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Research Article

In-Silico Studies of Phytoconstituents of *Allium sativum* for Anticancer Activity

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ABSTRACT

Lung cancer is major global health concern starts in the lungs, usually in the cells lining the air passages. It happens when these cells grow uncontrollably and form a tumor. Natural products, particularly medicinal plants, have gained significant attention as potential sources of anticancer agents due to their safety, availability, and therapeutic properties. Allium sativum (garlic) is a well-known medicinal plant widely used in traditional medicine and reported to possess various pharmacological activities, including anticancer effects. The present study aimed to evaluate the anticancer potential of selected phyto-constituents of Allium sativum using in silico molecular docking and drug-likeness analysis. Major bioactive compounds such as quercetin, kaempferol, β-sitosterol, stigmosterol, phloretin, and S-allyl mercaptocysteine were selected for computational analysis. The three-dimensional structures of ligands were obtained from public chemical databases, and molecular docking studies were performed against the cancer-related receptor EML4-ALK receptor (PDB ID: 2XP2) using molecular modeling software. Drug-likeness properties of the selected compounds were evaluated based on Lipinski's Rule of Five, including molecular weight, hydrogen bond donors and acceptors, lipophilicity (Log P), and rotatable bonds. The docking results indicated favorable binding interactions between the phyto-constituents and the receptor, suggesting potential inhibitory activity against cancer cell proliferation. In conclusion, Stigmasterol (−8.5 kcal/mol), β-sitosterol (−8.3 kcal/mol), and Quercetin (−8.0 kcal/mol) exhibited the highest binding affinities . The findings of this in silico study suggest that phyto-constituents of Allium sativum possess promising anticancer potential and may serve as lead compounds for the development of novel anticancer drugs. However, further in vitro and in vivo studies are required to validate their therapeutic efficacy and safety.

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INTRODUCTION

1.1 Drug Discovery and Development

"Drugs discovery is the process through which potential new medicines are identified. It involves a wide range of scientific disciplines, including biology, chemistry and pharmacology". In the most drugs have been discovered either by identifying the active ingredient from traditional remedies or by serendipitous discovery, but we know the disease controlled by the molecular and physiological level and shape of molecule at atomic level is well understood. The drugs discovery process mainly involves for the identification of candidates, characterization, screening and assay for therapeutic efficacy. The process of drug development is very expensive process due to high costs of R&D and human clinical tests. The average total cost for only drug development varies from USD 897 million to USD 1.9 billion. The typical development time is 10-15 years. The past most drugs have been discovered either by identifying the active ingredients from traditional remedies or by serendipitous discovery. At present a new approach is being tried to understand how disease and infection are controlled at the molecular and physiological level and to target specific entities based on the knowledge.

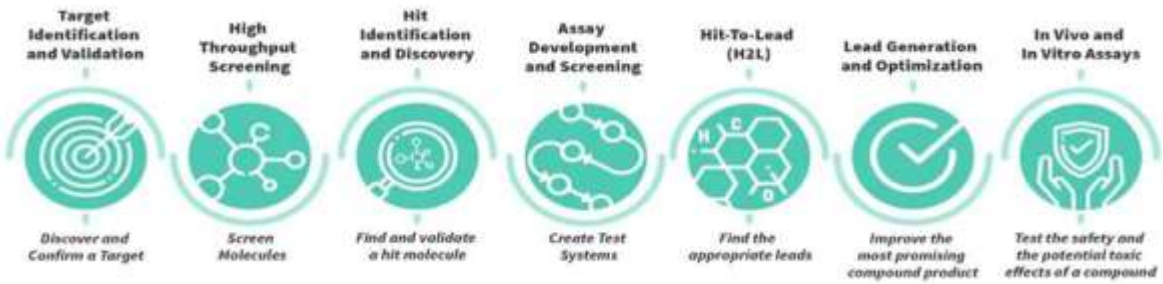


Figure 1: Stages of Drug Discovery and Development Process

Step 1: Target identification: Target identification is the first key stage in the drug discovery pipeline. Generally speaking, a drug target is the specific binding site of a drug in vivo through which the drug exerts its action 6. The process is a chemistry based. Which mean produce compounds for screening. Approach of identification includes protein expression structural and

functional studies and study of biochemical pathways. For these modes of identification, recently several methods are available1. Sequence Analysis 2. Positional cloning 3. cDNA library generation Therefore process helps to find a huge number of target identification 7 A bio molecule may be involved in a disease process, but to be a drug target it has to be validated. In other words, shown to be critical in the disease process useful technique available are to validate a target such as gene knockout and RNA interference.

Step 2: Target validation: new target validation is the basis of completely new drug exploration and the initial step of drug discovery. Research scientists can they identify compounds that have an effect on the target selected. Test are conducted to confirms that interaction with the drugs target is associated with a desired change in the behavior of diseased cells, researcher analyze and compare each drug target to other based on their association with a specific disease and their ability to regulate biological and chemical compounds in the body8. New drug research and development but also provide more insight into the pathogenesis of target related disease.

Basically, the target validation process might include six steps:

1. Discovering a molecule of interest.

2. Evaluating its potential as a target

3. Designing a bioassay to measure biological activity.

4. Constructing a high-through put screen.

5. Performing screening to find hits.

6. Evaluating the hits

Step 3: Lead discovery: This is accomplished primarily with knock-out or knock-in animal models; small molecular target in vitro usually precedes the validation of the therapeutic concept in vivo; together this defines in clinical potential. Validation involve studies in molecular target in vitro usually proceeds with the target protein and modulate its activity10. Synthetic chemicals, peptides, natural or engineered protein, or antibodies are exposed to the target in a manner that will detect and isolate those members of the library that interact with and preferably have an effect on the target 11. For example, if the goal is to inhibit a protein that is involved in activating the expression of a particular gene or set of genes, the assay can include readout to determine if the expression of the gene is reduced by the compound. The assay can be cell based, but more often they are enzymatic assay that can be performed in a high-throughput manner.

