"Identification of Therapeutic Compounds for Breast Cancer: An In-Silico Approach Exploring EGFR Targeting Interactions of Kinase Inhibitors and Bioactive Phytocompounds"

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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CANDIDATE'S DECLARATION

I **Srishti Satija (23/MSCBIO/48)** hereby certify that the scientific work which is being demonstrated here in this thesis named "**Identification of Therapeutic Compounds for Breast Cancer: An In-Silico Approach Exploring EGFR Targeting Interactions of Kinase Inhibitors and Bioactive Phytocompounds**" is in contribution to the requirements for the award of the Master of Science degree submitted by me to the Department of Biotechnology, Delhi Technological University, New Delhi-42, India is a credible record of my own work carried out during the time period between January 2025 to May 2025 under the active supervision of our supervisor Dr. Asmita Das. The matter demonstrated in this thesis has not been presented and submitted by me for award of any degree from this institute or any other institute.

My conference paper has cleared its review round and has been accepted in a Scopus Indexed journal whose details are as follows:

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CERTIFICATE BY THE SUPERVISOR

This is to certify that the thesis entitled "**Identification of Therapeutic Compounds for Breast Cancer: An In-Silico Approach Exploring EGFR Targeting Interactions of Kinase Inhibitors and Bioactive Phytocompounds**" submitted by Srishti Satija (23/MSCBIO/48) to the Delhi Technological University (DTU), New Delhi-42, in partial fulfilment of the requirements for the award of the Master's degree, is the outcome of authentic and sincere research work, performed by her in the Immunotherapeutics Laboratory, Department of Biotechnology under my guidance, support and mentorship. To the best of my knowledge, the matter presented in this thesis has not been submitted to any other university or institution with the objective of receiving any degree or diploma.

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ABSTRACT

Introduction - One of the leading contributors in cancer-related mortality within female population is Breast Cancer (BC), which is a progressive disease and a lifelong susceptibility among females characterized by unrestrained cell proliferation, metastasizing to different parts of the body, impacting overall health and organ function. It stands out as the most predominant malignancies among female population across the globe, with millions of fresh cases getting diagnosed each year. Given the worldwide predominance of breast cancer, there is a crucial demand for innovative, potent and effective treatment strategies. Several overexpressed genes have been discovered in association with breast cancer, among them is EGFR (Epidermal Growth Factor Receptor). EGFR demonstrates pivotal role in progression or advancement of tumor and resistance to therapy. EGFR inhibition has been reported for therapeutic purpose in several variants of BC especially involving triple negative breast cancer (TNBC) making it one of the promising target molecule.

Methodology - In this investigation, we executed the docking of EGFR protein utilizing FDA approved kinase inhibitor effective against cancers, alongside certain phytocompounds with anti-cancer properties as the target ligands, aiming for identification of potential therapeutic drug and comparative analysis among the natural compounds and TKIs. Molecular docking was executed utilizing PyRx and Biovia Discovery studio software for interpreting the potent drug candidate among the chosen ligands. Lipinski's criteria of 5 and toxicity analysis was employed for assessing the drug-likeness and pharmacokinetic characteristic against breast cancer activity. The standard reference compound used in response to EGFR were Panaxadiol, Lapatinib, and Gefitinib.

Results - Our investigation revealed, Psoralidin, Capmatinib, and Tucatinib as best docked and stably bound upon docking with chosen breast cancer target protein and TKIs performed better than phytocompounds.

Limitations - The study proposes that the shortlisted phytocompounds and kinase inhibitors must undergo further in vitro and in vivo evaluation to unfold their effectiveness against breast cancer, demonstrating their potency to downregulate EGFR-linked cascades.

Keywords- Breast Cancer, EGFR receptor, Bioactive Phytocompounds, FDA-recognized Tyr kinase inhibitors, Docking, Pharmacokinetic Assessment

LIST OF PUBLICATIONS

1. Our conference paper “In – Silico Discovery of Druga for Breast Cancer Therapy: Investigating Kinase Inhibitors and Phytocompounds Interactions Targeting EGFR Protein” has cleared the review round and has been accepted at the International Conference on Emerging Technologies in Science and Engineering (ICETSE-2025), which will be held in the month of June, 2025.

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LIST OF SYMBOLS & ABBREVIATIONS

Abbreviation & Symbol	Expanded Form
ADME	Absorption, Distribution, Metabolism, Excretion
ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicity
AI	Artificial Intelligence
AKT	Akt serine/threonine kinase
ATP	Adenosine Triphosphatase
BBB	Blood Brain Barrier
BC	Breast Cancer
BLAST	Basic Local Alignment Search Tool
CID	PubChem ID
CML	Chronic Myelogenous Leukaemia
EGCG	Epigallocatechin Gallate
EGF	Epidermal Growth Factor Receptor
EGFR	Epidermal Growth Factor Receptor
ER	Estrogen
ESOL	Estimated Solubility
FASTA	Fast-All
FDA	Food and Drug Administration
GI	Gastrointestinal
HBA	Hydrogen-Bond Acceptor
HBD	Hydrogen-Bond Donor
HCC	Hepatocellular Carcinoma
HER-2	Human Epidermal Growth Factor Receptor 2
IBC	Inflammatory Breast Cancer
ICMR	Indian Council of Medical Research
IMPPAT	Indian Medicinal Plants, Phytochemistry and Therapeutics
JAK	Janus Kinase
JNK	Jun-N-Terminal Kinase
MAPK	Mitogen-Activated Protein Kinase
MD	Molecular Docking
ML	Machine Learning
MW	Molecular Weight

NCBI	National Centre for Biotechnology Information
NF-Kβ	Nuclear Factor Kappa-B
NIH	National Institutes of Health
NLM	National Library of Medicine
NSCLC	Non-Small Cell Lung Cancer
PD	Panaxadiol
PDB	Protein Data Bank
PR	Progesterone
RB	Rotatable Bonds
RCC	Renal Cell Carcinoma
SDF	Structure Data File
SGI	Structural Bioinformatics Group
SMILES	Simplified Molecular Input Line Entry System
STAT	Signal Transducer and Activator of Transcription
TKI	Tyrosine Kinase Inhibitor
TNBC	Triple Negative
TPSA	Topological Polar Surface Area
VEGFR	Vascular Endothelial Growth Factor

CHAPTER 1
INTRODUCTION

1.1 INTRODUCTION

Breast Cancer (BC) stands as a serious concern for global health, characterized by aberrant growth of cancerous cell within tissue of breast[1]. Majorly it affects female population, but can also affect male population but rarely[2], [3] and exists in varied subtypes with different range of severity[4] [5]. It can impact women health severely if not diagnosed and treated within time as it exhibits metastasis i.e, spreads within body, thus degrading overall health [6].

Breast cancer occurs in varied subtypes involving hormone receptor-positive, HER-2 positive TNBC, IBC, and various others each having unique attributes, prognosis, and response towards treatment[4]. Among them, TNBC stands as the most aggressive subtypes characterized by absence of ER, PR, HER2 receptors [7]. Aggressive behaviour within BC subtypes involving TNBC, IBC and basal-like forms and, have witnessed overexpression of oncogenic marker EGFR aiding in progression of tumor mass and offering resistance towards conventional standard treatment [8], [9]. EGFR overexpression leads to activation of intracellular downstream signalling cascades resulting in cancerous cell progression and proliferation, increased metastasis as well as enhanced angiogenesis [10] [11]. Attacking EGFR provides a therapeutic strategy in overcoming cancer.

Developing EGFR inhibitors is a potent approach in direction of cancer treatment but their effectiveness within breast cancer, to be specific in TNBC stays limited. Earlier detected therapeutic compounds offered modest outcomes due to lowered rates of response [12]. Therefore, further intensive research for identifying effective and potent EGFR inhibitors is crucial.

Our investigation revealed Psoralidin, Capmatinib, and Tucatinib as effective drugs for purpose of downregulating intracellular EGFR-linked cascades in tackling advancement of BC. Computational approach of biomolecular docking was conducted to assess binding energy value and interactions of promising drug candidates with target EGFR [13] . Combinatorial therapy involving both phytocompounds and TKIs can yield effective therapy strategy. However, further in vitro and in vivo evaluation is necessary to unfold their effectiveness against breast cancer.

1.2 OBJECTIVES OF THE STUDY

The key goals of our present investigation are:

- Assessment of natural bioactive phytocompounds effective against BC
- Assessment of FDA-recognized TKIs showing potent activity against BC

Exhibiting effective functional activity, reduced adverse reactions as well as generating profound impact upon BC receptors utilizing bioinformatics approach leading to identification of efficient therapeutic strategy in response to the disease. Standard established drugs were employed as a reference in order to authenticate or validate results of our assessment.

- Comparing performance of phytocompounds and TKIs against selected target receptor of BC.

CHAPTER 2
LITERATURE REVIEW

2.1 Breast Cancer (BC)

Breast cancer with varied subtypes or forms exhibiting unique molecular properties and distinct clinical behaviour, continues to be categorized as the most predominant as well as risk-laden malignancies diagnosed within the female population across the globe[4], [14], [15]. Millions of new cases are emerging throughout the world with each progressing year, indicating enhanced incidences of cancer occurrence as well as rates of mortality[16]. HER2-positive, Luminal A, Luminal B, and Triple Negative BC are among the several subtypes into which BC is broadly categorized[4], [17].

Incidences of BC has been on a steady surge with each passing year among the female population of India[18]. The number of cases has escalated to such a level that BC forms the most predominant form of cancer among women of India[19] [20]. ICMR organization state that 1 in every twenty-two women of India is highly likely to suffer BC during their journey of life. Trends show a difference within rate of occurrence of BC among rural and urban females[21]. Due to urban lifestyle involving inactive routines, food habits, late pregnancies, as well as decreased breastfeeding, urban females are more prone to BC when compared with rural females[22]. Though incidence of occurrence is more in urban area females , mortality rate is higher among rural females due to absence of adequate medical facility and awareness [23]. Addressing the burden as well as escalating incidences of BC is a crucial step and pressing need to curb the disorder and maintaining the well-being.

BC occurs when genetic mutation disrupts normal functioning within the cell, resulting in an uncontrolled division yielding formation of tumor mass (either benign or malignant in nature)[24]. Via metastasis, tumor mass invades towards different parts in our body either through blood or lymph[25]. Certain key attributes possessed by cancerous cells in BC[26]:

- Unchecked proliferation
- Irresponsive towards cell death
- Persistent blood-vessel build-up
- Invasive
- Bypass apoptosis
- Escape immune system
- Self- propagating

These cancer cells can be put to an end by targeting specific receptors present upon cell surface. Certain subtypes of cancer including TNBC and IBC have been linked either with overexpression or malfunctioning of EGFR, a Tyr kinase transmembrane receptor participating in cancerous cell survival, progression and invasion within other body parts[8] [9]. Targeting of EGFR utilizing Tyr kinase inhibitors as well as phytocompounds can showcase potent prospect among preclinical and clinical investigations via suppression of downstream signalling cascades critical in supporting growth and proliferation among cancerous cells.

Attacking EGFR in subtypes of cancer which showcase its uncontrolled expression can prove promising in the treatment. Inhibiting EGFR can hinder or block growth of tumor, reduced invasion, and aids in overcoming resistance towards traditional therapeutic strategy[27].

