WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

In *Silico* Docking of Jackfruit *(Artocarpus Heterophyllus)* Phytochemicals as Competitive Inhibitors for PD-1 Binding to PD-L1 (PDB ID: 2M2D) in *Cancer Cells*

Camasura, Jean Ymerzon A.[1], Medalle, Justin D.[2], Edward Brian M. Ranjo[3], Sherwin S. Fortugaliza, PhD[4]

[1,2] Proponents, [3]Research Consultant, [4]Research Adviser
Davao City National High School, F. Torres St., Davao City
DOI : https://doi.org/10.55248/gengpi.6.0425.1376

ABSTRACT

Cancer cells often evade the immune system by exploiting the PD-1/PD-L1 interaction, which inhibits T cell activity. Current treatments that target this pathway aim to block PD-1 or PD-L1, thus reactivating the immune response against cancer cells. This study investigates the potential of jackfruit (Artocarpus heterophyllus) phytochemicals as natural inhibitors of the PD-1/PD-L1 interaction using in silico molecular docking. Phytochemicals from jackfruit were screened for drug-likeness using Lipinski's rule of five, toxicity evaluated through ProTox, and their ability to penetrate biological barriers was assessed via the boiled-egg model. Molecular docking simulations using Chimera were performed to analyze binding interactions between the phytochemicals and PD-L1. Among the tested compounds, Artocarpesin demonstrated the highest binding affinity, followed by Urolithin D and Artocarpin. These findings suggest that jackfruit-derived phytochemicals could serve as potential natural inhibitors of the PD-1/PD-L1 pathway, warranting further in vitro and in vivo studies to explore their therapeutic potential in cancer treatment.

Keywords: Cancer, PD-1, PD-L1, Jackfruit, Artocarpus heterophyllus, Phytochemicals, Molecular docking, Immunotherapy, Natural inhibitors, Drug-likeness

I. Introduction

According to the World Health Organization (WHO), cancer remains a leading global health challenge, responsible for approximately 10 million deaths in 2020 alone (Sung et al., 2021). This complex disease arises when abnormal cells grow uncontrollably, invading surrounding tissues and potentially spreading throughout the body. Despite advancements in medical treatments such as chemotherapy, radiation, and immunotherapy, cancer continues to pose significant difficulties due to its ability to develop resistance to therapies and its multifaceted nature.Cancer remains largely incurable, highlighting the urgent need for further research and study. Scientists are actively investigating innovative treatment methods, exploring genetic factors, and improving early detection techniques. The ultimate goal is to enhance treatment efficacy and eventually discover a cure. As the global cancer burden grows—with projections of over 35 million new cases by 2050—continued scientific exploration is critical to address this pressing health crisis (World Health Organization, 2024).

Cancer cells are especially problematic because of their superior skill at evading the body's natural defenses. Normally, the body's immune system is able to detect and remove abnormal or foreign cells, keeping any uncontrolled growth in check. But cancer cells have found elaborate ways to hide from immune detection, enabling them to grow and metastasize unchecked. One of the main methods by which they do this is by exploiting the PD-1/PD-L1 pathway. PD-1 (programmed cell death protein 1) is an immune checkpoint receptor expressed on T-cells, which play a key role in detecting and eliminating faulty cells. When PD-1 is engaged with its ligand, PD-L1 (programmed death-ligand 1), it transmits an inhibitory signal that slows down the activity of T-cells to avoid unwanted immune activation and safeguard normal cells against autoimmunity. But cancer cells often overexpress PD-L1 on their surfaces and essentially 'capture' this pathway. In the process, they establish an immunosuppressive milieu that lets them evade immune surveillance. This mechanism of immune evasion helps cancer cells to survive and develop tumors without being attacked by the immune system. The PD-1/PD-L1 axis is now a prime target in cancer immunotherapy. Immune checkpoint inhibitors (ICIs) like pembrolizumab and nivolumab inhibit the binding between PD-1 and PD-L1, reactivating T-cell function and permitting the immune system to target cancer cells. This method has demonstrated marked efficacy in the treatment of numerous cancers, including melanoma, non-small cell lung carcinoma, and Hodgkin lymphoma (Topalian et al., 2012; Ribas & Wolchok, 2018). Yet, while the results are encouraging, a few patients become resistant to checkpoint inhibitors, indicating the intricacy of cancer's immune evasion mechanisms (Sharma et al., 2017).

The PD-1/PD-L1 pathway is an important immune checkpoint pathway controlling the body's immune response and avoiding excessive activation and autoimmune conditions. PD-1 (Programmed Cell Death Protein 1) is a receptor on T-cells, and PD-L1 (Programmed Death-Ligand 1) is its ligand, which exists on antigen-presenting cells as well as in normal tissues. In the normal situation, the interaction of PD-L1 with PD-1 conveys an inhibitory signal that decreases T-cell activity to avoid chronic inflammation and autoimmune responses (Pardoll, 2012). In cancer, the malignant cells take advantage of this mechanism by overexpressing PD-L1 to avoid immune recognition. When PD-L1 expressed on tumor cells interacts with PD-1 receptors on T-cells, it inhibits their activity, resulting in an immunosuppressive environment that facilitates tumor growth (Chen & Mellman, 2013). Such an immune escape strategy makes cancer especially hard to eliminate, and hence immune checkpoint inhibitors (ICIs) like pembrolizumab and nivolumab have been created that inhibit the PD-1/PD-L1 interaction, reviving the action of T-cells and improving the capability of the immune system to attack cancer cells (Ribas & Wolchok, 2018). Although these treatments have been largely successful, resistance is seen in some patients, and thus continuous research into combination regimens and new drug therapies is vital.

