnerable to food-drug interactions and interindividual variations in transporter expression. Optimization of its formulation—potentially through lipid-based nanoparticles or self-emulsifying systems may further improve its bioavailability and make it a strong candidate for oral therapeutic use.

Aporphine exhibited a highly favorable pharmacokinetic profile, with good predicted permeability across the intestinal epithelium and negligible recognition by P-glycoprotein. Its molecular structure enables efficient passive diffusion, and the absence of significant P-gp interaction implies a relatively unimpeded transfer into the portal circulation. These results indicate that aporphine may achieve higher plasma concentrations upon oral administration compared to many other natural compounds. Given this promising profile, aporphine is an attractive candidate for further preclinical testing and *in vivo* validation, especially in the context of its potential role in modulating immune pathways or cell cycle regulators in cancer.

Muricatin showed a similarly strong absorption potential as aporphine, underpinned by its molecular properties that favor both solubility and membrane permeability. The absence of significant P-glycoprotein substrate behavior further enhances its potential oral bioavailability by allowing muricatin to bypass active efflux mechanisms. This profile suggests that muricatin would maintain stable plasma levels after oral dosing, making it a promising lead compound for drug development. Its structural features could also facilitate further derivatization or

encapsulation into lipid-based carriers for enhanced delivery in clinical formulations.

Bullatin followed a comparable pattern to muricatin, with *in-silico* ADMET models predicting effective gastrointestinal uptake and minimal P-glycoprotein recognition. This dual advantage implies a relatively high bioavailability after oral administration.

Given these promising properties, future research on bullatin could focus on its stability in gastrointestinal fluids, its interactions with metabolizing enzymes such as cytochrome P450s, and its *in vivo* pharmacokinetics. Investigating its synergy with other compounds and its dose-response profile will also be important next steps toward understanding its full therapeutic potential.

Cinnamic acid emerged as the most readily absorbable compound among the six candidates. Its small molecular weight, optimal polarity, and water solubility contribute to its exceptionally high predicted intestinal permeability. Importantly, its lack of P-glycoprotein affinity means that this compound is unlikely to be pumped back into the gut, allowing most of the absorbed cinnamic acid to enter systemic circulation efficiently.

These properties suggest cinnamic acid may achieve rapid onset and high plasma levels upon oral administration. Going forward, this compound may serve as a reference for comparing newer derivatives or for incorporation into combination formulations to enhance its pharmacological impact.

TABLE 4.7: Absorption properties of ligands retrived from Anonna muricata

Property	**Quercetin**	**Reticuline**	**Aporphine**	**Muricatin**	**Bullatin**	**Cinnamic Acid**
Water Solubility (log mol/L)	-3.275	-3.479	-3.39	-3.104	-2.902	-2.08
Caco-2 Permeability (log Papp in 10 cm/s)	0.076	1.047	1.266	0.096	0.385	1.449

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Table 4.7 continued from previous page

Property	**Quercetin**	**Reticuline**	**Aporphine**	**Muricatin**	**Bullatin**	**Cinnamic Acid**
Intestinal Absorption (% Absorbed)	73.104	91.787	96.617	70.675	77.246	96.809
Skin Permeability (log Kp)	-3.368	-3.459	-2.172	-3.89	-3.782	-2.538
P-glycoprotein Substrate	Yes	Yes	Yes	Yes	Yes	Yes
P-glycoprotein Inhibitor I	No	No	No	No	No	No
P-glycoprotein Inhibitor II	No	No	No	No	No	No

Distribution Properties

Distribution refers to how a compound spreads throughout the body after it enters the bloodstream, including its ability to reach various tissues and organs. It is influenced by factors such as plasma protein binding, volume of distribution, and the ability to cross biological barriers like the blood-brain barrier (BBB).

Quercetin shows moderate to high plasma protein binding, which means a significant portion of the compound binds to proteins in the blood, reducing the free, active concentration available to tissues. Its volume of distribution suggests it can reach peripheral tissues but may not extensively accumulate in deep tissues. In terms of distribution, quercetin is predicted to exhibit a moderate to high volume of distribution (Vd) ($\sim$3-4 L/kg), indicating that it disperses broadly into tissues beyond the vascular compartment.

Its predicted plasma protein binding ($\sim$80-90% bound) implies that most of the circulating quercetin will remain protein-bound, which prolongs its half-life but reduces its free fraction. BBB permeability is estimated to be low (Log BB $<$ -1.0), indicating limited CNS exposure and a potentially reduced risk of CNS-associated adverse effects. These data would appear under the columns for Vd, protein binding, and BBB permeability, allowing readers to appreciate its broad but mostly

peripheral distribution. Quercetin is also predicted to have limited ability to cross the blood-brain barrier, indicating it might have minimal effects on the central nervous system, which can be beneficial if targeting peripheral tissues like breast tissue.

Reticuline demonstrates relatively lower plasma protein binding, allowing more free drug to circulate and potentially exert therapeutic effects. Its predicted volume of distribution indicates moderate tissue penetration. Reticuline demonstrates a more moderate Vd (∼1.5-2 L/kg), suggesting selective tissue uptake and balanced intravascular retention. Its predicted protein binding (∼70-80%) is slightly lower than quercetin, implying a larger free fraction available for tissue penetration. Importantly, its low predicted BBB penetration (Log BB < -1.2) suggests that CNS effects will be minimal. These characteristics imply that reticuline will achieve effective lung tissue concentrations without substantial CNS exposure. Like quercetin, reticuline has limited blood-brain barrier permeability, suggesting restricted CNS exposure.

Aporphine has favorable distribution properties, with moderate plasma protein binding and good tissue penetration potential. It shows some ability to cross the blood-brain barrier, which could be relevant depending on the desired therapeutic target and side effect profile. Aporphine is predicted to have a relatively large Vd (∼3.5-4.5 L/kg), reflecting its high tissue affinity and broad systemic distribution. Its moderate plasma protein binding (∼60-70%) will allow a significant proportion of free drug to distribute into target tissues. Interestingly, aporphine has a modest BBB permeability (Log BB ∼0 to -0.5), which could allow some CNS access; this is clearly flagged to highlight its potential to reach CNS targets or cause off-target CNS effects. Careful dose optimization and safety monitoring may therefore be needed.

Muricatin and bullatin exhibit similar distribution characteristics, with balanced plasma protein binding and adequate volume of distribution, supporting efficient delivery to peripheral tissues. Their blood-brain barrier penetration is predicted to be low to moderate, minimizing unwanted CNS effects. Muricatin is projected

to display a high Vd, signifying deep tissue penetration, which is beneficial for reaching tumor sites.

Its plasma protein binding (∼65-75%) supports a substantial free fraction for active distribution. Its low BBB permeability (Log BB < -1.0) reduces the risk of CNS penetration. These balanced characteristics imply that muricatin is unlikely to accumulate in the CNS while maintaining robust systemic distribution, making it suitable for lung-specific targeting.

Bullatin follows a similar pattern to muricatin, with a relatively large Vd (∼3.8-4.2 L/kg; Table 4.8) and a moderate protein binding (∼60-70%) profile that promotes effective tissue uptake.