Step 4: Lead optimization : A lead compound is a compound from a series of related compounds that has some of a desired biological activity. Leads compounds that survive the initial screening are then "optimized'" or altered to make them more effective and safer. By changing the structure of a compound, scientists can give it different properties. For example, they can make it less likely to interact with other chemical pathway in the body, thus reducing the potential for side effects. Researchers begin to think about how the drug will be made, considering formulation (the recipe for making a drug, including inactive ingredient used to hold it together and allow to perturbation in condition has been the basic approach for establishing potential targets suitable in large quantities.

Step 5: Pre-clinical and clinical and development: Pre-clinical development: the pre-clinical development includes the following develop large scale synthesis; animal safety studies; carcinogenicity tests; drug delivery; elimination and metabolism studies; drug formulation experiments; dose-ranging studies in animals. Wide ranging dosages of the compounds are introduced to the cell line or animal in order to obtain preliminary efficacy and pharmacokinetic information.

Figure 2: Stages of Development Process

Clinical development The NH organizes clinical trial in to 5 different types:

- Treatment trials: test experimental treatments or a new combination of drugs.
- Prevention trials: look for ways to prevent a disease or prevent it from returning.
- Diagnostic trials: find better test or procedures for diagnosing a disease.
- Screening trials: test methods of detecting disease.
- Quality of life trials: explore way to improve comforts of quality of life for individuals with a chronic illness Pharmaceutical clinical trials are commonly classified into 4 phases.

1. Phase 0/Micro dosing-

- To obtain preliminary data pharmacokinetics data.
- Sub acute toxicity study in one species by two routes of administration.

2. Phase 1 –

- 20-25 healthy volunteers; duration 6-12 months.
- The aim of a phase 1 trial is to determines the maximum tolerated dose (MTD) of the new treatment.

3. Phase 2-

- New action of a marketed drug, start with phase 2
- Designed to study efficacy and assess dosing requirements.

4. Phase 3 –

- Its a therapeutics confirmatory trial.
- Target population: several 100 are to 3000 patients.
- Duration: takes a long time, up to 5 years.

5. Phase 4-

- Post marketing surveillance (PMS).
- Confirms the efficacy and safety profiles in large population during practice.

The ADR can be reported to a formal reporting system such as:

- WHO international system
- USFDA- medwatch
- UK- yellow card system
- India-national pharmacovigilance programme (CDSCO)

1.2. Computer Aided Drug Design :

Computer-aided drug design is a pc era that designs a product and files the design's technique. CADD can also facilitate the manufacturing system through shifting unique diagrams of a products materials, methods, tolerances and dimensions with unique conventions for the product in query (2). It can be used to supply both two-dimensional or 3-dimensional diagrams, that can then when rotated to be considered from any attitude, even from the inside searching out. The channel of drug discovery from concept to marketplace includes seven fundamental steps: ailment choice, target selection, lead compound identification, lead optimization, pre-medical trial trying out, medical trial checking out and pharmacogenomic optimization. In practice, the closing five steps required to skip again and again. The compounds for trying out may be obtained from herbal supply (Plants, animals, microorganisms) and by using chemical synthesis. These compounds can be rejected as perspectives owing to absence or low hobby, life of toxicity or carcinogenicity, complexity of synthesis, inadequate performance etc. As a result simplest

one of a hundred thousand investigated compounds may be delivered to the market and one average fee of improvement of latest drug rose as much as 800 million bucks. The discount of time ingesting and value of the last ranges of drug trying out is not likely due to kingdom popular on their cognizance . Therefore essential efforts to increasing performance of development of medicine are directed to levels of discovery and optimization of ligands.