Phytochemicals are bioactive compounds isolated from diverse plant food products, such as fruits, vegetables, grains, legumes, nuts, and herbs. While nutrients provide the body's basic needs, phytochemicals are groups of non nutrient molecules that have powerful health-promoting and disease-preventing effects. Some of the potential groups include flavonoids, carotenoids, glucosinolates, saponins, and polyphenols, each with unique properties for health promotion. For example, flavonoids, found in fruits and vegetables as well as in beverages like tea, have antioxidant properties that could decrease inflammation and oxidative stress. Carotenoids, abundant in brightly colored crops like carrots and spinach, are also supposed to lower risks for a number of cancers and eye diseases. The major health benefits afforded by phytochemicals originate from their ability to fight oxidative stress, a process whereby free radicals damage the cell and contribute to chronic conditions such as cardiovascular disease, cancer, and neurodegenerative disorders. A high proportion of phytochemicals have antioxidant activity, thus preventing free radicals from contributing to further cell damage. Furthermore, their anti-inflammatory activities may, at least reduce chronic inflammation known to be a major risk factor for many conditions; specific phytochemicals have been reported also to ensure hormone equilibrium levels and stimulate the immune system. While thousands of phytochemicals have been identified so far, about half are not well characterized; more are, thus, urgently needed in order to complete their characterization profiles for the discovery of specific health benefits and mechanisms of action associated with each of these compounds. As more is known about the intricacies of phytochemicals, it becomes more sensible to include a broad range of plant-based foods as a component of any diet. A variety of colorful fruits and vegetables add a boost of nutritional value to meals while maximizing the intake of phytochemicals, which are further associated with a healthy condition and reduction in chronic diseases (Liu, 2003).

The PD-1/PD-L1 pathway is a key mechanism of immune evasion by tumor cells and therefore an ideal candidate for therapeutic intervention. In physiological conditions, the engagement of PD-1, a T-cell receptor, and its ligand PD-L1, which is present on antigen-presenting cells and normal tissues, serves as a brake against hyperimmune activation. Yet, it is used to its advantage by most cancer cells that overexpress PD-L1, the latter interacting with PD-1 on T-cells and triggering inhibitory signals that impede T-cell activity and proliferation. Immune suppression due to this enables cancer cells to escape immune surveillance and continue to proliferate undeterred (Chen & Mellman, 2013). Blocking this axis with ICIs has proven efficacy in cancer therapy, reviving T-cell functionality and allowing the immune system to target tumor cells (Ribas & Wolchok, 2018). Existing ICIs, including nivolumab and pembrolizumab, are though costly and feature immune-related toxicities, for which reason developing more affordable natural alternatives with minor side effects has become a high priority (Postow et al., 2018).

Considering the imminent necessity for new and affordable PD-1/PD-L1 inhibitors, jackfruit (Artocarpus heterophyllus) appears as a highly valuable candidate in view of its diverse phytochemical content and reported bioactivity. Jackfruit is well-known to contain flavonoids, phenolics, stilbenoids, and lectins, all of which exhibit antioxidant, anti-inflammatory, and anti-cancer activity (Baliga et al., 2011). A number of studies suggest that plant-derived phenolic compounds and flavonoids are able to modulate immune checkpoints, such as suppressing PD-L1 expression or interfering with PD-1/PD-L1 binding, and are thus promising candidates for cancer immunotherapy (Li et al., 2021). For instance, flavonoids can suppress PD-L1 expression on tumor cells, thus preventing them from hiding from immune attack (Zhao et al., 2019). Likewise, lectins, which are carbohydrate-binding proteins present in jackfruit, have exhibited cytotoxicity towards cancer cells through apoptosis induction and immune system modulation, thus holding promise as immunotherapeutic compounds (Ng, 2014).

In addition, the availability and accessibility of jackfruit in tropical areas render it a convenient and sustainable source of future PD-1/PD-L1 inhibitors. In comparison to artificially synthesized drugs, which are costly to manufacture and deliver, natural products from jackfruit may provide a cheaper and more accessible option, especially in low- and middle-income economies where access to advanced immunotherapies is restricted. By examining jackfruit's bioactive molecules as possible inhibitors of the PD-1/PD-L1 axis, this study hopes to identify a natural, low-cost therapeutic agent that can be used in conjunction with current immunotherapies. With effectiveness demonstrated, jackfruit-based inhibitors would not only augment outcomes in cancer treatment, but also alleviate the economic strain on patients and the health care system.

Furthermore, the antioxidant nature of compounds isolated from jackfruit could yield additional advantages when it comes to cancer treatment. Oxidative stress generated through the presence of reactive oxygen species (ROS) within the tumor microenvironment triggers cancer progression and facilitates immune suppression through damage to T-cell function (Sullivan & Chandel, 2014). Flavonoids and phenolics in jackfruit possess intense free radical-scavenging capacities that might eliminate oxidative stress and strengthen the tolerance of T-cells, subsequently augmenting immunity and decreasing immune evasion properties in the tumor (Baliga et al., 2011).