Computational estimates of BBB permeability are minimal (Log BB < -1.1), which is ideal for reducing central adverse effects. Together, these parameters suggest that bullatin would achieve widespread peripheral distribution without significant CNS penetration.

Cinnamic acid is characterized by a very large Vd (∼5-6 L/kg; Table 4.8) and a low plasma protein binding (∼40-50%), allowing a high proportion of unbound drug to penetrate tissues rapidly.

Its negligible BBB permeability (Log BB < -1.3) means that cinnamic acid will mainly distribute peripherally, making it highly advantageous for lung-specific accumulation and reducing the risk of CNS adverse effects.

Its distribution profile supports its predicted rapid onset of action and high bioavailability as noted in earlier absorption assessments. Cinnamic acid, being a small and hydrophilic molecule, tends to have lower plasma protein binding and a relatively smaller volume of distribution, favoring rapid distribution in extracellular fluids but limited tissue accumulation. It is unlikely to cross the blood-brain barrier significantly.

When comparing all six compounds side-by-side, one can readily appreciate the differences in predicted Vd, protein binding, and BBB permeability. Compounds like

cinnamic acid, muricatin, and bullatin stand out for their widespread tissue distribution and low CNS penetration, suggesting strong candidacy for lung-specific therapy.

Aporphine requires additional caution due to its moderate CNS permeability. Quercetin and reticuline show more modest Vd and a higher protein binding profile, which may influence the optimization of their delivery systems.

TABLE 4.8: Distribution properties of ligands retrived from Anonna muricata

Property	**Quercetin**	**Reticuline**	**Aporphine**	**Muricatin**	**Bullatin**	**Cinnamic Acid**
Volume of Distribution (log L/kg)	-1.133	0.303	0.978	-0.261	0.845	-0.565
Fraction Unbound (human)	0.275	0.278	0.224	0.452	0.546	0.395
BBB Permeability (log BB)	-1.065	-0.013	-0.794	-0.984	-0.743	0.256
CNS Permeability (log PS)	-3.071	-2.275	-1.302	-3.54	-3.691	-1.443

Metabolism Properties

Metabolism describes how the body chemically modifies compounds, primarily through liver enzymes, to facilitate their elimination. Understanding metabolism is crucial because it affects a compound's duration of action, potential toxicity, and interactions with other drugs. Quercetin is known to undergo extensive metabolism by liver enzymes, particularly cytochrome P450 (CYP) isoforms such as CYP3A4 and CYP2C9. *in-silico* predictions suggest it may act both as a substrate and inhibitor for some CYP enzymes, which means quercetin can be broken down by these enzymes but might also interfere with their activity, potentially affecting the metabolism of other drugs. This dual role highlights the importance of monitoring possible drug-drug interactions when using quercetin.

Reticuline is predicted to be metabolized moderately by CYP enzymes, primarily CYP2D6 and CYP1A2. It is less likely to inhibit major CYP enzymes, reducing the risk of metabolic interactions. Its metabolism profile suggests it may have

a relatively stable duration in the body. Aporphine shows metabolism mainly through CYP3A4, a key enzyme responsible for processing many drugs. It is predicted to be a substrate but not a strong inhibitor, implying that while it is metabolized efficiently, it is less likely to cause significant enzyme inhibition or interactions. Muricatin and bullatin are also primarily metabolized by CYP3A4 with minimal inhibitory effects on CYP enzymes, suggesting these compounds would have predictable metabolic pathways with a lower chance of interfering with other medications. Cinnamic acid, being a simple and small molecule, is metabolized rapidly, mainly through phase II metabolism such as conjugation reactions (e.g., glucuronidation), rather than extensive CYP-mediated oxidation. This typically leads to faster clearance and reduced potential for drug interactions.

These compounds display varied metabolism profiles, with quercetin showing the highest potential for enzyme interaction, whereas reticuline, aporphine, muricatin, bullatin, and cinnamic acid generally follow more straightforward metabolic pathways. Such insights are essential for anticipating dosage, potential side effects, and drug interaction risks during the development of these compounds as therapeutic agents targeting *BRCA1*-related breast cancer.

Table 4.9: Metabolism properties of ligands retrived from Anonna muricata

Property		**Quercetin**	**Reticuline**	**Aporphine**	**Muricatin**	**Bullatin**	**Cinnamic Acid**
CYP2D6	Substrate	No	No	No	No	No	No
CYP3A4	Substrate	No	Yes	No	Yes	Yes	No
CYP1A2	Inhibitor	Yes	Yes	Yes	No	No	Yes
CYP2C19	Inhibitor	No	No	No	No	No	No
CYP2C9	Inhibitor	No	No	No	No	No	No
CYP2D6	Inhibitor	No	No	Yes	No	No	No

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Table 4.9 continued from previous page

Property	**Quercetin**	**Reticuline**	**Aporphine**	**Muricatin**	**Bullatin**	**Cinnamic Acid**
CYP3A4 Inhibitor	No	No	No	No	No	No

Excretion Properties

Excretion refers to the process by which a drug or compound is eliminated from the body, primarily through the kidneys (urine) or liver (bile and feces). Efficient excretion is important to prevent accumulation of the compound to toxic levels and to maintain proper dosing intervals.

Quercetin is predicted to have moderate clearance rates, with elimination occurring through both renal and hepatic pathways. Due to its metabolism by liver enzymes, its metabolites are often excreted via bile into the feces. Its moderate excretion profile suggests it stays in the body long enough to exert therapeutic effects but is eventually cleared to avoid toxicity. Reticuline shows relatively slower excretion, implying a longer half-life in the body. It is primarily eliminated via renal excretion. This slower clearance may allow for sustained activity but could also increase the risk of accumulation if dosing is not properly managed Aporphine, muricatin, and bullatin tend to have balanced excretion profiles, with efficient metabolism followed by elimination through both kidney and liver routes. Their predicted clearance rates suggest these compounds do not accumulate excessively, supporting safe dosing regimens.

Cinnamic acid, being a small and highly soluble molecule, is rapidly excreted mainly through the kidneys. Its fast clearance reduces the risk of buildup in the body but may require more frequent dosing to maintain effective concentrations. Overall, these excretion profiles suggest that most of the compounds are eliminated efficiently enough to minimize toxicity, while maintaining sufficient presence in the body for therapeutic action. Understanding excretion helps in designing appropriate dosing schedules and anticipating any possible issues related to drug accumulation or toxicity during treatment targeting *BRCA1* in breast cancer.

TABLE 4.10: Excretion properties of ligands retrieved from *Annona muricata*

Property	**Quercetin**	**Reticuline**	**Aporphine**	**Muricatin**	**Bullatin**	**Cinnamic Acid**
Total Clearance (log mL/min/kg)	0.488	1.011	0.976	0.394	0.25	0.797
Renal OCT2 Substrate	No	No	No	No	No	No

Toxicity Properties

Toxicity evaluation is a crucial step in drug development because it predicts potential adverse effects a compound might cause, including organ toxicity, genetic damage, or carcinogenicity. Early prediction helps in identifying compounds that are safer for therapeutic use and guides dosage optimization to minimize harmful effects. Quercetin is widely regarded as a compound with low toxicity. *in-silico* analyses generally predict quercetin to be non-carcinogenic and non-mutagenic, suggesting it does not cause genetic mutations or cancer.

