In-silico studies on phytochemical, Carpaine in papaya leaves

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ABSTRACT

Cyclin dependent kinases are critical molecules that control cell cycle progression from one phase to the other. In humans, mutations in cyclin dependent kinase 2 (1GII) is responsible for nearly 50% of cancers. This study evaluates in-vitro and in-silico study of a phytochemical carpaine obtained from Carica papaya leaves.. Computational docking was used to predict conformations and free binding energy for ligand carpaine with CDK4 protein 1GII. In silico studies were done on different conformers of ligand carpaine and receptor 1GII, a CDK4 protein which gives binding free energy of -7.44 Kcal/ mole. This study reveals the pharmacokinetic properties and Lipinski's rule of five. Two hydrogen bonds were also obtained between them. Docking results for carpaine may be promising towards it as anticancer drug candidate.

Keyword: - *Phytochemical, Carica papaya leaves, anti-cancer, Ligand, protein interactions, Carpaine, binding affinity, structure-based drug design.*

1. INTODUCTION

1.1 Papaya Plant

Papaya Leaves Papaya (Carica papaya L.) belongs to the family Caricaceae and is the most economically important species of the genus Carica. It is also known as 'papaw' or 'pawpaw'.
Scientific classification: Kingdom: Plantae Order: Brassicales Family: Caricaceae Genus: Carica Species: C. Papaya It is known that plants and trees are very important to us in our life for oxygen, fruits, vegetables, fresh air and for medicinal uses. Earlier, people use all parts of trees and plants such as leaves, stems, roots, flowers and even fruits for medicinal purposes. It is well known that it has properties such as anti-oxidants, anti-inflammatory, anti-bacterial, anti-cancer that is useful to cure the cancer cell. It was originated in Mesomerica, within modern-day Southern Mexico and Central America. Today, Papaya is one of the highest cultivated crops not only in India but also in the world. It's all parts like roots, leaves, stem, flowers, fruits; seeds are used as a medicine. Especially the papaya leaf contains medicinal properties such as anti-cancer etc. They contain active components such as alkaloids (capraine), tannins, saponins, flavonoids, glycosides which is responsible for the medicinal activity. (1)

1.2 Global scenario of cancer

According to 2023 cancer statistics, 1,958,310 new cancer cases and 609,820 cancer deaths are projected to occur in the United States. Cancer mortality rates continue to decline, future progress may be attenuated by rising incidence for breast, prostate, and uterine corpus cancers.(2)

1.3 Oral Cancer

Oral cancer is the 3rd largest common cancer in the world. It is also called as 'Mouth Cancer' occurs on lining of the lips, mouth and upper part of the throat. Mouth Cancer includes cancer of lips, oral cavity, and pharynx. It starts when cells in the mouth develop the changes in the DNA. It is white, thick patches that look like an ulcer

and continuous to grow with a pain. The symptoms include difficulty in swallowing, lumps and bumps in the mouth and neck, numbness in the mouth. The most common cause of oral cancer is excess use of tobacco and intake of alcohols containing products. Other risk factors include HPV infection (Human Papilloma virus Infection), chewing paan and sun exposure. The diagnostic method is Tissue biopsy. The treatment of this cancer include surgery, radiation, chemotherapy if found at early stage.(3)

1.4 Cancer and phytochemicals

In some cases, antibacterial compounds can also have anticancer properties. This occurs because certain antibacterial compounds can target and inhibit the growth of cancer cells. Some examples of antibacterial compounds with anticancer activity include:

1. Doxorubicin: This antibiotic is commonly used in cancer chemotherapy. It acts by inhibiting DNA replication and is effective against a wide range of cancer types.
2. Mitomycin C: This antibacterial compound is used in the treatment of various cancers, including bladder and gastric cancers. It works by damaging DNA in cancer cells, preventing their replication and growth.
3. Taxanes (e.g., paclitaxel): Although primarily used as anti-mitotic agents to treat bacterial infections, taxanes have also shown efficacy against various cancers. They work by stabilizing microtubules, preventing cell division and eventually leading to cancer cell death.
4. Plitidepsin: Originally developed as an antifungal agent, plitidepsin has demonstrated potent anticancer activity. It inhibits the growth of cancer cells by interfering with important cellular proce5)sses.
5. Vinca alkaloids (e.g., vincristine): Originally derived from the periwinkle plant, these antibiotics have potent anticancer effects. They disrupt microtubule formation in cancer cells, halting their division and proliferation. [4]

It is evident that some antibacterial compounds may have anticancer properties, not all antibacterial compounds possess this attribute. Additionally, the efficacy and safety of using antibacterial compounds as anticancer agents are to be investigated and more research is needed to fully understand their potential in cancer treatment. In order to know the potentiality of an alkaloid phytochemical Capraine was selected and receptor 1GII was taken for studies.