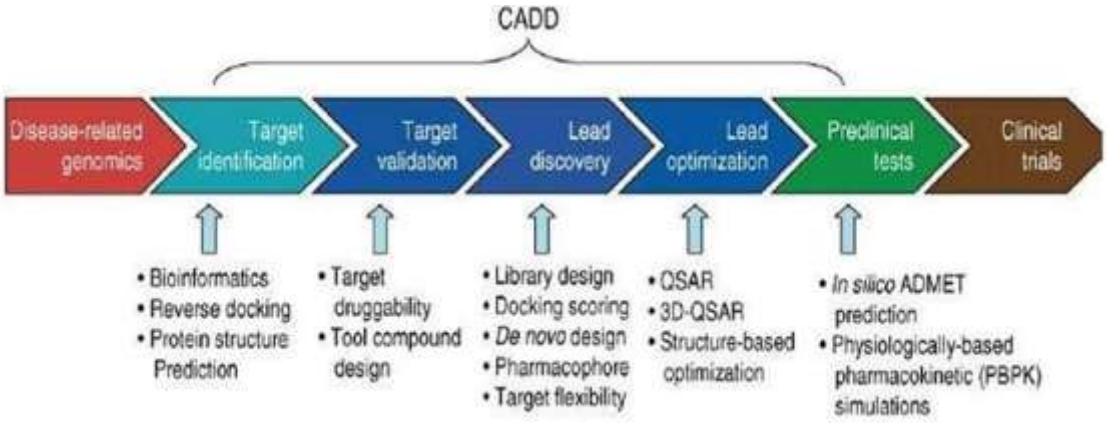


Figure 3: Computer Aided Drug Design

A. LIGAND-BASED DRUG DESIGN

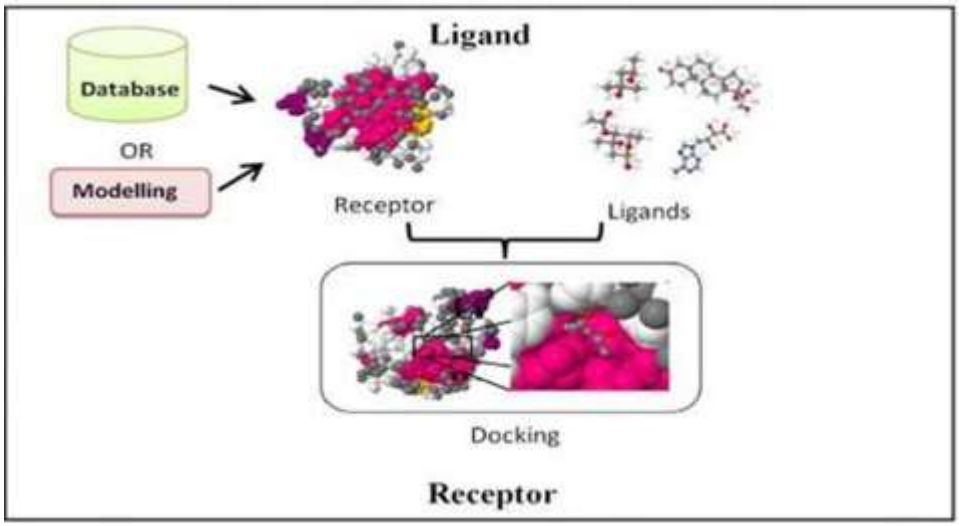


Figure 4 : Structure of Ligand based Drug Design

The ligand-based drug design approach involves the analysis of ligands known to interact with a target. These methods use a set of reference structure collected from compounds known to interact with the target of interest and analysis their 2D or 3D structure In some cases, usually in which data pertaining to the 3D structure of a target protein are not available, drug design can instead be based on process using the known ligands of a target protein as the starting point. This approach is known as "ligand-based drug design

B. STRUCTURE- BASED DRUG DESIGN (LBDD)

Structure-based drug design is the technique to be used in drug design. Structure-based drug design helps in the discovery process of new drugs . The structure is usually obtained from databases like

the Protein Data Bank. In SBDD, scientists analyze the binding site of the target protein and design molecules that can fit into that site and interact strongly, leading to better therapeutic effects.

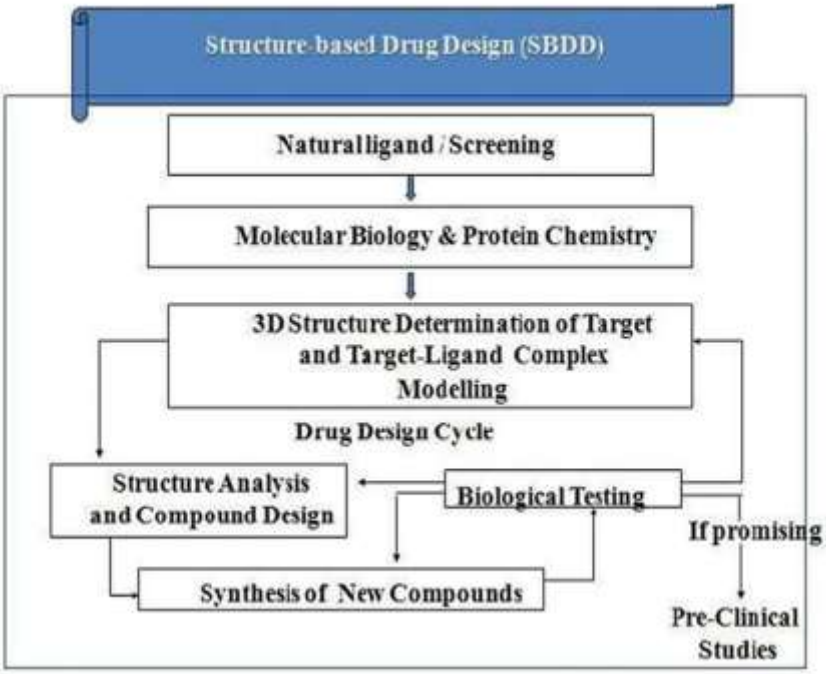


Figure 5: Structure Based Drug Design

1.3. Molecular Docking: Molecular Docking is the study of how two or more molecular structures (e.g., drug and enzyme or protein) fit together [50]. In a simple definition, docking is a molecular modeling technique that is used to predict how a protein (enzyme) interacts with small molecules (ligands). The ability of a protein (enzyme) and nucleic acid to interact with small molecules to form a supramolecular complex plays a major role in the dynamics of the protein, which may enhance or inhibit its biological function. The behavior of small molecules in the binding pockets of target proteins can be described by molecular docking. The method aims to identify correct poses of ligands in the binding pocket of a protein and to predict the affinity between the ligand and the protein. Based on the types of ligands, docking can be classified as

- Protein–small molecule (ligand) docking
- Protein–nucleic acid docking
- Protein–protein docking

Protein–small molecule (ligand) docking represents a simpler end of the complexity spectrum, and there are many available programs that perform particularly well in predicting molecules that may potentially inhibit proteins. Protein–protein docking is typically much more complex. The reason is that proteins are flexible and their conformational space is quite vast.

Docking can be performed by placing the rigid molecules or fragments into the protein's active site using different approaches like clique-searching, geometric hashing, or pose clustering. The performance of docking depends on the search algorithm [e.g., MC methods, genetic algorithms (GAs), fragment-based methods, Tabu searches, distance geometry methods, and the scoring functions like force field (FF) methods and empirical free energy scoring functions]. The first

step of docking is the generation of composition of all possible conformations and orientations of the protein paired with the ligand. The second step is that the scoring function takes input and returns a number indicating favorable interaction [51].