2.2 EGFR Protein: Structure, Function, Overexpression

A cell surface bound or transmembrane Tyr kinase receptor, EGFR (also known as ErbB1or HER1) belongs the receptor family of ErbB. HER 2, HER 3, HER 4 belonging to class of receptor ErbB2, ErbB3, and ErbB4 respectively, are among 3 related receptor type included within ErbB family[28] [29]. Upregulated protein EGFR expression though commonly observed within all the cancer subtypes or variants, however more significant overexpression happens in case of TNBC as well as IBC[30] [31]. Estimating around 10-15 % BC cases stands TNBC an aggressive form of BC, characterized via collective absence or lack of ER, PR, EGFR2 (estrogen, progesterone and epidermal growth factor) receptors[32]. Distinct molecular attributes marking TNBC involves an aggressive

Fig 1. 3D Structure of EGFR

behaviour, unique metastatic distribution as well as limited effective targeted therapeutic strategy[30], [32]. IBC stands as another rare but still an aggressive cancer subtype accounting around 1-5% BC cases. One interesting fact regarding IBC occurrence is that it is more prevalent within American African female population[33].

EGFR transmembrane glycoprotein is among the member of ErbB receptor family Tyr kinases[34]. The structure consists of an extracellular ligand-binding region, a transmembrane helix hydrophobic in nature and Tyr kinase domain present intracellularly[35]. Upon association of EGFR with ligands like EGF, it undergoes dimerization either in homodimer or heterodimer configuration[36]. This leads to autophosphorylation among specific Tyr residues within cytoplasmic tail region, initiating multiple pathways including, PKC, MAPK, JAK/STAT, PI3K/AKT/m-TOR and JNK cascades regulating principal cellular mechanism involving cancer survival, advancement, and differentiation[8], [37]. Signalling cascades switched on via EGFR activation ultimately control gene expression as well as coordinate major biological processes which determine fate of cell[38]. Dysregulation among signalling EGFR is implicated within various malignancies involving, breast, colorectal, and lung cancers, which correlates to enhanced survival as well as progression of tumor, poor prognosis, and resistance towards therapy[39].

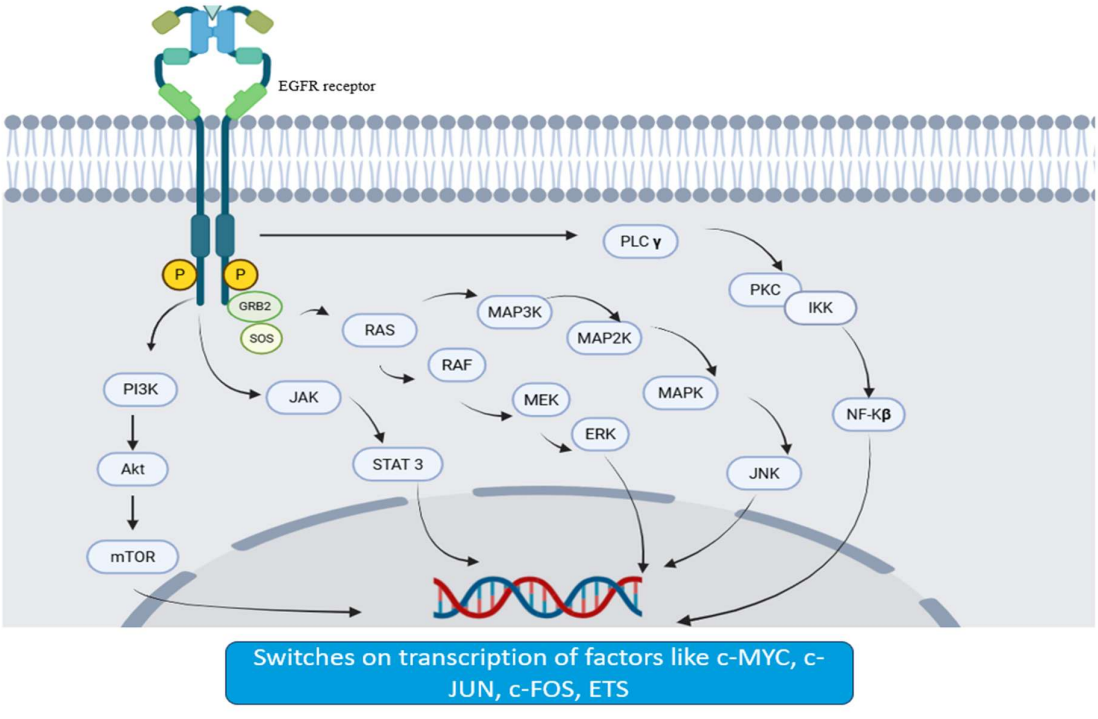


Fig 3. EGFR-linked Signaling Cascades and Transcriptional Activation

Uncontrolled expression among EGFR is associated with increased risk of cancer advancement, reduced survival, greater tumor size as well as reduced differentiation. Activation of downstream signalling pathways as demonstrated in Fig 3, aids to cancerous cell progression and proliferation, increased metastasis as well as enhanced angiogenesis[8], [38], [40].

Targeting receptor protein EGFR utilizing an appropriate and target focused therapeutic can inhibit and further block these signalling cascades, thus hindering cancerous growth. Clinical-trial investigations assessing EGFR-attacking strategy yield a mix of results, furthermore creating a pressing need for gaining more intense insights concerning EGFR's mechanism of resistance and yielding more effective, efficient and impactful strategy for therapy.

Irrespective of the progress in direction of early diagnosis and towards targeted therapeutic strategy, cancer-associated fatality persists to be a leading and major reason behind it. Current investigations highlight contribution of genetic mutations, hormonal influence, and microenvironment of tumor towards advancement of disease as well as response to therapy[41]. Present therapies seem quite expensive, inappropriate therapeutic impact, momentary relief, and hindered via adverse or severe side reactions, resistant to therapy, as well as tumor heterogeneity.

Leveraging computational strategy could screen potential and effective drug candidate which seem faster as well as cost effective approach, reduced dependence upon animal testing and hence aid to improve researcher's success. Observing the sustained adverse impacts of cancer, ineffective therapy and aggressive pattern, this bioinformatics strategy is better to utilize for safety assurance for animals under testing[42], [43].

2.3 Bioactive Phytocompounds

Nature originated bioactive phyto-compounds extracted via plants, are produced as defence mechanism in response to biotic and abiotic stress conditions[44]. Categorized into various classes involving terpenoids, alkaloids, polyphenols, saponins, flavonoids etc. have proven as effective therapeutic option for fighting against cancer [45]. Along with their ecological activity, plant-based chemicals are of considerable interest owing to their increased stability, antioxidant, anti-inflammatory, anti-cancerous, enhanced effectiveness, less toxic as well as increased bioavailability [46][47].

Unlike standard conventional drugs impacting healthy as well as diseased cells, they target specific molecular regions linked with diseased cell and hence effective in reducing side effects, and building of safer, advanced and more effective therapeutic option. When utilized in combination with other drugs, can yields better medical result by improving and increasing efficacy of standard drugs and reduced resistance towards therapy.

Wide variants of isothiocyanates are significantly impactful in preventing cancer[48]. Nature-derived curcuminoids showcasing potent antimicrobial, anticancer, antioxidant, antiarthritic, anti-inflammatory and various other, whose therapeutic impacts have been studied and confirmed to some extent[49]. Curcumin can improve destruction of cancerous cell via aiding sensitivity enhancement of diseased cells in response to drugs including cisplatin as well as paclitaxel[50], [51], [52]. Plant-derived campothecin derived phytochemicals, taxane diterpenoids, vinca alkaloids and epipodophyllotoxin showcase anti-cancerous attributes within clinical settings[46]. Intensive studies suggest role of genistein a key isoflavone belonging to Leguminosae family (involving soybean), might prove effective for cancer prevention as well as treatment[53]. Investigations have revealed green tea catechin namely EGCG belonging to flavonoid class via their antioxidant behaviour, offers protection from oxidative damage inflicted upon biomolecules linked with cancer advancement and development[54]. Immunomodulatory impact of polyphenols helps in determination and knocking-down cancerous cells via antiangiogenic activity[55]. A phytoalexin, resveratrol extracted via grapes exhibits cancer preventive effects[56]. Natural bioactive dietary phytochemicals (phytosterol, flavonoids, stilbenes, carotenoids, phenolic compound) have shown greater potential against cancer[47].

2.4 Tyr Kinase Inhibitors (TKIs)

Tyr Kinase Inhibitors are potent pharmacologic inhibiting and target therapeutic compound aiding blocking of enzyme, tyrosine kinase which catalyse downstream signalling transduction cascades. This enzyme performs major function in controlling cellular activities involving growth, proliferation, differentiation as well metabolism. Dysregulation of Tyr kinase results in development, advancement as well as progression of cancer[57]. TKIs inhibit these activities by targeted binding at ATP-binding domain of Tyr kinase which prevents phosphorylation thus blocking downstream signalling as well as induction of cell death and apoptosis. 50 TKIs till now have been introduced and recognized for cancer by FDA[58]. TKIs introduction have led

to revolution in treatment of malignancies such as CML (chronic myeloid leukemia)[50], RCC (renal cell carcinoma), and NSCLC (non-small cell lung cancer)[59].

Within targeted treatment, TKIs offers more specific, efficient, and effective strategy for fighting cancer when compared with traditional standard therapeutic agents. Upon focusing on specific targeted region associated with disease, TKIs aid to limit the damage inflicted upon healthy cells and decreasing toxicity. Upon combining other therapies along with TKIs, these inhibitors aid in overcoming resistance. They offer specificity, create effective impact and can be tailored according to individual genetic composition, thus showing impact in personalized medicine field.

Multi kinase inhibitor Sorafenib is utilized among high-risk patients of HCC (Hepatocarcinoma) and have proven effective via delaying its progression. Recognized for initial therapy against chronic disease CML are TKIs nilotinib, dasatinib, imatinib mesylate, bosutinib[60]. TKI Capmatinib utilized for metastatic (NSCLC) non-small cell lung cancer has been examined competently and validated[61]. Research suggests oral targeted treatment strategy utilizing imatinib exhibited potency to curb cancer through targeted approach[62]. Successful validation of lapatinib as well as neratinib in response towards breast cancer treatment[63]. Apatinib by attacking VEGFR2 receptor protein in cervical cancer, yield anti-tumor activity and can work synergistically with Paclitaxel for effective tumor-growth suppression[64].

Fig 3. Advantages of Phytochemicals and TKIs In Drug Discovery Study

2.5 Drug discovery utilizing bioinformatics approach

Computational strategies or approaches within drug discovery involves utilization of bioinformatic tools as well software for identification, designing, and optimization of new found drugs which are therapeutic[42]. It aids to analyse greater datasets involving biological data, model ligand-target or biomolecular interactions and predict fast and in an efficient manner the behaviour of our drug compound. Offers to interpret 3D configuration, molecular docking utilizing Schrodinger, AutoDock 4.2, and PyRx like software, assess docking value (binding affinity) of potent compounds against a specific target, and simulation study to gain insights within stability as well flexibility of drug-target interacting complex at molecular level, thus supporting modern drug discovery approach[65]. In-silico approach overall saves our time as well as cost at preliminary stage of drug development and thus allows targeted investigations for novel therapeutics generation. ML and AI because of their ability to predict ADMET attributes more efficiently and with much greater accuracy have been incorporated in modern drug discovery strategy[50].

In cancer disease, computational approach allows modelling and prediction of interaction among potent drug candidate with specific target like kinase receptor (EGFR), HER2, etc. Different strategies involving screening to find lead compounds, docking, as well pharmacophore modelling helps investigator to test large ligand library against specific target before performing clinical studies.

2.6 Molecular Docking in Drug Discovery Studies

MD predicts most-favoured orientation of ligand upon binding with specific target either enzyme or receptor by estimating binding affinity value. Finally, to enhance reliability of results, simulation are conducted. It helps to increase our knowledge with regard to how ligand fits within active site of our target. It overall saves our time as well as cost at preliminary stage of drug development and decreasing need for thorough laboratory investigations.