Despite the significant global health challenge posed by cancer, particularly in regions with high incidence rates, effective treatments targeting the PD-1/PD-L1 pathway remain limited. The PD-L1 protein plays a crucial role in tumor immune evasion by interacting with the PD-1 receptor on T cells, thereby inhibiting the immune response against cancer cells. However, there is a lack of research on natural compounds that could inhibit PD-L1 and

potentially serve as a basis for new therapeutic agents. Given the known medicinal properties of jackfruit (Artocarpus heterophyllus) phytochemicals, this study aims to investigate whether these compounds can effectively bind to and inhibit the PD-1 protein (PDB: 2M2D), offering a potential natural treatment approach for cancer immunotherapy.

This study aims to assess the potential of selected phytochemicals from Jackfruit (*Artocarpus heterophyllus*) in competitively inhibiting the PD-1/PD-L1 interaction (PDB: 2M2D) through molecular docking analysis utilizing Chimera. Specifically, the researchers' objectives are:

1. **Phytochemical Screening and Toxicity Assessment.** To screen the phytochemicals from *Artocarpus heterophyllus* using SwissADME for Lipinski's rule of five and the boiled-egg model, and to evaluate their toxicity using ProTox to ensure their safety for potential therapeutic use.
2. **3D Model Generation and Molecular Docking Simulations.** To generate 3D models of the selected phytochemicals using Avogadro software and perform molecular docking simulations using Chimera to assess the binding interactions between the chosen phytochemicals and the PD-L1 protein (PDB: 2M2D).
3. **Binding Energy Analysis.** To analyze the binding energy of the phytochemicals with PD-11 and compare it to the PD-1/PD-L1 interaction to determine the effectiveness of the compounds in competitive inhibition.
4. **Competitive Inhibition Assessment.** To evaluate the potential of the selected phytochemicals to inhibit the PD-1/PD-L1 interaction based on the outcomes of the molecular docking simulations..

II. Methodology

A. Screening and Preparation of Jackfruit (Artocarpus heterophyllus)

1. Lipinski's rule of 5 screening through SwissADME

Fifty-three main phytochemicals from *Artocarpus heterophyllus* (Zhang et al., 2017) were initially tested before undergoing a docking process. Each compound was screened for drug-likeness using Lipinski's rule of five through SwissADME (http://www.swissadme.ch). Lipinski's rule predicts poor absorption or permeability when there are more than five hydrogen bond donors, ten hydrogen bond acceptors, a molecular weight (MWT) over 500, and a calculated Log P (CLogP) greater than 5 (or MLogP higher than 4.15). The computational process for calculating Moriguchi Log P (MLogP) based on these rules is explained, alongside discussions on turbidimetric solubility testing, which is applied to established drugs. High-throughput screening (HTS) leads tend to exhibit a higher MWT and Log P with lower solubility compared to leads identified before HTS became common. During the development phase, solubility calculations focus on precise predictions but can be complicated by factors like polymorphism. Recent advances in linear free energy relationships and Log P methods are critically examined, demonstrating that effective predictions for closely related analogs can be achieved when combined with experimental thermodynamic solubility data (Lipinski et al., 1997). This comprehensive drug-likeness evaluation verified each compound's bioavailability, solubility, and chemical stability, as well as the likelihood of successfully developing these molecules into drugs for commercial production and use.

2. Boiled-egg model from SwissADME

After undergoing a screening process through Lipinski's rule of five, the phytochemicals that pass this criterion will proceed to another screening process called the boiled-egg model, available on the SwissADME website (http://www.swissadme.ch). According to a research (Daina et al., 2017), this model is a predictive tool used to evaluate and predict the absorption and brain penetration potential of compounds based on their lipophilicity and polarity, visualizing whether a compound can cross the blood-brain barrier (BBB) or be absorbed in the human intestinal tract (HIA). The boiled-egg model incorporates key parameters such as PGP+ (P-glycoprotein positive substrate), which identifies compounds that may be actively removed from cells by efflux transporters, and PGP-, indicating those that are not substrates and thus more likely to be absorbed. For PD-L1 inhibitors, PGP- compounds are preferable because they are less likely to be expelled from cells by P-glycoprotein, allowing for better intracellular retention, though PGP+ compounds can still be used, albeit with potentially reduced efficacy due to active efflux by the transporter. The model measures the topological polar surface area (TPSA), indicating a molecule's polarity—with lower TPSA values suggesting better absorption and brain penetration potential—and includes the Wildman-Crippen Log P (WLOGP), reflecting a compound's lipophilicity, with moderate values being optimal for membrane permeability. The model distinguishes compounds into regions, where the yolk represents molecules predicted to cross the BBB, and the white represents those likely to be absorbed in the intestines, aiding in the evaluation of drug-likeness for research.

3. Screening for toxicity through ProTOX

ProTox, a comprehensive tool for assessing the toxicological profiles of compounds, will be used for toxicity screening in order to guarantee the safety of the chosen phytochemicals. Protox evaluates a range of toxicity endpoints and assigns a score according to how likely it is that a certain substance would be harmful to people and other living things. A score of 4-6 indicates non-toxicity and good consumption potential, while a score of 1-3 indicates various levels of toxicity. The grading system runs from 1 to 6. ProTox assists researchers in assessing the safety of novel chemicals by assessing potential toxicity across a variety of organs, such as the liver, kidney, and nervous system (Banerjee et al., 2018).