To identify the active site of the protein, first, selection of the required X-ray cocrystallized structure from the protein data bank (PDB) is performed, and then extracting the bound ligand, one can optimize the protein active site of interest. But the process of identification of the active site in a protein is critical when the bound ligand is absent in the crystal structure. In that case, one has to do the following procedures:

a. One can perform comprehensive literature review of the source papers (from which the X-ray crystal structure has been included in PDB) to identify the active site of residues.

b. If any established drug giving the same pharmacological action of interest is available for the protein, then the active sites for this drug should be identified. In the initial phase of analysis, one can try these residues as active binding sites for the test ligands.

c. Every docking software program usually has a particular algorithm to identify the active site of the protein by allowing binding of the ligand in different parts of the protein and exploring the best possible binding position of the ligands with the protein.

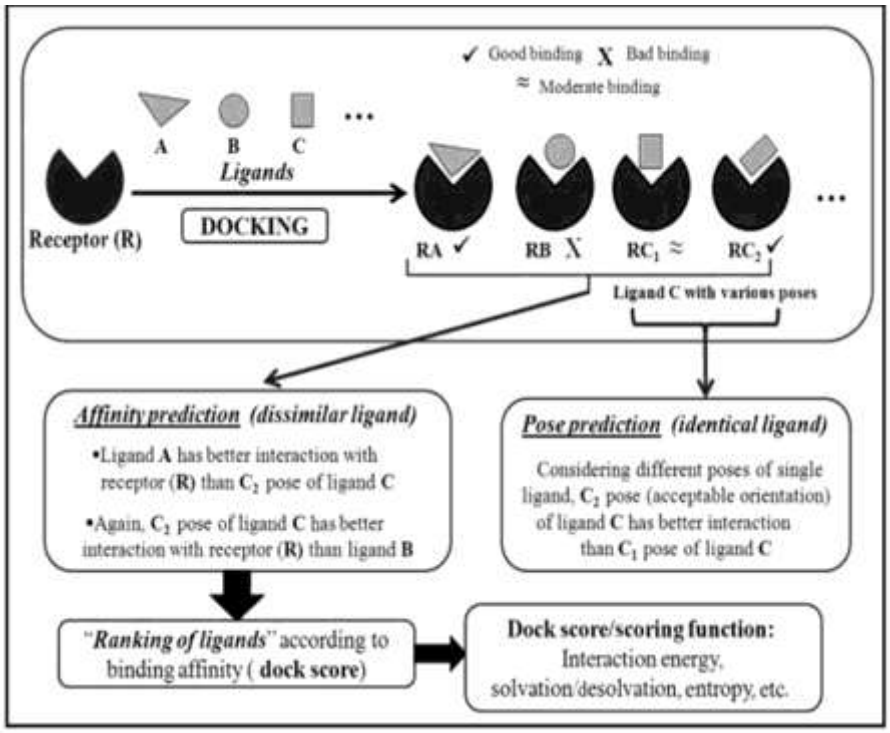


Figure 6 :Molecular Docking

Receptor: A receptor is a protein molecule or a polymeric structure in or on a cell that distinctively recognizes and binds a molecule (ligand) acting as a molecular messenger. When such ligands bind to a receptor, they cause some kind of cellular response.

Ligand: A ligand is the complementary partner molecule that binds to the receptor for effective bimolecular response. Ligands are most often small drug molecules, neurotransmitters, hormones, lymphokines, lectins, and antigens, but they could also be another biopolymer or

macromolecule (in the case of protein–protein docking).

Docking: Docking is a molecular modeling technique designed to find the proper fit between a ligand and its binding site (receptor).

Dock pose: A ligand molecule can bind with a receptor in a multiple positions, conformations, and orientations. Each such docking mode is called a dock pose.

Binding mode: Binding mode is the orientation of the ligand relative to the receptor, as well as the conformation of the ligand and receptor when they are bound to each other.

Dock score: The process of evaluating a particular pose by counting the number of favorable intermolecular interactions such as hydrogen bonds and hydrophobic contacts. In order to recognize the energetically most favorable pose, each pose is evaluated based on its compatibility to the target in terms of shape and properties such as electrostatics and generate corresponding dock score. A good dock score for a given ligand signifies that it is potentially a good binder.

Ranking: Ranking is the process of classifying which ligands are most likely to interact favorably to a particular receptor based on the predicted free energy of binding. After completion of docking, all ligands are consequently ranked by their respective dock scores (i.e., their predicted affinities). This rank-ordered list is then employed for further synthesis and biological investigation only for those compounds that are predicted to be most active.

Pose prediction: Pose prediction can be defined as searching for the accurate binding mode of a ligand, which is typically carried out by performing a number of trials and keeping those poses that are energetically best. It involves finding the correct orientation and the correct conformation of the docked ligand due to their flexible nature.

Scoring or affinity prediction: Affinity prediction or scoring functions are applied to the energetically best pose or n number of best poses found for each ligand, and comparing the affinity scores for different ligands give their relative rank ordering. [52]

Scoring functions are generally divided into two main groups. One main group comprises knowledge-based scoring functions that are derived using statistics for the observed interatomic contact frequencies, distances, or both in a large database of crystal structures of protein–ligand complexes. The other group contains scoring schemes based on physical interaction terms [53]. These so-called energy component methods are based on the assumption that the change in free energy upon binding of a ligand to its target can be decomposed into a sum of individual contributions: (10.8)

$$\Delta G_{bind} = \Delta G_{int} + \Delta G_{solv} + \Delta G_{conf} + \Delta G_{motion}$$

The terms defined for the main energetic contributions to the binding event are as follows: specific ligand–receptor interactions (ΔGint), the interactions of ligand and receptor with solvent (ΔGsolv), the conformational changes in the ligand and the receptor (ΔGconf), and the motions in the protein and the ligand during the complex formation (ΔGmotion).