MD nowadays is performed via varied platforms involving Schrodinger, PyRx as well as AutoDock[65]. Helps in predicting compounds effective in blocking cancer-linked downstream pathways in cancer-research field, which can be prioritized for experimental validation and synthesis purpose. It forms a vital step in cancer drug discovery pipeline for specific, focused and effective generation of therapeutics which are novel[66].

2.7 Pharmacokinetic Assessment in Drug Discovery Studies

PK assessment offers major function in drug discovery as well as development's early stages by emphasizing mainly how drugs are absorbed, distributed, metabolized, and excreted within the body. By evaluating these parameters investigator gain insight into the behaviour of drug within biological settings, crucial for drug's safety and efficacy[67].

Major PK attributes involving bioavailability, lipophilicity, Lipinski rule of 5, half-life, clearance, distribution volume, solubility, absorption via GI, BBB etc are estimated for interpreting right amount of dosage, appropriate frequency and efficient delivery method[68]. Drug candidates with good PK attributes are taken further with an estimate of their success in clinical studies. However, ligands possessing poor PK parameters often fail in biological system in spite of having significant efficacy in vitro.

Early assessment of PK characteristics helps in focusing on lead drug candidates, identify the potent weaknesses (like poor absorption or rapid metabolism) and thus decrease last-stage losses. It can also guide to optimize the drug's structure and behaviour within the body. PK analysis makes sure that our target attacks at the desired domain and stays there for optimum time period thus producing effective impact.

Toxicity analysis is also performed to gain knowledge about the toxicity profiles of drug-candidates[69]. It helps to assess possible harm a drug can cause, and mitigate the harmful impact prior to clinical studies. It ensures safety, aids to fulfil regulatory requirement, prevent expensive late-stage research failures, and helpful in maintaining public health.

CHAPTER 3
METHODOLOGY

SOFTWARES UTILISED FOR OUR ASSESSMENT

Throughout our assessment involving docking interactions and analysis of our results, employment of subsequent tools occurred.

I. **NCBI**

For extraction of FASTA format of specific protein sequence for 3D modelling it and proceeding further in our assessment.

II. **Phyre2.0**

For modelling 3D configuration of extracted FASTA sequence of specific protein of our interest.

III. **PubChem**

For extracting 2D as well as 3D configuration as well as SMILES of chosen ligand library for execution of docking in drug discovery studies.

IV. **IMPPAT**

For extracting 2D as well as 3D configuration as well as SMILES of several Indian Medicinal Plant varieties, and other natural phytochemicals.

V. **Biovia Disc. Stu. Visualizer**

Employed in the processing or preparation of protein of interest performed with the removal of molecules which are non-essential. Offers visualization of docked or associated target-ligand complex for assessment of interactions or bond within them.

VI. **PyRx**

Applied for molecular docking assessment between our ligand library and target protein, involving AutoDock Vina as the primary engine for docking purpose. Also applied for ligand processing or preparation involving minimization of energy as well as conversion to PDBQT file format.

VII. **Swiss ADME Server**

For predicting physiochemical and pharmacokinetic attributes, drug-likeness property, Lipinski's rule of 5, water solubility, lipophilicity (Log P), and various other parameters for predicting drug's effectiveness.

VIII. pk CSM Server

For conducting ADME assessment as well as predicting toxicity profile of drug candidates.

IX. MS Excel

For documenting down our results in tabulated form and arranging our data appropriately.

X. MS Power Point

Enabled clarity and effectiveness in presenting our concepts, findings and data.

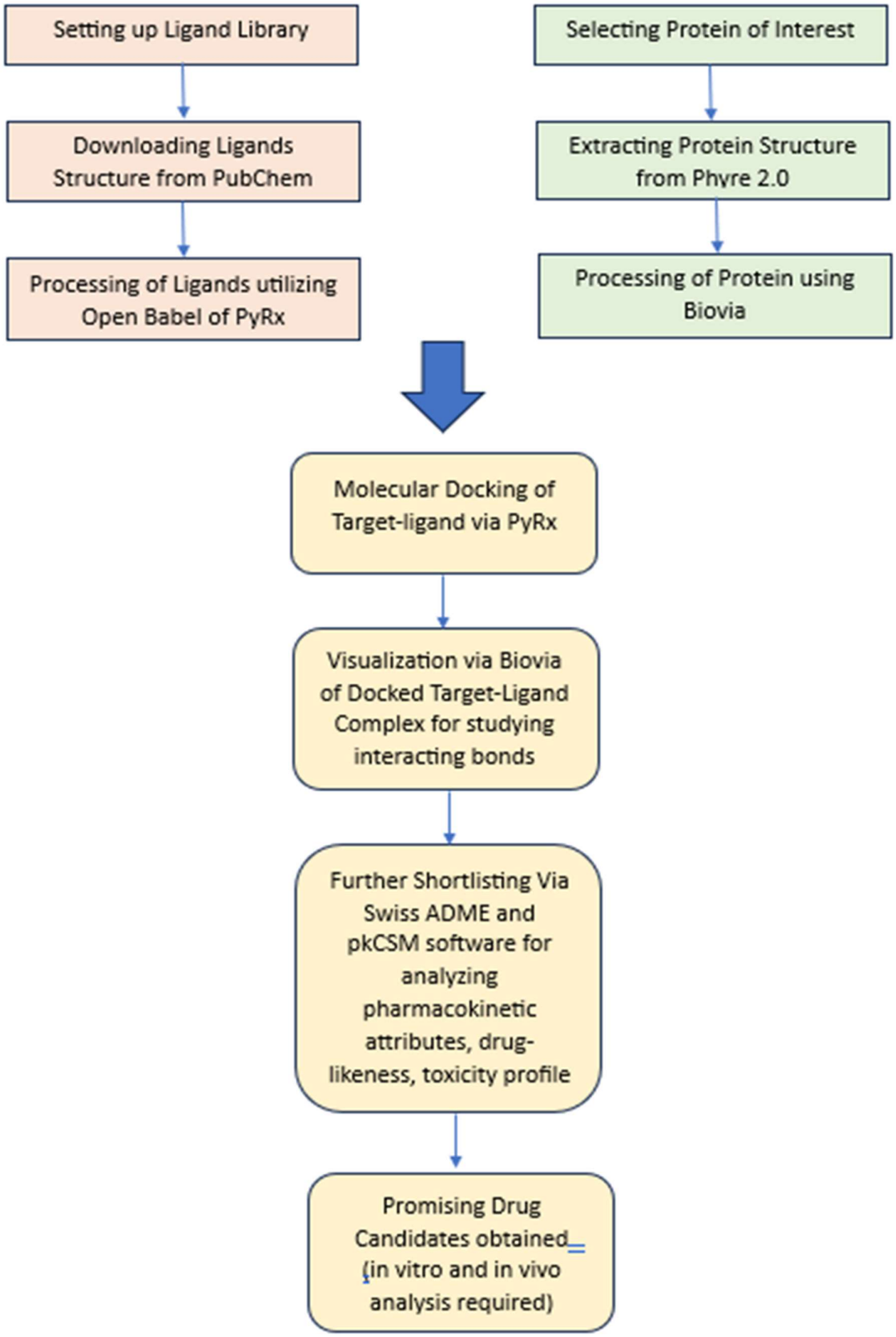


Fig 4. Protocol Involved in Assessment

3. METHODOLOGY

3.1 Acquisition of Sequence for EGFR Protein and 3D Modelling utilizing Bioinformatics Software

3.1.1 Acquisition of Polypeptide Sequence of EGFR Protein from database of NCBI:

National Centre for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/), is a well-recognized branch of U.S. NLM (National Library of Medicine), which is a division of NIH (National Institutes of Health). It offers database for genomic as well as biomedical information. It gives us an access to a wide variety of repositories as well as software involving PubMed for biological research literature[70], GenBank for accessing sequences of nucleotides[71], BLAST for alignment of sequences[72], PubChem for gaining insights into chemical information [73]and various other. By offering extensive data related to genes, proteins, etc., NCBI assists research work in the biological research domain, which is extensively applied by research scholars, scientists, and healthcare workers.

NCBI database was browsed. For searching, protein was selected and EGFR was written in the search option. EGFR protein sequence having accession ID P00533.2, was acquired in FASTA configuration.

Fig 5. Extracting Polypeptide Sequence from NCBI

3.1.2 Retrieval of 3-Dimensional Conformation of EGFR Protein via Phyre2.0 Software

Phyre 2.0 is a free software developed by Structural Bioinformatics Group (SGI) at the Imperial College London, for predicting the 3D conformation of proteins utilizing homology modelling principle. By extensive analysis of polypeptide sequence, Phyre determines structural template using known protein configuration and finally builds a 3D model for our target protein[74]. It has wide applications in structural biology and can be accessed via https://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index.

The FASTA amino acid sequence of EGFR polypeptide was submitted for obtaining 3D configuration. After 8-9 hours, result was obtained on the e-mail id. Final 3D configuration structure was retrieved via the link sent by Phyre2.0 server.

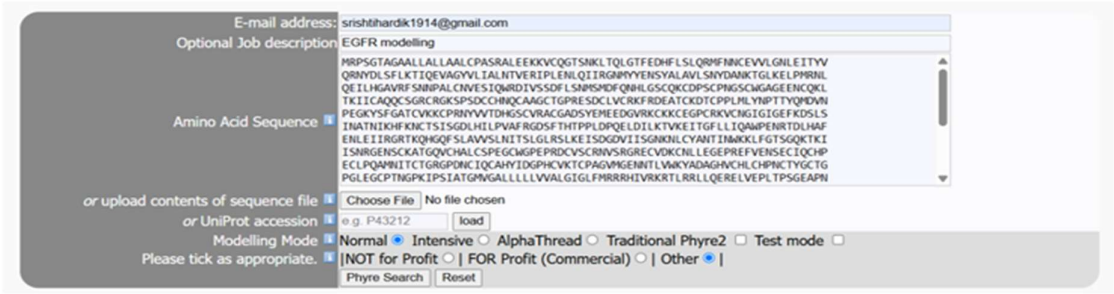


Fig 6. Submission of EGFR protein sequence for 3D modelling

3.2 Collection of Bioactive Phytocompounds and FDA-recognized TKIs via PUBCHEM

3.2.1 Retrieval of Anti-Cancerous Phytochemicals utilizing PubChem and IMPPAT databases

Maintained by NIH, PubChem database (https://pubchem.ncbi.nlm.nih.gov/) provides us comprehensive knowledge on chemical compounds involving structure, biological properties, safety profile and various other[73]. For accurate and easy identification and extraction of related information, a unique CID is provided to each compound. Widely utilised by researchers, investigators, healthcare professionals and analysts for drug discovery and screening studies, chemical data investigation. Free-access and easy to use interface, adds to its advantages for research purposes.

IMPPAT (https://cb.imsc.res.in/imppat/) is a curated repository, having open-access and offers detailed knowledge on 4000+ Indian medical plants, more than 17000 phytochemicals and their

correlated medical application[75]. It offers information regarding the 3D as well as 2D structures and utilizing in silico chemical approach provides insight on physicochemical attributes, ADMET analysis, and drug-likeness characteristics. Researchers worldwide are utilizing it for exploration of natural compounds with the intent of drug- discovery, analysing and understanding natural product based therapeutic strategy and gain knowledge about thousands of plant-based chemicals.

Anti-cancer characteristics possessing phytochemicals were chosen on the basis of earlier investigations and thorough literature review. Overall, 135 ligands were taken for the assessment of present work in association to anti-breast cancer outcome. 2D as well as 3D configuration of the chosen phytochemicals were loaded in .sdf file format utilizing the PubChem database and then verified utilizing another database IMPPAT for the purpose of target-ligand docking assessment. PubChem as well as IMPPAT unique ID's were recorded for the purpose of reference.