4. Preparation of the Screened Phytocompounds

Once the phytocompounds pass the multi-screening process, their isomeric SMILES, sourced from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/), can be used to generate 3D models of the compounds or ligands in Avogadro software, a molecular modeling tool that enables users to create, edit, and visualize 3D molecular structures (Hanwell et al., 2012). After generating the models, they are saved as "Sybyl Mol2 (*.mol2)" files and organized in a designated folder for ligands in preparation for further analysis.

B. Preparation of PD-1 Protein

The 3D structure of the target protein (RCSB PDB ID: 2M2D) was downloaded in PDB format from the RCSB Protein Data Bank (https://www.rcsb.org/). After downloading the PDB file, we choose the second model of the protein. All nonstandard amino acids, including water (HOH), were deleted. The protein then underwent a structure minimization process, also known as energy minimization, using Chimera software. This process aims to clean up small molecule structures and improve localized interactions within larger systems (UCSF Computer Graphics Laboratory, 2020). Finally, the minimized structure of the protein was saved as a PDB file and organized into a designated folder.

C. Receptor Gridbox Manual Generation

The active binding residues of the 2M2D PD-1 protein were predicted through CASTp. The receptor grid area was determined using Autodock Vina through UCSF Chimera. The center of the grid box was positioned according to the active binding site in correspondence to the active residues of the main pocket of the receptor protein: PRO 35, PHE 56, SER 57, ASN 58, THR 59, LYS 78, LEU 79, ALA 80, ALA 81, PRO 83, GLU 84, ASP 85, ARG 86, GLN 88, PRO 89, GLY 90, GLN 91, ASP 92, PHE 95, ARG 96, VAL 97, ALA 125, ILE 126, SER 127, GLN 133, LYS, 135 . The binding site size was minimized to 42.1101 , 74.815 , 54.5541 Angstrom to lessen the probability of the software producing less accurate results and unnecessary binding outcomes. The XYZ values of the coordinates of the center of the grid box (x:1.43086 , y: 1.92503, z: 0.480804), as well as the dimensions of the grid box (45.4503 , 30.7134 , 31.1952)

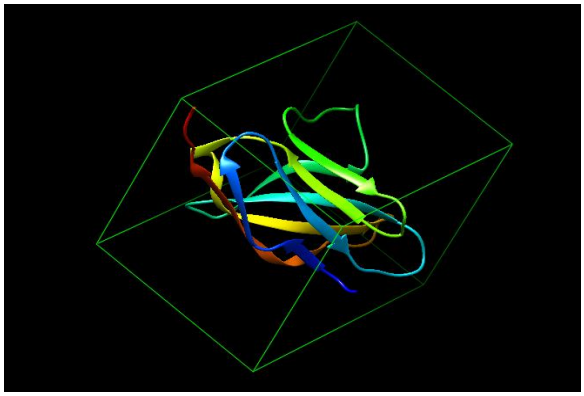

Figure 1. *PD-1 protein (PDB:2M2D)receptor grid box visualization through Biovia Discovery Studio Visualization.*

D. Analysis and Simulation of Molecular Docking

For the molecular docking analysis, the preparation of the ligands, protein, and working folder was completed before the actual docking process began. Dock prep was used to prepare the system, with Amber ff14SB set for the residues and Gasteiger for other residues. The docking process was carried out using the AutoDock Vina tool integrated with UCSF Chimera. The executable location was specified as

"C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe."

The phytocompounds were docked to the PD-1 receptor (PDB ID: 2M2D), and the binding scores were computed. Once the docking was completed, the output and protein files were saved in PDBQT format. Finally, Biovia Discovery Studio Visualizer was used to visually display the interaction sites between the compounds and the receptor, helping to understand the binding interactions more clearly.

E. Scoring and Analysis

The results of the docking computation were already saved and set to a specific file before the scores were given. The conformation and orientation are assessed using a scoring function of the ligand in the protein's binding site and quantifies binding affinity, or how strongly a ligand and protein interact. Stronger contact between the ligand and the protein and a higher chance of effective binding are indicated by a more negative binding affinity score (Pantsar & Poso, 2018). According to Ali et al. (2018) the stronger the connection between the ligand and the protein, the lower the binding affinity value.

The visual analysis was conducted using the structure-based virtual screening approach, and the 3D outputs of the protein-ligand docking were viewed using UCSF Chimera. Biovia was used to observe the 2D visualization of the hydrogen bonds, hydrophobic interactions, and receptor-ligand interactions. These Connections are essential in ligand-protein interactions. docking as they ascertain the binding and specificity of the protein-ligand complex's affinity (Hudait et al., 2019). Additionally, the interaction residues are assessed. following docking via Biovia.

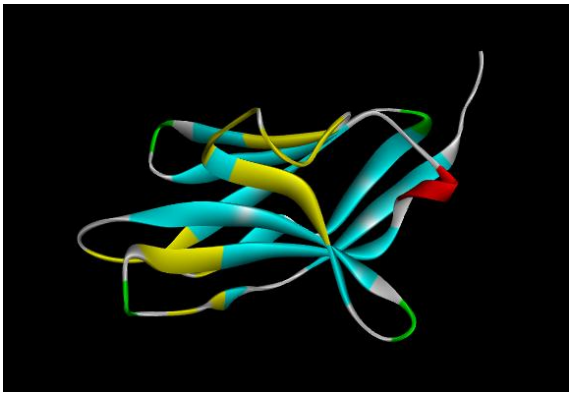

Figure 2. *Human host cell PD-1 interacting residues of cancer/tumor cells (PDB ; 2M2D) (yellow).*

After docking, the interacting residues of the binding sites in the protein are evaluated using Biovia. These residues, which interact with the ligand, will be compared to the natural binding residues of PD-1 (Programmed Cell Death Protein 1), including PRO35, PHE37, LEU42, THR51, CYS54, PHE56, SER57, ASN58, THR59, LEU65, ASN66, ASP77, ARG86, ASP92, VAL111, SER127, and LYS135.