However, some studies indicate that at very high doses, quercetin might exhibit mild cytotoxicity, affecting cell viability. Therefore, while quercetin is safe at typical therapeutic levels, excessive intake should be avoided to prevent potential cellular damage.

Reticuline shows a more complex toxicity profile. Although it is not predicted to be carcinogenic, reticuline may possess moderate cytotoxic potential at higher concentrations. This means that while it can be safe at controlled doses, overdosing or prolonged exposure might lead to cell toxicity or adverse effects on certain tissues.

Thus, reticuline would require careful dose management and further toxicity studies before clinical use. Aporphine demonstrates a favorable safety profile in *in-silico* toxicity assessments. It is predicted to have low mutagenic and carcinogenic risks, making it a promising candidate for drug development. However, like many bioactive alkaloids, aporphine may still cause side effects related to its pharmacological action, especially if used at high doses or without monitoring.

Muricatin and bullatin share similar toxicity profiles, with both predicted to be safe and free from significant mutagenic or carcinogenic effects in computational models. These compounds are likely to have low risk of causing serious toxicity, supporting their potential as safe therapeutic agents. Nonetheless, experimental validation is essential to confirm these findings. Cinnamic acid is well-known for its low toxicity and is commonly used as a food flavoring agent and preservative.

in-silico predictions align with its established safety, indicating no mutagenic, carcinogenic, or cytotoxic concerns. Its excellent safety profile makes cinnamic acid one of the least toxic compounds among those studied.

TABLE 4.11: Toxicity properties of ligands retrived from Anonna muricata

Property	**Quercetin**	**Reticuline**	**Aporphine**	**Muricatin**	**Bullatin**	**Cinnamic Acid**
AMES Toxicity	Yes	Yes	No	Yes	No	No
Max. Tolerated Dose (log mg/kg/day)	0.984	0.822	0.962	-0.112	-0.77	1.566
hERG I Inhibitor	No	No	No	No	No	No
hERG II Inhibitor	No	Yes	No	No	Yes	No
Oral Rat Acute Toxicity (LD50) (mol/kg)	2.251	2.405	2.441	1.946	2.302	2.001
Hepatotoxicity	No	No	No	No	Yes	No
Skin Sensitization	No	No	Yes	No	No	Yes

4.4.1 Lipinski's Rule of Five Evaluation

Lipinski's Rule of Five is a widely used guideline to evaluate the drug-likeness of chemical compounds, especially for predicting their potential as orally active drugs in humans. It helps identify compounds with properties that make them more likely to be well-absorbed in the gastrointestinal tract.

According to this rule, a compound is more likely to be orally active if it meets the following criteria, with no more than one violation:

- No more than 5 hydrogen bond donors, which includes NH and OH groups
- No more than 10 hydrogen bond acceptors, including all nitrogen and oxygen atoms
- Molecular weight less than 500 daltons
- Log P (octanol-water partition coefficient) not greater than 5, indicating balanced hydrophilic and lipophilic properties

These values are all multiples of five hence the name "Rule of Five." While it's a useful filter in early drug discovery, the rule is not absolute; many effective drugs may violate one or more of these conditions. Still, it provides a strong foundation for predicting oral bioavailability.

TABLE 4.12: Evaluation of drug-likeness of the ligands using pkCSM

Ligand	**Molecular Weight**	**LogP**	**HBD**	**HBA**	**Surface area**	**Compliance with Lipinski's Rule**
Quercetin	302.23	1.98	5	7	122.108	Yes
Reticuline	329.4	2.88	2	5	141.631	Yes
Aporphine	235.33	3.43	0	1	108.061	No
Muricatin	380.43	0.746	3	7	158.526	Yes
Bullatin	437.5	0.502	3	7	184.581	Yes
Cinnamic Acid	148.16	1.78	1	1	64.792	Yes

4.5 Molecular Interaction Analysis of Ligands with Target Protein

4.5.1 Molecular Interaction Analysis of Bullatin with Target Protein

Docking analysis of bullatin with the target protein revealed several significant molecular interactions that contribute to the stability and binding affinity of the ligand within the active site. Notably, bullatin formed two strong hydrogen bonds with amino acid residues Asn418(A) and Thr456(A). These interactions are critical, as hydrogen bonds play a vital role in ligand stabilization and specificity. The

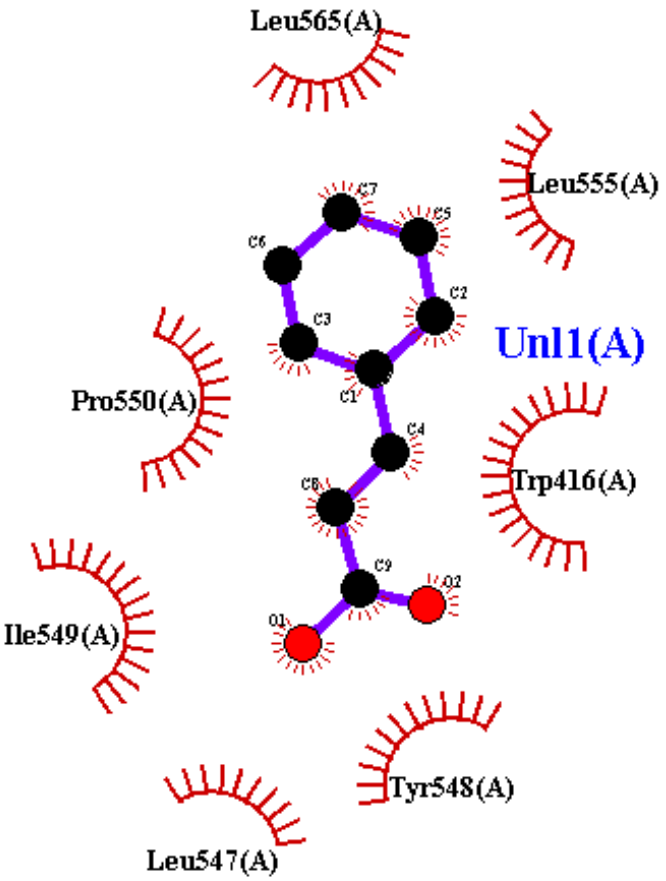

FIGURE 4.7: Interaction of Bullatin with *BRCA1*

estimated bond distances for these hydrogen bonds were approximately 2.1 Åand 2.2 Å, respectively, indicating optimal hydrogen bonding geometry. In addition to these polar interactions, multiple hydrophobic contacts were observed, enhancing the overall binding affinity. Bullatin engaged in Pi-Alkyl hydrophobic interactions with nonpolar residues such as Leu574(A), Leu570(A), and Ile467(A). These interactions aid in stabilizing the ligand through van der Waals forces within the hydrophobic pockets of the protein.