1.4. Lung Cancer

Lung cancer is a type of cancer that starts in the lungs, usually in the cells lining the air passages. It happens when these cells grow uncontrollably and form a tumor.

Symptoms

- Persistent cough that does not go away
- Coughing up blood (even small amounts)
- Chest pain that worsens while breathing or coughing
- Shortness of breath
- Hoarseness (change in voice)
- Unexplained weight loss
- Loss of appetite
- Constant tiredness (fatigue)

Causes

The main factors that increase the risk include:

- Smoking (most common cause)
- Secondhand smoke (breathing others' smoke)
- Air pollution
- Exposure to harmful substances like asbestos, radon gas, or chemicals
- Family history of cancer
- Long-term lung diseases

Diagnosis

- Doctors use different tests to detect lung cancer:
- Chest X-ray – first basic test to spot abnormal growth
- CT scan (Computed Tomography)– gives detailed images of lungs
- Sputum cytology – checks mucus for cancer cells
- Biopsy – small tissue sample taken to confirm cancer
- Bronchoscopy – a thin tube is inserted into airways to examine lungs
- PET scan – helps detect spread of cancer

Treatment options

1. Surgery

- Used when cancer is in an early stage
- The tumor or part of the lung is removed

2. Chemotherapy

- Uses strong medicines to kill cancer cells
- Can be given before or after surgery

3. Radiation therapy

- Uses high-energy rays to destroy cancer cells
- Often used when surgery is not possible

4. Targeted therapy

- Special drugs that attack specific cancer cells
- Causes less damage to normal cells

5. Immunotherapy

- Helps the body's immune system fight cancer

1.5. Anti-cancer Drug

Anti-cancer drugs (also called anticancer agents or chemotherapy drugs) are medicines used to treat cancer by destroying cancer cells or stopping their

growth and spread. They work in different ways depending on the type of drug and cancer.

The mechanisms of action, resistance, and toxicities of anticancer drugs will be reviewed here. Use of these agents for prevention and treatment of cancer is discussed in detail separately.

Plant work in anticancer drug:

1. GARLIC

The *Allium sativum (Garlic*) has a vital role in various problems associated with human health.

Taxonomical Classification

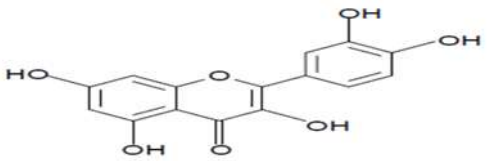
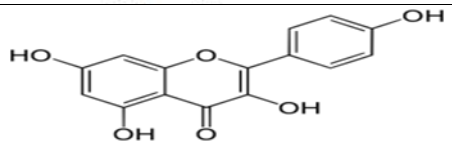
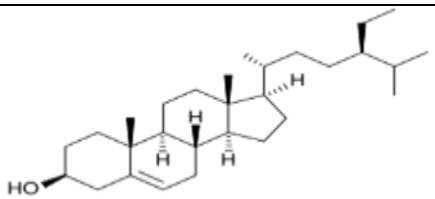
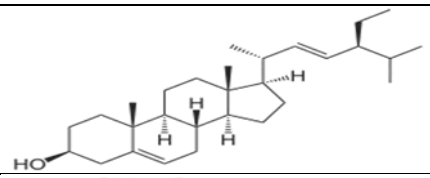
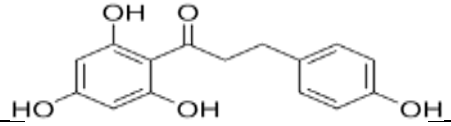
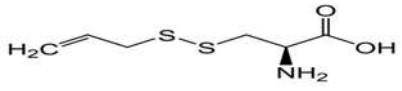
- **Kingdom: Plantae**
- **Division (Phylum): Angiosperms (Magnoliophyta)**
- **Class: Monocotyledonae (Liliopsida)**
- **Order: Asparagales**
- **Family: Amaryllidaceae**
- **Genus: Allium**

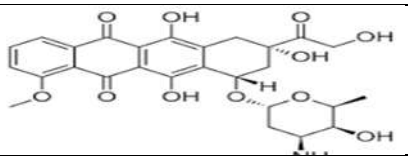
Biological Source

The drug garlic consists of the fresh or dried bulbs of *Allium sativum*

Family: Amaryllidaceae

Table 1: Chemical Constituents of Garlic with their structure

Sr No	Chemical Constituents	Structure
1	Quercetin	
2	Kaempferol	
3	B-sitosterol	
4	Stigmasterol	
5	Phloretin	
6	S-allyl mercaptocysteine	

7	Doxorubicin (Standard)	

Uses:

1. Antibacterial Activity

- Fights harmful bacteria
- Useful in infections (e.g., throat, skin)

2. Antifungal Activity

- Effective against fungal infections like ringworm

3. Antiviral Activity

- Helps the body fight viruses (e.g., cold, flu)

4. Anticancer Activity

- Contains compounds like allicin that may help prevent cancer cell growth

5. Cardioprotective Activity

- Reduces blood pressure
- Lowers cholesterol levels
- Helps prevent heart diseases

6. Antioxidant Activity

- Protects cells from damage caused by free radicals

7. Antidiabetic Activity

- Helps in controlling blood sugar levels

8. Anti-inflammatory Activity

- Reduces inflammation and swelling

9. Anthelmintic Activity

- Helps remove intestinal worms

2. AIM AND OBJECTIVES

AIM

To Perform In-silico studies of phytoconstituents of Allium sativum for anticancer activity

OBJECTIVES

- Design structure of Phytoconstituents of Allium sativum
- Study of physiochemical property of phytoconstuents
- Study of ADME
- Study of toxicity
- Molecular docking of phytoconstituents on the 2XP2 receptor (Echinoderm Microtubule-Associated Protein-Like 4 – Anaplastic Lymphoma Kinase fusion receptor).

