Preparation of tumor targeting combinatorial therapy using nanoparticle conjugated natural compounds

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

MASTER OF TECHNOLOGY

IN

BIOMEDICAL ENGINEERING

Submitted by:

SHALEEN JAIN

M.tech, BME

2K18/BME/02

Under the supervision of

Dr. Asmita Das

Assistant Professor

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi-110042

JUNE, 2020

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi-110042

CANDIDATE'S DECLARATION

I SHALEEN JAIN, 2K18/BME/02 of M.tech. in Biomedical Engineering , hereby declare that the project entitled "**Preparation of tumor targeting combinatorial therapy using nanoparticle conjugated natural compounds**" which is submitted by me to the Department of Biotechnology, Delhi Technological University in partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title or recognition.

29/6/20

Place: Delhi

Date: 29.06.2020

SHALEEN JAIN

M.Tech., BME

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, Delhi-110042

CERTIFICATE

I hereby certify that the Project Dissertation titled "**Preparation of tumor targeting combinatorial therapy using nanoparticle conjugated natural compounds**" which is submitted by **Mr. SHALEEN JAIN**, Roll No. 2K18/BME/02, Department of Biotechnology, Delhi Technological University, Delhi, in partial fulfilment of the requirement for the award of the degree of Master of technology, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Dr. Asmita Das
(SUPERVISOR)
Department of Biotechnology

Prof. Jai Gopal Sharma
HoD
Department of Biotechnology

Head of the Department
Department of Biotechnology
Delhi Technological University
(Formerly Delhi College of Engg.)
Bawana Road, Delhi-110042

ACKNOWLEDGEMENT

I wish to express my gratitude towards my supervisor **Dr. ASMITA DAS** for her kind guidance, support, encouragement and cooperation during the course of this work. Working under her has indeed been an enriching scientific experience.

I am extremely grateful to Professor Jai Gopal Sharma, Dean, Department of Biotechnology, DTU for providing us with best of the resources. I would also express my gratitude towards Dr. BD Malhotra, Dr. Pravir Kumar, Dr. Prakash Chandra, Dr. Saurabh Saxena, Dr. Yasha Hasija for skillfully guiding me through different fields of biotechnology and biomedical engineering.

I would also like to give my sincere regards and thanks to my Ph.Ds Ms. Shine, Ms. Niharika, Mr. Sunil for constantly helping me at all times and guiding me in planning, executing and compiling the experiment. Without their support and encouragement, this project could not have not been possible.

I would like to mention a deep hearted thanks to my seniors Ms. Vani, Ms. Aditi, Ms. Ritu, Ms. Nidhi who have been extremely helpful during planning and execution of my project work as well as imparting their valuable suggestions and knowledge in the middle of his busy schedule.

I express my gratitude towards all the laboratory staff members Mr. CB Singh, Mr. Jitender for their assistance throughout the project.

This work would not have been possible without the support of my colleagues Sachin, Hrash, Bhawana.

Thanks would be a very insignificant word to express my gratitude towards them they have made it all possible.

-Shaleen Jain

CONTENTS

Preparation of tumor targeting combinatorial therapy using nanoparticle conjugated natural compounds

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

VEGFR- Vascular Endothelial growth factor Receptor

HER2- Human Endothelial growth factor Receptor

ER- Estrogen Receptor

PR- Progesterone receptor

CXCR 4- Chemokine

FR alpha- Folate receptor

HGFR/c-Met- Hepatocyte growth factor receptor

RTK- Receptor tyrosine kinase

AR- Androgen *receptor*

PPARγ- Peroxisome Proliferator-Activated Receptor Gamma

AhR- Aryl hydrocarbon receptor

IMPPAT- Indian Medicinal plants, Phytochemicals and Therapeutics

Nps- Nanoparticles

AuNps- Gold Nanoparticles

MAbs- Monoclonal Antibodies

EPR- Enhance Permeability and retention effect

DI- Deionised

BSA- bovine serum albumin

ABSTRACT

Cancer, a multifactorial disease, caused by several genetic and environmental factors and their interactions in which normal cells become progressively transformed to malignant cells, is currently the most studied topic. Numerous receptors and pathways have been identified for cancer that play a crucial role in its progression. Natural compounds obtained from various medicinal plants have shown a promising potential in targeting cancer in multiple ways. In the present project we are exploring the design of a combinatorial cancer therapy. At first, different bioactive compounds were screened using literature reviews and researches conducted previously and molecular docking simulations were performed against different receptors involved in cancer associated pathways, particularly breast cancer as India's most prevalent cancer. In this project, AutoDock was used for molecular docking and later, PyMol 2.3.2 was used for identifying the binding amino acid residues of ligands against targeting receptors. From the results conducted, the binding energies were assessed and it is hereby proposed that, combination of **Withferin A, Curcumin, Rutin and Selicib** shows highest binding energies against selected key receptors in breast cancer, which can be further explored for combinatorial efficacy. Furthermore, for effective drug delivery, BSA nanoparticles were designed using desolvation process in which the effective size was found to be 160nm with a charge of -100mV. Thus this project holds significant implications for the development of combinatorial therapy using natural compounds and designing new protocols for their in-vivo delivery using nanoparticles, which eventually may have exciting potential for applicability in various cancers.

OBJECTIVES

1. Screening of the known medicinal plants and their single or multiple components (Bioactive compounds) used in cancer therapy using literature reviews and researches conducted previously.
2. To identify the known targeting receptors/ligands and associated pathways of those components.
3. In-silico analysis of protein-protein interactions in breast cancer.
4. Perform Molecular Docking Simulations of selected ligands with selected target protein in Breast cancer.
5. Study interactions of selected ligand with the target protein
6. Screening of different nanoparticles for effective drug delivery of these designed combinatorial drugs.

INTRODUCTION

Cancer etiology is multifactorial, i.e. it is caused by the interaction of genetic factors, environmental factors, lifestyle factors etc., the interaction of whom leads to the development of malignancy. When their associations become strong, that the increased exposure period, then these interactions are visible among the population. [1]

Among the different cancers worldwide, it is seen that around 90 percent are sporadic i.e. they are not inheritable in families and nearly 10 percent have been recognized as run into families or familial cancers. Some cancers on other hand are inherited as autosomal dominant way. Therefore cancer is influenced by certain genetic and environmental factors like ultraviolet radiations, viruses, smoking, carcinogenic agents, etc. It is seen that cancer arises due to the role of several receptors, molecules and pathways participating in its development. [2]

Combinatorial thereby has been developed in recent years in which two or more drugs are combined for targeting different receptors in cancer, thereby treating cancer in a synergistic way. It has been proved as a cornerstone in cancer thereby as it enhances drug efficiency and rate of apoptosis by targeting multiple pathways and receptors of mitotically active tumor cells, tumor stem cells. [3]. Natural compounds have been used at a large scale due to their cytotoxic effects and cytotoxic and chemosensitizing effects. They are widely extracted from different plants worldwide and has been used in several researches for anti-cancerous effects. Moreover, they are involved in targeting cell death pathways (extrinsic and intrinsic), autophagic pathways. [4], [5]

Nanoparticles as drug delivery systems have shown a huge success in recent years and have been explored for therapeutic uses in conjugation with natural compounds, as they possess useful properties like bioavailabilty, increasing drug's stability, nano-sized in nature etc.

This project focuses on existing natural drugs and designing combinatorial therapies from them. In light of limitations of monotherapies and growing demand for combinatorial immunotherapies, this project explores the development of new cancer combinatorial drugs based on synergistic combinations of bioactive compounds which may provide a robust therapy for cancer in the near future.

REVIEW OF LITERATURE

A. Cancer: a multifactorial disease

Cancer results from abnormal proliferation of normal cells in an unregulated manner, and responds inappropriately to the signals controlling the normal behavior of cells. They grow and divide in an uncontrolled fashion and invade normal tissues and organs. Eventually, the loss of controlled growth in normal cells result into abnormalities in cell regulatory systems. Therefore, Cancer is considered as a multifactorial disease, in which many receptors and pathways are associated and is caused by numerous genetic factors, certain environmental factors like ultraviolet radiations, viruses, smoking, carcinogenic agents, etc. Cancers occur due to the mutations in several classes of tumor suppressing genes and oncogenes. Also, product of genes participating in genome surveillance, play role in causing cancer, eg. DNA damage repairing. Furthermore, mutations in the above mentioned genes leads to somatic mutations also, thereby predisposing individuals to the cancer spontaneously. These mutations appear later in an individual's life and do not inherit in families. [6]

B. Types and prevalence of cancers

There are more than hundred types of cancers have been discovered which vary in their response to treatment and behavior. They may be either benign or malignant tumor. Benign tumor remain confined to a particular location while metastatic tumors show the property of metastasis by invading surrounding tissues through lymphatic and circulatory systems.[7]

Most cancers are grouped into mainly three groups: carcinomas, leukemias, sarcomas, and lymphomas. Carcinomas are present in epithelial cells and occur in around 90% population. Sarcomas, are related to connective tissues, like bone, cartilages, muscles, etc. and are rare. Leukemias and lymphomas are related with blood borne tumors and are also related with immune system cells. On the basis of tissue origin, tumors can also be classified as erythroid leukemias (related to erythrocytes), fibrosarcomas related to fibroblasts, etc.

Cancer is a major cause of mortality all over the world. According to 2008 data worldwide, 8 million deaths have been found due to cancers and this data is expected to reach 11 million by 2030. Four most prevalent cancers have been recorded worldwide: breast cancer, lung cancer, prostate cancer and colon cancer, among which lung cancer is lethal, accounting for 30% cancer deaths. According to United States data, 5 million Americans are affected by Cancers every year. These incidence data are obtained through several studies like surveillance, end results program, epidemiology. According to 2020 data, being recent one, around 6 million deaths have been reported due to cancer. Skin cancer being another fatal cancer has been reported declined to 2.2% from 2016 to 2017 data. [8]

Cancer has been identified as a major disease in India through epidemiological transitions and analysis. This has increased the overall economic burden of cancer in India. The prevalence of cancer according to age standardization shows that there are 97 individuals get affected per 1 lakh persons, urban areas being the most prevalent.

According to WHO report, cancer accounts for 6% of total deaths in India, i.e. 79 per 1 lakh individuals. This data has been considered as harmful for future which may rise up to 9 million deaths by 2030. These prevalent studies have been conducted according to gender dimensions also. [9]

C. Breast cancer: India's most prevalent cancer

Breast cancer is identified as the most prevalent cancer in India and is diagnosed as most common cancer in women worldwide, that accounts one or more in ten cases diagnosed every year. Breasts are anatomically designed as milk-producing glands located anteriorly, in front of chest which are supported by ligaments. They are arranged on pectoralis major muscle. There are 2 lobes circularly arranged to form breasts, under which lobules are present that possess glands for milk production in response to particular hormones.

The diagnosis of breast cancer is considered as typical as it evolves silently. Mostly, it is diagnosed during routine screening, while some are accidently diagnosed during formation of lump, nipple discharge, increment in size, etc. There are certain imaging techniques involved in this, like mammography, tissue biopsy for its diagnosis. The metastasis is a condition of spreading of tumor

cells hematologically and lymphatically to distant places, making it difficult for diagnosis. [10] [11]

The treatment of breast cancer involves certain therapies and which undergoes a multidisciplinary approach. It includes, radiotherapy, chemotherapy, surgery, adjuvant therapy, etc., but there are lots of side effects associated with this. So, an effective therapy is required for maximum efficiency with minimal unconditional side-effects.

D. Cancer therapies

Improvements in cancer therapies have been done in various researches for developing an effective targeted therapy. Conventional therapy is focused on using pharmacological compounds for inhibition of growth and cell death in a non-specific way. Moreover, there are other problems associated with this like drug hydrophobicity, stability etc. Which need to be improved. So, modern therapies are focused on targeted treatment for identifying specific receptors and molecules rather using broad base of treatment. The different targeted therapies involve monoclonal antibody- based, immunotoxin- based, small inhibitory molecule- based treatments. New treatments benefiting individual patients has been developed in recent years and shown a remarkable success. Modern ways of treating cancer is evolving rapidly. For instance, development in understanding of the normal cell mechanisms, that how they subverted from normal behaviour, lead to the development of various therapies like therapies based on DNA-repair mechanisms, cell apoptotic pathways etc. Conventional therapies like radiotherapy often damage the DNA. Furthermore, hormone-based therapies like estrogen antagonists (e.g. Tamoxifen) are available for blocking estrogen synthesis in breast cancer. Moreover, for blocking HER2, a blocking monoclonal antibody agent has been designed for targeting and shutting off the HER2. [12]–[14]

Another therapies involve new modalities developed in past decade. The molecular targeted therapy (MTT) has received US FDA approval for cardiac toxicities. In addition, the immune-based therapies include ICIs(Immune checkpoint inhibitors), CART (chimeric antigen receptor) have also been used as potential cancer therapies.