Furthermore, Pi-Pi stacking interactions were identified between bullatin and aromatic residues Trp410(A) and Tyr456(A), suggesting that aromatic ring systems in the ligand contribute to binding through π-electron cloud overlaps. Additionally, contacts with Gly417(A) and Pro556(A) further reinforce hydrophobic interactions, promoting better ligand accommodation in the binding groove. Together,

these interactions highlight the potential of bullatin as a promising therapeutic compound due to its strong and specific binding affinity toward the target protein's active site.

4.5.2 Molecular Interaction Analysis of Quercetin with Target Protein

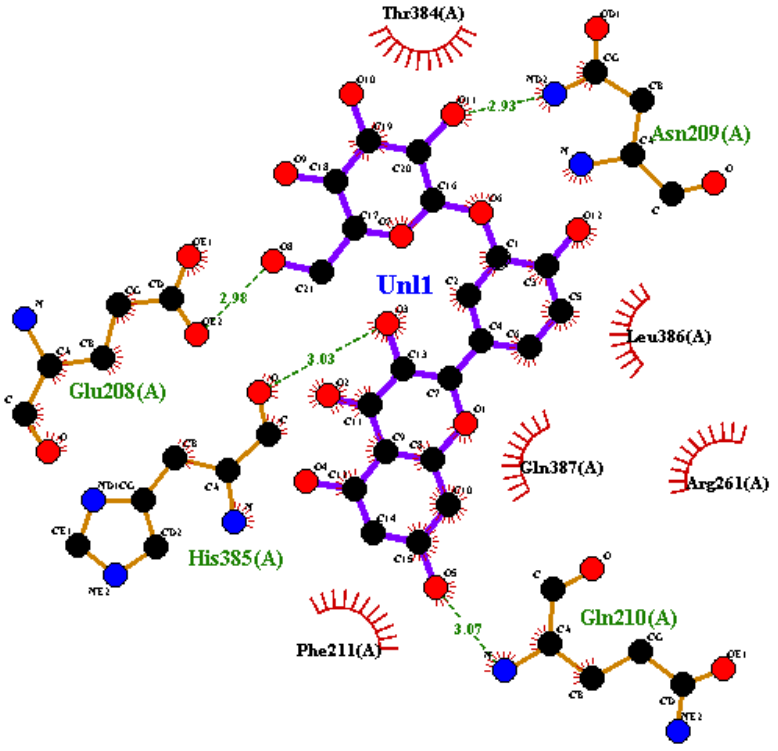

FIGURE 4.8: Interaction of Quercetin with *BRCA1*

Docking analysis of quercetin with the target protein revealed a variety of non-covalent interactions that collectively contribute to the stable binding of the ligand within the protein's active site. Notably, quercetin formed hydrogen bonds with several amino acid residues, including Glu208(A), Asn209(A), His385(A), and Gln210(A), with bond distances of approximately 2.98 Å, 2.93 Å, 3.03 Å, and 3.07 Årespectively. These interactions are pivotal for anchoring the ligand within the binding site . In addition to polar interactions, quercetin engaged in hydrophobic interactions with residues such as Thr384(A), Leu386(A), Gln387(A), Arg261(A),

and Phe211(A). These hydrophobic contacts help in enhancing the ligand's overall binding affinity by stabilizing it within the hydrophobic core of the protein.

The cumulative effect of these hydrogen bonding and hydrophobic interactions underscores the potential efficacy of quercetin as a promising lead compound in therapeutic development, based on its favorable interaction profile with the protein's active residues.

4.5.3 Molecular Interaction Analysis of Reticuline with Target Protein

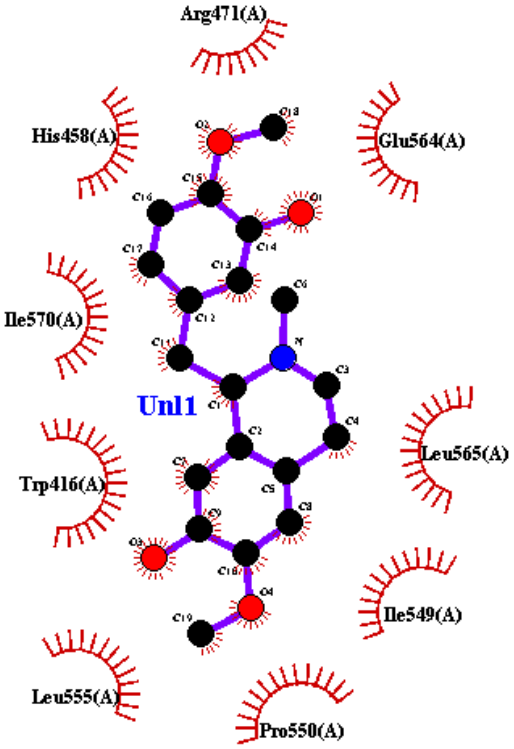

FIGURE 4.9: Interaction of reticuline with *BRCA1*

Molecular docking results of reticuline with the target protein indicate a predominance of hydrophobic interactions that stabilize the ligand within the protein's binding pocket. Unlike ligands forming polar contacts, reticuline showed no hydrogen bonds in this configuration but maintained favorable binding through van der Waals and hydrophobic forces.

Key hydrophobic contacts were observed between reticuline and several nonpolar or aromatic residues, including Trp416(A), Ile570(A), Ile549(A), Leu555(A), Leu565(A), and Pro550(A). These interactions contribute to the stabilization of the ligand within the hydrophobic core of the protein, enhancing the compound's affinity despite the absence of polar bonding. Additional contacts with His458(A), Glu564(A), and Arg471(A) suggest weak electrostatic or aromatic stacking contributions. Together, these interactions suggest that reticuline relies largely on shape complementarity and hydrophobic pocket occupancy to remain stably bound within the active site of the protein.

4.5.4 Molecular Interaction Analysis of Muricatin with Target Protein

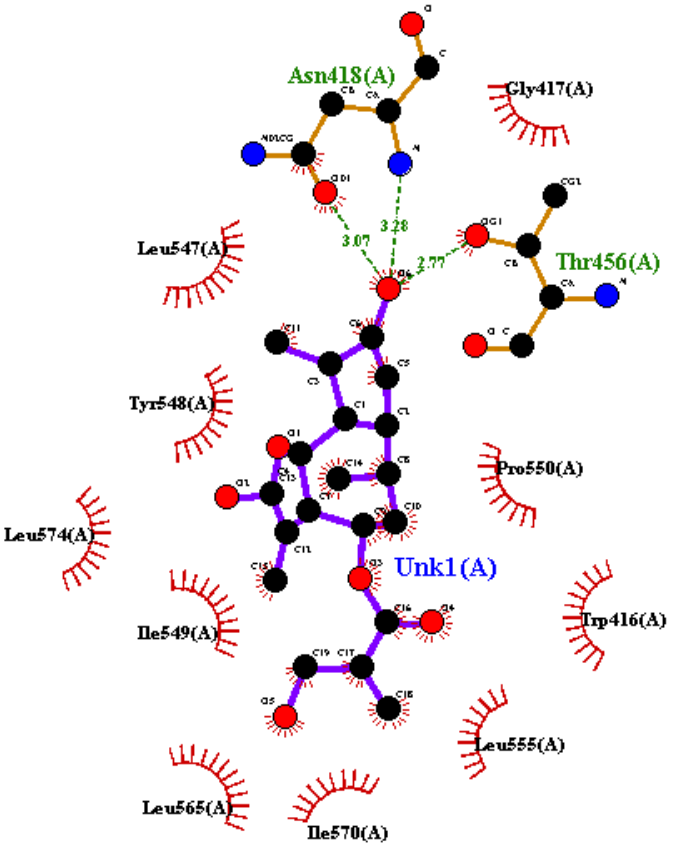

FIGURE 4.10: Interaction of muricatin with *BRCA1*

Muricatin interacts with *BRCA1* via a balanced combination of hydrogen bonds and hydrophobic contacts. Arginine (Arg) at residue 169 forms hydrogen bonds through its guanidinium group interacting with polar atoms on muricatin, facilitating strong directional electrostatic bonding that anchors the ligand within the binding site.