3. EXPERIMENTAL WORK

3.1. CHEMSKETCH

Chem sketch is an open-source software is a chemical molecule or molecular modeling program used to create, draw and modify images of chemical structures or compounds and there is software that allows molecule and molecular models displayed in two and three dimensions, to

understand the structure of chemical bonds and nature of the functional groups.

3.2. MOLINSPIRATION

Molinspiration provides a comprehensive suite of cheminformatics tools for molecular manipulation and processing, including SMILES and SD file conversion, molecule normalization, tautomer generation, fragmentation, and calculation of molecular properties essential for QSAR, molecular modeling, and drug design.

3.3. SWISS ADME

This website allows you to compute physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties, drug like nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery**.**

3.4. TARGET PREDICTION

This website allows you to estimate the most probable macromolecular targets of a small molecule, assumed as bioactive. The prediction is founded on a combination of 2D and 3Dsimilarity with a library of 370'000 known actives on more than 3000 proteins from three different species.

3.5. TOXICITY PREDICTION

Determining the toxicity of chemicals is necessary to identify their harmful effects on humans, animals, plants, or the environment. It is also one of the main steps in drug design.

3.6. PREPARATION OF LIGAND

The major active phytoconstituents from selected plants were identified, and their SMILES notations were retrieved from PubChem. Using Chem Sketch, 2D structures were drawn and saved as .mol files. All ligands were imported into Avogadro, optimized using the UFF force field, and prepared by detecting torsion roots, correcting torsion angles, assigning charges, and converting them into .pdb format for further use.

3.7. PREPARATION OF RECEPTOR

The Structure of Echinoderm Microtubule-Associated Protein-Like 4 – Anaplastic Lymphoma Kinase fusion (PDB ID: 2XP2) was retrieved from the RCSB Protein Data Bank in PDB format . The downloaded protein structure was imported into BIOVIA Discovery Studio for receptor preparation.

All water molecules, co-crystallized ligands, and unwanted ions were removed from the protein structure to avoid interference during docking. Hydrogen atoms were added to the receptor to stabilize the structure and ensure proper bonding interactions.

The structure was visualized in Discovery Studio, with unnecessary chains removed, and prepared for docking using AutoDock in PyRx. The final structure was saved in .pdb format.

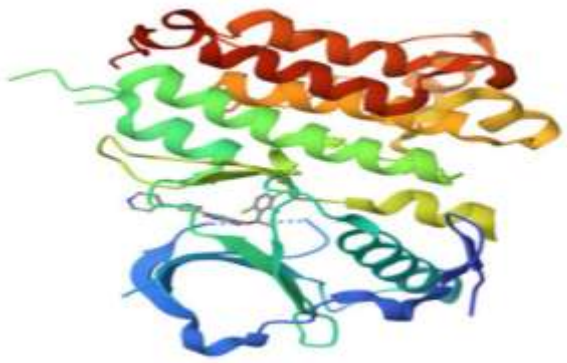


Figure 7. 3D Structure of 2XP2 receptor

3.8. MOLECULAR DOCKING :-

Computational chemistry is the mathematical description or chemistry data used to analyze the

interaction between drug molecules and targeted molecules of microbial, cancer cell etc. molecular docking is a powerful approach to detecting new structure-based drugs. molecular docking studies can be done at minimum cost, save time, and produce fast result in computational studies, which are used to predict the active sites, binding angle, binding targeted protein.

4. RESULT AND DISCUSSION

4.1. DRUG LIKELINESS STUDY: The results of Drug Likeliness study given in table 2.

Table 2: Drug Likeliness Study Results

Sr. No.	Chemical Constituents	Molecular Weight	Rotatable Bond	H-Bond Acceptor	H-Bond Donar	LogP	Follow Lipinski rule	Violations
1.	Quercetin	302.24 g/mol	1	7	5	1.8	Yes	0
2.	Kaempferol	286.24 g/mol	1	6	4	1.9	Yes	0
3.	B-sitosterol	414.71 g/mol	6	1	1	5.05	yes	MLOG>4.15
4.	Stigmasterol	412.69 g/mol	5	1	1	5.08	yes	MLOG>4.15
5.	Phloretin	274.27 g/mol	3	5	4	2.4	yes	0
6.	S-allyl mercaptocysteiene	177.29 g/mol	4	3	2	0.6	yes	0
7.	Doxorubicin (Standard)	543.52 g/mol	5	12	6	1.27	yes	3

DISCUSSION BASED ON DRUG LIKENESS STUDY:

The evaluated phytoconstituents showed acceptable drug-likeness properties according to Lipinski's Rule of Five. Most compounds such as quercetin, kaempferol, phloretin, and S-allyl mercaptocysteine exhibited zero violations, indicating good oral bioavailability potential. Beta-sitosterol and stigmasterol showed one violation due to high lipophilicity (Log P > 5), but remained within acceptable limits. The standard drug doxorubicin showed multiple violations; however, it is clinically effective due to its strong pharmacological activity.