2. MATERIALS AND EXPERIMENTAL TECHNIQUES

2.1 Collection of plant material

Fresh green leaves of papaya were collected and washed with distilled water thoroughly. It was then air-dried under shade at room temperature for about 20-25 days. Dried leaves are to grind to fine powder.

Fig -1: Ethanol extract of Carica papaya leaves

Fig -2: Powdered leaves of Carica papaya leaves

2.2 Preparation of extract

The amount of prepared papaya leaf powder is dissolved in suitable solvent (i.e., methanol, ethanol, etc.) in a beaker. Keep the prepared solution undisturbed for 2-3 days. Filter the solution with Whatman filter paper no.42. The filtrate was kept for 1hr in a water bath at 600 ℃. The prepared extract was kept at cold condition until use.

2.3 Phytochemical Analysis (Test for extract)

Phytochemical analysis was done with required reagents to test the alkaloid, carbohydrates, reducing sugar, flavonoids, glycosides, phenolic compounds, saponin, steroids, amino acids and tannins.

- **Test for Alkaloid**

Extracts were mixed with dilute hydrochloric acid and following tests were done.
Mayer's Test: Extracts were treated with Mayer's Reagent (Mercuric chloride & Potassium iodide). Formation of a cream indicates the presence of alkaloids.
Wagner's Test: Extracts were treated with Wagner's Reagent (Iodine & Potassium iodide). Formation of a reddish-brown precipitate indicates the presence of alkaloids. Test for Flavonoids Aqueous extracts was treated with dilute ammonia and conc. Sulphuric acid was added. Yellow coloration indicates the presence of flavonoids. Test for Terpenoids To the extracts 2ml of chloroform was added, conc. Sulfuric acid was carefully added to form a layer. Reddish brown coloration at the interface indicates the presence of terpenoids. Test for Steroids Extracts were mixed with 2ml of chloroform, 2 ml of conc. sulphuric acid and 2ml of acetic acid were poured into the mixture. Development of greenish coloration indicates the presence of steroid.
Test for Phenols Ferric chloride Test: Extracts were treated with 3 to 4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

- **Test for Glycosides**

Libermann Burchard's Test:
Extracts were treated with 2 ml of chloroform and 2 ml of acetic acid. The mixture was cooled in ice and conc. sulphuric acid was added. Color change from violet to blue to green the indicates the presence of glycosides
Salkowski's Test:
Extracts were mixed with 2ml of chloroform. Then 2 ml of conc. sulphuric acid was added carefully and shaken gently. A reddish-brown color indicates the presence of steroidal ring.
Keller-kilani Test:
Extracts were mixed with 2ml of glacial acetic acid containing 1 to 2 drops of ferric chloride solution. The mixture was then poured into another test tube containing 2 ml of concentrated sulphuric acid. A brown ring at the interface indicates the presence of cardiac glycosides.

- **Test for Protein**

1. Ninhydrin Test: Extracts were boiled with 2 ml of ninhydrin solution. Appearance of violet color indicates the presence of proteins.
2. Xanthoproteic test: Extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

- **Test for Carbohydrates**

Benedict's solution: Extracts were treated with 2 ml of Benedict's reagent and gently heated. Formation of orange red precipitate indicates the presence of reducing sugar.9
Fehling's Test: Equal volume of Fehling A and Fehling B reagents were mixed together and 2 ml of it was added to the extract and gently boiled. A brick red color indicates the presence of reducing sugar.
Iodine Test: Extracts were mixed with 2 ml of Iodine solution; a dark blue or purple color indicates the presence of Carbohydrates.

- **Test for Tannins**

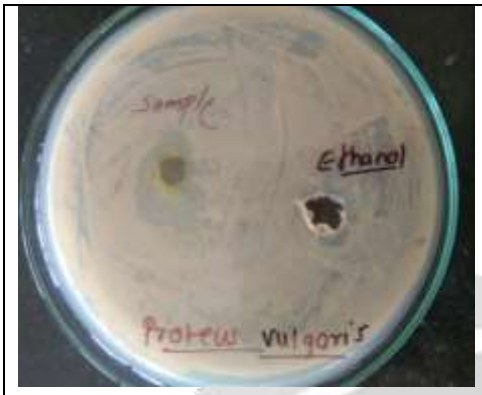
Extracts were boiled with 10 ml of water in test tubes. A few drops of ferric chloride were added and observed for blue black coloration.