Biocompatible nanoparticles as cancer Nanomedicines are being used for effective drug delivery systems in a controlled way. Eg. Doxorubicin has been widely used since a long time, ThermoDox is used for release of doxorubicin in response to temperature increment. Other egs. for nanomedicines include SPION (Supermagnetic iron oxide nanoparticles) based formulation named ' Nanotherm' has been approved fro glioblastoma in which iron oxide is coated with aminosilane. Other egs. - myocet, daumoxone, abraxane, etc.[15]–[17]

Liquid biopsies have shown a great significance recently for detecting circulating tumor cells. In this, the identification of Ds DNA, m- RNA, lnc RNA, microRNA, are detected in the plasma and serum of Cancer cells. It has been validated as an important tool. Gene therapy, like ADA (Adenosine deaminase) therapy in which a defective gene is repaired, is another promising therapy for gene triggered apoptosis. Eg - RNAi, RISC (RNA induced silencing complex) for targeted gene silencing, delivery of Thymidine kinase (TK) gene and administration of gamciclovir, a Prodrug, has been given in prostate and glioma cancers. Other immunotherapies involve antibodies for active targeting, ACT (Adoptive cell transfer) in which T-lymphocytes are isolated from patient's blood for activity against cancer CAR-T (chimeric antigen receptor) in which autologous T- cell are genetically engineered against cancer has shown a huge success in acute lymphocytic leukemia. [18]

Radiotherapy is also paving ways since a long time, and radiomics and pathomics are well studied areas. Egs. Include, IGRT (Image-guided radiotherapy), IMRT (Intensity modulated radiotherapy), SABR (stertactic ablation radiotherapy), ractination (radiotherapy given in fractionated regime based on radiobiomolecular properties of cancer, 3D conformal radio therapy (3DCRT), etc. [19] [14]

E. Combinatorial therapy

One approach for overcoming conventional therapy challenges is by designing a combinatorial therapy using multiple drugs. The rationale behind this method is to target and suppress more than one receptors and pathways in a particular tumor to synergistically eradicate the tumor cells. This

method of combining two or more drugs has been proved as a cornerstone in cancer treatment in recent years. Different combinatorial drugs have been approved, belonging to natural compounds, monoclonal antibodies, synthetic compounds etc. Moreover, they are also designed to target specific genetic targets in various subtypes of cancer. [20]

F. Natural compounds in cancer therapy in general and combinatorial cancer therapy

Natural compounds have been used in treatment of several diseases since time immemorial, because of their natural origin with minimal side effects. The vast majority of natural compounds is present in plants which are available in market today in the form of drugs used for anti-cancerous effects, nutrition supplements for balancing macro and micro nutrients inside body for good health. So, numerous bioactive compounds, chemicals etc. have been explored and are underway.[5]

Some of the examples for anti-cancerous natural compound based medicines include paclitaxel (from pacific yew plant), camptothecin, vinblastine (*Vinca rosea),* etoposide etc. Around 600 or more natural compounds have been reported for exhibiting anti-cancerous effects. Among them, curcumin (phenolic compound), obtained from *Curcuma longa* has been found to possess powerful anti-cancerous effects. So, believed to be promising chemotherapeutic agent. [4], [5]

Combining two or more natural compounds and assessing their multi-targeting anti-cancerous effects has been studied since a long time, but identifying specific and effective natural compound is challenging as they possess low specificity. In addition, they have capacity of targeting multiple receptors and pathways. So, it makes a long-term planning and procedure for their chemotherapeutic treatment. Moreover, they also possess low toxicity. For instance, these natural bioactive compounds possess the ability to target multiple receptors and pathways. So, they have been proved advantageous by surpassing limitations like signaling feedback and cross-talk in which if one pathway blocks, then natural compound may select the other pathway inside tumor microenvironment. [5], [21].

There is a vast majority of these bioactive compounds present in different plants. So, makes them easier to select for synergistic/combinatorial treatments. On other hand, it also makes it difficult for searching a specific natural compound from a huge library. [22].

Many combinations which are selected from a huge library doesn't give the results. So, to avoid the hectic experimental evaluations for exploring combinatorial agents, there has a lot of methods developed in recent years aimed at identifying possible synergistic agents like identifying a specific inhibitor/receptor for a single ligand. [23], [24]

Combinatorial thereby (may be herbal and synthetic drug combination) possess several advantages like increasing bioavailability, stability, overcoming MDR (Multi-drug resistance) in autoimmune diseases and cancer, facilitating drug transport, increasing permeability etc. some egs. Include curcumin (Natural) and Cyclophosphamide and Paclitaxel (Synthetic), has been used in MCF-7, MCF-12F, MDA-MB-231 cancer cell lines. [25] [26].

G. Breast cancer as a prototype

In this project, Breast cancer was taken as prototype as it has been a frequently diagnosed cancer and the most prevalent in India, that accounts one or more in ten cases diagnosed every year. The number of breast cancer cases in India has been estimated to 25.8 per 100,000 women as incidence rate. A vast majority of receptors play role in occurrence of a particular cancer. Numerous receptors has been discovered which are responsible for expansion of breast cancer, which has been successfully evaluated in several researches. These include, HER2, PR, which are being the most common ones, while other includes insulin-like growth factor receptor (IGFR). [27]

H. Combinatorial therapy for Breast cancer

The combinatorial natural therepy for breast cancer involves predicting the various combinations of natural bioactive compounds which gives maximum efficiency, with minimal side effects, resistance to toxic effects in which they are selected and designed in a way to target cancer receptors and pathways in multiple ways. In this project, breast cancer is selected as India's most prevalent cancer and a combinatorial natural therepy is proposed.

The combinatorial therepy majorly attacks different receptors and pathways and reduces the risk of recurrence with effective dose response rates due to these beneficial advantages, this therepy is

preferred since a long time and is being practiced clinically. It involves certain multi-disciplinary fields like omics, isolation techniques, cell biology, immunology, etc.[28] [29] [30]

I. Receptors of breast cancer

A vast majority of receptors play role in occurrence of a particular cancer. Numerous receptors has been discovered which are responsible for expansion of breast cancer. This includes, Er, HER2, PR, which are being the most common ones, while other includes insulin-like growth factor receptor (IGFR). The method of breast cancer subtyping has been into picture recently which has been proved remarkable. It is based on gene expression, DNA microarray evaluation and it relies on several receptors like HER2, ER, PR receptors etc. So, subtyping is done as Luminal A ER+, Luminal B ER+, Triple negative (ER-, HER2-, PR-), thereby making novel therapy based on subtyping. [31] [32] [33]

HER2 and other hormonal receptors like ER, PR (Estrogen and progesterone receptors) play a major role by overexpressing and downregulating respectively in breast cancer cells and has been found of prognostic importance. The ratios of ER/PR is therefore a concerning factor for identification of ER+ breast cancer. They are also responsible for pleural metastatic cancer.

Among receptors, in addition to ER, PR and HER2, EGFR also play a crucial role in the proliferation of metastasis. HER3, on other hand is associated with downstream pathways, like MAP kinase pathway [34] [35]

There are four major types identified for HER/EGF receptors: HER1, HER2, HER3 and HER4, which are implicated in the onset regulation of cell growth and differentiation.

Among them, HER2 (ErbB2) serve as an important biomarker as it doesn't induces activity by interacting with a specific ligand. It is a kind of tyrosine kinase transmembrane receptor. It also plays a role in other cancers like ovarian, bladder, colorectal cancers. So, HER2 has been recognized as the important biomarker and beneficial tool for breast cancer by the 'American association of Clinical Oncology and European Group'.

Anti-HER2 therapy is now interest to many scientists around the world. So, various techniques has been developed in a past decade which includes molecular imaging, which is highly preferable. PET and SPET are the two radioimaging techniques which are in current clinical use. Eg. 99mTc

radionuclide is used for HER2 molecular imaging. In this project, various receptors were screened from reviews and researches and further evaluations were done on them. [36] [37] [38]

In this project, the interactions of different receptors in breast cancer was analyzed among themselves using STRING database, in which below mentioned receptors showed the highest interaction scores. Thereafter, these selected receptors underwent molecular docking simulations with different ligands. They are:

1. **HER2 (Human Epidermal growth factor Receptor 2)**

- Most commonly expressed
- Associated with other aggressive diseases
- Its extracellular domain has no discovered ligand reported yet.
- It is present in active conformation, so can undergo dimerization.
- HER2/HER3 dimer- identified as most active tumor generating combination.

2. **ER alpha (Estrogen receptor)**

- Present in majority of breast cancers.
- Plays a critical role in biology of mammary glands
- Critical role in progression of breast cancer
- ER-coregualtory proteins are produced which are tightly regulated under the normal conditions.
- Structure- N-terminal AF1 domain, a C-terminal AF2 domain contained by a ligand-binding region and DNA-binding domain.
- Upon binding, the ligand-ER*α complex* binds to target gene promoter in nucleus, thereby stimulating gene transcription.

3. **HGFR (Hepatocyte growth factor Receptor)/c-MET**

- Important role in physiological processes.
- Role in tumorigenesis.
- Acts on wide variety of epithelial cells as mitogen, motogen and morphogen. Key molecule for construction of normal tissue structure during organogenesis, embryogenesis.
- Multi-domain protein

4. Aryl hydrocarbon receptor (AhR)

- According to preclinical and clinical studies, AhR is overexpressed in triple negative and advanced breast cancers.
- Several flavonoids have shown inhibitory effects on AhR.
- Central role in mammary gland development, and in breast cancer progression
- Elevated levels of AhR is seen in human breast carcinoma cell lines like MDA-MB231, MDA-MB435s , MDA-MB468, etc.

5. Integrin alpha V beta 6

- Heterodimeric transmembrane receptors serve as cell adhesion molecules.
- Upregulated during cancers and wound healing.
- Modulate invasion, inhibit apoptosis.
- Bidirectional signaling molecules- controlling vital functions like differentiation, cell division, migration.
- Crucial roles in embryogenesis, hair follicles development etc.

J. In-silico approach in Drug development

As the search space is huge, so identification of synergistic combinatorial agents is very challenging. Therefore, in recent years, a computational based approach has been in picture for evaluating the different parameters of synergistic combinations, thereby predicting the best

possible or exact targets for natural compounds. In some cases, the genetic information of patient is also needed in this approach. [39] [40]

In a research, a method has been developed for predicting synergistic drug combinations called 'DREAM AstraZeneca' which assess the interactions of biological drugs with particular cell lines and their targets using machine learning techniques. [41]

In another research, a bioinformatics tool has been developed called 'Combenefit' for the prediction of personalized synergistic drugs based on synergistic binding energies and scores obtained. In this, a predictive pipeline is constructed in which quantifications are done on the basis of certain models, i.e. comparing difference between actual effect for a specific drug dose response and additive effect (based on Loew model). [42]

K. Molecular docking

In recent years, many bioinformatics tools have shown a huge success for various biological studies like Drug discovery, drug designing, etc. The structure-based in-silico analysis ensures the specificity of a particular entity which is to be analyzed. Molecular docking is an in silico technique for studying protein-ligand interactions. It enables predictions of novel therapeutic compounds and their interactions with different receptors at molecular level. The various applications and upgrading in this technique is underway.

It can be applicable in various fields like polypharmacology, target profiling, drug repurposing, etc. In recent years, it has also been combined with other emergent techniques like Artificial intelligence for assisting in drug discovery task in a better way. [43]

High-Throughput Screening is emergent experimental screening of natural compounds against their targets which has emerged as a potential technique. However, they are not cost-effective which hamper the use for drug discovery.

In molecular docking, different algorithms are used and scoring functions are obtained, based on which analysis is done. Some eg.s., of molecular docking includes, LMMC, a recently developed approach for potential flexible receptor docking. [44] [45] [46]

In current project, different molecular docking softwares were used for performing molecular docking simulations on the breast cancer receptors selected. The ligands (Natural bioactive compounds) were selected from different databases discussed below.

L. **Nanoparticles for drug delivery of combinatorial drugs**

Although providing a promising strategy over monotherapy, the combinatorial drugs designing faces a lot of undesirable in-vivo effects as they have to coordinate with several routes. The challenges include poor solubility, less bioavailability, stability in biological surroundings, systemic toxicity, etc.

Nanoparticles have been designed in worldwide research using nano-technological principles for optimizing drug delivery. Uptil now several kinds of nanoparticles has been developed. In combinatorial thereby, nanoparticles can be used for eliciting synergism among drug combinations, thereby enhancing physical stability, decreasing side-effects and increasing pharmacokinetics and its uptake cellularly. These carriers can improve the accumulation of drugs in tumor microenvironment by passive and active targeting mechanisms.

Numerous kinds of nanoparticle-based on metals, natural compounds, etc. have been developed. Gold nanoparticles has shown a promising success in several researches conducted. AuNp shows passive accumulation and retainment at the tumor area through EPR effect which is based on the leaky vasculature of tumor tissue. Moreover, surface functionalization can also be done easily on AuNp by conjugating active moieties like MAbs, peptides etc. Furthermore high surface to area voume ratio, inherent bioinertness adds to their properties, allowing them to be effective candidates of nanocarrier drug delivery. [47] [48]

Calcium carbonate nanoparticles

Calcium carbonate (CaCO3) Nps are developed widely because CaCO3 is present in earth's crust abundantly in the form of limestone. They have been shown as a good drug delivery agents due to its low toxicity, slow biodegradability. Moreover they are also used on a large scale at industrial level as fillers. [49]

Liposols

Liposols are the lipid –based nanocarriers which are exploited due to the biostability nature of lipids. Silica-based liposomes, TiO2-liposomes based on sol-Gel process within lipid bilayer has been also developed in a research. [50]

Albumin Nanoparticles

Numerous protein-based nanoparticles has been designed for drug-delivery systems. They serve as a naturally self-assembled subunits pf proteins which shows remarkable properties like stability, low toxicity, shelf-life, natural origin, protection from opsonization in-vivo., etc. [51]

Albumin Np have been attracting a considerable interests to the scientists worldwide in comparison to other protein nanoparticle due to their unique properties like, high binding capacities, non-toxicity and well tolerated immunogenicity in-vivo. Their surface possess multiple drug-binding sites and the binding is reversible. So, they serve as a better transport and release systems inside the cell. Due to their high content of amino acids present on the surface (eg. lysine). They show other properties like electrostatic adsorption. Moreover, certain functional groups are attracted over its surface (like carboxylic acids, amino groups) that allows the covalent attachment. [52], [53], [51]

There are many methods of preparing Albumin nanoparticles for eg., Desolvation process, Emulsion-solvent evaporation method, Self-assembly etc. In this project we are using 'Desolvation process' also called 'coacervation method' to synthesize albumin nanoparticles, which is being considered as a simple process. [54]

Desolvation process is a kind of self assembly process for polymeric materials to prepare nanoparticles. In this, a desolvating agent (e.g., alcohol or acetone) is added dropwise to an aqueous solution of protein with provided stirring conditions which leads to dehydrate the protein resulting in conformational change. During the addition of ethanol into the aqueous solution of albumin (pH 5.5), albumin is phase separated. After this, 8% glutaraldehyde or other cross-linking agent in water (v/v) is added for cross-linking the albumin nanoparticles which has been desolvated. [55]

MATERIALS AND METHODS

I. Screeing of ligands and receptors and Molecular docking simulations

A. Materials:

a) **Databases used**

In this project following Databases were used for identification and downloading the structures/files of Proteins/Receptors and Ligands/Bioactive compounds:

1. ZINC15
2. IMPPAT
3. Pubchem
4. PDB
5. STRING Database

b) **Softwares used**

In this project, molecular docking was carried out using following softwares:

1. Autodock Tools 1.5.6
2. Autodock Vina
3. UCSF Chimera
4. OpenBabel GUI v2.4.1
5. PyMol 2.3.2

AutoDock

AutoDock is a useful tool for predicting protein-ligand interactions by binding the ligands to the particular inhibitory sites of receptors. The studies includes virtual screening of compounds, analysis of binding of ligands to protein, catalytic properties of natural compounds and proteins,

etc. There are grid maps generated in results for analysing the binding energies, rates etc. The available and developed computer science techniques enables the screening of entire libraries of compounds against the target. There are different versions of AutoDock available, they are: AutoDock tools, Vina, AutoDock 4 which are open source tools. [56] [57] [58] [59]

PyMol

After performing molecular docking simulations using AutoDock, the visualization of interactions is challenging. So, different tools have been developed. PyMol is a software developed for molecular visualization of Protein-Ligand interactions by protein modeling softwares, crystallographic analysis and molecular dynamics evaluation.