Methodological Framework

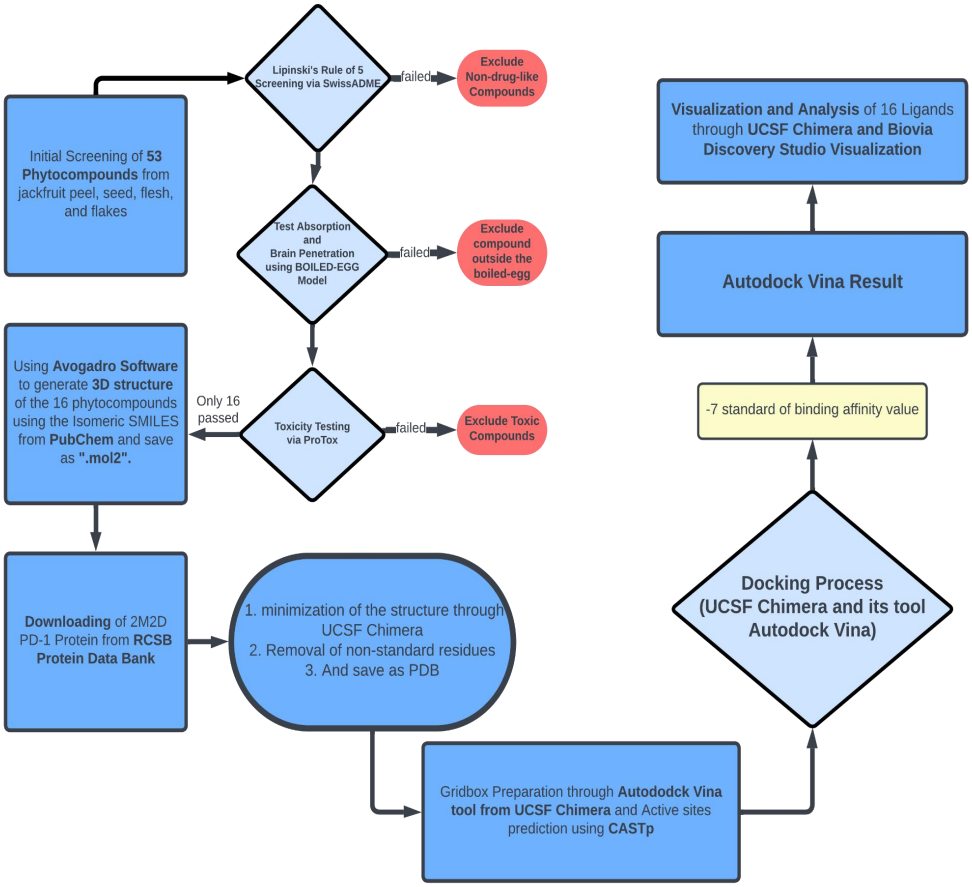

Figure 3. *Methodological Framework of the study.*

III. RESULTS AND DISCUSSIONS

This work sought to identify and evaluate the 53 major phytochemical components—leaves, seeds, peel, pulp, and flake—obtained from Artocarpus heterophyllus that target the active binding sites of PD-L1. Following the Lipinski rule, which requires that the physicochemical characteristics of the phytochemical ligands be assessed before the molecular docking process, the 53 phytochemicals were submitted to SwissADME. Of the 53 phytochemicals, 16 were found to have passed Lipinski's rule of fifth.

Table 1: *Phytochemicals found in Artocarpus heterophyllus (Jackfruit)*

a	***b***	***c***	***d***	***e***	***f***
Cyanomaclurin	288.25	4	6	0.24	0
Linoleic Acid	280.45	1	2	0.24	0
Chorismic Acid	226.18	3	6	-0.75	0
Valtrate	422.47	0	8	1.87	0
Furofuranone	124.09	0	3	0.02	0
Chlorophorin	380.48	4	4	3.83	0
Morachalcone A	340.37	4	5	2.14	0
Naphthalenedicarboxylic Acid	216.19	2	4	2.19	0
Ferulic Acid	194.18	2	4	1.00	0
Malic Acid	134.09	3	5	-1.37	0
Artocarpin	436.50	3	6	2.30	0
Artocarpesin	354.35	4	6	1.09	0
Urolithin D	260.20	4	6	0.30	0
Caffeic Acid	180.16	3	4	0.70	0
Pentenylnaringenin	340.37	3	5	1.82	0
1-Hexylglycerol	176.25	2	3	0.73	0

a *= Ligands;* ***b*** *= Molecular Weight (g/mol,* ***<500 Da****);* ***c*** *= Number of Hydrogen bond donors* ***(<5)****;* ***d*** *= Number of Hydrogen bond acceptors* ***(<10)****;* ***e*** *= M Log* $P_{o/w}$ ***(≤4.15)****;* ***f*** *= Number of Violations* ***(<1)***

Out of the 53 phytocompounds screened, 16 successfully passed Lipinski's rule, indicating their drug-likeness. The remaining compounds were then evaluated using the boiled-egg model to predict their potential for absorption and brain penetration, based on their lipophilicity and polarity.