- **Test for Saponins**

Extracts were boiled with distilled water. The solution was shaken vigorously for a stable, persistent, froth or foam. (5)

2.4 In vitro studies
The alkaloid carpaine which is present in papaya leaf extract was subjected to in vitro analysis. The pure clinical isolates used in the study and maintained in the nutrient agar plants before use. A control well was filled with used solvent. The petri plates were incubated at 370℃ for 48 hrs. It is determined on the basis of diameter of zone of inhibition around the well. The bacteria Proteus vulgaris nitrate reducing bacteria showed the maximum inhibition

from 12 to 23 mm. Inhibition activities of different microorganisms against the selected phytochemical was studied and their results were obtained. The microorganisms taken for the test were Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus and candida albicans.



Proteus vulgaris

Staphylococcus aureus

Candida albicans

Pseudomonas aeruginosa

Fig -3 Antimicrobial activities of alkaloid Capraine against different bacteria

2.5 In silico studies

General Methodology: Molecular docking is a computational technique used in drug discovery and molecular biology to predict the binding affinity and orientation of a small molecule (ligand) within a target protein or other macromolecule (receptor). It helps in understanding the interaction between the ligand and receptor at the molecular level, which is crucial for designing new drugs or optimizing existing ones. The process of molecular docking typically involves the following steps:

Preparation of ligand and receptor: The ligand and receptor structures are prepared by removing any unwanted molecules or solvent molecules and optimizing the structures using molecular modelling software. The ligand is a small phytochemical molecule drug candidate or a library of compounds, while the receptor is usually a protein structure derived from experimental techniques like X-ray crystallography or NMR spectroscopy.

Grid generation: A three-dimensional grid is generated around the receptor, representing the search space for the ligand. This grid is used to evaluate the ligand's potential binding positions and orientations within the receptor.

Scoring function: A scoring function is defined to estimate the binding affinity between the ligand and receptor. The scoring function calculates a numerical value that represents the predicted strength of the interaction. Various scoring functions exist, ranging from empirical scoring functions that use simple physics-based terms to more advanced force-field-based scoring functions.

Ligand docking: The ligand is systematically docked into the receptor's binding site by exploring different positions and orientations. Docking algorithms use various search algorithms, such as genetic algorithms or Monte Carlo simulations, to explore the conformational space and find the most favorable binding pose. The scoring function is used to evaluate the fitness of each docked pose.
Analysis and visualization: The generated docking poses are analyzed and ranked based on their predicted binding affinity. Visualizing the docked poses of helps to understand the interaction between the ligand and receptor ie, Carpaine and 1GII and identify molecular interactions, such as hydrogen bonds, hydrophobic interactions, or electrostatic interactions. [6]