Furthermore, different amino acid residues to which the ligand is bound within its nearer distances can also be identified using PyMol. So, makes it an important tool. [60] [61]

c) Computer Configurations

This research was conducted by computational approach where Personal Computer (PC) with Intel® Core™ i3 3110M CPU @2.4GHz Processor with 4GB of RAM and Windows 8.1 Professional as Computer Operating System was used.

B. Methodology

1. Screening of different medicinal plants, their natural compounds, target receptors and pathways.
2. Structure preparation of protein and ligand.
3. Molecular docking simulations of selected compounds.
4. Comparison of ligands through molecular interaction.

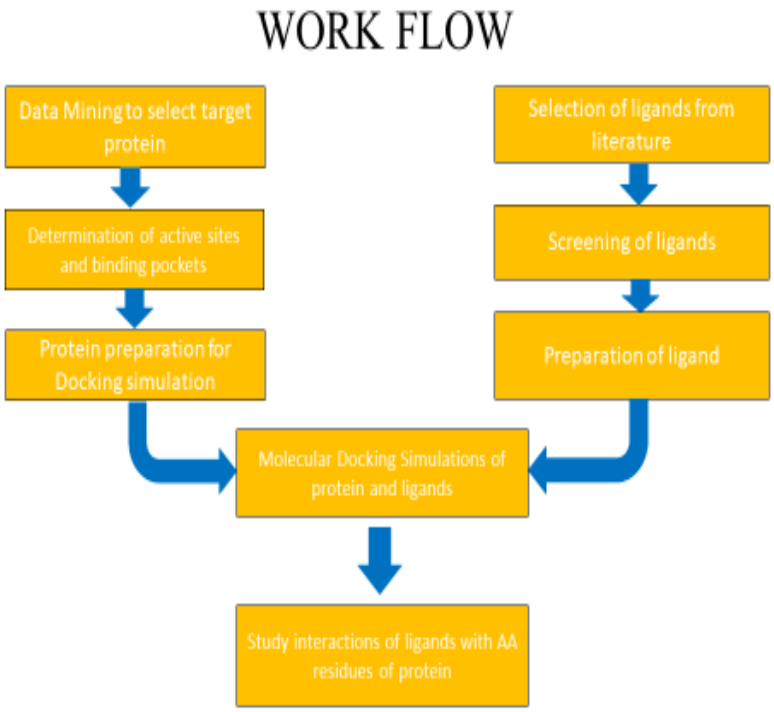


Figure 1 : Conducted work flow for molecular docking simulations.

II. Screening of nanoparticles for effective size

In this project screening of three different nanoparticles was proposed. i.e, Albumin nanoparticles, calcium carbonate nanoparticles, Liposol (Lipid- sol-gel). Initially synthesis of albumin nanoparticles was taken into consideration due to simple synthesizing process with effective size and other properties.

A. Materials required

a) Chemicals and apparatus

1. Spirit
2. DI water (Ultrapure)
3. Acetone
4. Quartz cuvette 3ml

5. BSA- SIGMA ALDRICH (min. 98% electrophoresis)
6. Ethanol- Merck (EMSURE- ACS,ISO,reag. PhEur)
7. Glass vials- 15 ml
8. Glass vials- 30 ml
9. Glycerol
10. D-Glucose
11. Micropipettes

b) **Software used**: SigmaPlot 2000 for plotting graphs using obtained values.

B. Protocol

Here nanoparticles were prepared using biodegradable bovine serum albumin (BSA) as polymer. Following protocol was followed:

- Briefly, bovine serum albumin between 50 mg and 200 mg was dissolved in 2.0 ml of purified distilled water.
- Then 8 ml of Ethanol was added dropwise with stirring (500 rpm) at a rate of 1 ml/min. This resulted in the formation of an opalescent suspension spontaneously at room temperature. Volume can also be varied for making samples more diluted.
- Different cross-linking agents were added for making different samples and placed on magnetic stirrer overnight for 24 hrs.
- The resulting suspension was purified by 5 cycles of differential centrifugation (12,000*g, 8 min) and pellet was redispersed to the original volume in distilled water.
- Each redispersion step was performed in a bath sonicator over 5 mins.
- Solution was stored at 4 ˚C.
- The solution obtained was proceeded for characterization.

a) D-Glucose as cross linker

- After adding 8ml ethanol dropwise to the sample, UV (254nm) treatment was given for 30min. followed by addition of 115ul of 6mM D-Glucose solution.
- Solution placed on magnetic stirrer overnight.
- Rest process is same as above.

b) Glycerol as cross linker

- After adding 8ml ethanol dropwise to the sample, 115ul of glycerol was added.
- Solution placed on magnetic stirrer overnight.
- Rest process is same as above.

C. Characterization

- The formation of nanoparticles using this technique was confirmed by Zetasizer (Using the principle of 'Dynamic light scattering'), for the estimation of size.
- 3 ml of the nanoparticle solution was taken in Zetasizer vials and placed in the unit.
- Readings were taken and the graphs and histograms were plotted using 'Sigma Plot' software for size estimation.
- The charge on nanoparticle was estimated by using zetapotential.

RESULTS AND DISCUSSIONS

1. Screening of Natural compunds, Receptors, Ligands and Approved Drugs

Medicinal Plant	**Uses**	**Compounds**	**Targeting receptor**	**Targeted pathways**
Tinospora cardifolia (Guduchi)	lymphoma	• Columbin • 20 β-hydroxyecdysterone, Cordioside	• Topoisomerase • NF–κB • **Tumor** necrosis factor-α (TNF-α) • COX–II and Nrf2 Interleukin-2 (IL-2), IL-6, IL-8	• Notch • Wnt • Mitochondrial-related apoptotic **pathway**
*Phyllanth*us amarus	Blood cancer, prostate cancer	• Nirtetralin • Phyltetralin • Niranthrin • phyllanthin	Toll-like ***receptor***	Hypoxia **pathway**
Indian pennywort *(Centella asiatica)*	Liver tumors	• Asiaticoside • Vallerine • pectic acid • hydrocotyline • stigmasterol	TLR4 **receptor**	• TGF-β/Smad ***pathway*** • Mitochondrial apoptotic **pathway**

*Andrographis paniculata (*Creat)	Esophageal cancer	Andrographolide	• COX-2, • CXCR3 • CXCR7	• p300 pathway • VEGF ***pathway***
*Ziziphus nummularia (*jujube bush)	Breast cancer, leukaemia, ovarian cancers	Betulin, Betulinic acid	• Estrogen-**receptor** • AMFR • P53 and CD95	• ROS • MAP kinase pathways
Common turmeric/ *curcumin (Curcuma longa)*	Breast, bowel, stomach and skin cancer	Curcumin	Estrogen **receptor** HER2 EGFR NFk-B AP-1 COX-2 LOX MMP-9 TNF EGR-1 NOs	• JNK pathway • serine/threonine kinase pathway
Mappia foetida	Breast, prostate cancer	Camptothecin	• Estrogen **receptor** • Topoisomerase-1 (topo −1)	• p53 • Caspase-3 mediated pathway
*Anno*na atemoya	Several types of cancer	Bullatacin	• Fas • TNF **receptor**.	• Bax- and caspase-3 **pathways**
Cedrus deodara	Leukemia	• Lignans • Matairesinol Wikstromol • Dibenzyl butyrolactol	CCK-B	• Wnt/Wg pathway
Withania somnifera	Oral cancers	Withaferin A	Estrogen **receptor**-α	• ROS • JAK/STAT3

Table 1: List of Medicinal plants and their multiple natural compounds, their cancer targeted receptors and pathways. This table represents some medicinal plants which have been used in various cancer researches. Their multiple bioactive compounds have been found targeting one or more receptors and pathways. Moreover there can be similar bioactive compounds present in two or more plants.

Approved drugs	**Cancer**	**Targeting receptor**
Brigatinib	Anaplastic lymphoma kinase-positive non-small cell lung cancer (NSCLC)	ALK EGFR
Olaparib	Prostate cancer Pancreatic adenocarcinoma	**PARP**- PARP1, PARP2, and PARP3 Neuropilin 1 (NRP1)
Atezolizumab	Lung cancer	PD-L1
Ripretinib	GIST	KIT PDGFRA
Rucaparib	Prostate cancer	PARP
Pomalidomide	AIDS-Kaposi sarcoma	PD-L1
Selpercatinib	Lung cancer Thyroid cancer	VEGFR1 VEGFR3 RET protein
Capmatinib	Lung cancer	MET EGFR
Niraparib	Fallopian tube, or primary peritoneal cancer	PARP1 and PARP2
Pemigatinib	Cholangiocarcinoma	FGFR2
Mitomycin	LG-UTUC	Folate ***receptor,***
Selumetinib	Plexiform neurofibromas	MEK
Luspatercept-aamt	Anemia	CAR

MONOCLONAL ANTIBODY DRUGS	**Cancer**	**Targeting receptor**
Fam-trastuzumab deruxtecan-nxki	HER2-positive breast cancer	HER2 (ERBB2)
Abciximab	Non-small Cell Lung ***Cancer***	αIIbβ3-Integrin (GP IIb/IIIa)
Adalimumab	Skin cancer	TNF ***receptor***
Alefacept	**T-cell** lymphoid malignancies	CD2 ***receptor*** on T cells
Alemtuzumab	Chronic lymphocytic leukaemia (CLL)	CD52
Basiliximab		IL-2 ***receptor***
Belimumab	skin **cancers**	BAFF
Cetuximab	metastatic colorectal ***cancer***	EGFR
Daclizumab	Lymphoid Malignancies	CD25
Pembrolizumab	NMIBC	CPI
Ramucirumab	NSCLC	VEGFR-2 EGFR
SYNTHETIC ANTI-CANCEROUSNANOMEDICINES	**Cancer**	**Targeting receptor**
Doxil (Caelyx)	Ovarian/breast cancer	Estrogen receptor
Ontak	Cutaneous T-cell	IL-2
Genexal-PM	Breast cancer/small cell lung cancer	Folate ***receptor***
Nimotuzumab	Head and neck cancer, Glioma, Nasopharyngeal cancer	EGFR
Ipilimumab	metastatic melanoma	CTLA4
Catumaxomab	EPCAM-positive tumour	CD3 and EPCAM
Pertuzumab	slowed tumor growth	HER2

Myocet	Breast cancer	folate ***receptor***
Abraxane	Pancreatic and breast cancer	Gp60 ***receptor,*** Mitotic inhibitor
Imatinib mesylate, Sunitinib and Sorafenib	ABL tyrosine kinase	RTK PDGF-Rs VEGFRs
Rituximab, Ibritumomab tiuxetan	NHL and chronic lymphocyticleukaemia	CD20 IgG1
Alemtuzumab	CLL, CTCL and T-cell lymphoma.	CD52
Bevacizumab	Metastatic colon cancer	VEGF-A

Table 2: List of Approved natural, synthetic and antibody chemotherapeutic drugs with their targets. This table infers that similar receptors and multiple receptors for a particular cancer can be targeted by two or more approved drugs. So, analyzing combinations from these drugs can be useful in designing combinatorial therepy, as their effects has been successfully seen.