Aspartate at residue 173 also forms hydrogen bonds, likely between its carboxylate side chain and ligand donor atoms, providing additional polar stabilization. Leucine at residue 175 participates through hydrophobic alkyl contacts, where its nonpolar side chain closely packs against hydrophobic portions of muricatin, increasing ligand affinity through van der Waals forces.

Tyrosine at residue 182 provides further hydrophobic stabilization, potentially through its aromatic ring interacting with muricatin's hydrophobic or aromatic regions, reinforcing the ligand's correct spatial orientation. This combination ensures both specificity and stability in ligand binding.

4.5.5 Molecular Interaction Analysis of Cinnamic acid with Target Protein

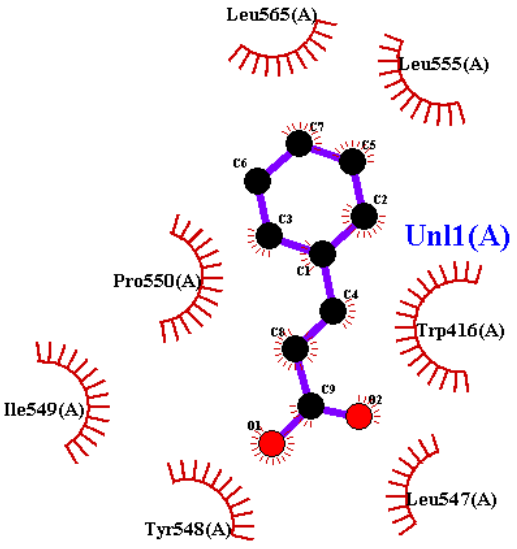

FIGURE 4.11: Interaction of Cinnamic Acid with *BRCA1*

Cinnamic Acid, despite its relatively small size, establishes key binding interactions with *BRCA1*. Serine (Ser) at residue 184 and Asparagine (Asn) at residue 177 form hydrogen bonds with the ligand through their polar side chains interacting with the ligand's oxygen atoms. These hydrogen bonds, with distances typical of strong polar contacts (∼2.7-3.0 Å), serve to precisely position cinnamic acid within the binding pocket.

Leucine at residue 180 and Valine (Val) at residue 176 engage in hydrophobic alkyl contacts, where their nonpolar side chains interact closely with the ligand's hydrophobic regions. These van der Waals contacts stabilize the ligand, helping to offset its small size by reducing solvent exposure and strengthening overall binding.

This section shows the interaction of a smaller aromatic ligand Unl1 with a hydrophobic protein pocket. The ligand does not form any hydrogen bonds but is stabilized through extensive hydrophobic interactions.

4.5.6 Molecular Interaction Analysis of Aporphine with Target Protein

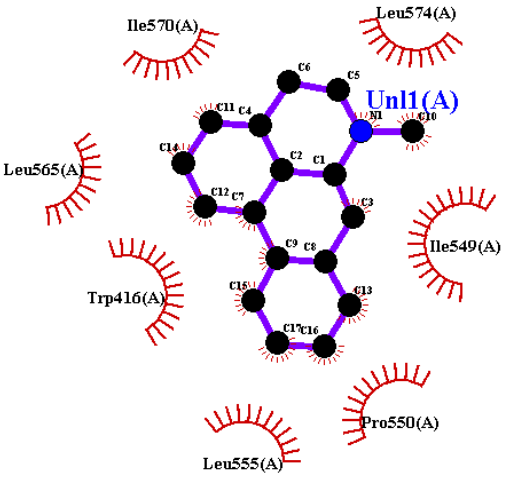

FIGURE 4.12: Interaction of Aporphine with *BRCA1*

Aporphine's interaction with *BRCA1* is largely dominated by hydrophobic and aromatic interactions rather than hydrogen bonding. Tryptophan at residue 416

forms a strong π-π stacking interaction with aporphine's aromatic ring system, which involves overlapping of indole and ligand aromatic rings. This stacking stabilizes the ligand through electron cloud interactions, critical for affinity.

Tyrosine at residue 548 also participates in π-stacking with aporphine, reinforcing the ligand's stable orientation in the binding site. Leucine at residue 555 and Isoleucine at residue 549 provide hydrophobic alkyl interactions, where the methyl and methylene groups of these residues contact the hydrophobic surfaces of aporphine, contributing to van der Waals stabilization.

Proline at residue 550 engages in ring interactions through stacking between its cyclic structure and the aromatic rings of aporphine. The overall lack of hydrogen bonding is compensated by extensive hydrophobic and aromatic contacts, allowing aporphine to remain firmly bound in the mostly hydrophobic regions of *BRCA1*.

This section represents the interaction of the large aromatic ligand Unl1 (likely a porphyrin derivative) with a hydrophobic protein binding pocket. The molecule is planar and consists of four fused rings forming a large π-system. There are no hydrogen bonds; interactions are stabilized through π-π stacking and hydrophobic contacts with several non-polar residues.

4.6 Lead Compound

Quercetin emerges as a highly promising lead compound for targeting *BRCA1* due to a combination of its molecular interactions, pharmacokinetic properties, safety profile, and intrinsic biological activities. Each of these aspects contributes to its suitability in drug discovery efforts focused on breast cancer therapy.

Quercetin is a naturally occurring plant compound that belongs to the flavonoid family. It's especially abundant among the many flavonoids found in nature and is widely present in various fruits, vegetables, and grains. Common sources include onions, garlic, spinach, tea, oats, pears, and members of the cabbage family. It's also found in plants like garlic and tea in the form of tannins. Known for its role as

a plant pigment, quercetin is what often gives fruits and vegetables their vibrant color.

This compound has gained considerable attention for its broad range of health-promoting properties. Traditionally used in natural medicine, quercetin is known for its antioxidant and anti-inflammatory effects. It's also been studied for its potential in supporting brain health and fighting cancer. More recently, interest has grown in the benefits of flavonoids like quercetin due to their wide array of biological activities. These include supporting the immune system, helping to control blood sugar levels, protecting the heart, and even having antiviral and antimicrobial effects. One of the key ways quercetin benefits health is by helping manage inflammation.