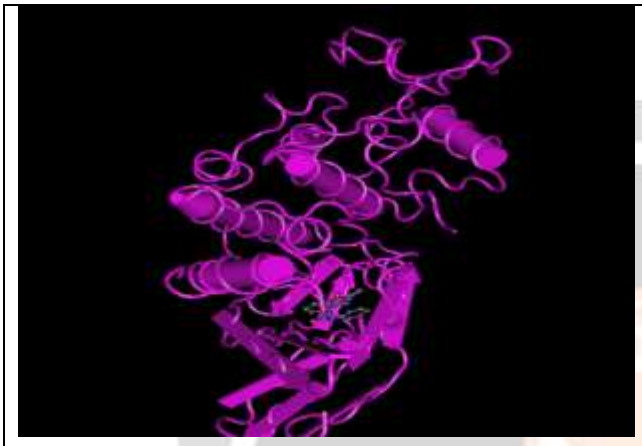


Fig-4: The selected receptor 1GII

Fig-5: The 3D structure of selected Ligand Carpain

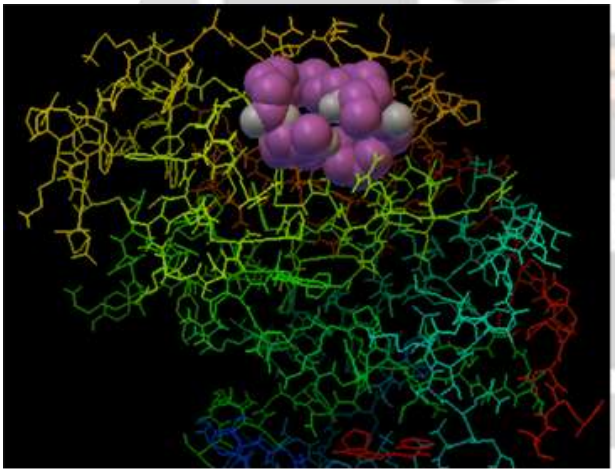


Fig-6: Docked structure of receptor 1GII and Carpaine in cpk and wireframe mode

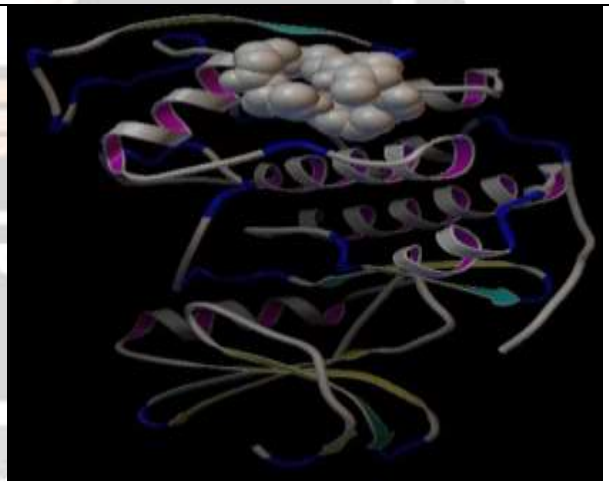


Fig-7: Docked structure of receptor 1GII and Carpaine in ribbon mode

Validation and refinement: Docking results are further validated and refined through experimental techniques like biochemical assays or structural determination methods such as X-ray crystallography or NMR spectroscopy. This helps in validating the predicted binding poses and optimizing the ligand's structure to enhance its binding affinity and specificity. Molecular docking is a valuable tool in drug discovery as it provides insights into the binding mechanisms of small molecules to their target proteins. It can be used for virtual screening of large compound libraries to identify potential drug candidates, lead optimization to improve the binding affinity of existing

compounds, and understanding the binding modes of ligands to aid in the design of novel drug molecule. The ligand Caprine was downloaded from PubChem website which was 3D structure in SDF format.

3.1 In-vitro results

Antimicrobial activities were obtained for Proteus vulgaris, Staphylococcus aureus, Candida albicans and Pseudomonas aeruginosa. From the results it can be seen that the sample which shows good antimicrobial activity are Proteus vulgaris and candida albicans as compared to Staphylococcus aureus and Pseudomonas aeruginosa.

Name of the microorganisms	**Control zone diameter of ethanol sample (mm)**	**Test zone of the Sample (mm)**
Proteus vulgaris	12	23mm
Staphylococcus aureus	17	No zone
Candida albicans	15	19mm
Pseudomonas aeruginosa	No zone	No zone

3.2 In-Silico results

The Capraine molecule is studied in silico using autodock software. It is reported that this ligand capraine successfully docked with the enzyme 1GII and it gave **- 7.44 Kcal/mol** binding energy. This value indicates that the capraine may be a drug candidate for the CDK based inhibition. Out of 50 conformations, the 6th conformer gives the low binding free energy which is – 7.44 Kcal/mol. The different poses of conformations in docking analysis are shown in Fig-8 which forms one hydrogen bond with arginine of protein.