Cancer type	**Receptors**	**Medicinal plants used**	**Bioactive compounds used**	
Breast cancer	**Most common**- EGFR and HER2 (erbB-2), Estrogen receptor (ERα) **Others:** 1. Hepatocyte growth factor receptor- HGFR/c-Met 2. Aryl hydrocarbon receptor (AhR) 3. Receptor tyrosine kinase (RTK)- AXL 4. Adenosine receptor 2B Toll like receptors (TLRs) 5. LRP6 and FZD7 receptors 6. PARP 7. VEGF 8. Integrin alpha V 9. Tumor necrosis factor related apoptosis-inducing 10. Folate (FR alpha) 11. Chemokine (CXCR 4) 12. Interleukin (IL-6)	*Allium sativum* *Echinacea* *Camellia sinensis* *Curcuma longa* *Arctium lappa* *Panax ginseng* *Withania somnifera* *Amoora rohituka* *Dysoxylum binectariferum*	Fucoxanthin Punicalagin Curcumin, Triterpenoid Cucurbitacin I Phenolic CAPE Geraniin Tocotrienol SFN Plumbagin Camptothecin	Withaferin A Epigallocatechin-3-gallate (EGCG) Genistein Resveratrol BITC Honokiol NITC PEITC
			In Vivo-effects Roscovitine (Seliciclib), Rutin, Quercetin, eriodictyol-7-*O*-glucoside, Apigenin, Kaempferol, gallic acid, catechin, cyanidin-3-*O*-galactoside, epicatechin.	
Prostate cancer	1. Androgen *receptor* (AR)-NR3C4	*Kalanchoe gastonis-bonnieri* *Ficus deltoidea*	**<u>Bioactive compounds which have both in-vivo and in-vitro anti-prostate cancer effects</u>**	

	2. Prostate specific membrane antigen (PSMA) 3. LH/CG receptors 4. IL-11 5. *Sigma-1* receptor (σ1R) 6. Orphan nuclear receptor-COUP-TFII 7. BAG1L 8. Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) 9. EphB4 (B4)- a receptor kinase	*Morus nigra* *Acacia hydaspica* *Trimeria grandifolia* *Azadirachta indica* (neem) *Wissadula periplocifolia* *Arabidopsis thaliana*	**Alkaloids** • Emetine • Lycorine **Phenolic compounds** • α-Mangostin • Curcumin • Delphinidin • Ellagic acid • Fisetin • Flavokawain A • Flavopiridol • Garcinol • Ginkgetin • Mangiferin • Paeonol • Plumbagin • Quercetin • Resveratrol **Protein** • Agglutinin • Diffusa cyclotide 3 **Terpenoids** • α-Santalol • (20R)-Dammarane-3β,12β,20,25-tetrol (25-OH-PPD) • Andrographolide • Celastrol

Table 3: Receptors and bioactive compounds related to prevalent cancers in India. This table represents some key receptors of India's two prevalent cancers on which several researches have been conducted previously. Also, the mentioned natural compounds have shown their effects in-vitro as well as in-vivo. Using this data, further molecular docking simulations were performed for designing combinatorial therapy in breast cancer.

Combinatorial drug	**Uses**	**Receptors**	
Ursolic acid, resveratrol and curcumin	Prostate cancer	ASCT2 STAT3	mTORC1
Quercetin and Doxorubicin	Breast cancer	P-gp HIF-1a	MRP1 BCRP
Curcumin and Paclitaxel	Breast cancer	IκBα NF-κB	
Mangiferin and Doxorubicin	Breast cancer	P-gp	
Furanodiene and Tamoxifen	Breast cancer	p-cyclin D1 CDK2 CDK6	p-Rb p-p44
Furanodiene and Doxorubicin	Breast cancer	Integrin aV b-catenin	PARP-1
Xanthohumol and Doxorubicin	Breast cancer	STAT3 EGFR	MDR1
Garcinol and Paclitaxel	Breast cancer	MMP-2 MMP-9	
Genistein and Cisplatin	Breast cancer	NF-kB	

Table 4: Combinatorial drugs and their targets successfully seen in india's most prevalent cancers: prostate cancer and breast cancers. This table infers that similar receptors and multiple receptors for a particular cancer can be targeted by cominations of two or more

approved drugs. So, analyzing combinations from these drugs can be useful in designing combinatorial therepy, as their effects has been successfully seen.

2. Protein-protein interactions and Molecular docking simulations in breast cancer.

A. Protein-protein interaction study using STRING Database.

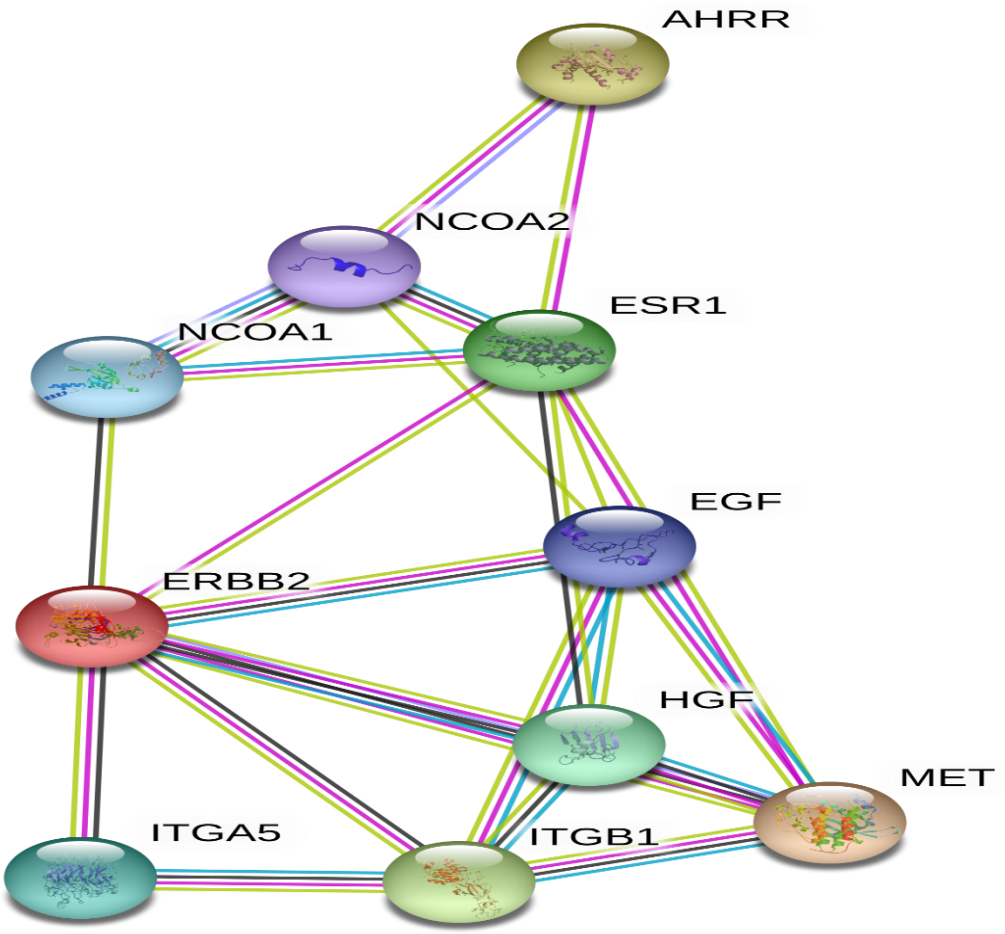


Figure 2 : Selected protein-protein interactions analysis using STRING database. Coloured nodes depicts the query protein and Lines depicts the known interactions. This picture depicts the interactions between the selected receptors in breast cancers. These interactions can be seen by colored lines between two proteins, which shows gene neighborhood, gene fusions, gene co-occurrence, textmining, co-expression, protein homology. Experimentally

determined and curated database interactions are also well shown here. This network shows many interactions between selected key receptors, so molecular docking simulations can be performed on these.

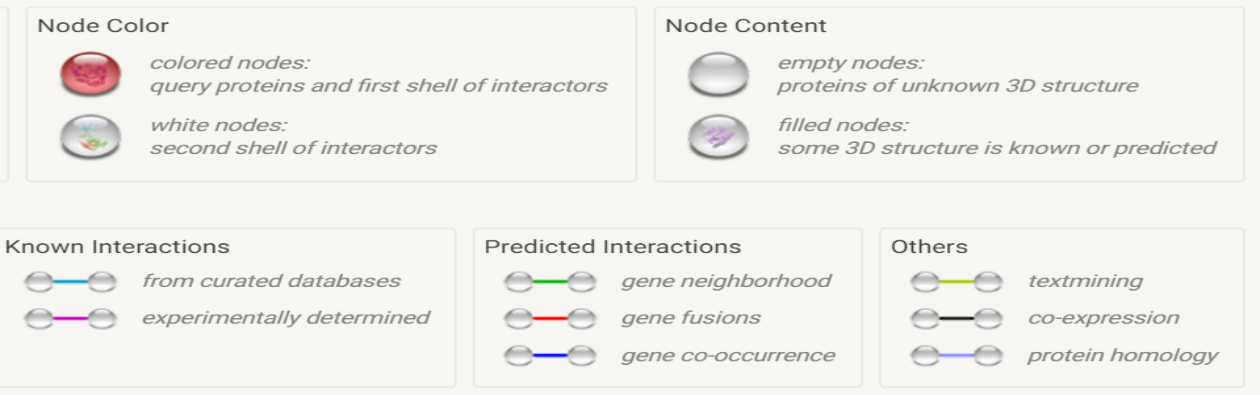


Figure 3: Key to Protein-Protein interactions using STRING database. These interactions are gene neighborhood, gene fusions, gene co-occurrence, textmining, co-expression, protein homology, experimentally determined and curated database interactions.

node1	node2	node1 accession	node2 accession	node1 annotation	node2 annotation	score
AHRR	ESR1	ENSP00000323816	ENSP00000405330	Aryl hydrocarbon receptor repressor; Medi...	Estrogen receptor; Nuclear hormone rece...	0.498
AHRR	NCOA2	ENSP00000323816	ENSP00000399968	Aryl hydrocarbon receptor repressor; Medi...	Nuclear receptor coactivator 2; Transcripti...	0.525
EGF	ERBB2	ENSP00000265171	ENSP00000269571	Pro-epidermal growth factor; EGF stimulat...	Receptor tyrosine-protein kinase erbB-2; P...	0.996
EGF	ESR1	ENSP00000265171	ENSP00000405330	Pro-epidermal growth factor; EGF stimulat...	Estrogen receptor; Nuclear hormone rece...	0.836
EGF	HGF	ENSP00000265171	ENSP00000222390	Pro-epidermal growth factor; EGF stimulat...	Hepatocyte growth factor; Potent mitogen...	0.984
EGF	ITGB1	ENSP00000265171	ENSP00000379350	Pro-epidermal growth factor; EGF stimulat...	Integrin beta-1; Integrins alpha-1/beta-1, al...	0.957
EGF	MET	ENSP00000265171	ENSP00000317272	Pro-epidermal growth factor; EGF stimulat...	Hepatocyte growth factor receptor; Recep...	0.871
EGF	NCOA2	ENSP00000265171	ENSP00000399968	Pro-epidermal growth factor; EGF stimulat...	Nuclear receptor coactivator 2; Transcripti...	0.524
ERBB2	EGF	ENSP00000269571	ENSP00000265171	Receptor tyrosine-protein kinase erbB-2; P...	Pro-epidermal growth factor; EGF stimulat...	0.996
ERBB2	ESR1	ENSP00000269571	ENSP00000405330	Receptor tyrosine-protein kinase erbB-2; P...	Estrogen receptor; Nuclear hormone rece...	0.947
ERBB2	HGF	ENSP00000269571	ENSP00000222390	Receptor tyrosine-protein kinase erbB-2; P...	Hepatocyte growth factor; Potent mitogen...	0.867
ERBB2	ITGA5	ENSP00000269571	ENSP00000293379	Receptor tyrosine-protein kinase erbB-2; P...	Integrin alpha-5; Integrin alpha-5/beta-1 is ...	0.505
ERBB2	ITGB1	ENSP00000269571	ENSP00000379350	Receptor tyrosine-protein kinase erbB-2; P...	Integrin beta-1; Integrins alpha-1/beta-1, al...	0.622
ERBB2	MET	ENSP00000269571	ENSP00000317272	Receptor tyrosine-protein kinase erbB-2; P...	Hepatocyte growth factor receptor; Recep...	0.447
ERBB2	NCOA1	ENSP00000269571	ENSP00000385216	Receptor tyrosine-protein kinase erbB-2; P...	Nuclear receptor coactivator 1; Nuclear re...	0.591
ESR1	AHRR	ENSP00000405330	ENSP00000323816	Estrogen receptor; Nuclear hormone rece...	Aryl hydrocarbon receptor repressor; Medi...	0.498
ESR1	EGF	ENSP00000405330	ENSP00000265171	Estrogen receptor; Nuclear hormone rece...	Pro-epidermal growth factor; EGF stimulat...	0.836
ESR1	ERBB2	ENSP00000405330	ENSP00000269571	Estrogen receptor; Nuclear hormone rece...	Receptor tyrosine-protein kinase erbB-2; P...	0.947
ESR1	HGF	ENSP00000405330	ENSP00000222390	Estrogen receptor; Nuclear hormone rece...	Hepatocyte growth factor; Potent mitogen...	0.443
ESR1	MET	ENSP00000405330	ENSP00000317272	Estrogen receptor; Nuclear hormone rece...	Hepatocyte growth factor receptor; Recep...	0.473

Figure 4: Scores of selected Protein-protein interactions. Page-1. This data shows that there are firm interactions present between the studied receptors. The interactions between HER2 and Estrogen receptor, EGFR receptors are strong giving high scores.