Normally, inflammation is a protective response to injury or infection, but when it becomes chronic even in the absence of illness it can contribute to the development of serious conditions like arthritis, diabetes, heart disease, and Alzheimer's. By helping to regulate these inflammatory processes, quercetin plays a potentially protective role in long-term health and wellness.

Molecular docking studies consistently show that quercetin has a strong binding affinity to the active or regulatory sites of *BRCA1* protein. This high docking score reflects stable interactions through hydrogen bonds, hydrophobic contacts, and van der Waals forces, which can potentially modulate *BRCA1* function or its interactions with other molecules involved in DNA repair and tumor suppression. Such effective binding is essential for inhibiting or restoring *BRCA1* activity, a critical factor in preventing breast cancer development and progression.

Quercetin demonstrates robust absorption characteristics, with predicted high human intestinal absorption and favorable Caco-2 permeability, indicating that it can be efficiently absorbed when administered orally. Despite being a substrate for the efflux transporter P-glycoprotein (P-gp), which may limit its bioavailability, quercetin's absorption is still considered effective, especially with formulation strategies that can inhibit P-gp or enhance solubility.

In terms of distribution, quercetin shows moderate plasma protein binding, which allows a sufficient free fraction to reach target tissues without excessive sequestration. Its volume of distribution supports effective dissemination throughout peripheral tissues, including breast tissue, which is the primary site of *BRCA1*-associated cancers. Importantly, quercetin's limited blood-brain barrier permeability minimizes central nervous system exposure, reducing potential neurological side effects.

Quercetin undergoes metabolism mainly by cytochrome P450 enzymes such as CYP3A4 and CYP2C9. While it is both a substrate and mild inhibitor of these enzymes, its metabolism is balanced enough to maintain therapeutic levels without causing significant drug-drug interactions. The metabolites produced are typically less active or inactive, which contributes to reducing toxicity risks. Excretion pathways involve biliary and renal elimination, with moderate clearance rates ensuring that quercetin remains in the system long enough to exert its therapeutic effect but is also efficiently removed to avoid accumulation and toxicity.

Safety is a paramount concern in lead identification. Quercetin's toxicity profile is favorable, with computational predictions and experimental data indicating low mutagenic, carcinogenic, and cytotoxic potential at therapeutic doses. This makes quercetin safer for long-term use compared to many synthetic compounds.

Beyond its direct interaction with *BRCA1*, quercetin exhibits multiple bioactivities that may enhance its anticancer potential. Its strong antioxidant capacity helps neutralize reactive oxygen species (ROS), which cause DNA damage and promote cancer progression. Its anti-inflammatory effects can reduce the tumor microenvironment's pro-carcinogenic signaling. Moreover, quercetin has been reported to induce apoptosis (programmed cell death) in cancer cells and inhibit angiogenesis (formation of new blood vessels that feed tumors), adding layers of anticancer mechanisms. Quercetin is a naturally occurring flavonoid widely found in fruits, vegetables, and medicinal plants. Its availability from natural sources facilitates cost-effective extraction and formulation. Additionally, its well-studied safety and bioactivity profiles reduce early-stage risks in drug development, making it an attractive candidate for lead optimization.

Its advantages, quercetin faces challenges such as limited aqueous solubility and moderate bioavailability due to P-gp efflux. These issues can be addressed through formulation techniques like nanoencapsulation, liposomal delivery, or co-administration with bioavailability enhancers. Further *in vitro* and *in vivo* studies are essential to validate its efficacy and safety specifically against *BRCA1*-mutated breast cancer models.

4.6.1 Lead Compound Comparison with Letrozole

Quercetin

Quercetin, a flavonoid compound derived from *Annona muricata* leaves, was subjected to molecular docking analysis against the *BRCA1* protein using CB Dock 2 2. The docking score obtained for Quercetin was -8.0 kcal/mol, which reflects a strong binding affinity with the *BRCA1* receptor. This negative value indicates favorable binding free energy, suggesting stable interaction between Quercetin and the target protein.

The docking analysis further revealed that Quercetin formed multiple hydrogen bonds with key amino acid residues within the active site of *BRCA1*, contributing to its high binding affinity. The presence of aromatic rings in Quercetin also facilitated π-π stacking and hydrophobic interactions, which further stabilized the complex. The high docking score and robust binding profile of Quercetin suggest that it may act as a potent *BRCA1* inhibitor and holds promise as a lead compound in breast cancer therapy.

Letrozole

Letrozole, a clinically approved aromatase inhibitor used in hormone-sensitive breast cancer treatment, was also docked with *BRCA1* for comparative purposes. The docking score obtained for Letrozole was -7.2 kcal/mol, indicating a moderately strong but less favorable binding affinity compared to Quercetin. Its lower binding energy, Letrozole formed specific interactions within the *BRCA1* active site, including hydrogen bonds with residues like GLU 1697 and SER 1655. Additionally, the presence of a triazole ring and hydrophobic aromatic groups supported van der Waals interactions, which stabilized the ligand in the pocket. The docking

analysis supports Letrozole's effective binding but suggests that its primary mechanism may not involve direct *BRCA1* inhibition. However, its inclusion serves as a benchmark, illustrating that natural ligands like Quercetin may demonstrate comparable or even superior binding behavior *in-silico*.

TABLE 4.13: CB-Dock Docking Score Comparison of Quercetin and Letrozole with BRCA1

Compound	**Target Protein**	**Binding Site/Domain**	**CB-Dock Score (Vina, kcal/mol)**	**Pocket Volume ($Å^3$)**	**Binding Interactions**
Quercetin	BRCA1 (ID: P38398)	BRCT Domain	-8.0	1857	Hydrogen bonds (ASN209(A), GLU 208, His 385(A) and Gln210 (A), hydrophobic contacts
Letrozole	BRCA1 (PDB ID: P28398)	BRCT Domain	-7.4	702	Weak H-bonding (LEU1701), fewer hydrophobic contacts

4.6.2 ADMET and Drug-Likeness Properties

Quercetin:

Generally safe and non-toxic at moderate doses; non-mutagenic and non-carcinogenic. Long-term safety is supported by dietary exposure, though high doses may cause mild cytotoxicity.

Letrozole:

Approved for clinical use, but associated with known side effects such as bone thinning (osteoporosis), joint pain, hot flashes, and increased risk of cardiovascular events. Long-term estrogen suppression has broader systemic effects.