3.2.2 Retrieval of Anti-Cancerous FDA-Approved Tyr Kinase Inhibitors utilizing PubChem

Anti-cancer properties exhibiting FDA-recognised Tyr Kinase inhibitors were chosen on basis of literature review. In total, 50 FDA-recognized TKIs were picked for the assessment of our study. Both 2D as well as 3D configuration were loaded in .sdf file format using PubChem database and unique IDs of the compounds were also recorded.

3.3 Preparation for setting up Target and Ligands for Docking

3.3.1 Target Protein Processing

Biovia Disc. Stu. Visualizer (https://discover.3ds.com/discovery-studio-visualizer-download), an impactful software providing free access for molecular modelling as well as visualization established via Dassault Systemes[76]. It has wide usage among molecular structural biology studies, bioinformatics, and studying drug discovery for assessment and visualization of complex structures of biomolecules like polypeptides, interactions involving macromolecules etc. Easy to use graphical interface offers researchers to demonstrate visualization of macromolecules and preparation, docked molecules visualization, drug designing based upon

structures. It facilitates better understanding of mechanisms at molecular level and aids in acceleration of pharmaceutical research.

3D configuration of Tyr Kinase EGFR receptor polypeptide was acquired from Phyre2.0 software and non-essential molecules were eliminated while visualizing in the Biovia Discovery Studio software and later uploaded in the PDB file format. PDB configuration of specific target receptor protein EGFR was uploaded utilizing Open Babel in the Vina wizard section of PyRx docking software, transformed into PDBQT configuration format and selecting target polypeptide molecule as the macromolecule.

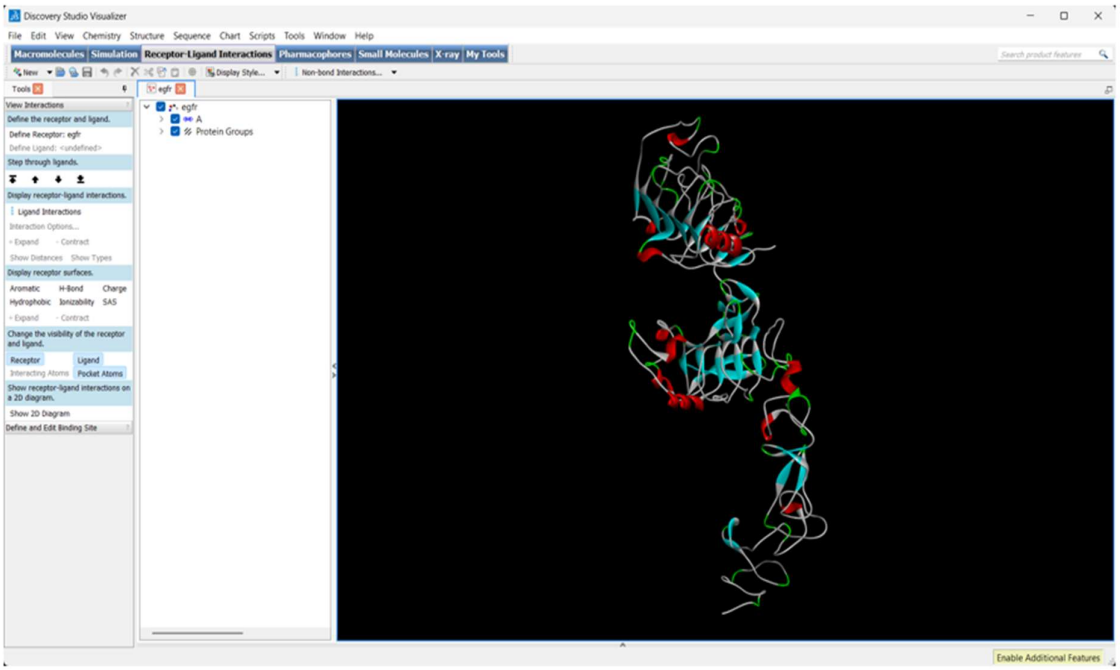


Fig 7. Processing or Preparation of Target Protein EGFR in Biovia Discovery Studio

3.3.2 Processing of Bio-active Plant-Based and FDA-Recognized TKI Ligands

.sdf format files of chosen FDA-approved TKIs as well as plant-derived chemicals were submitted to PyRx following minimization of energy execution and further utilising Open Babel application ligands were transformed into PDBQT file format for our assessment of target-ligand docking.

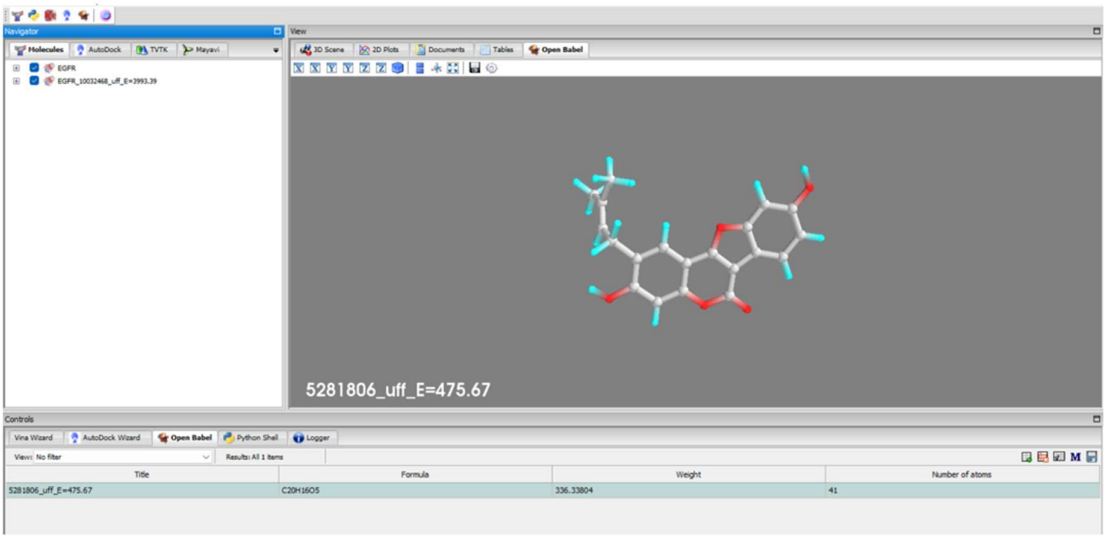


Fig 8. Showcasing An Example of Ligand Preparation in PyRx

3.4 Execution of Docking

3.4.1 Performing Docking Simulation

PyRx (https://pyrx.sourceforge.io/) is among widely utilized software for computational screening as well as biomolecular docking studies having a user-friendly graphic interface for the purpose of drug discovery studies and computational biology purpose. It offers screening of selected ligands against our target proteins utilizing its impactful docking platforms AutoDock Vina and AutoDock[77]. It simplifies the whole docking process just by providing tools for processing of ligands, importing specific macromolecule as target, setting grid box around our target and finally visualization of our result, all within one software. Supporting different file format as well as involving Open Babel like tool for conversion of structure in appropriate format, it aids in docking assessment.

All shortlisted plant-based ligands were picked and uploaded alongside target protein within AutoDock VinaWizard tool of PyRx docking software, grid box was defined around 3D EGFR target polypeptide structure, and further docking simulation executed. Similar steps repeated for TKIs separately. Biovia Disc. Stu. Visualizer aided in visualization of bounded ligand-target complexes for analysing hydrogen bonds, hydrophobic bonds and various other interactions operating between docked target and ligand.

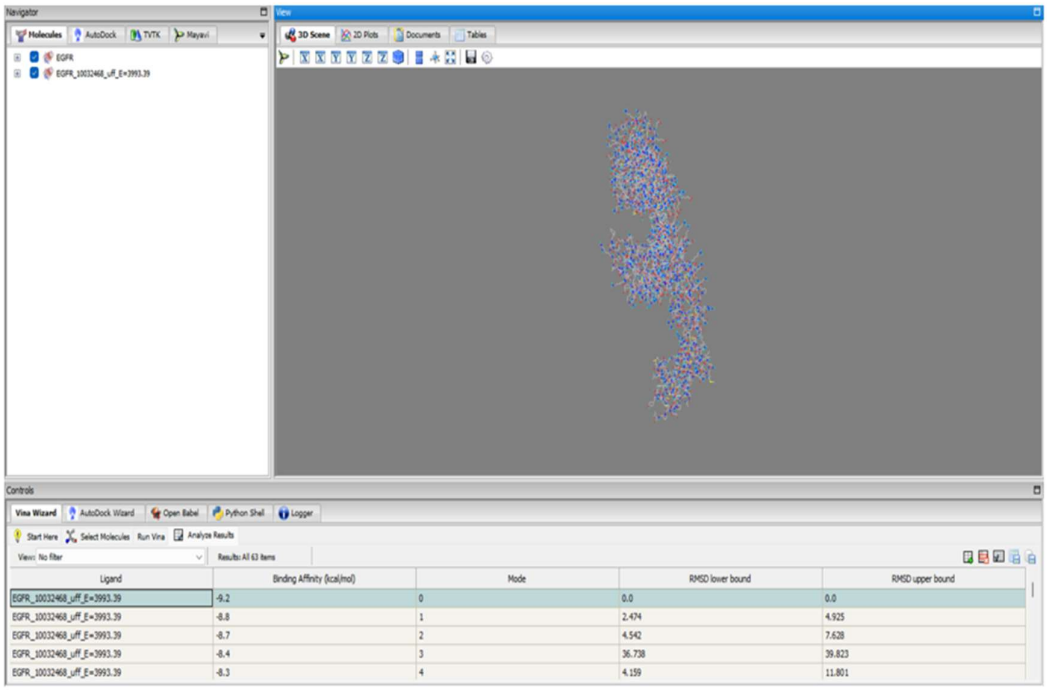


Fig 9. Docking Simulation in PyRx

3.4.2 Documenting B.E. Scores and Shortlisting Phytochemicals as well as TKIs

MS Excel utilized for documenting docking studies result and for arranging our result data in tabulated format. Upon completion of docking, binding energy scores obtained in kcal/mol were documented. Only those compounds were taken into consideration further which had binding energy lesser than our reference molecule. For our assessment we took Panaxadiol (plant-based phytochemical) and Lapatinib as well as Gefitinib (Tyr kinase inhibitor) for our consideration.

3.5 Screening Selected Ligands for Drug-Like Attributes and Assessment of Potent Lead Compounds

3.5.1 Assessment of Potent lead compounds via Screening utilizing SwissADME and pkCSM

SwissADME (https://www.swissadme.ch/) server, helps to evaluate pharmacokinetic properties, drug-likeness characteristics and chemical properties of ligands effective against the target [78]. By submitting SMILES upon the server, one can predict ADME characteristics,

Lipinski's rule of 5, absorption via gastrointestinal tract, permeability via blood-brain barrier and biological availability. It aids in silico assessment by analysing promising and effective drug from the library.

pkCSM (https://biosig.lab.uq.edu.au/pkcsm/) server, offers prediction of pharmacokinetic attributes as well as toxicity profile of drug candidates[79]. It aids in drug discovery at initial stages. Major ADMET properties, and toxicity risks can be assessed using this software by simply submitting SMILES notation to the server, which helps in focusing on certain drug candidate prior to in vitro (experimental) validation.

Further evaluation of drug-likeness, ADME assessment as well as toxicity characteristics for most stable-docked compounds with aid of appropriate bioinformatics software. SMILES notation for shortlisted ligands was acquired using PubChem or IMPPAT database before proceeding ahead with analysis. For ADME analysis and drug-likeness assessment, SMILES were submitted on SwissADME server and for evaluating toxicity of compounds SMILES notation were submitted on pkCSM server.