node1	node2	node1 accession	node2 accession	node1 annotation	node2 annotation	score
ESR1	NCOA1	ENSP00000405330	ENSP00000385216	Estrogen receptor; Nuclear hormone rece...	Nuclear receptor coactivator 1; Nuclear re...	0.997
ESR1	NCOA2	ENSP00000405330	ENSP00000399968	Estrogen receptor; Nuclear hormone rece...	Nuclear receptor coactivator 2; Transcripti...	0.995
HGF	EGF	ENSP00000222390	ENSP00000265171	Hepatocyte growth factor; Potent mitoge...	Pro-epidermal growth factor; EGF stimulat...	0.984
HGF	ERBB2	ENSP00000222390	ENSP00000269571	Hepatocyte growth factor; Potent mitoge...	Receptor tyrosine-protein kinase erbB-2; P...	0.867
HGF	ESR1	ENSP00000222390	ENSP00000405330	Hepatocyte growth factor; Potent mitoge...	Estrogen receptor; Nuclear hormone rece...	0.443
HGF	ITGB1	ENSP00000222390	ENSP00000379350	Hepatocyte growth factor; Potent mitoge...	Integrin beta-1; Integrins alpha-1/beta-1, a...	0.954
HGF	MET	ENSP00000222390	ENSP00000317272	Hepatocyte growth factor; Potent mitoge...	Hepatocyte growth factor receptor; Recep...	0.999
ITGA5	ERBB2	ENSP00000293379	ENSP00000269571	Integrin alpha-5; Integrin alpha-5/beta-1 is...	Receptor tyrosine-protein kinase erbB-2; P...	0.505
ITGA5	ITGB1	ENSP00000293379	ENSP00000379350	Integrin alpha-5; Integrin alpha-5/beta-1 is...	Integrin beta-1; Integrins alpha-1/beta-1, a...	0.999
ITGB1	EGF	ENSP00000379350	ENSP00000265171	Integrin beta-1; Integrins alpha-1/beta-1, a...	Pro-epidermal growth factor; EGF stimulat...	0.957
ITGB1	ERBB2	ENSP00000379350	ENSP00000269571	Integrin beta-1; Integrins alpha-1/beta-1, a...	Receptor tyrosine-protein kinase erbB-2; P...	0.622
ITGB1	HGF	ENSP00000379350	ENSP00000222390	Integrin beta-1; Integrins alpha-1/beta-1, a...	Hepatocyte growth factor; Potent mitogen...	0.954
ITGB1	ITGA5	ENSP00000379350	ENSP00000293379	Integrin beta-1; Integrins alpha-1/beta-1, a...	Integrin alpha-5; Integrin alpha-5/beta-1 is...	0.999
ITGB1	MET	ENSP00000379350	ENSP00000317272	Integrin beta-1; Integrins alpha-1/beta-1, a...	Hepatocyte growth factor receptor; Recep...	0.944
MET	EGF	ENSP00000317272	ENSP00000265171	Hepatocyte growth factor receptor; Recep...	Pro-epidermal growth factor; EGF stimulat...	0.871
MET	ERBB2	ENSP00000317272	ENSP00000269571	Hepatocyte growth factor receptor; Recep...	Receptor tyrosine-protein kinase erbB-2; P...	0.447
MET	ESR1	ENSP00000317272	ENSP00000405330	Hepatocyte growth factor receptor; Recep...	Estrogen receptor; Nuclear hormone rece...	0.473
MET	HGF	ENSP00000317272	ENSP00000222390	Hepatocyte growth factor receptor; Recep...	Hepatocyte growth factor; Potent mitogen...	0.999
MET	ITGB1	ENSP00000317272	ENSP00000379350	Hepatocyte growth factor receptor; Recep...	Integrin beta-1; Integrins alpha-1/beta-1, a...	0.944
NCOA1	ERBB2	ENSP00000385216	ENSP00000269571	Nuclear receptor coactivator 1; Nuclear re...	Receptor tyrosine-protein kinase erbB-2; P...	0.591

page 2 of 3

Figure 5: Scores of selected Protein-protein interactions. Page-2. This data shows that there are firm interactions present between the studied receptors. The interactions between HER2 and HGFR, Integrin receptors are strong giving high scores.

node1	node2	node1 accession	node2 accession	node1 annotation	node2 annotation	score
NCOA1	ESR1	ENSP00000385216	ENSP00000405330	Nuclear receptor coactivator 1; Nuclear re...	Estrogen receptor; Nuclear hormone rece...	0.997
NCOA1	NCOA2	ENSP00000385216	ENSP00000399968	Nuclear receptor coactivator 1; Nuclear re...	Nuclear receptor coactivator 2; Transcripti...	0.958
NCOA2	AHRR	ENSP00000399968	ENSP00000323816	Nuclear receptor coactivator 2; Transcript...	Aryl hydrocarbon receptor repressor; Medi...	0.525
NCOA2	EGF	ENSP00000399968	ENSP00000265171	Nuclear receptor coactivator 2; Transcript...	Pro-epidermal growth factor; EGF stimulat...	0.524
NCOA2	ESR1	ENSP00000399968	ENSP00000405330	Nuclear receptor coactivator 2; Transcript...	Estrogen receptor; Nuclear hormone rece...	0.995
NCOA2	NCOA1	ENSP00000399968	ENSP00000385216	Nuclear receptor coactivator 2; Transcript...	Nuclear receptor coactivator 1; Nuclear re...	0.958

page 3 of 3

Figure 6: Scores of selected Protein-protein interactions. Page-3. This data shows that there are firm interactions present between the studied receptors. The interaction between NCOA1 and Estrogen receptor is strong giving high scores.

Protein1	Protein 2	Score
HER2	Estrogen receptor (ERα)	**0.947**
	Hepatocyte growth factor receptor- HGFR/c-Met	**0.867**
	Aryl hydrocarbon receptor (AhR)	**0.720**

	Integrin alpha V beta 6	**0.622**
Estrogen receptor (ERα)	HER2	**0.996**
	Hepatocyte growth factor receptor- HGFR/c-Met	**0.984**
	Aryl hydrocarbon receptor (AhR)	**0.498**
	Integrin alpha V beta 6	**0.957**
Hepatocyte growth factor receptor- HGFR/c-Met	HER2	**0.867**
	Estrogen receptor (ERα)	**0.984**
	Aryl hydrocarbon receptor (AhR)	**0.691**
	Integrin alpha V beta 6	**0.954**
Aryl hydrocarbon receptor (AhR)	HER2	**0.487**
	Estrogen receptor (ERα)	**0.512**
	Hepatocyte growth factor receptor- HGFR/c-Met	**0.717**
	Integrin alpha V beta 6	**0.621**
Integrin alpha V beta 6	HER2	**0.622**
	Estrogen receptor (ERα)	**0.957**
	Hepatocyte growth factor receptor- HGFR/c-Met	**0.954**

	Aryl hydrocarbon receptor (AhR)	**0.525**

Table 5: Score obtained from protein-protein interactions of selected key receptors of breast cancer. It infers that all the receptors show significant interaction score among themselves.

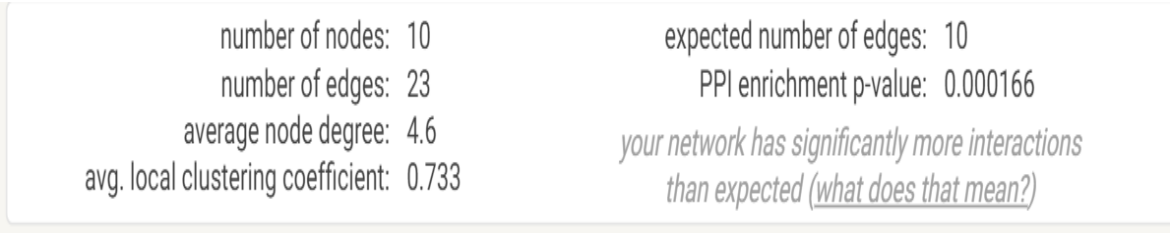


Figure 7 : Result values of selected Protein-protein interactions. This network has significantly many interactions. The efficacy of interactions can be found out by PPI enrichment p-value, which is 0.000166, which is strong enough to elucidate that these receptors can be further studied for molecular docking simulations.

B. Molecular docking simulations using AutoDock and PyMol

Ligand-receptor complex		**Binding energy (Kcal/mol)**
Ligand	**Receptor**	
Roscovitine (Seliciclib)	HER2	-9.05
	Estrogen receptor (ERα)	-9.04
	Hepatocyte growth factor receptor- HGFR/c-Met	-8.71
	Aryl hydrocarbon receptor (AhR)	-9.47
	Integrin alpha V beta 6	-8.69
Quercetin	HER2	-7.62
	Estrogen receptor (ERα)	-7.46
	Hepatocyte growth factor receptor- HGFR/c-Met	-7.78
	Aryl hydrocarbon receptor (AhR)	-7.71
	Integrin alpha V beta 6	-7.15
Apigenin	HER2	-7.45
	Estrogen receptor (ERα)	-8.09
	Hepatocyte growth factor receptor- HGFR/c-Met	-7.74
	Aryl hydrocarbon receptor (AhR)	-7.68
	Integrin alpha V beta 6	-7.05
Kaempferol	HER2	-7.57
	Estrogen receptor (ERα)	-7.98
	Hepatocyte growth factor receptor- HGFR/c-Met	-7.72

	Aryl hydrocarbon receptor (AhR)	-7.44
	Integrin alpha V beta 6	-7.11
Rutin	HER2	-9.45
	Estrogen receptor (ERα)	-9.39
	Hepatocyte growth factor receptor- HGFR/c-Met	-10.23
	Aryl hydrocarbon receptor (AhR)	-8.74
	Integrin alpha V beta 6	-8.87
Catechin	HER2	-7.44
	Estrogen receptor (ERα)	-7.18
	Hepatocyte growth factor receptor- HGFR/c-Met	-7.53
	Aryl hydrocarbon receptor (AhR)	-7.70
	Integrin alpha V beta 6	-7.31
Curcumin	HER2	-7.87
	Estrogen receptor (ERα)	-8.54
	Hepatocyte growth factor receptor- HGFR/c-Met	-9.03
	Aryl hydrocarbon receptor (AhR)	-8.15
	Integrin alpha V beta 6	-8.28
Withaferin A (WFA)	HER2	-8.07
	Estrogen receptor (ERα)	-8.86
	Hepatocyte growth factor receptor- HGFR/c-Met	-8.49
	Aryl hydrocarbon receptor (AhR)	-8.22
	Integrin alpha V beta 6	-7.49

Resveratrol	HER2	-7.37
	Estrogen receptor (ERα)	-7.72
	Hepatocyte growth factor receptor- HGFR/c-Met	-7.54
	Aryl hydrocarbon receptor (AhR)	-7.57
	Integrin alpha V beta 6	-7.31
Honokiol (HNK)	HER2	-7.35
	Estrogen receptor (ERα)	-7.04
	Hepatocyte growth factor receptor- HGFR/c-Met	-7.77
	Aryl hydrocarbon receptor (AhR)	-7.79
	Integrin alpha V beta 6	-7.25
Plumbagin	HER2	-6.49
	Estrogen receptor (ERα)	-6.72
	Hepatocyte growth factor receptor- HGFR/c-Met	-6.65
	Aryl hydrocarbon receptor (AhR)	-6.52
	Integrin alpha V beta 6	-6.74
Camptothecin	HER2	-7.26
	Estrogen receptor (ERα)	-6.99
	Hepatocyte growth factor receptor- HGFR/c-Met	-7.74
	Aryl hydrocarbon receptor (AhR)	-7.06
	Integrin alpha V beta 6	-6.88

Table 6: Observed binding energies in molecular docking of selected Receptors and ligands in Breast cancer using AutoDock. It shows that Rutin is showing highest binding efficiency with all these receptors among all the ligands. Moreover, Selicib, Withferin A, Curcumin

has also shown potential binding energies. So, this combination of natural compounds can be further explored using more computational aided drug designing softwares.

Receptor	Ligand	Binding Energy (kcal/mol)
HER2	Roscovitine (Seliciclib)	-9.05
	Rutin	-9.45
Estrogen receptor (ERα)	Withferin A	-8.86
	Rutin	-9.39
	Curcumin	-8.54
	Selicib	-9.04
Hepatocyte growth factor receptor- HGFR/c-Met	Withferin A	-8.49
	Curcumin	-9.03
	Rutin	-10.23
	Selicib	-8.71
Aryl hydrocarbon receptor (AhR)	Withferin A	-8.22
	Curcumin	-8.15
	Rutin	-8.74
	Selicib	-9.47
Integrin alpha V beta 6	Curcumin	-8.28
	Rutin	-8.87
	Selicib	-8.69

Table 7 : Table showing ligands with most effective binding energy. Hence, combination of Roscovitine (Seliciclib), Rutin, Withferin A and Curcumin is proposed for further studies. It shows that Rutin is showing highest binding efficiency with all these receptors among all the ligands. Moreover, Selicib, Withferin A, Curcumin has also shown potential binding energies. So, this combination of natural compounds can be further explored using more computational aided drug designing softwares.

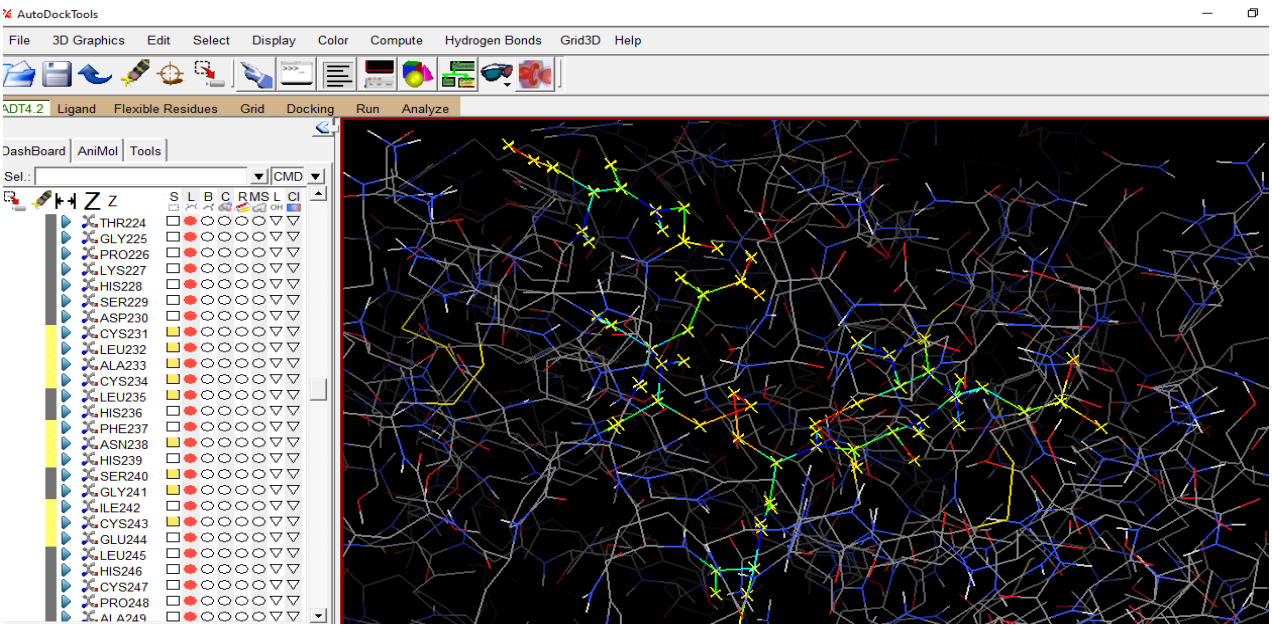


Figure 8: HER2 Protein preparation and Docking simulations by selecting active sites/inhibitory sites and generating grid maps using AutoDock tools 1.5.6. Here, binding sites are selected manually using previous liturature reviews. This is needed for protein preparation for performing molecular docking simulations. Polar hydrogens, and charges are also added in this process.