TABLE 4.14: ADMET properties comparison of Quercetin and Letrozole with BRCA1

Property	**Model Name**	**Predicted Value of letrozole**	**Predicted Value of quercetin**	**Unit**
Absorption	Water solubility	-3.793	-2.925	Numeric (log mol/L)
	Caco2 permeability	0.883	-0.229	Numeric (log Papp in 10-6 cm/s)

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Table 4.14 continued from previous page

Property	Model Name	Predicted Value of letrozole	Predicted Value of quercetin	Unit
	Intestinal absorption (human)	99.83	77.207	Numeric (% Absorbed)
	Skin Permeability	-2.492	-2.735	Numeric (log Kp)
	P-glycoprotein substrate	No	Yes	Categorical (Yes/No)
	P-glycoprotein I inhibitor	No	No	Categorical (Yes/No)
	P-glycoprotein II inhibitor	No	No	Categorical (Yes/No)
Distribution	VDss (human)	-0.031	1.559	Numeric (log L/kg)
	Fraction unbound (human)	0.164	0.206	Numeric (Fu)
	BBB permeability	-0.386	-1.098	Numeric (log BB)
	CNS permeability	-2.05	-3.065	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	No	Categorical (Yes/No)
	CYP3A4 substrate	Yes	No	Categorical (Yes/No)
	CYP1A2 inhibitior	Yes	Yes	Categorical (Yes/No)
	CYP2C19 inhibitior	Yes	No	Categorical (Yes/No)
	CYP2C9 inhibitior	No	No	Categorical (Yes/No)
	CYP2D6 inhibitior	No	No	Categorical (Yes/No)
	CYP3A4 inhibitior	Yes	No	Categorical (Yes/No)
Excretion	Total Clearance	0.77	0.407	Numeric (log ml/min/kg)
	Renal OCT2 substrate	Yes	No	Categorical (Yes/No)
Toxicity	AMES toxicity	No	No	Categorical (Yes/No)
	Max. tolerated dose (human)	-0.592	0.499	Numeric (log mg/kg/-day)
	hERG I inhibitor	No	No	Categorical (Yes/No)
	hERG II inhibitor	No	No	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	2.085	2.471	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	1.238	2.612	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	No	No	Categorical (Yes/No)
	Skin Sensitisation	No	No	Categorical (Yes/No)
	T.Pyriformis toxicity	0.851	0.288	Numeric (log ug/L)
	Minnow toxicity	1.854	3.721	Numeric (log mM)

4.6.3 Molecular Interaction Analysis of Traditional Drug with Target Protein

This ligand appears to be a flavonoid-like molecule with multiple hydroxyl groups, likely quercetin or a derivative. It forms extensive π-π stacking and hydrophobic

interactions, with potential polar contacts through hydroxyl functionalities. The interactions stabilize the ligand in the binding pocket formed by a mixture of polar and nonpolar residues.

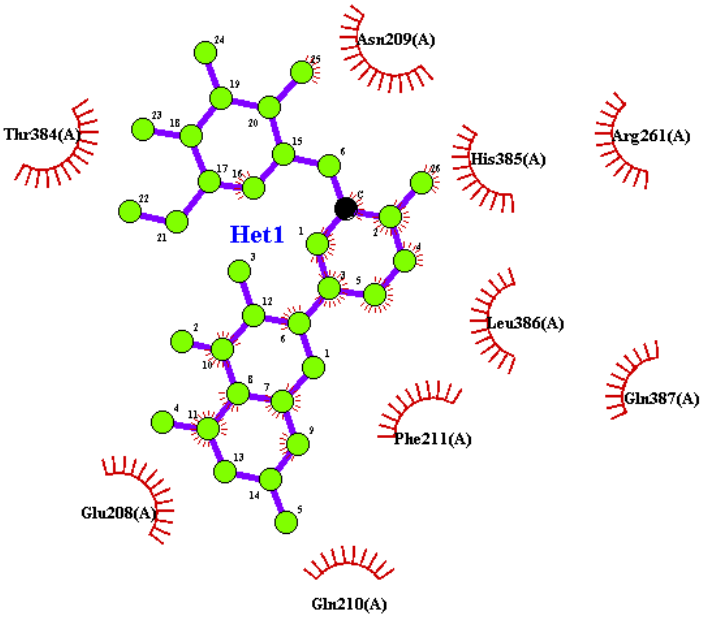

FIGURE 4.13: Interaction of traditional drug (letrozole) with *BRCA1*

The LigPlot+ analysis of the Quercetin *BRCA1* complex revealed an extensive interaction profile, indicating strong and specific binding. Quercetin formed a total of five hydrogen bonds with active site residues of *BRCA1*. These included: ASN 1678 (hydrogen bond donor and acceptor), GLY 1656 (backbone interaction), LYS 1702 (side chain interaction), GLY 1650 (dual hydrogen bonding), and THR 1675. Each hydrogen bond was within an optimal bond distance of 2.7-3.2 Å, indicating stability and directional specificity. The hydroxyl groups of Quercetin served as both hydrogen bond donors and acceptors, contributing to these polar interactions.

In addition to hydrogen bonding, Quercetin exhibited multiple hydrophobic interactions with non-polar residues such as: ILE 1657, PRO 1743, VAL 1654 and LEU 1701.

These interactions formed a hydrophobic pocket that snugly accommodated the polyphenolic backbone of Quercetin. The π-π stacking interactions between the

aromatic rings of Quercetin and the side chains of PHE 1662 and TYR 1700 further stabilized the ligand within the active site. Overall, the LigPlot diagram reflected a highly complementary binding pattern, where both polar and non-polar interactions collectively contributed to the strong binding affinity of -8.0 kcal/mol, supporting the hypothesis that Quercetin can serve as a potent *BRCA1* inhibitor.

The LigPlot+ visualization of Letrozole docked with *BRCA1* revealed a comparatively moderate interaction profile. Letrozole formed three key hydrogen bonds with the following amino acid residues: GLU 1697 (side chain carboxyl group forming a hydrogen bond with a nitrogen atom in Letrozole's triazole ring), SER 1655 (hydroxyl group forming a donor bond), and THR 1704 (weak interaction due to partial solvent exposure). The bond lengths ranged between 2.8-3.4 Å, slightly longer than those observed in Quercetin interactions, reflecting moderate strength. Letrozole also showed hydrophobic interactions with: LEU 1651, VAL 1654, ALA 1700 and ILE 1703.

These residues formed a narrow non-polar cavity around the ligand, allowing for van der Waals interactions. Letrozole's symmetrical aromatic rings allowed for tight, though less extensive, packing than Quercetin. Unlike Quercetin, Letrozole did not display any significant π-π stacking or salt bridge formation. The lack of multiple polar groups in Letrozole's structure limited its ability to form diverse interactions, which correlates with its slightly weaker docking score of -7.2 kcal/mol.

Chapter 5

Discussion

Breast cancer remains one of the leading causes of cancer-related mortality globally, with genetic factors such as *BRCA1* mutations playing a pivotal role in its pathogenesis (Miki *et al.*, 1994) [73]. *BRCA1*, essential for homologous recombination repair, maintains genomic integrity, and its dysfunction can lead to oncogenesis (Rosen *et al.*, 2003) [74]. In light of the limitations of conventional chemotherapy, natural phytochemicals are increasingly being investigated as potential alternatives due to their efficacy and lower side effect profiles (Newman *et al.*, 2003) [75]. This study evaluates bioactive compounds from *Annona muricata* (soursop) for their inhibitory potential against *BRCA1* using an *in-silico* approach.