For further selection of right compounds effective against our target protein, Lipinski's criteria was employed aiding in predicting ligand's capability in demonstrating effective drug-likeness characteristics satisfying the selective criteria:

- M. wt. <500g/mol,
- logP < 5 (effective lipophilicity),
- at most 5 H-Bonds,
- at most 10 H-Bond acceptors,
- less than 10 rotatable bonds and
- TPSA ≥ 140 $Å^2$.

Pharmacokinetic attributes as well as toxicity assessment proves to be effective in drug discovery studies offering valid and reliable results. The analysis of ADMET characteristics such as ESOL Log S (solubility), GI value (absorption by GI pathway), BBB permeability (blood-brain barrier), Log Kp (permeation through skin), Pgp (binding with p-glycoprotein) of shortlisted compounds was done utilizing SwissADME and pkCSM server respectively.

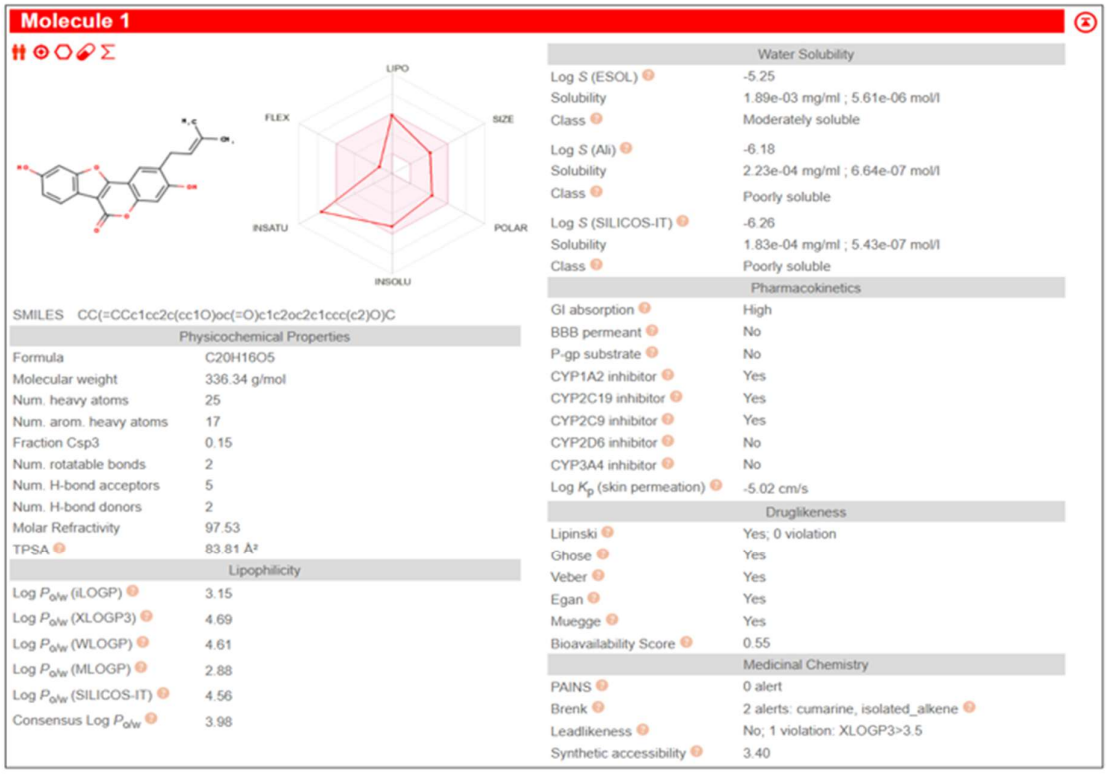


Fig 10. An example of Swiss ADME assessment result

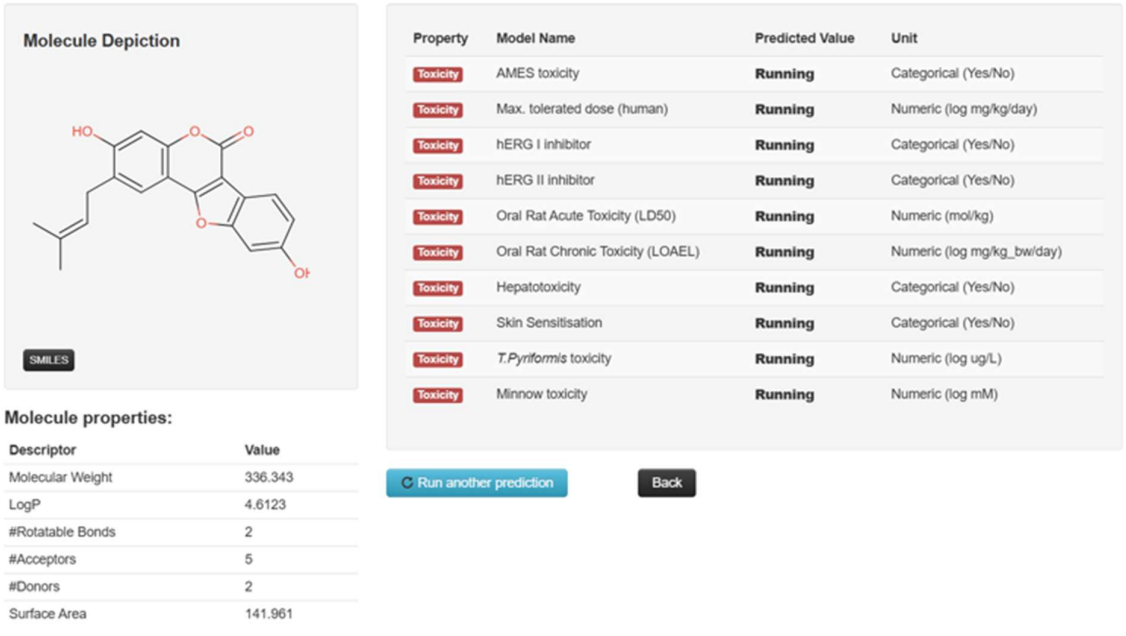


Fig 11. An example of Toxicity Analysis via pkCSM software

CHAPTER 4

RESULTS

4. RESULTS

Panaxadiol drug (PD), a well-recognised tri-terpene sapogenin arising from the species Panax (ginseng), known for its effective health-boosting effects, involving anti-cancer attribute, cardiovascular health-promoting impact, anti-arrhythmic and antioxidative capabilities[80]. For combating Triple Negative BC which demonstrates aggressive behaviour and marked with the lack of 3 receptors involving progesterone, estrogen, and HER2, panaxadiol has proved it's effectiveness. PD exhibits inhibitory impacts on EGFR mediated cascades or pathways including PI3K/Akt/mTOR and NF-Kβ signalling cascades displaying important role in the survival as well as progression of cancer[81], [82]. PD exhibited a substantial binding energy of approx. -7.8 kcal/mol along with target protein EGFR. This binding association within PD and target EGFR is necessary for the drug's ability in hindering or blocking the EGFR signalling cascade aiding in promotion of survival and advancement of cancer.

Targeting two key receptors EGFR and HER2, Lapatinib drug having CID 208908 (PubChem ID) stands among transmembrane Tyr kinase inhibitor (TKI) aiding in treatment of breast cancer [83]. EGFR which is overexpressed commonly among the aggressive types of breast cancer, is inhibited by this effective inhibitor Lapatinib. Docking Lapatinib and target EGFR together yielded a significant binding affinity score of -8.5kcal/mol, displaying good binding strength and hence demonstrated effectiveness in inhibiting kinase action of target EGFR efficiently blocking signalling cascades leading to tumor progression.

Another potent EGFR Tyr kinase inhibitor is Gefitinib having CID 123631(PubChem ID) was taken as reference. FDA-approved TKI for different EGFR mutant cancer, Gefitinib binds efficiently to the ATP-binding site of EGFR polypeptide, resulting in suppression of downstream signalling pathway[84]. Binding energy of amount -7.9 kcal/mol was achieved upon docking of gefitinib with EGFR leading to inhibition of tumor expansion as well as advancement of disease within patients. Table I displays docking scores for reference compounds.

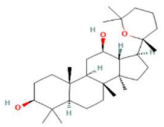
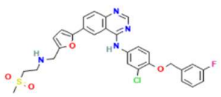
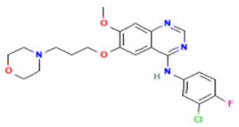
Phyto-chemicals	Panaxadiol (CID 73498)	Lapatinib (CID 208908)	Gefitinib (CID 123631)
Structure			
Binding Affinity (kcal/mol)	-7.8	-8.5	-7.9

Table I: Docking Scores of Reference Compounds

Upon completion of docking of reference compounds against our target, we further docked our library of ligands involving 135 bioactive phytocompounds, and 50 FDA-recognized kinase inhibitors against selected target EGFR. In total, 6 natural bioactive phytochemicals and 5 (TKIs) kinase inhibitors were finalized as they demonstrated best docking scores and appropriate ADMET assessment. Shortlisted natural plant-based compounds belonged to different class of phytocompounds including flavonoids, anthraquinones, lactones, limonoids, and diterpenoids as well.

Psoralidin of flavonoid class extracted from plant *Psoralea corylifolia*[85], demonstrates significant binding or association with EGFR target protein, yielding a binding affinity score of -8.2 kcal/mol, showing immense ability of downregulating EGFR-associated signalling cascades. Nimbolide belonging to limonoid class of phytocompound and extracted out of *Azadirachta indica*[86], exhibited effective docking value of -8.1 kcal/mol upon docking with target EGFR. Extracted out of *Rheum palmatum* and a trihydroxyanthraquinone, Emodin [87]displayed moderate binding association resulting in -8 kcal/mol binding affinity value, aiding in downregulation of the target. Other phytocompounds Bryophilin A, WithaferinA, as well as Capillarisin which belonged to lactone, diterpenoid, and flavonoid phytocompound class respectively[46] yielded a docking value of -7.9 kcal/mol, displaying reasonable binding association with our target EGFR. All 6 shortlisted natural plant-based compounds demonstrated binding affinity value greater than reference drug PD. This underscores their effectiveness in employing inhibitory impact on EGFR-associated signalling pathway, which might prove useful in BC treatment as shown in.