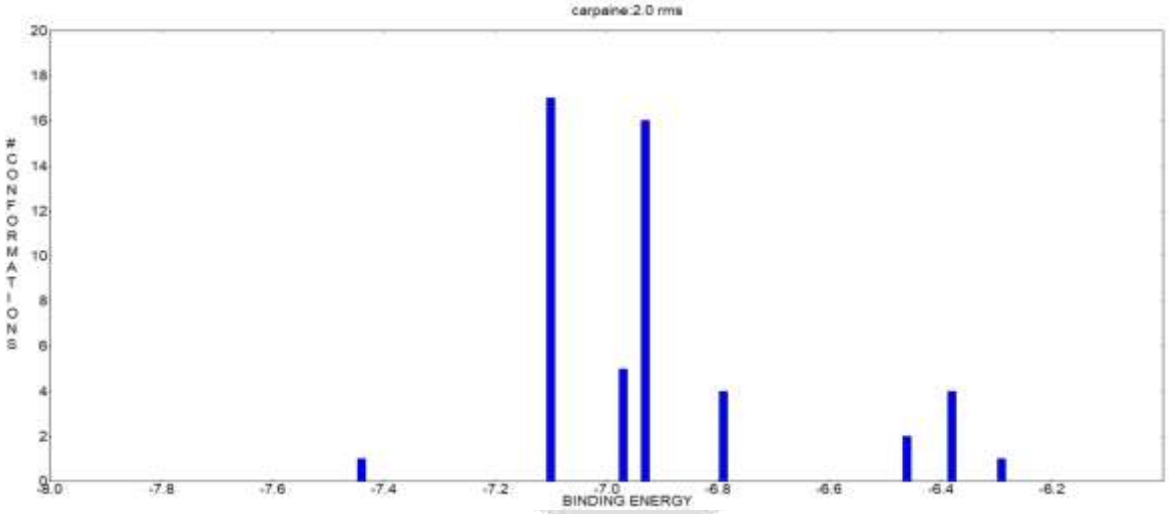


Chart-1: Binding Energies of conformers of ligand and the receptor

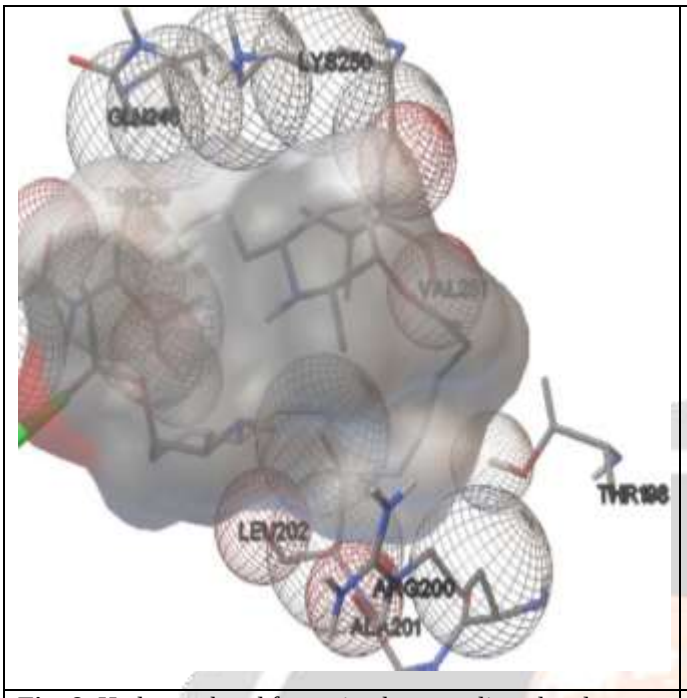


Fig -8: Hydrogen bond formation between ligand and receptor

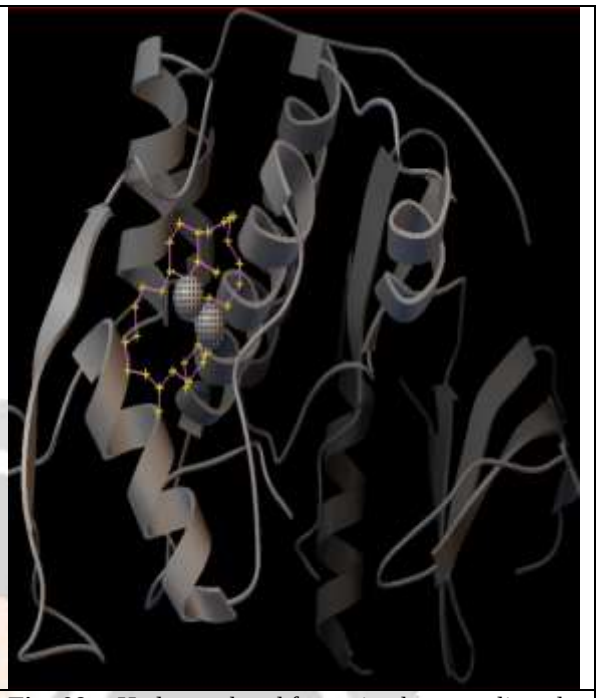


Fig -09: : Hydrogen bond formation between ligand and receptor

4. CONCLUSIONS

This research evaluates the results of in vitro and in silico study of selected ligand molecule which is a phytochemical named carpaine, obtained from papaya leaf. 1GII is responsible for nearly 50 percent of cancers so, in-silico studies were done on different conformers of ligand carpaine and receptor 1GII, a CDK4 protein which gives binding free energy of **-7.44** kcal/mole. This research evaluated the docking studies of 1GII with carpaine. This can be further docked with various proteins of CDK, alkaloid, or diseases like urinary tract infection

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