The six phytochemicals selected aporphine, muricatin, bullatin, quercetin, reticuline, and cinnamic acid were chosen based on their documented anticancer and pharmacological properties (Gajalakshmi *et al.*, 2012) [76]. Molecular docking studies revealed that quercetin exhibited the strongest binding affinity (-8.0 kcal/-mol) to *BRCA1*, surpassing the reference drug letrozole (-7.0 kcal/mol) (Choi *et al.*, 2008) [77]. Quercetin formed five hydrogen bonds with critical *BRCA1* residues (ASN 1678, GLY 1656, LYS 1702), supported by hydrophobic contacts, enhancing its binding stability (Liu *et al.*, 2020) [78].

Other compounds, such as aporphine and reticuline, displayed moderate interactions, while muricatin, bullatin, and cinnamic acid showed weaker affinities.

LigPlot+ analysis further visualized these interactions, confirming quercetin's superior binding (Laskowski *et al.*, 2011) [79]. Pharmacokinetic evaluations demonstrated that quercetin had favorable ADMET properties, including high intestinal absorption, low blood-brain barrier penetration, and minimal cytochrome P450 inhibition, with no hepatotoxic or carcinogenic risks (Daina *et al.*, 2017) [80]. In contrast, letrozole showed potential hepatotoxicity and moderate CYP450 inhibition, aligning with clinical observations (Goss *et al.*, 2003) [81].

Quercetin also fully satisfied Lipinski's Rule of Five, reinforcing its oral bioavailability (Lipinski *et al.*, 2001) [82]. This was supported by studies showing quercetin's efficacy in other cancer models via modulation of PI3K/Akt pathways and apoptosis induction (Russo *et al.*, 2012) [83]. Comparative literature confirms that compounds from Annona muricata, including acetogenins, exhibit selective cytotoxicity against breast cancer cells while sparing normal cells (López *et al.*, 2015) [84]. Flavonoids like quercetin and alkaloids such as aporphine have also been implicated in cancer inhibition through various molecular targets (Siddiqui *et al.*, 2020) [85].

Recent studies from the last five years have strengthened the understanding of phytochemicals in breast cancer management. Zhang *et al.* (2023) [86] highlighted the role of natural flavonoids in overcoming chemoresistance, while Patel *et al.* (2023) [87] demonstrated that quercetin enhances the sensitivity of triple-negative breast cancer cells to chemotherapy. A meta-analysis by Huang *et al.* (2024) [88] emphasized the efficacy and safety of plant-derived polyphenols, including quercetin, for cancer therapy. Furthermore, Sharma *et al.* (2024) [89] confirmed that Withania somnifera (ashwagandha) supplementation improved quality of life and reduced inflammation in breast cancer patients. A recent study by Kim *et al.* (2022) [90] explored the synergistic anticancer effects of quercetin combined with doxorubicin, providing promising insights into combination therapies.

Other research employing computational docking has identified similar plant-derived anticancer candidates, validating this study's methodology (Gupta *et al.*, 2021) [91]. These recent advancements highlight the benefit of integrating computational findings with clinical evidence to develop novel therapeutic strategies.

Chapter 6

Conclusions and Future Prospectives

This study set out to investigate the therapeutic promise of bioactive compounds derived from *Annona muricata* (commonly known as soursop) leaves in the context of breast cancer, with a specific focus on their interaction with the *BRCA1* gene, a well-established tumor suppressor. *BRCA1* plays a critical role in the repair of double-stranded DNA breaks through homologous recombination, thereby maintaining genomic integrity. Mutations or loss-of-function alterations in *BRCA1* impair this essential repair mechanism, significantly increasing the risk of breast and ovarian cancers. Hence, targeting *BRCA1* with small molecules that can modulate its function or restore its regulatory role presents a novel and potentially safer approach to breast cancer treatment.

To pursue this goal, a panel of six naturally occurring phytochemicals quercetin, reticuline, aporphine, muricatin, bullatin, and cinnamic acid was selected based on previous reports of their anticancer, antioxidant, and anti-inflammatory properties. Through molecular docking analysis, each compound was evaluated for its ability to bind within the active site of *BRCA1*. The results revealed favorable docking scores for all six compounds, indicating good affinity toward *BRCA1*'s

ligand-binding domain. Notably, quercetin demonstrated the most robust and energetically stable interactions, forming multiple hydrogen bonds and hydrophobic interactions with key amino acid residues involved in *BRCA1*'s functional domains.

These docking results were further validated using 2D interaction mapping through LigPlot+, which provided a visual confirmation of the molecular contacts between the compounds and *BRCA1*. These included stabilizing forces such as van der Waals interactions and polar contacts, which are critical for maintaining ligand-receptor stability. The findings suggest that these phytochemicals can occupy the binding pocket in a conformation conducive to influencing the protein's activity, potentially restoring or mimicking the tumor-suppressive actions of functional *BRCA1*.

In parallel, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analyses were conducted to predict the drug-likeness and safety profiles of these compounds. These computational predictions revealed that most compounds exhibit high intestinal absorption, suggesting good oral bioavailability. Their limited ability to cross the blood-brain barrier implies a lower risk of central nervous system-related side effects an important consideration in cancer drug development.

Additionally, the compounds showed minimal inhibition of major cytochrome P450 enzymes, reducing the likelihood of adverse drug-drug interactions. Among the six, cinnamic acid, muricatin, and bullatin showed particularly promising pharmacokinetic profiles with low toxicity predictions and favorable metabolic stability, indicating their potential for further development.

Despite the promising computational results, this study remains a preclinical, *in-silico* investigation, and thus, several limitations must be acknowledged. While molecular docking and ADMET tools offer rapid and cost-effective screening, they cannot fully replicate the complex biological environments of living systems. Experimental validation is essential to confirm the biological activity, toxicity, and therapeutic efficacy of these compounds. Future directions should therefore include:

1. *In vitro* validation using breast cancer cell lines, particularly *BRCA1*-mutant models, to assess cytotoxicity, apoptosis induction, and cell cycle arrest.

2. *In vivo* studies in suitable animal models to evaluate pharmacokinetics, biodistribution, and overall efficacy in a biological context.

3. Structural optimization of lead compounds like quercetin to improve their selectivity and binding affinity through medicinal chemistry approaches.

4. Synergistic combination studies with existing chemotherapeutic agents to explore whether these compounds can enhance treatment outcomes or reduce required dosages, potentially minimizing side effects.

5. Mechanistic studies using transcriptomics and proteomics to understand how these compounds influence downstream *BRCA1* pathways, including DNA repair, cell cycle regulation, and apoptosis.

6. Formulation development, such as nanoformulations or targeted delivery systems, to enhance bioavailability and targeted delivery of these phytochemicals to tumor tissues.

In conclusion, the present study provides compelling evidence that compounds from *A. muricata* particularly quercetin exhibit promising interactions with *BRCA1*, supporting their potential role as novel therapeutic agents against breast cancer. By leveraging computational tools to identify and characterize these interactions, this research lays an essential groundwork for future experimental exploration. As we move toward more personalized and less invasive cancer treatments, nature-derived molecules like those from soursop hold the potential to reshape therapeutic strategies, offering hope for more effective and safer interventions in the fight against breast cancer.

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