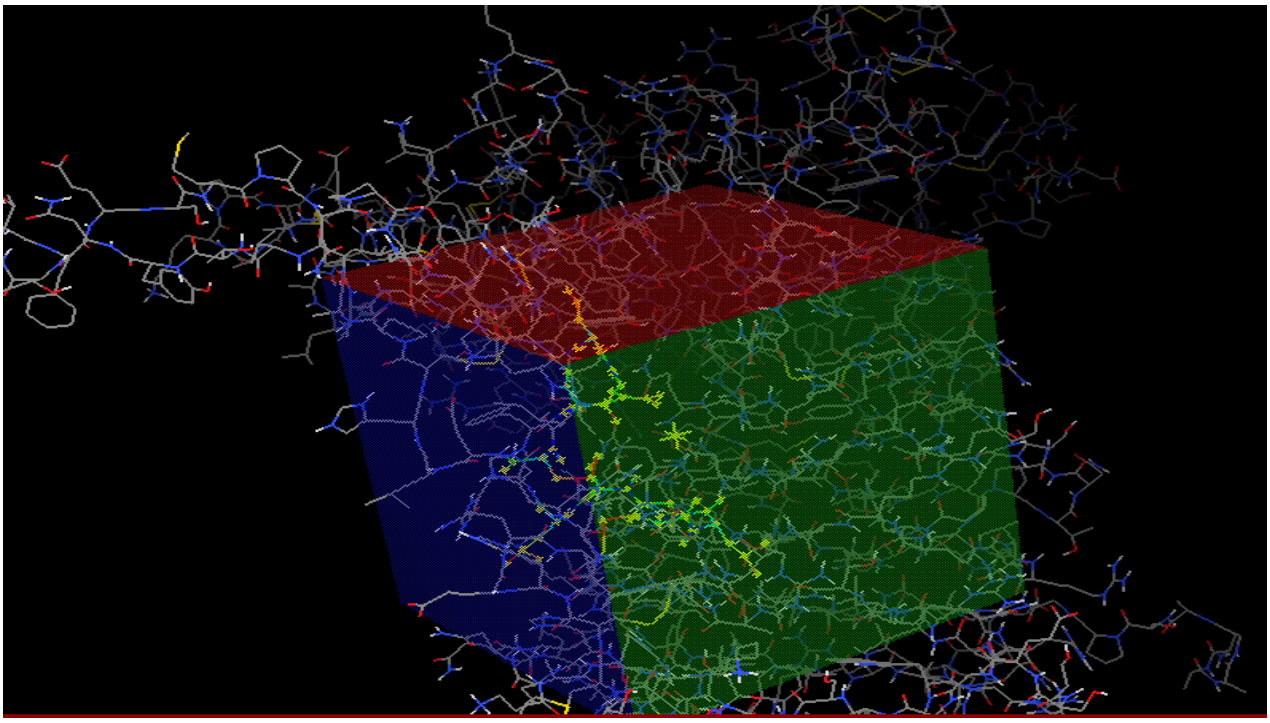


Figure 9: HER2 Protein preparation and Docking simulations by selecting active sites/inhibitory sites and generating grid maps using AutoDock tools 1.5.6. This colored grid box is arranged in a way, so that all selected inhibitory sites are covered within the box, thereby targeting the ligand to this particluar site only.

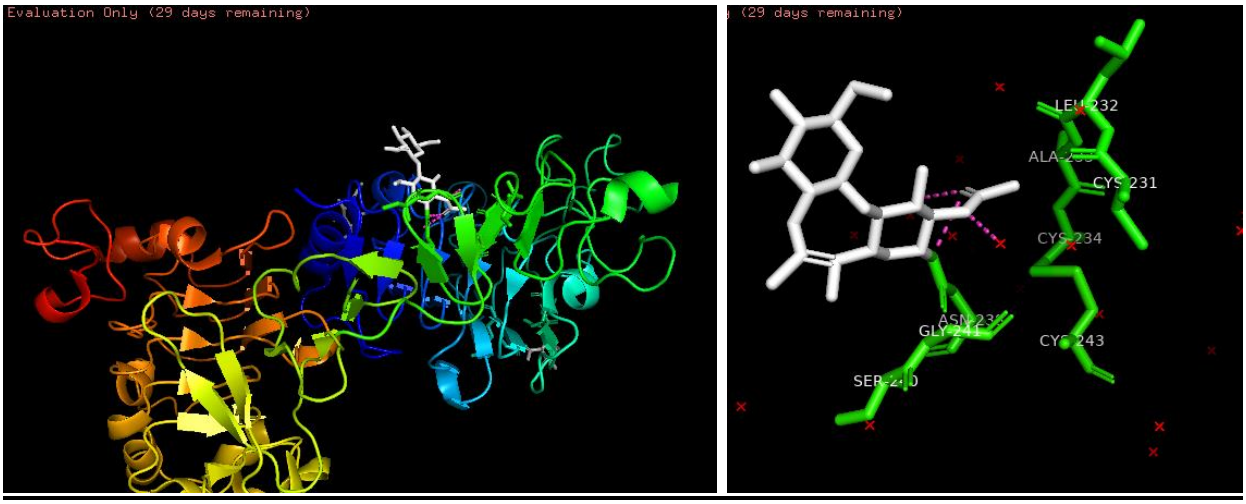
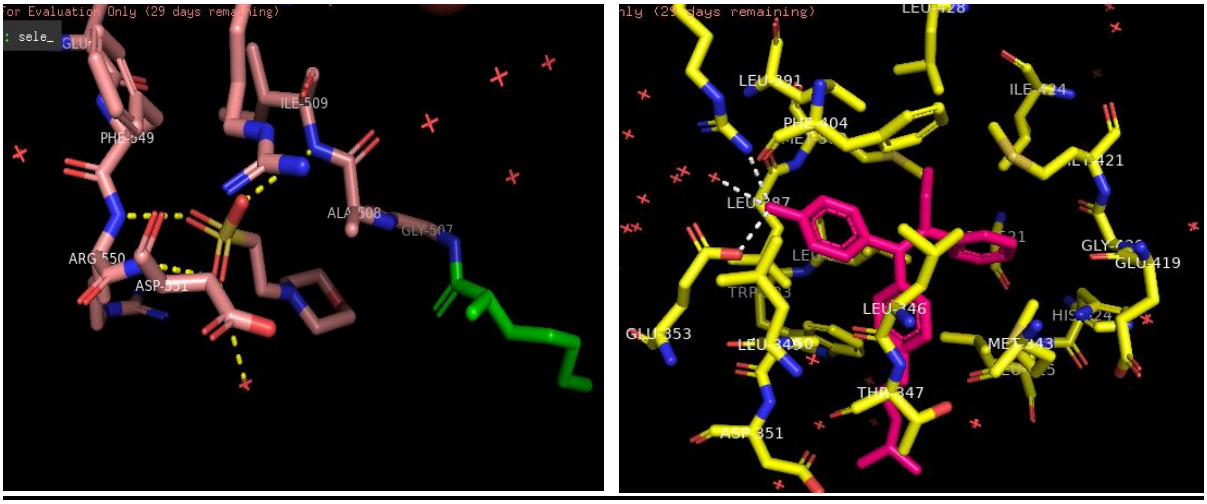


Figure 10: Study interactions of Rutin with amino acid residues of HER2 using PyMol 2.3.2. Potential binding can be infered by identifying the AA residues.



A **B**

Figure 11: Study interactions of A. Curcumin with amino acid residues of Estrogen receptor (ERα) and B. Withferin A with amino acid residues of *Integrin alpha V beta 6* using PyMol 2.3.2. Potential binding can be infered by identifying the AA residues.

Receptor	Binding/Inhibitory sites	Effective ligand bounded	Binding sites common to Receptor Inh. sites
HER2	LEU 235, ALA 233, CYS 234, ASN 238, CYS 243, CYS 231, SER 240, GLY 241, ASN 166, ASN 47, PRO, 45, GLN 135, ARG, 136, ASP 164	Rutin	CYS 231, ALA 233, LEU 235, SER 420
Estrogen receptor (ERα)	GLY 420, GLY 521, MET 343, ALA 350, ASP 351, TRP 383, THR 347, LEU 346, GLU 419, PHE 404, LEU 349, ARG 394	Withferin A	LEU 346, GLU 419
Hepatocyte growth factor receptor-HGFR/c-Met	PHE 1223, MET 1211, MET 1131, ILE 1130, PHE 1134, PHE 1200, LEU 1195	Rutin	PHE 1134, ILE 1130
Aryl hydrocarbon receptor (AhR)	MET 226, GLY 227, LYS 230, LEU 232, ILE 270, GLU 268	Selicib	ILE 270
Integrin alpha V beta 6	PHE 549, ILE 509, ARG 550, GLU 548, ALA 508, GLU 548, GLY 507, ARG 550, ASP 551, ARG 511, LYS 506, GLY 507	Curcumin	ARG 550, ALA 508, GLY 507

Table 8: Common binding AA residues of Receptors and ligands indicating Rutin as strongly bound natural agent to HER2 and HGFR with more AA residues. Other lignads also showing potential binding. Initially inhibitory AA binding residues are found using literature reviews, then prepared molecular docking file from AutoDock is put in PyMol for study interaction. This table infers that all these ligands have some of the actual binding residues with receptors. Further studies using more computer aided drug designing softwares and more wet lab experiments are needed to explore its efficiency.

3. NANOPARTICLE SYNTHESIS

Figure 12 : BSA nanoparticle solution after adding ethanol dropwise and cross-linking agent, glycerol.

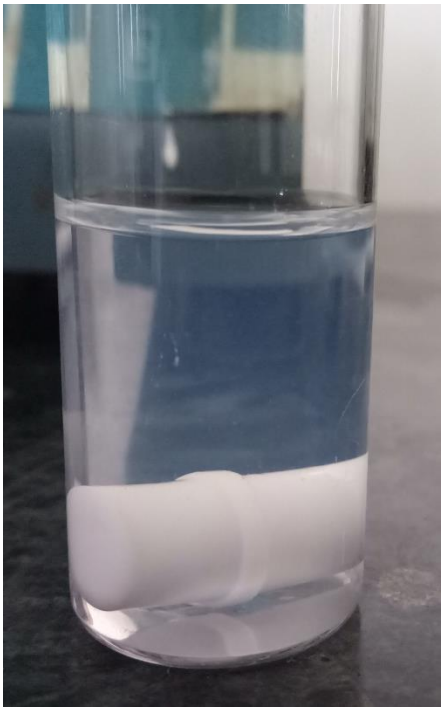


Figure 13 : BSA nanoparticle solution after centrifugation and redispersion of pellet in DI water. BSA conc.-50mg, Volume of ethanol used- 8ml. In most cases, 8ml ethanol is considered as appropriate for effective turbidity of solution. Further optimizations using different concentrations of BSA and volume of ethanol are needed for effective size.

Sample No.	Concentration of BSA added	Volume of ethanol added (ml)	Cross linker used	Size (nm)	Charge (mV)	Absorbance
1	50mg	8	None	1000	-78.0	0.786
2	50mg	8	Glycerol (Not centrifuged)	208	-72.0	0.448
3	50mg	8	UV+ Glucose	4000	-59.7	0.359
4	50mg	8	Glycerol	255	-95.2	0.334
5	30mg	8	Glycerol	190	-69.7	0.260
6	60mg	8	Glycerol	300	-112	0.564
7	50mg	8	Glycerol	235	-74.3	0.389
8	50mg	8	Glycerol	160	-100	0.312
9	30mg	10	Glycerol	-	-	0.247
10	50mg	10	Glycerol	-	-	0.126
11	20mg	10	Glycerol	-	-	0.481
12	50mg	12	Glycerol	-	-	0.396

Table 9: Size and charge estimation of Samples of BSA Np at different concentrations, different volumes of ethanol added, and different cross-linkers used. BSA at 50mg conc. shows the effective size of 160nm, having a charge of -100mV. In most cases, 8ml ethanol is considered as appropriate for effective turbidity of solution. Further optimizations using different concentrations of BSA and volume of ethanol are needed for effective size. More diluted solution may give more effective size.

GRAPHS

1. Size

In zetasizer and zetapotential, three runs take place for a particular sample and an average value is noted. Here three samples were selected, giving relevant results and their cumulative size distribution was plotted using SigmaPlot 2000 software.