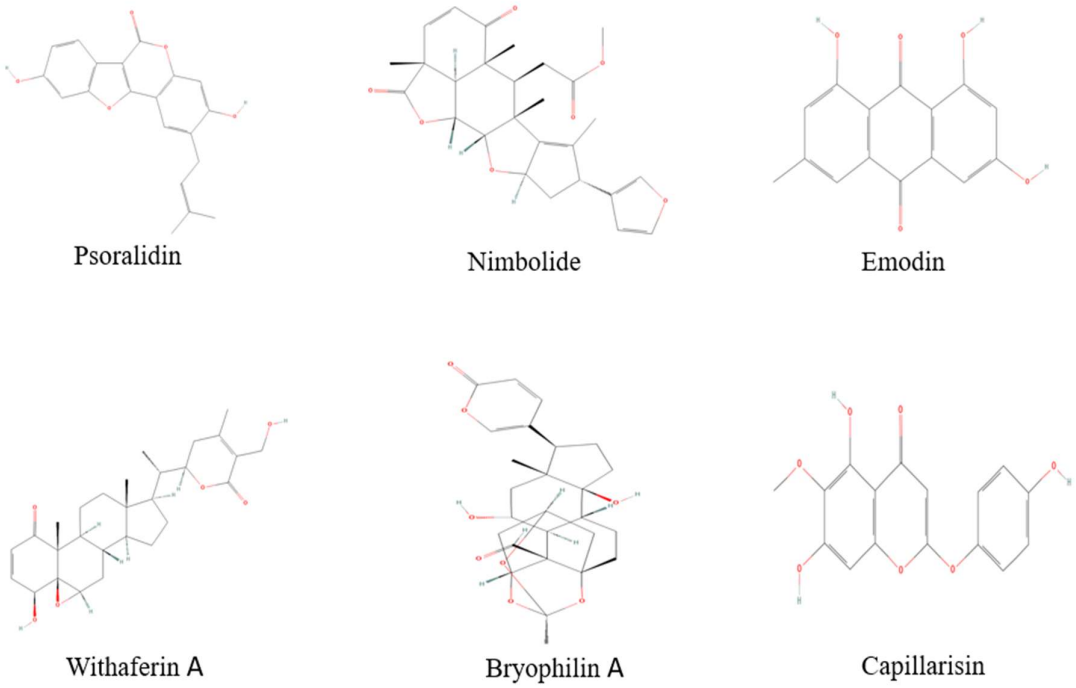
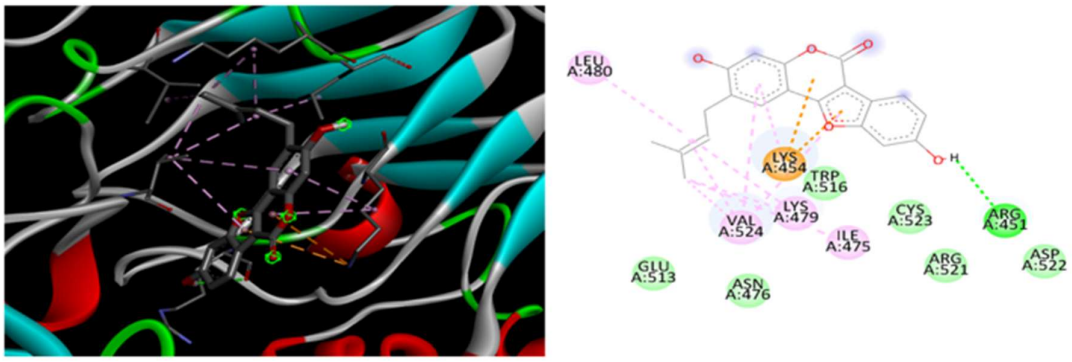
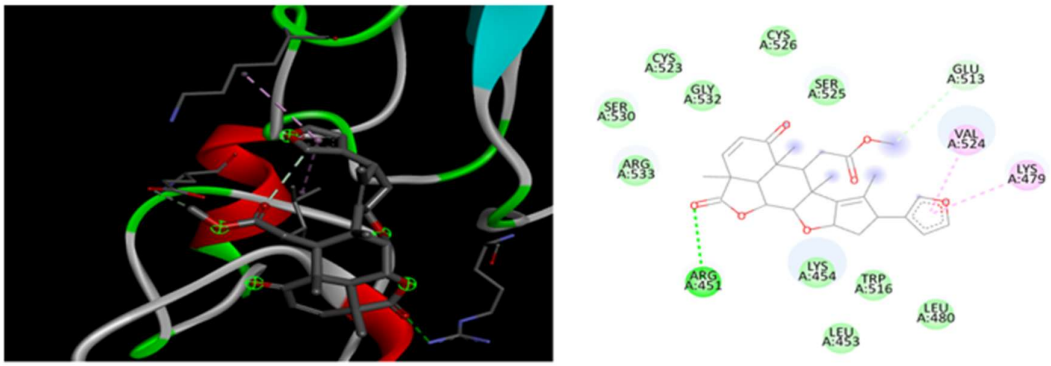


Fig 12. Structures of Selected Bioactive Natural Phytochemicals

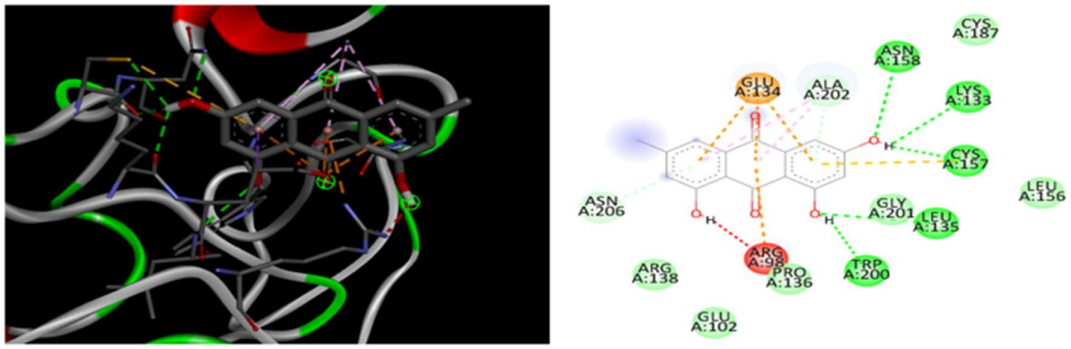


Psoralidin CID 5281806 (-8.2 kcal/mol)

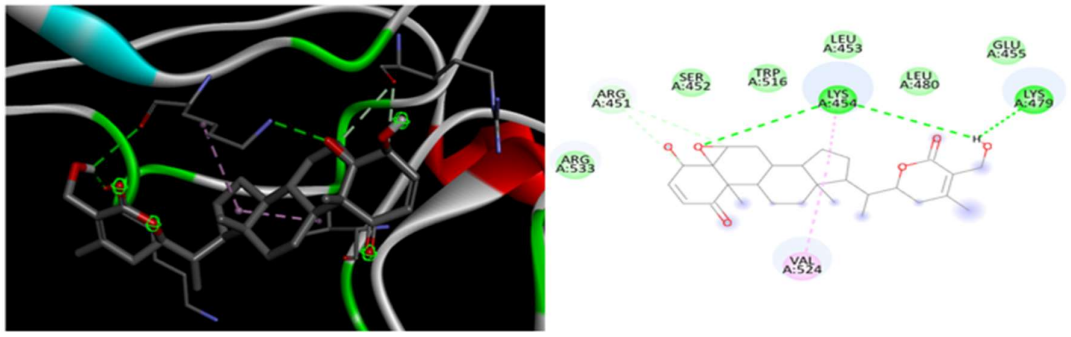


Nimbolide CID 12313376 (-8.1kcal/mol)

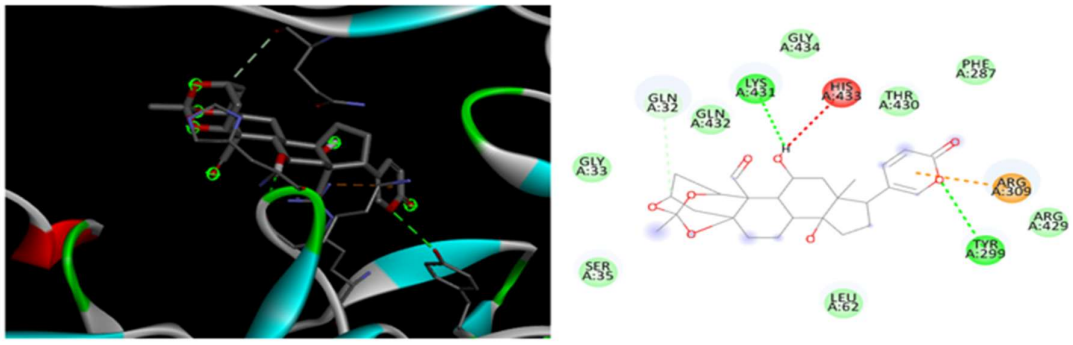
Fig 13. Docking of Selected Phytocompounds with target EGFR Protein



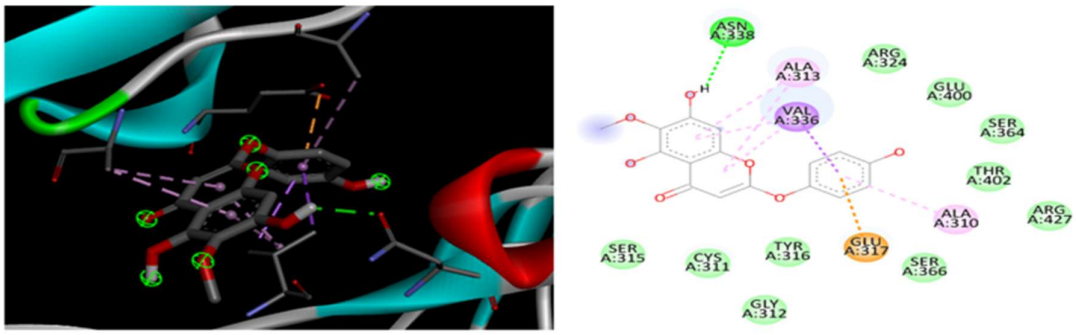
Emodin CID 3220 (-8 kcal/mol)



Withaferin A CID 265237 (-7.9 kcal/mol)

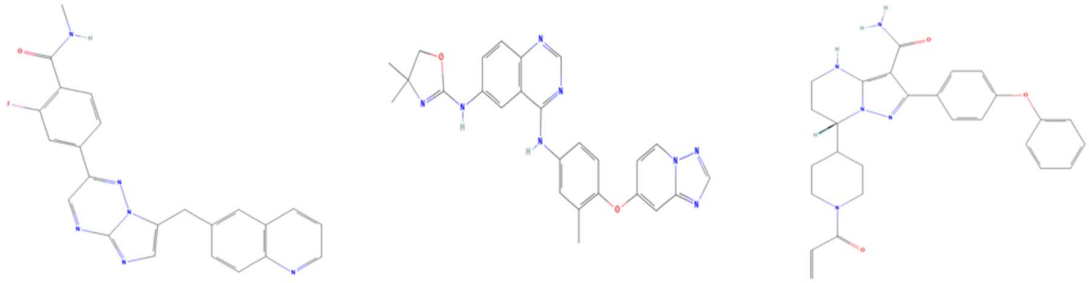


Bryophilin A CID 5488801 (-7.9 kcal/mol)

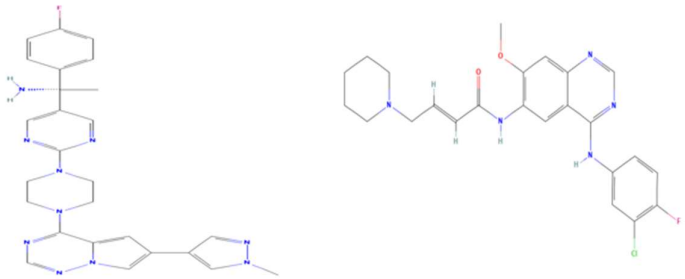


Capillarisin CID 5281342 (-7.9 kcal/mol)

Fig 13. Docking of Selected Phytocompounds with target EGFR Protein

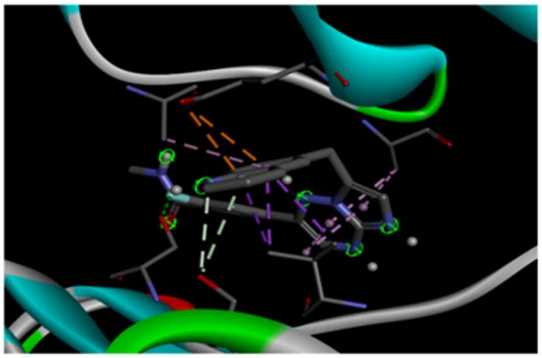


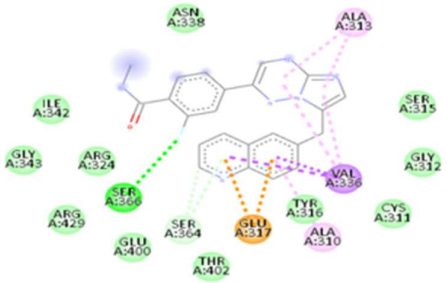
Capmatinib Tucatinib Zanubrutinib



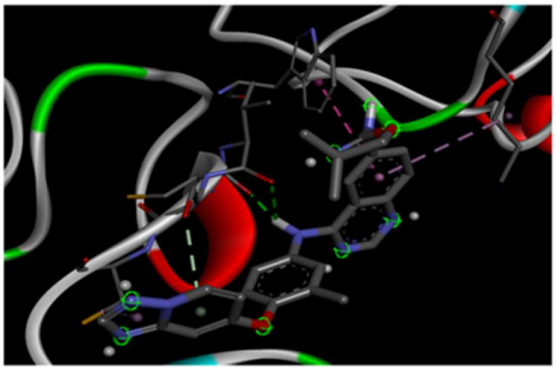
Avapritinib Dacomitinib

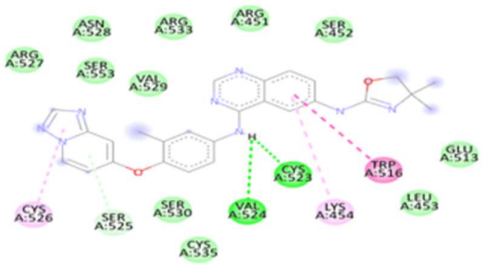
Fig 14. Structures of Selected Tyr Kinase Inhibitors