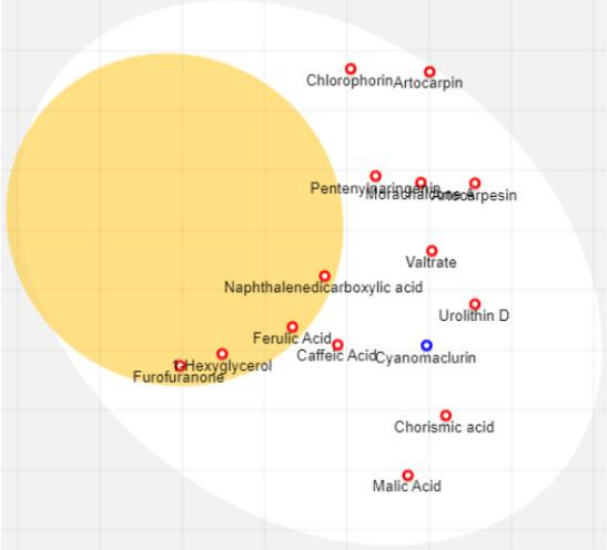

Figure 4. *Egg-boiled model from SwissADME*

The 16 phytochemicals derived from jackfruit underwent the boiled-egg process to predict whether a compound can cross the blood-brain barrier (BBB) or be absorbed in the human intestinal tract (HIA). We are aiming for the red dot which is PGP- (P-glycoprotein negative substrate) as it stays longer in the human system than the positive substrate and the compounds are at the egg yolk (yellow circle) for the reason that it can penetrate through the brain. However, we still consider them all and proceed to the last process.

Toxicity test of the ligand using ProTox

Toxicity evaluation is one of the most important phases in drug and chemical design. Therefore, there is a great need for computational predictive models to assess the possible harmful consequences in chemicals and medications (Banerjee et al., 2024). The identified phytochemicals from Jackfruit *(Artocarpus heterophyllus Lam)* underwent toxicity evaluation through ProTox to determine the toxicity value of the following phytochemicals.

a	***b***	***c***
Cyanomaclurin	C1=CC2=C(C=C1O)O[C@@H]3C([C@@H]2OC4=CC(=CC(=C34)O)O)O	5
Pentenylnaringenin	CCC/C=C/C1(CC(=O)C2=C(C=C(C=C2O1)O)O)C3=CC=C(C=C3)O	4
Urolithin D	C1=CC(=C(C2=C1C3=CC(=C(C=C3C(=O)O2)O)O)O)O	4
Naphthalenedicarboxylic acid	C1=CC=C2C(=C1)C=CC(=C2C(=O)O)C(=O)O	5
Artocarpin	CC(C)/C=C/C1=C(C=C2C(=C1O)C(=O)C(=C(O2)C3=C(C=C(C=C3)O)O)CC=C(C)C)OC	5
Artocarpesin	CC(=CCC1=C(C2=C(C=C1O)OC(=CC2=O)C3=C(C=C(C=C3)O)O)O)C	5

Valtrate	CC(C)CC(=O)O[C@H]1C=C2[C@@H]([C@@]13CO3)[C@@H](OC=C2COC(=O)C)OC(=O)CC(C)C	6
Morachalcone A	CC(=CCC1=C(C=CC(=C1O)C(=O)/C=C/C2=C(C=C(C=C2)O)O)O)C	4
Chorismic acid	C=C(C(=O)O)O[C@@H]1C=C(C=C[C@H]1O)C(=O)O	4
Malic acid	C(C(C(=O)O)O)C(=O)O	5
Linoleic acid	CCCCC/C=C\C/C=C\CCCCCCCC(=O)O	6
Ferulic acid	COC1=C(C=CC(=C1)/C=C/C(=O)O)O	4
1-Hexylglycerol	CCCCCCOC[C@H](CO)O	4
Furofuranone	C1C2=C(C=CO2)OC1=O	4
Caffeic acid	C1=CC(=C(C=C1/C=C/C(=O)O)O)O	5
Chlorophorin	CC(=CCC/C(=C/CC1=C(C=C(C=C1O)/C=C/C2=C(C=C(C=C2)O)O)O)/C)C	5

a** = Phytochemical Name; **b** = Phytochemical smiles; **c** = Predicted Toxicity Class **(≥4)

Scoring Functions

Chimera was used to visualize and identify the positions of the ten docking attempts that occupied the binding pocket. A standardized -7 affinity value was given to pinpoint the phytochemicals having a higher chance of interacting with the 2M2D protein. This process narrowed down the number to 16. Each ligand conformation (1st pose) was integrated into PDB files, generating 16 ligand complexes subjected to visual analysis. Among the phytochemicals, the ligand with the most negative binding affinity score was Cyanomaclurin, followed by Artocarpesin, and Morachalchone A

Table 2: *16 Phytochemicals with their binding affinity scores*

a	***b***	***c***
Cyanomaclurin	44257130	-6.6
Pentenylnaringenin	171393850	-5.8
Urolithin D	5482042	-7.6
Naphthalenedicarboxylic acid	14357	-6.2