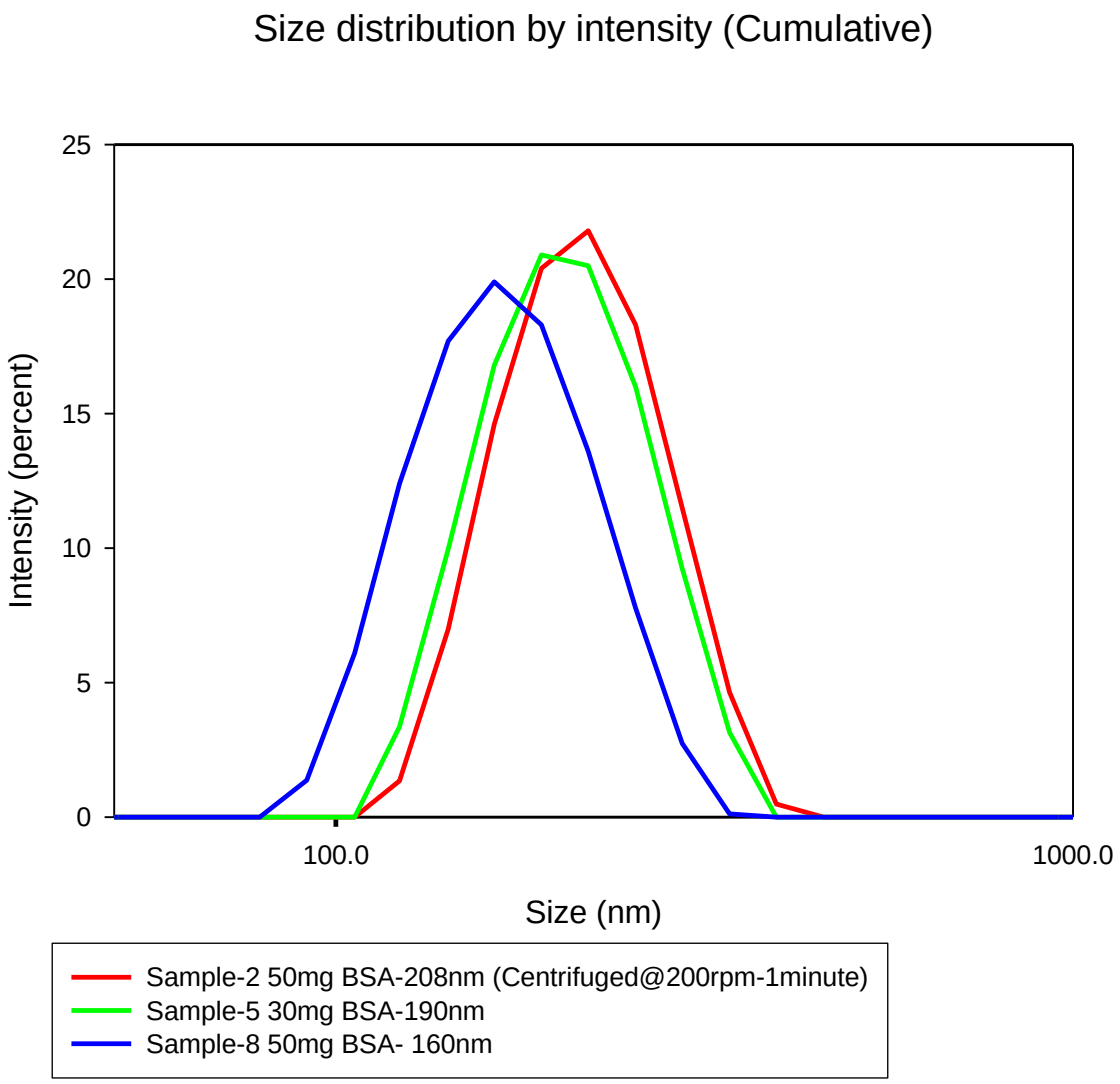


Figure 14: Cumulative size distribution for sample-2, 5 and 8, showing 208nm, 190nm, and 160nm size respectively. This graph shows Further optimizations using different

concentrations of BSA and volume of ethanol are needed for effective size. More diluted solution may give more effective size.

2. **Charge estimation using zetapotential**

Sample-5 (-69.7mV)

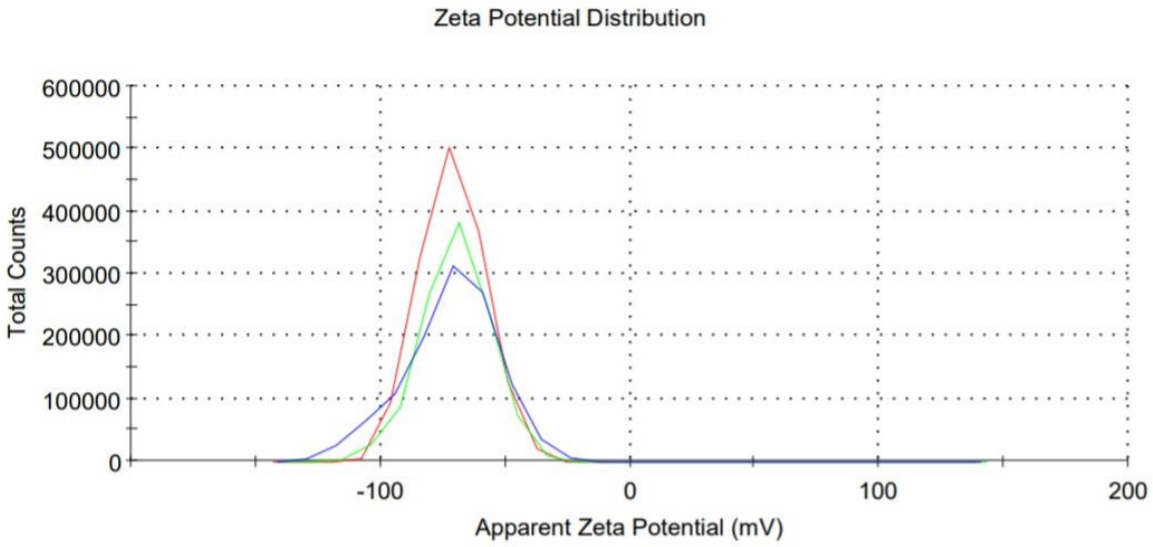


Figure: 15 This graph shows that sample 5 having 190nm size, possess asurface charge of -69.7mV. This shows that particle is hydrophilic. There are three runs take place in zetasizer and an average value is noted. The hydrophilicity of nanoparticle shows that it can be loaded with concerned drug effectively.

Sample- 8 (-100mV)

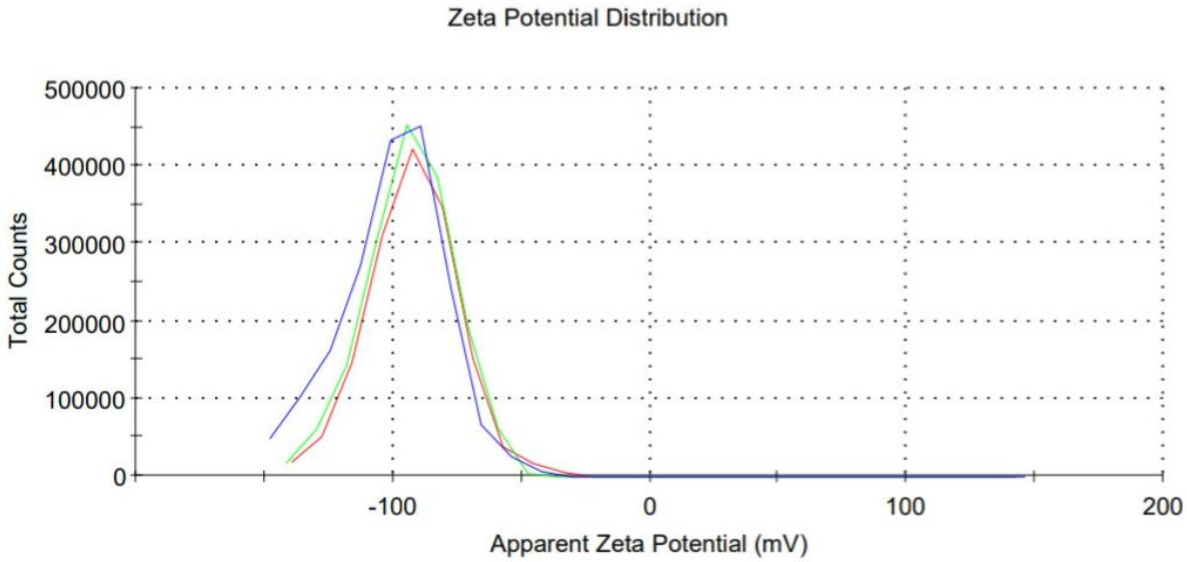


Figure 16: **This graph shows that sample 8 having 160nm size, possess asurface charge of -100mV. This shows that particle is hydrophilic. There are three runs take place in zetasizer and an average value is noted. The hydrophilicity of nanoparticle shows that it can be loaded with concerned drug effectively.**

CONCLUSION

Cancer is a multifactorial disease, caused by several factors and there are numerous receptors and pathways involved in its proliferation. In this research, different biogenic compounds and their associated receptors were screened using literature review and reserches conducted previously. Structural informations of proteins and ligands were obtained from ZINC15 database and IMPPAT database. Thereafter, ligand preparation and protein preparation was done to undergo molecular docking simulations using AutoDock tools 1.5.6 and AutoDock Vina softwares. Then, the binding sites were identified using PyMol 2.3.2. Here, the combination of **Withferin A, Selicib, Curcumin, Rutin** was observed to show highest binding energies with the selected breast cancer receptors, which can be further explored for its combinatorial efficiency in molecular dynamics studies. Since these selected receptors are key receptors in clinical expression of the disease, therefore these combinatorial drugs can be useful. Moreover, BSA nanoparticles for effective drug delivery systems were synthesized in this project of size **160nm and -100mV** charge on which more optimizations are needed. Thus, further studies on these compounds as well as synergistic applications in animal models are required to prove their combinatorial efficiency and their potential to act as a combinatorial therapeutic agents against cancer.

FUTURE PROSPECTS

Facing the worldwide challenges associated with several cancers, combinatorial therapies have been developed in recent years for targeting cancers in multiple ways. The improvement in bioinformatics tools for evaluating the predictions of designed combinatorial drugs is very useful in computer aided drug design strategies. Applications of CADD (Computer aided Drug designing), High-throughput screening, (QSTR) Quantitative structure-toxicity relationship, Homology modeling, SBDD (Structure based Drug design), etc. may mitigate some of the problems associated with drug development and lower the costs associated. In the current project, we designed and proposed a combinatorial natural drug therapy for Breast cancer using molecular docking simulations which was conjugated with nanoparticles for development of effective drug delivery systems. Thus, further studies on these compounds through molecular dynamic simulations, as well as in-vivo experiments are required to prove their synergistic efficacy and their potential to act as a combinatorial therapeutic agents against cancer. Moreover, in future to combat the challenges faced by combinatorial therapies for cancer, a better understanding of tumor biology, multi-disciplinary evaluations and collaborations, cost-effectiveness goals are needed.

REFERENCES

[1] P. B.-P. C. I. S. [Internet] and undefined 2006, "Cancer Genetics Overview (PDQ®)," *ncbi.nlm.nih.gov*, Accessed: Jun. 25, 2020. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK65761/.

[2] "Human genetic disease - Genetics of cancer | Britannica." https://www.britannica.com/science/human-genetic-disease/Genetics-of-cancer (accessed Jun. 25, 2020).

[3] R. B. Mokhtari *et al.*, "Combination therapy in combating cancer," *Oncotarget*, vol. 8, no. 23. Impact Journals LLC, pp. 38022–38043, 2017, doi: 10.18632/oncotarget.16723.

[4] T. N. Aung, Z. Qu, R. D. Kortschak, and D. L. Adelson, "Understanding the effectiveness of natural compound mixtures in cancer through their molecular mode of action," *International Journal of Molecular Sciences*, vol. 18, no. 3. MDPI AG, Mar. 17, 2017, doi: 10.3390/ijms18030656.

[5] A. Agbarya, N. Ruimi, R. Epelbaum, E. Ben-Arye, and J. Mahajna, "Natural products as potential cancer therapy enhancers: A preclinical update," *SAGE Open Med.*, vol. 2, p. 205031211454692, Jan. 2014, doi: 10.1177/2050312114546924.

[6] G. M. Cooper, "The Development and Causes of Cancer," 2000, Accessed: Jun. 29, 2020. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK9963/.

[7] Z. Momenimovahed and H. Salehiniya, "Epidemiological characteristics of and risk factors for breast cancer in the world," *Breast Cancer: Targets and Therapy*, vol. 11. Dove Medical Press Ltd., pp. 151–164, 2019, doi: 10.2147/BCTT.S176070.

[8] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2020," *CA. Cancer J. Clin.*, vol. 70, no. 1, pp. 7–30, Jan. 2020, doi: 10.3322/caac.21590.

[9] S. Rajpal, A. Kumar, and W. Joe, "Economic burden of cancer in India: Evidence from cross-sectional nationally representative household survey, 2014," *PLoS One*, vol. 13, no. 2, Feb. 2018, doi: 10.1371/journal.pone.0193320.

[10] "Cancer, Breast - PubMed." https://pubmed.ncbi.nlm.nih.gov/29493913/ (accessed Jun. 25, 2020).

[11] M. Arslan, H. S. Küçükerdem, H. Can, and E. Tarcan, "Retrospective Analysis of Women with Only Mastalgia.," *J. breast Heal.*, vol. 12, no. 4, pp. 151–154, Oct. 2016, doi:

10.5152/tjbh.2016.2944.

[12] T. Baudino, “Targeted Cancer Therapy: The Next Generation of Cancer Treatment,” *Curr. Drug Discov. Technol.*, vol. 12, no. 1, pp. 3–20, Aug. 2015, doi: 10.2174/1570163812666150602144310.

[13] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter, “Cancer Treatment: Present and Future,” 2002, Accessed: Jun. 29, 2020. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK26811/.

[14] L. Nakashima, “Evolution of cancer treatment and evolving challenges,” *Healthc. Manag. Forum*, vol. 31, no. 1, pp. 26–28, Jan. 2018, doi: 10.1177/0840470417722568.