4.2. TOXICITY STUDIES: The results Toxicity study given in table 3.

Table 3: Toxicity Studies

Sr. No.	Ligands	Predicted Toxicity	Predicted LD-50	Carcinoge-nicity	Immuno-toxicity	Hepa-toxicity	Nephro -toxicity
1.	Quercetin	4	1190mg/kg	Inactive	Active	Active	Inactive
2.	Kaempferol	4	1190mg/kg	Inactive	Active	Active	Inactive
3.	B-sitosterol	4	1190mg/kg	Inactive	Active	Active	Inactive
4.	Stigmasterol	4	1190mg/kg	Inactive	Active	Active	Inactive
5.	Phloretin	4	1190mg/kg	Inactive	Active	Active	Inactive
6.	S-allyl mercaptocyst-eine	4	1190mg/kg	-	-	-	-

7.	Doxorubicin (Standard)	4	1190mg/kg	Inactive	Active	Active	Inactive

DISCUSSION BASED ON TOXICITY STUDIES:

The present study was conducted to evaluate the anticancer potential of selected phytoconstituents through molecular docking and in silico toxicity analysis. The selected compounds, namely Quercetin, Kaempferol, β-sitosterol, Stigmasterol, Phloretin, and S-allyl mercaptocysteine, demonstrated favorable binding interactions with the target receptor, indicating their potential inhibitory activity.

The toxicity studies revealed that all compounds exhibited predicted toxicity class 4 with an LD_{50} value of 1190 mg/kg, suggesting moderate toxicity and acceptable safety profiles. The compounds were predicted to be non-carcinogenic and non-nephrotoxic, while showing possible immunotoxic and hepatotoxic effects. These findings indicate that the selected phytoconstituents possess promising biological activity with manageable toxicity risks.

Overall, the results suggest that the studied compounds may serve as potential candidates for anticancer drug development. However, further experimental validation through in vitro and in vivo studies is required to confirm their safety and therapeutic efficacy.

4.3. ADME STUDY: The results ADME Study given in table 4.

Table 4: ADME Study Result

Ligands	GI Absorption	BBB Permanent	P-gp Substrate	CYPIA2 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor	Log Kp
Quercetin	High	No	No	Yes	Yes	Yes	-7.05 cm/s
Kaempferol	High	No	No	Yes	Yes	Yes	-7.05 cm/s
B-sitosterol	Low	No	No	No	No	No	-2.20cm/s
Stigmasterol	Low	No	No	No	No	No	-2.74 cm/s
Phloretin	High	No	No	Yes	No	Yes	-6.11cm/s
S-allyl mercaptocysteine	High	No	No	No	No	No	-8.93 cm/s
Doxorubicin (Standard)	Low	No	Yes	No	No	No	-8.71 cm/s

DISCUSSION BASED ON ADME STUDY:

The ADME analysis of selected phytoconstituents revealed that most compounds demonstrated favorable pharmacokinetic properties. Quercetin, Kaempferol, Phloretin, and S-allyl mercaptocysteine showed high gastrointestinal absorption, indicating good oral bioavailability, whereas β-sitosterol and Stigmasterol exhibited low absorption. All compounds were predicted to have no blood-brain barrier permeability, suggesting limited central nervous system exposure.

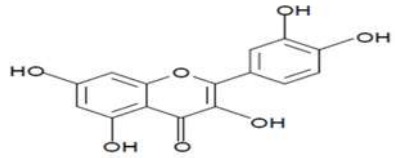
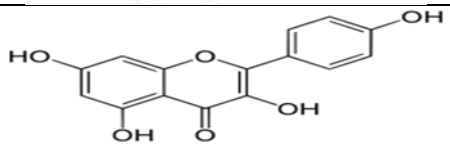
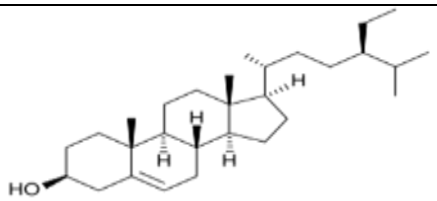
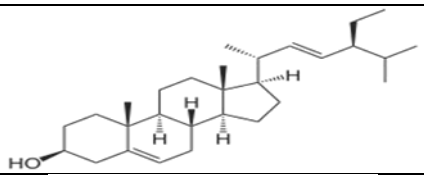
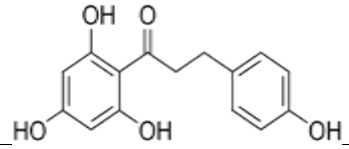
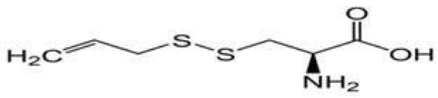
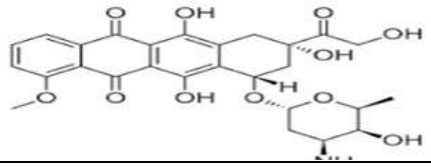
Most of the compounds were not identified as P-glycoprotein substrates, indicating a lower risk of drug resistance. Some compounds showed inhibitory activity against cytochrome P450 enzymes, suggesting the possibility of drug interactions during metabolism. Overall, the ADME results indicate that the selected compounds possess acceptable pharmacokinetic

characteristics and may serve as promising candidates for further drug development, although additional experimental validation is required.

4.4. BINDING AFFINITY OF DIFFERENT CHEMICAL CONSTITUENTS.:

The results Binding Affinity of different Chemical Constituents given in table 4.

Tabel No. 5: Binding Affinity of different Chemical Constituents

LIGAND	STRUCTURE	BINDING AFFINITY
Quercetin		-8
Kaempferol		-7.9
B-sitosterol		-8.3
Stigmosterol		-8.5
phloretin		-7
S-allyl mercaptocysteine		-4.5
Doxorubicin (Standard)		-8.7

DISCUSSION BASED ON BINDING AFFINITY OF DIFFERENT CHEMICAL CONSTITUENTS:

The docking study revealed variations in binding affinities among the tested ligands, indicating differences in their interactions with the target receptor. Stigmasterol (−8.5 kcal/mol), β-sitosterol (−8.3 kcal/mol), and Quercetin (−8.0 kcal/mol) exhibited the highest binding affinities, which are closely comparable to the standard drug Doxorubicin (−8.7 kcal/mol). This suggests their strong potential as inhibitors.