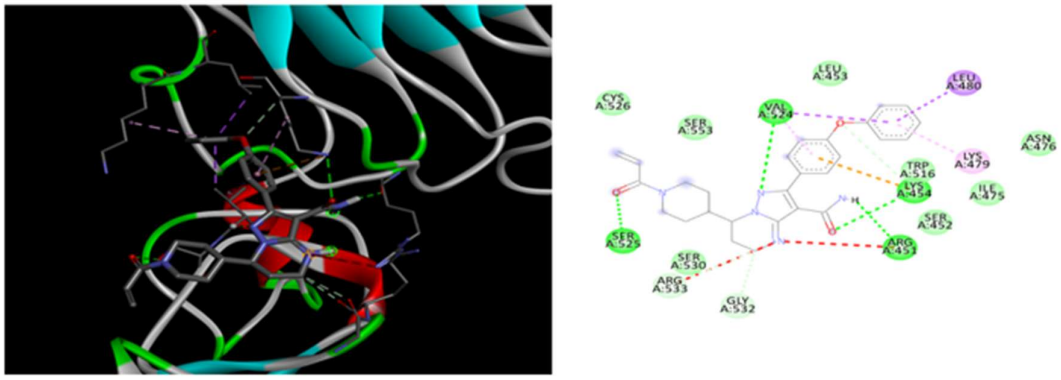
Capmatinib CID 25145656 (-9.3kcal/mol)



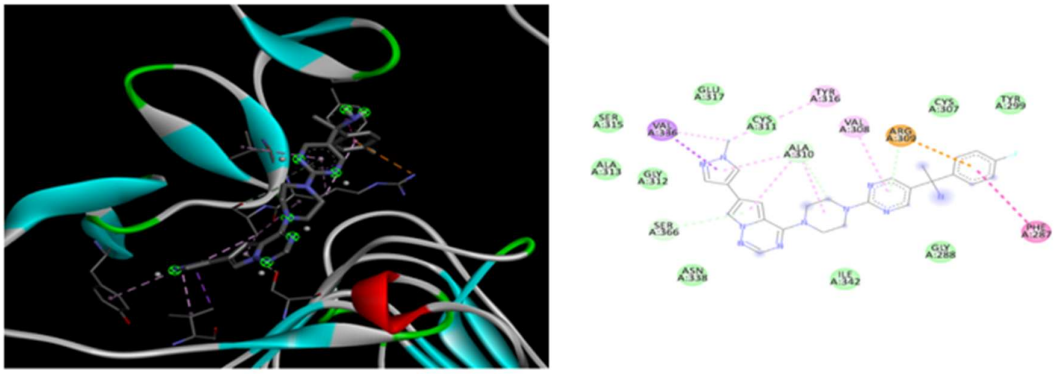


Tucatinib CID 51039094 (-9.3 kcal/mol)

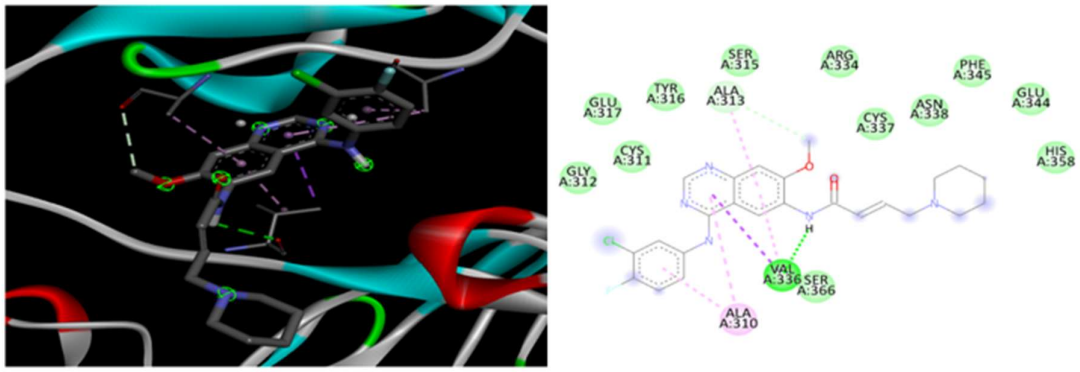
Fig 15. Docking of Selected TKIs with target EGFR



Zanubrutinib CID 135565884 (-8.9 kcal/mol)



Avapritinib CID 118023034 (-8.6 kcal/mol)



Dacomitinib CID 11511120 (-8.6 kcal/mol)

Fig 15. Docking of Selected TKIs with target EGFR

Out of all TKIs, Capmatinib as well as Tucatinib exhibited the most stable binding association and yielded a docking value of -9.3 kcal/mol against EGFR. With a moderate docking value of -8.9 kcal/mol, another FDA-recognized TKI Zanubrutinib displayed moderately stable association with EGFR target. Dacomitinib as well as Avapritinib yielded a docking value of -8.6 kcal/mol with EGFR. All 5 TKIs are effective in downregulating the EGFR-associated signalling pathways. These TKIs were shortlisted for exhibiting binding association more than reference drugs, lapatinib and gefitinib. Binding affinity values for bioactive phytochemicals and TKIs are documented below in Tables II, and III.

Bioactive Phytocompounds	PubChem ID (CID)	Binding Affinity Value (kcal/mol)
Psoralidin	5281806	-8.2
Nimbolide	12313376	-8.1
Emodin	3220	-8
Withaferin A	265237	-7.9
Bryophilin A	5488801	-7.9
Capillarisin	5281342	-7.9

Table II: Binding Affinity Value of Natural Bioactive Phytochemicals

Kinase Inhibitors	PubChem ID (CID)	Binding Affinity Value (kcal/mol)
Capmatinib	25145656	-9.3
Tucatinib	51039094	-9.3
Zanubrutinib	135565884	-8.9
Avapritinib	118023034	-8.6
Dacomitinib	11511120	-8.6

Table III: Binding Affinity Value of FDA-recognized TKIs

Docked compounds were visualized for understanding the existing interaction within target-ligand complex in Biovia Disc. Stu. Visualizer. 2D representation of docked ligand-target complex was obtained from Biovia and then studied further for gaining insights about the interactions.

Pharmacokinetic and toxicity assessment results obtained via Swiss ADME and pkCSM server were obtained. Pharmacokinetic attributes and toxicity assessment results were documented as described in Table IV, V, VI, and VII.

Phytocompounds	M Wt. (g/mol)	HBD	HBA	RB	TPSA (Å²)	Lipinski violation	Log P
Psoralidin	336.34	2	5	2	83.81	0	3.98
Nimbolide	466.52	0	7	4	92.04	0	3.04
Emodin	270.24	3	5	0	94.83	0	1.87
Withaferin A	470.6	2	6	3	96.36	0	3.42
Bryophilin A	472.53	2	8	2	115.43	0	1.97
Capillarisin	316.26	3	7	3	109.36	0	2.09

HBD: H-Bond Donors; HBA: H-Bond Acceptors; RB: Rotatable Bond Num.; TPSA: Topological Surface Area

Table IV: Shortlisted Bioactive Phytochemicals Complying Lipinski's Criteria

Kinase inhibitors	M Wt. (g/mol)	HBD	HBA	RB	TPSA (Å²)	Lipinski violation	Log P
Capmatinib	412.428	1	6	4	85.07	0	3.09
Tucatinib	480.52	2	7	6	141.66	0	3.77
Zanubrutinib	471.55	2	4	7	102.48	0	3.17
Avapritinib	498.56	1	7	5	106.29	0	2.22
Dacomitinib	469.94	2	6	8	79.38	0	4.34

HBD: H-Bond Donors; HBA: H-Bond Acceptors; RB: Rotatable Bond Num.; TPSA: Topological Surface Area

Table V: Shortlisted Tyr Kinase Inhibitors Complying Lipinski's Criteria

Kinase inhibitors	Log S	Log Kp (cm/s)	GI absp.	Pgp binding	BBB	AMES/Hepatotoxicity
Capmatinib	-4.52	-6.73	High	Yes	No	Yes/Yes
Tucatinib	-5.45	-6.4	High	Yes	No	No/Yes
Zanubrutinib	-4.9	-6.66	High	Yes	No	Yes/Yes
Avapritinib	-4.29	-8.02	High	Yes	No	No/Yes
Dacomitinib	-5.38	-6.02	High	No	No	No/Yes

GI: Gastrointestinal Absorption; Pgp: P glycoprotein; BBB: Blood Brain Barrier; AMES Toxicity

Table VI: Pharmacokinetic and Toxicity Assessment of Shortlisted Tyr Kinase Inhibitors

Phytocompounds	Log S	Log Kp (cm/s)	GI absp.	Pgp binding	BBB	AMES/Hepatotoxicity
Psoralidin	-5.25	-5.02	High	No	No	Yes/ No
Nimbolide	-3.94	-7.61	High	Yes	No	No/No
Emodin	-3.67	-6.02	High	No	No	No/ No
Withaferin A	-4.97	-6.45	High	Yes	No	No/No
Bryophilin A	-3.14	-8.76	High	Yes	No	No/ Yes
Capillarisin	-3.84	-6.28	High	No	No	No/ No
GI: Gastrointestinal Absorption; Pgp: P glycoprotein; BBB: Blood Brain Barrier; AMES Toxicity						

Table VII: Pharmacokinetic and Toxicity Assessment of Shortlisted Bioactive Phytocompounds

CHAPTER 5

DISCUSSIONS

5.1 Results of Docking Simulation

From initial 135 phytocompounds, only 6 were shortlisted based on docking as well as ADMET assessment. All 6 showed moderate binding or interaction with target resulting in suppression of EGFR-linked cascades. For FDA-recognized TKIs, only 5 were chosen utilizing docking as well as ADMET analysis, which showed significant binding or association with target aiding in inhibiting downstream EGFR-associated signalling cascades. Our assessment revealed, Capmatinib (TKI), Tucatinib (TKI) and Psoralidin (phytocompound) among the best drugs to downregulate EGFR-associated cascades for tackling advancement of BC. Phytocompounds and TKIs exhibited significant interactions like H-bonds, etc, upon docking with our target EGFR.

The docking results clearly demonstrated better association of TKIs with our target when compared with natural plant-based compounds. However, we can utilize phytocompounds along with TKIs to improve effectiveness and impact of drugs, lowering down drug resistance and much reduced side effects.