Artocarpin	5458461	-7.1
Artocarpesin	399491	-8.2
Valtrate	442436	-4.8
Morachalcone A	9862769	-6.6
Chorismic acid	12039	-5.0
Malic acid	525	-4.4
Linoleic Acid	5280450	-4.8
Ferulic acid	445858	-5.3
1-Hexylglycerol	10313344	-4.5
Furofuranone	70499527	-4.1
Caffeic Acid	689043	-4.7
Chlorophorin	5281713	-6.3

***a** = phytochemical name; **b** = CID from PUBCHEM (<5); **c** = binding affinity values*

Protein-Ligand Interactions between interacting residues

This comprehensive molecular docking study examined the interactions between 16 phytochemicals docked with the active binding site of PD-1. The results indicated that each of the 16 ligands in their highest predicted conformations made at least one interaction with critical amino acid residues located in the active binding site. Among the various ligands, the binding affinities of the twelve most prominent compounds were above or equal to -5 kcal/mol. In addition to exhibiting robust binding, these ligands interacted with PD-1

Table 3: *Biovia analysis of 16 Phytochemicals*

a	***b***	***c***
Cyanomaclurin	**THR51**	PHE37
	[4.62]	PRO39
		LEU42
		THR51
		CYS54
		LEU65
		ASN66
		VAL97
		TYR121
		LEU122
		ALA140

Pentenylnarengenin	**GLN91**	**ASP77**
	[2.20]	LYS78
	ASP92	LEU79
	[2.68]	ARG86
		GLN91
		ASP92
Urolithin D	N/A	LEU79
		ALA80
		ALA81
		GLU84
		ASP85
		GLY90
		GLN91
		ASP92
		PHE95
		ARG96
Naphthalenedicarboxylic Acid	N/A	LEU79
		ALA80
		ALA81
		GLU84
		ASP85
		GLY90
		GLN91
		ASP92
		PHE95
		ARG96
Artocarpin	N/A	GLU84
		ASP85
		ARG86
		ARG96
Artocarpesin	N/A	**ALA80**
		ALA81
		GLU84
		ASP85
		ARG86

		GLN91 ASP92 ARG96 VAL97
Valtrate	**LYS78** [4.90]	**LYS78** LEU79 ARG86 GLN91
Morachalcone A	**LEU79** [5.45]	LYS78 **LEU79** ASP92 PHE95
Chorismic acid	**ARG86** [3.96]	**LYS78** LEU79 **ARG86** GLN91
Malic acid	N/A	ASN58 THR59 PHE63 SER127
Linoleic Acid	N/A	**ARG86** GLN91 ASP92 SER93 ARG94 PHE95 **ARG96** **VAL111**
Ferulic acid	N/A	LEU79 **ALA80** ALA81 GLU84 ASP85 GLY90

		GLN91
		ASP92
		PHE95
1-Hexylglycerol	N/A	**ALA80**
		ALA81
		GLU84
		ASP85
		ARG86
		GLN91
		ASP92
		PHE95
		ARG96
		VAL97
Furofuranone	**THR59**	PRO35
	[2.13]	PHE56
		SER57
		ASN58
		THR59
		SER127
		LYS135
Caffeic Acid	**ARG86**	LYS78
	[3.75	**ARG86**
		GLN91
Chlorophorin	**LEU79**	ASP77
	[5.29]	LYS78
		LEU79
		GLN91
		ASP92
		PHE95

***a** = phytochemical name; **b** = hydrogen bonds with interacting residues; **c** = Hydrophobic interactions with interacting residues; **bold** = binding sites of PD-1*

Based on this ranking, the top ligands are Artocarpesin, Urolithin D, and Artocarpin. The ligands displayed a wide range of impressive binding affinities, with Artocarpesin showing the highest value at -8.2 kcal/mol and Urolithin D coming in second at -7.6 kcal/mol followed by Artocarpin at -7.1 kcal/mol.

The binding affinities of the remaining ligands, including Cyanomaclurin, Naphthalenedicarboxylic Acid, Chlorophorin varied between -6.6 and -6.0 kcal/mol. The consistent results highlight the compounds' strong binding and unique interaction with residues involved in the binding process of the Program Cell Death 1 (PD-1) cell receptor. The information this data provides is extremely valuable for furthering the study of these compounds as potential inhibitors, improving our understanding of their potential applications in competitive inhibition therapy. The evaluation of each inhibitory

potential of the chemical depended on assessing each of their levels of binding affinity and interaction between interacting residues, including hydrophobic and hydrogen bonding interactions between molecules. Binding affinity within molecular docking denotes the robustness of the interaction occurring at a specific binding site between a ligand and a receptor, foretelling the probability and strength of their association. Notably, the effectiveness of the phytochemicals from Artocarpus heterophyllus Lam binding to the PD-1 Protein is greatly impacted by the considerable contributions of hydrogen bonds and hydrophobic interactions between molecules. Artocarpesin demonstrated interaction with 2 pivotal binding sites on the Program Cell Death 1 (PD-1) cell receptor—specifically, ALA 80, ARG 86—engaging in hydrophobic interactions with all 2 of these interacting residues. It interacted with six prominent amino acids from within the 22 critical residues of 2M2D to PD-1 receptor

Top Three Phytochemicals with most interacting residues

Such identification of Artocarpesin, Urolithin D, and Artocarpin as potential ligands for the PD-1 protein (2M2D) opens promising avenues for identifying novel therapeutic strategies. Upon its consideration as the core constituent in maintaining the balance of immune responses, this key immune checkpoint protein is crucial for the proper functioning of the body. Overexpression, underexpression, or aberrant function of PD-1 signaling is linked to various diseases such as cancers and autoimmune disorders. Of the three, Artocarpesin has the highest binding affinity, which could play a role in modulating PD-1-induced immune responses. Further into detail, though, it would be necessary to understand its interactions with PD-1. Knowing what residues in the interaction would be involved and what kind of interactions-hydrogen bonding, hydrophobic interaction, or otherwise-will be keys to understanding how Artocarpesin will work therapeutically.