Kaempferol also demonstrated a favorable binding affinity (−7.9 kcal/mol), supporting its effectiveness as a bioactive compound. Phloretin showed moderate binding affinity (−7.0 kcal/mol), indicating a stable but comparatively weaker interaction with the receptor.

In contrast, S-allyl mercaptocysteine exhibited a significantly lower binding affinity (−4.5 kcal/mol), suggesting weaker interactions and reduced inhibitory potential.

Overall, ligands with more complex molecular structures demonstrated better binding efficiency. These findings highlight the potential of selected compounds as promising candidates for further investigation in drug development studies.

4.5. 2D STRUCTURE OF LIGAND INTERACTION WITH RECEPTOR 2XP2

1. Quercetin

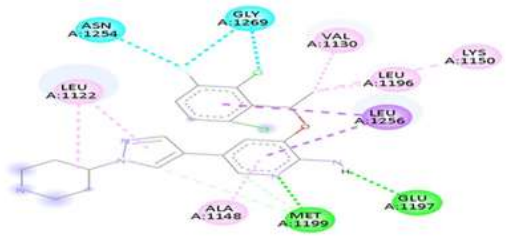


Figure 8. 2D Structure of Interaction of Quercetin with 2XP2 Receptor

2. Kaempferol

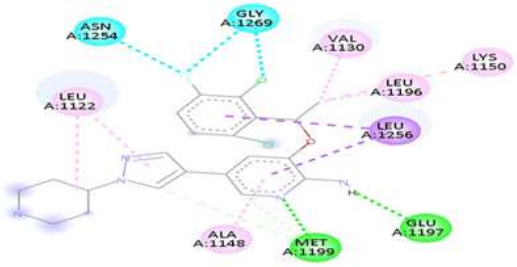


Figure 9. 2D Structure of Interaction of Kaempferol with 2XP2 Receptor

3.B-sitosterol

Figure 10. 2D Structure of Interaction of B-sitosterol with 2XP2 Receptor

4. Phloretin

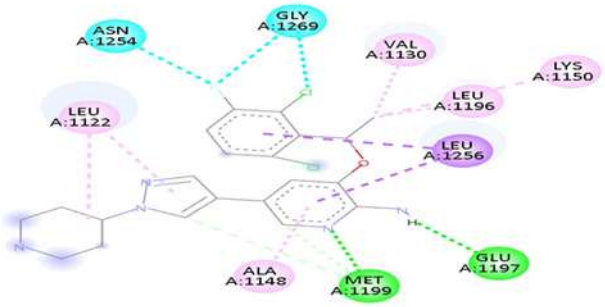


Figure 11. 2D Structure of Interaction of Phloretin with 2XP2 Receptor

5. S-allyl mercaptocysteine

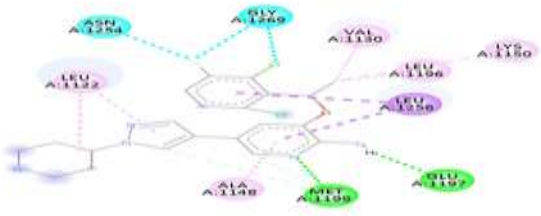


Figure 12. 2D Structure of Interaction of S-allyl mercaptocysteine with 2XP2 Receptor

6 . Doxorubicin

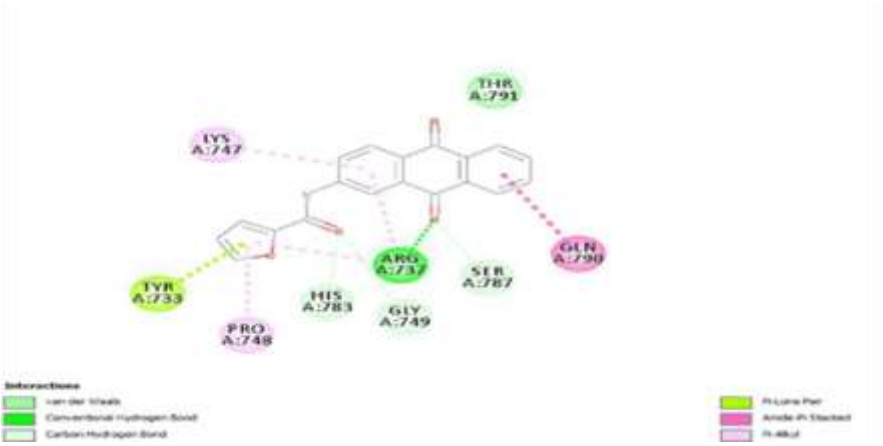


Figure 13. 2D Structure of Interaction of Doxsorubicin with 2XP2 Receptor

5. CONCLUSION

This study evaluated the anticancer potential of phytoconstituents derived from *Allium sativum* through molecular docking analysis targeting the Echinoderm Microtubule-Associated Protein-Like 4 – Anaplastic Lymphoma Kinase (EML4–ALK) fusion receptor (2XP2). Among the tested compounds, stigmasterol and β-sitosterol exhibited strong binding affinities, comparable to the standard drug doxorubicin, suggesting significant inhibitory activity against the target receptor.

Additionally, drug-likeness, toxicity, and ADME assessments indicated that several of these phytocompounds possess favorable pharmacokinetic characteristics along with relatively low toxicity profiles. Collectively, these results suggest that *Allium sativum* could serve as a promising source of potential anticancer agents. Nevertheless, further in vitro and in vivo studies are necessary to validate these computational findings**.**

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