5.2 ADMET Assessment

As discussed above, we understood that out of our chosen library of plant-derived compounds as well as FDA-recognized transmembrane TKI, the final shortlisted ones display a potent and effective interaction with our protein receptor target EGFR. Originating from the family of flavonoids Psoralidin [85] , exhibited most stable along with highest value of binding affinity with target EGFR, out of 135 chosen phytocompound ligands from the library. It followed Lipinski's rule of 5 criteria and thus depicting therapeutic potency as a drug. Though this compound has AMES toxicity, it can possibly be modified utilizing rational drug designing approach or some other modification strategy and thus correcting toxicity issue. Other selected plant-derived compounds Emodin, Nimbolide, Withaferin A, Capillarisin obeyed Lipinski's rule of 5 criteria. One advantage of all these compounds making them an ideal and promising drug candidate with effectiveness is that they do not exhibit AMES and hepatotoxicity. Our other potent candidate Bryophillin A followed all Lipinski's rule of 5 criteria and did not exhibit AMES toxicity. However, it exhibited hepatotoxicity, which could be effectively addressed utilizing drug designing strategies, structural optimization or prodrug development like approaches. Our chosen reference standard drug Panaxadiol exhibited one violation within the criteria of Lipinski's rule of 5. However, our recommended phytocompounds offered no

Lipinski's violation and thus effective as a therapeutic option targeting EGFR target against BC.

We finally can conclude based on docking interaction studies and pharmacokinetic assessment, Psoralidin offered to be the best drug candidate and effective in binding stably with EGFR target protein receptor. Flavonoids have been characterized as vital bioactive plant-derived natural compounds holding therapeutic potency as well as application via extensive pharmacological investigations. Certain flavonoids are being explored for their anti-cancer properties and for therapeutic purposes stands baicalein, chrysin, liquirtin, kampferol, flavokawain B,etc[88].

Among selected Tyr kinase inhibitors recognized by FDA for our study, Capmatinib and Tucatinib displayed most stable interaction upon docking with our BC target protein EGFR and hence can be considered for further research for determining potent effectiveness against BC. Though they did not violate any Lipinski's criteria and cleared them all, toxicity possessed by them is concerning. However, toxicity can be corrected via optimization of drug structure or prodrug development approaches. Demonstrating effective binding or stable association and potency to inhibit EGFR-linked cascades for tackling survival, growth and development of BC, are drug candidate Zanubrutinib, Dacomitinib and Avapritinib. Based upon Lipinski's selective criteria and ADMET assessment, out of the above three dacomitinib appears to be best and effective. However, it's hepatotoxicity is concerning which needs to be corrected before use as a drug which can be achieved via modification in drug structure. No Lipinski's violation, high GI absorption as well as MW <500 in above selected 5 TKIs, make them an effective therapeutic compound when compared with standard reference compound Lapatinib.

Hepatotoxicity stays as a challenge among TKIs for their utilization as potential therapeutics, but structure optimization and modification strategies could effectively sort this problem, liver-targeting and varied prodrug formulations strategy which could ensure and enhance the safety of patients [89]. Out of TKIs, ultimately Capmatinib and Tucatinib showed excellent docking and binding association with EGFR and proved as most potent drug candidate.

TKIs performed better than natural bioactive phytocompounds against our specific target EGFR protein.

CHAPTER 6

CONCLUSION AND FUTURE PERSPECTIVE

6.1 Conclusion of Study

Indian medicinal plants derived bioactive phytocompounds and FDA-recognized transmembrane Tyr-kinase inhibitors holding anti-cancer therapeutic potency were assessed for their effectiveness towards inhibiting our opted target EGFR against breast cancer for our present investigation. Fabrication of library containing our chosen anti-cancerous ligands from databases of PubChem as well as IMPPAT, constituting TKIs holding FDA approval and phytocompounds of varied classes. For studying interaction within ligand and target, MD was employed utilizing AutoDock VinaWizard of PyRx software. Further for analysing pharmacokinetic and toxicity attributes to identify effective and promising drug candidate capable of suppressing our target, SwissADME and pkCSM server were employed.

From the assessment, we deduce following phytocompounds: Psoralidin, Nimbolide, Emodin, Withaferin A, Bryophilin A, and Capillarisin and following TKIs: Capmatinib, Tucatinib, Zanubrutinib, Dacomitinib, and Avapritinib are among promising therapeutic drug candidate for BC therapy via suppression of our principal target EGFR receptor. Pharmacokinetic assessment concludes that they all are endowed with strong pharmacokinetic attributes[8], however drug resistance continues to remain a significant challenge further pressing on the need for creating alternative therapy options which are effective and impactful[90]. Combinatorial approaches utilizing both phytocompounds and TKIs can yield effective outcomes.

Our approach of assessment incorporates comparison of phytocompounds and TKIs interaction with target EGFR, hence estimating efficiency of binding association as well as stability upon interaction with target [42]. Our study will further help in evaluating potent bioactive phyto-compounds and TKIs as a therapeutic approach against BC.

Our investigation proposed to evaluate our shortlisted phytocompounds and kinase inhibitors for further in vitro as well as in vivo assessment for unfolding of their effectiveness against BC, underscoring their ability to downregulate EGFR-linked pathways.

6.2 Future Perspectives (Looking Ahead)

- Multiple avenues for further assessment emerge out from our instigation study involving MD (Molecular dynamics) simulations can be performed to authenticate or

verify the predicted interactions and profile of stability for our best bounded or associated compounds to EGFR protein. Simulation plays major role among drug discovery purpose via modelling dynamic behaviour (motion of atoms and molecules over time) of our chosen drug showcasing interaction along with protein of interest. It further aids refinement of binding poses, assessment of stability, and interpreting binding affinity values, thus increasing our understanding of molecular associations as well as picking the right drug.

- Investigation into the effects of natural plant-based chemicals as well as TKIs upon EGFR mediated signalling cascades within BC cell lines can be performed. Cell lines model in drug screening or discovery are utilized as an in vitro model for testing the efficiency and toxicity profile of our drug candidate. It offers researchers a platform for studying affects of drug compounds on specific types of cell, under controlled environment. Helpful in successfully screening compounds before proceeding on to animal and clinical studies.

- One among the most effective approach can be of utilizing combination strategies for which assessment can be done for effective and potent synergistic impacts of EGFR targeting phytochemicals and TKIs with conventional standard drugs for therapy of breast cancer. Combinatorial therapy serves to enhance effectiveness of treatment, reduce side effects as well as lowers resistance of drugs. Combinatorial approach allows targeting multiple mechanisms or cascades, thus improving overall outcome of treatment.

- Structural optimization of lead TKIs can be performed to reduce its hepatotoxicity and improve its drug likeness without compromising their binding efficacy. Optimization of structure includes modification within chemical structure of our lead candidates, hence increasing overall potential, selectivity, stability as well as pharmacokinetic attributes. This can be performed via computational modelling and various other strategies for improving efficacy of drugs.

- We evaluated cancer therapy option utilizing bioinformatics tool, however there is a need to validate the results which can be performed via conducting in-vivo as well as in-vitro analysis.

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LIST OF PUBLICATIONS

1. Our conference paper "In – Silico Discovery of Druga for Breast Cancer Therapy: Investigating Kinase Inhibitors and Phytocompounds Interactions Targeting EGFR Protein" has cleared the review round and has been accepted at the International Conference on Emerging Technologies in Science and Engineering (ICETSE-2025), which will be held in the month of June, 2025.

Submission of Paper for Second International Conference on Emerging Technologies in Science and Engineering (ICETSE) Inbox ×

Srishti Satija Mon, Apr 14, 5:01 PM

Respected ICETSE Conference Committee, I hope this message finds you well. I am writing to submit my research paper titled "In Silico Discovery of Drugs for Bre

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DELHI TECHNOLOGICAL UNIVERSITY

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

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CURRICULUM VITAE

Srishti Satija

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2K23/MSCBIO/48

EDUCATION

Msc. Biotechnology	2023-2025	Delhi Technological University	92.7% (1 Sem)
Bsc. Life Sciences	2020-2023	Hansraj College, Delhi University, New Delhi	91.8%
AISSCE/CBSE (Class XII)	2019	GTB 3rd Centenarian School	95 %
AISCE/CBSE (Class X)	2017	S.D. Public School	95 %

INTERNSHIPS

March 2024 -August 2024 (6 Month Internship)

Biotech Researcher, Vitalth Forgers Pvt. Ltd. – Internship (Present Member)

- Teamwork
- Leadership skills
- Research on Medtech devices
- Scientific writing
- Management skills
- Entrepreneurship skills

April 2021 (Present)

Self Employed Teacher

- Teach complex concepts in an easy way.
- Teach Maths, Science to Class 9 and 10.
- Teach Biology to Class 11 and 12.

ACADEMIC PROJECT

JUNE 2022-JULY 2022

DSKC, Miranda House, University of Delhi - undergraduate research fellow

- Project on amplification, cloning, and expression of plant virus gene
- Conducted in-depth research on groundnut bud necrosis virus
- Learnt about various equipment used in the laboratory
- Extracted the RNA

- Primer designing
- Performed PCR and Gel Electrophoresis
- Did Bacterial cell culture plating
- Performed blue-white screening and tried to express the gene.

2023-2024

Botany Department, Hansraj College - Apprenticeship training

- Learning about different bioinformatics Software.
- Scientific Writing
- PCR
- Molecular Docking
- Structure prediction (I-Tasser, Phyre2, Swiss, Uniprot, Pymol, KEGG, etc.)
- Protein Interaction (String)
- Omics box
- Sequence Analysis
- Database Searching using BLAST, FASTA
- Ramachandran Plot Analysis
- Attended Molecular Biology and Biochemistry Technique workshop organized by EDUFABRICA
- Research and Review Paper Writing

21 December2023 - 4 January 2024

Department of Biotechnology, Delhi technological University -

Trainee Learnt culture of Bacillus brevis

- HPLC
- Learnt about various equipment in biotech lab

OCTOBER 2021

Delhi Biodiversity Park with Centre of Management of Degraded Ecosystem - Project Intern

- Under the guidance of Professor CR Babu
- Butterfly Assessment Week
- In-depth analysis of the number and identification of the different species of butterflies in biodiversity parks of Delhi.

ACADEMIC ACHIEVEMENTS AND AWARDS

- IIT-JAM, GATE, CUET Qualified
- Secured 95% marks in PCB stream (Stream Topper in school)
- Student of the Year Award by Times of India (Times NIE) (2016-17)
- Secured 10 C.G.P.A (School Topper)

TECHNICAL SKILLS

MS Excel, MS Word, etc.	Canva, Editing Software	R (BEGINNER)

POSITIONS OF RESPONSIBILITY

- BIOSOC-DTU – Technical Team Member (2 year)
- NSS, Hansraj College, DU- Rural Development Wing Member (1 year)
- Green Warriors Club, Hansraj College, DU- Social Media Wing Member (2 year experience)
- Placement Coordinator, 2024-25 (1 year)

EXTRA-CURRICULAR ACTIVITIES AND ACHIEVEMENTS

- First prize for Poster Presentation- GENE THERAPY held at RamLal Anand College, New Delhi.
- Participated in Poster Presentation – RNA THERAPEUTICS held at Hindu College, New Delhi.

OTHER INFORMATION

- I am a keen learner and passionate for exploring and learning new skills.
- Some of my skills are:
- Teamwork, Leadership skills, Researcher, Educator, Scientific writing, Management skills
- Crisp and concise knowledge of biology and chemistry concepts, Review paper writing, Scientific Writing, Content Strategy, Strong command over grammar, Excellent time management and organizational skills, teamwork, Presentations, Canva, MS Word, R language.
- Hands-on experience in Cloning, amplification, gene expression, primer designing, Database searching using BLAST, Sequence Analysis, Structure prediction, Pymol, Protein Interaction, Omics (bioinformatics), Ramachandran plot analysis, PCR, Gel electrophoresis (Agarose and SDS), Plating and Streaking, Spectroscopy, Molecular docking, good expertise in biology and chemistry, HPLC, Bacterial Culture, Spectrophotometer.

LINKED IN PROFILE

https://www.linkedin.com/in/srishti-satija