[15] C. Pucci, C. Martinelli, and G. Ciofani, “Innovative approaches for cancer treatment: Current perspectives and new challenges,” *ecancermedicalscience*, vol. 13. ecancer Global Foundation, Sep. 10, 2019, doi: 10.3332/ecancer.2019.961.

[16] P. P. Zheng, J. Li, and J. M. Kros, “Breakthroughs in modern cancer therapy and elusive cardiotoxicity: Critical research-practice gaps, challenges, and insights,” *Medicinal Research Reviews*, vol. 38, no. 1. John Wiley and Sons Inc., pp. 325–376, Jan. 01, 2018, doi: 10.1002/med.21463.

[17] M. E. Abd El-Hack *et al.*, “Microalgae in modern cancer therapy: Current knowledge,” *Biomedicine and Pharmacotherapy*, vol. 111. Elsevier Masson SAS, pp. 42–50, Mar. 01, 2019, doi: 10.1016/j.biopha.2018.12.069.

[18] R. R. Caspi, “Immunotherapy of autoimmunity and cancer: The penalty for success,” *Nature Reviews Immunology*, vol. 8, no. 12. NIH Public Access, pp. 970–976, Dec. 2008, doi: 10.1038/nri2438.

[19] R. Baskar, K. A. Lee, R. Yeo, and K. W. Yeoh, “Cancer and radiation therapy: Current advances and future directions,” *International Journal of Medical Sciences*, vol. 9, no. 3. Ivyspring International Publisher, pp. 193–199, Feb. 27, 2012, doi: 10.7150/ijms.3635.

[20] S. hong Chen and G. Lahav, “Two is better than one; toward a rational design of combinatorial therapy,” *Current Opinion in Structural Biology*, vol. 41. Elsevier Ltd, pp. 145–150, Dec. 01, 2016, doi: 10.1016/j.sbi.2016.07.020.

[21] A. M. L. Seca and D. C. G. A. Pinto, “Plant secondary metabolites as anticancer agents: Successes in clinical trials and therapeutic application,” *International Journal of Molecular Sciences*, vol. 19, no. 1. MDPI AG, Jan. 16, 2018, doi: 10.3390/ijms19010263.

[22] A. Lodi *et al.*, “Combinatorial treatment with natural compounds in prostate cancer inhibits prostate tumor growth and leads to key modulations of cancer cell metabolism,” *npj Precis. Oncol.*, vol. 1, no. 1, Dec. 2017, doi: 10.1038/s41698-017-0024-z.

[23] L. K. Caesar and N. B. Cech, “Synergy and antagonism in natural product extracts: When 1 + 1 does not equal 2,” *Natural Product Reports*, vol. 36, no. 6. Royal Society of Chemistry, pp. 869–888, Jun. 01, 2019, doi: 10.1039/c9np00011a.

[24] M. Schenone, V. Dančík, B. K. Wagner, and P. A. Clemons, “Target identification and mechanism of action in chemical biology and drug discovery,” *Nature Chemical Biology*, vol. 9, no. 4. NIH Public Access, pp. 232–240, Apr. 2013, doi: 10.1038/nchembio.1199.

[25] I. Górniak, R. Bartoszewski, and J. Króliczewski, “Comprehensive review of antimicrobial activities of plant flavonoids,” *Phytochemistry Reviews*, vol. 18, no. 1. Springer Netherlands, pp. 241–272, Feb. 15, 2019, doi: 10.1007/s11101-018-9591-z.

[26] V. Shriram, T. Khare, R. Bhagwat, R. Shukla, and V. Kumar, “Inhibiting bacterial drug efflux pumps via phyto-therapeutics to combat threatening antimicrobial resistance,” *Front. Microbiol.*, vol. 9, no. DEC, Dec. 2018, doi: 10.3389/fmicb.2018.02990.

[27] N. Manoharan, O. Nair, N. K. Shukla, and G. K. Rath, “Descriptive epidemiology of female breast cancer in Delhi, India,” *Asian Pacific J. Cancer Prev.*, vol. 18, no. 4, pp. 1015–1018, Apr. 2017, doi: 10.22034/APJCP.2017.18.4.1015.

[28] F. A. Fisusi and E. O. Akala, “Drug Combinations in Breast Cancer Therapy,” *Pharm. Nanotechnol.*, vol. 7, no. 1, pp. 3–23, Jan. 2019, doi: 10.2174/2211738507666190122111224.

[29] “Response rate for each metastatic site | Download Table.” https://www.researchgate.net/figure/Response-rate-for-each-metastatic-site_tbl3_23996513 (accessed Jun. 25, 2020).

[30] “First line chemotherapy of metastatic breast cancer | Request PDF.” https://www.researchgate.net/publication/6978142_First_line_chemotherapy_of_metastatic_breast_cancer (accessed Jun. 25, 2020).

[31] J. Wahler and N. Suh, “Targeting HER2 Positive Breast Cancer with Chemopreventive Agents,” *Current Pharmacology Reports*, vol. 1, no. 5. Springer International Publishing, pp. 324–335, Oct. 01, 2015, doi: 10.1007/s40495-015-0040-z.

[32] A. Rani, J. Stebbing, G. Giamas, and J. Murphy, “Endocrine resistance in hormone

receptor positive breast cancer–from mechanism to therapy," *Frontiers in Endocrinology*, vol. 10, no. MAY. Frontiers Media S.A., 2019, doi: 10.3389/fendo.2019.00245.

[33] T. Tanos, L. J. Rojo, P. Echeverria, and C. Brisken, "ER and PR signaling nodes during mammary gland development," *Breast Cancer Res.*, vol. 14, no. 4, p. 210, Aug. 2012, doi: 10.1186/bcr3166.

[34] H. Mirtavoos-Mahyari, A. Khosravi, and Z. Esfahani-Monfared, "Human Epidermal Growth Factor Receptor 2 and Estrogen Receptor Status in Respect to Tumor Characteristics in Non-Metastatic Breast Cancer," *Tanaffos*, vol. 13, no. 1, p. 26, 2014, Accessed: Jun. 25, 2020. [Online]. Available: /pmc/articles/PMC4153272/?report=abstract.

[35] C. Gutierrez and R. Schiff, "HER2: Biology, detection, and clinical implications," *Arch. Pathol. Lab. Med.*, vol. 135, no. 1, pp. 55–62, Jan. 2011, doi: 10.1043/2010-0454-RAR.1.

[36] T. V. Sekar, A. Dhanabalan, and R. Paulmurugan, "Imaging Cellular Receptors in Breast Cancers: An Overview," *Curr. Pharm. Biotechnol.*, vol. 12, no. 4, pp. 508–527, 2011, doi: 10.2174/138920111795164039.

[37] M. J. Wieduwilt and M. M. Moasser, "The epidermal growth factor receptor family: Biology driving targeted therapeutics," *Cellular and Molecular Life Sciences*, vol. 65, no. 10. NIH Public Access, pp. 1566–1584, May 2008, doi: 10.1007/s00018-008-7440-8.

[38] J. L. Hsu and M. C. Hung, "The role of HER2, EGFR, and other receptor tyrosine kinases in breast cancer," *Cancer Metastasis Rev.*, vol. 35, no. 4, pp. 575–588, Dec. 2016, doi: 10.1007/s10555-016-9649-6.

[39] M. Jeon, S. Kim, S. Park, H. Lee, and J. Kang, "In silico drug combination discovery for personalized cancer therapy," *BMC Syst. Biol.*, vol. 12, Mar. 2018, doi: 10.1186/s12918-018-0546-1.

[40] N. O. Carragher, A. Unciti-Broceta, and D. A. Cameron, "Advancing cancer drug discovery towards more agile development of targeted combination therapies," *Future Medicinal Chemistry*, vol. 4, no. 1. pp. 87–105, Jan. 2012, doi: 10.4155/fmc.11.169.

[41] R. Celebi, O. Bear Don't Walk, R. Movva, S. Alpsoy, and M. Dumontier, "In-silico Prediction of Synergistic Anti-Cancer Drug Combinations Using Multi-omics Data," *Sci. Rep.*, vol. 9, no. 1, Dec. 2019, doi: 10.1038/s41598-019-45236-6.

[42] M. Jeon, S. Kim, S. Park, H. Lee, and J. Kang, "In silico drug combination discovery for

personalized cancer therapy," *BMC Syst. Biol.*, vol. 12, no. Suppl 2, 2018, doi: 10.1186/s12918-018-0546-1.

[43] E. G. Puigjané, "A new ligand-based approach to virtual screening and profiling of large chemical libraries," p. 172, 2008.

[44] L. Pinzi and G. Rastelli, "Molecular docking: Shifting paradigms in drug discovery," *International Journal of Molecular Sciences*, vol. 20, no. 18. MDPI AG, Sep. 01, 2019, doi: 10.3390/ijms20184331.

[45] S.-S. Ou-Yang, J.-Y. Lu, X.-Q. Kong, Z.-J. Liang, C. Luo, and H. Jiang, "Computational drug discovery.," *Acta Pharmacol. Sin.*, vol. 33, no. 9, pp. 1131–40, Sep. 2012, doi: 10.1038/aps.2012.109.

[46] L. Pintilie and A. Stefaniu, " In Silico Drug Design and Molecular Docking Studies of Some Quinolone Compound ," in *Molecular Docking and Molecular Dynamics*, IntechOpen, 2019.

[47] J. Beik *et al.*, "Gold nanoparticles in combinatorial cancer therapy strategies," *Coordination Chemistry Reviews*, vol. 387. Elsevier B.V., pp. 299–324, May 15, 2019, doi: 10.1016/j.ccr.2019.02.025.

[48] R. Arvizo, R. Bhattacharya, and P. Mukherjee, "Gold nanoparticles: Opportunities and challenges in nanomedicine," *Expert Opinion on Drug Delivery*, vol. 7, no. 6. NIH Public Access, pp. 753–763, Jun. 2010, doi: 10.1517/17425241003777010.

[49] K. N. Islam *et al.*, "A novel method for the synthesis of calcium carbonate (aragonite) nanoparticles from cockle shells," *Powder Technol.*, vol. 235, pp. 70–75, Feb. 2013, doi: 10.1016/j.powtec.2012.09.041.

[50] S. Shen *et al.*, "A New Route to Liposil Formation by an Interfacial Sol-Gel Process Confined by Lipid Bilayer," *ACS Appl. Mater. Interfaces*, vol. 7, no. 45, pp. 25039–25044, Jul. 2015, doi: 10.1021/acsami.5b01386.

[51] A. MaHam, Z. Tang, H. Wu, J. Wang, and Y. Lin, "Protein-based nanomedicine platforms for drug delivery," *Small*, vol. 5, no. 15. Small, pp. 1706–1721, Aug. 03, 2009, doi: 10.1002/smll.200801602.

[52] F. F. An and X. H. Zhang, "Strategies for preparing albumin-based nanoparticles for multifunctional bioimaging and drug delivery," *Theranostics*, vol. 7, no. 15. Ivyspring International Publisher, pp. 3667–3689, 2017, doi: 10.7150/thno.19365.

[53] M. Karimi *et al.*, “Albumin nanostructures as advanced drug delivery systems,” *Expert Opinion on Drug Delivery*, vol. 13, no. 11. Taylor and Francis Ltd, pp. 1609–1623, Nov. 01, 2016, doi: 10.1080/17425247.2016.1193149.

[54] C. Weber, J. Kreuter, and K. Langer, “Desolvation process and surface characteristics of HSA-nanoparticles,” *Int. J. Pharm.*, vol. 196, no. 2, pp. 197–200, Mar. 2000, doi: 10.1016/S0378-5173(99)00420-2.

[55] K. Langer, S. Balthasar, V. Vogel, N. Dinauer, H. Von Briesen, and D. Schubert, “Optimization of the preparation process for human serum albumin (HSA) nanoparticles,” *Int. J. Pharm.*, vol. 257, no. 1–2, pp. 169–180, May 2003, doi: 10.1016/S0378-5173(03)00134-0.

[56] S. Cosconati, S. Forli, A. L. Perryman, R. Harris, D. S. Goodsell, and A. J. Olson, “Virtual screening with AutoDock: Theory and practice,” *Expert Opinion on Drug Discovery*, vol. 5, no. 6. NIH Public Access, pp. 597–607, Jun. 2010, doi: 10.1517/17460441.2010.484460.

[57] S. Forli, R. Huey, M. E. Pique, M. F. Sanner, D. S. Goodsell, and A. J. Olson, “Computational protein-ligand docking and virtual drug screening with the AutoDock suite,” *Nat. Protoc.*, vol. 11, no. 5, pp. 905–919, May 2016, doi: 10.1038/nprot.2016.051.

[58] N. T. Nguyen *et al.*, “Autodock Vina Adopts More Accurate Binding Poses but Autodock4 Forms Better Binding Affinity,” *J. Chem. Inf. Model.*, vol. 60, no. 1, pp. 204–211, Jan. 2020, doi: 10.1021/acs.jcim.9b00778.

[59] G. M. Morris, R. Huey, and A. J. Olson, “UNIT using AutoDock for ligand-receptor docking,” *Current Protocols in Bioinformatics*, vol. Chapter 8, no. SUPPL. 24. John Wiley and Sons Inc., 2008, doi: 10.1002/0471250953.bi0814s24.

[60] B. H. M. Mooers, “Simplifying and enhancing the use of PyMOL with horizontal scripts,” *Protein Sci.*, vol. 25, no. 10, pp. 1873–1882, Oct. 2016, doi: 10.1002/pro.2996.

[61] R. E. Rigsby and A. B. Parker, “Using the PyMOL application to reinforce visual understanding of protein structure,” *Biochem. Mol. Biol. Educ.*, vol. 44, no. 5, pp. 433–437, Sep. 2016, doi: 10.1002/bmb.20966.