Urolithin D shows significant binding affinity to PD-1 as well. This intermediate is derived from the degradation of ingested polyphenols, which indicates it could be a natural immune response modulator. The mode of interaction of Urolithin D with PD-1 will uncover potential use in therapies, especially in metabolic diseases and aging. Artocarpin, while interacting with a very poor affinity versus the binding site in comparison to Artocarpesin and Urolithin D, warrants interest as a potential PD-1 ligand given its possible mechanism of action and future therapeutic benefit.

Visualization of the top three Phytocompounds with the most interacting residues

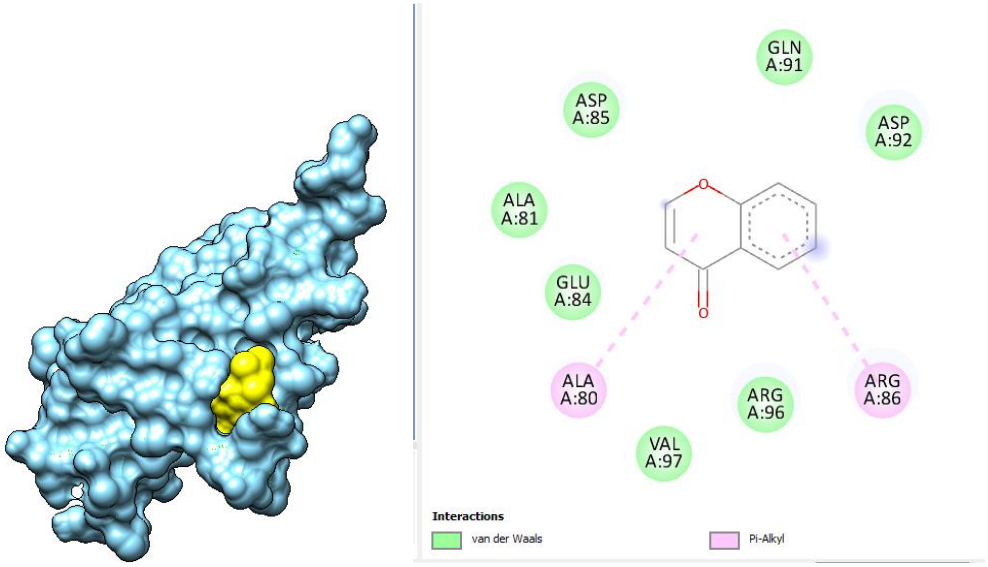

Figure 5. *Artocarpesin - PD-1Chimera 3D visualization (left); Receptor-Ligand interaction Biovia 2D visualization (right).*

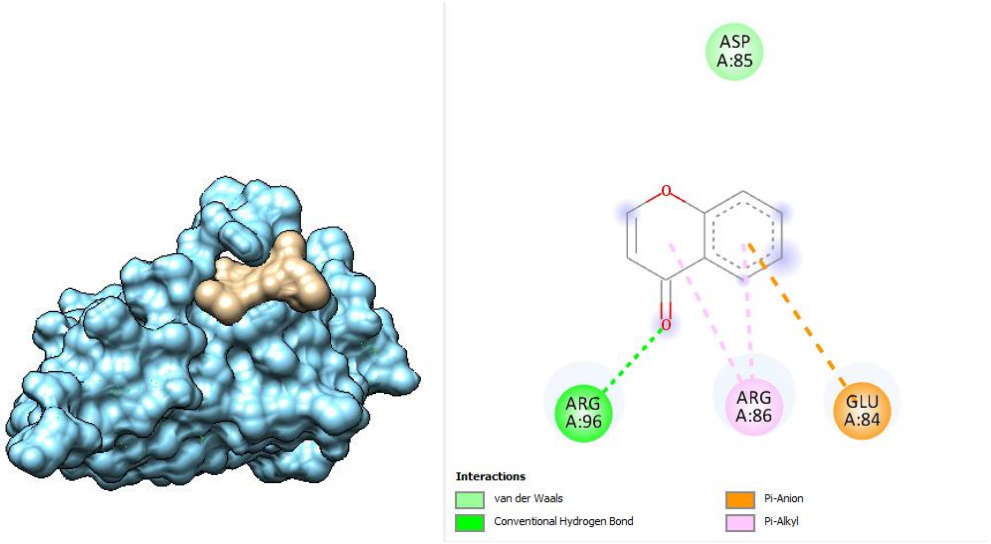

Figure 6. *Artocarpin - PD-1 Chimera 3D visualization (left); Receptor-Ligand interaction Biovia 2D visualization (right).*

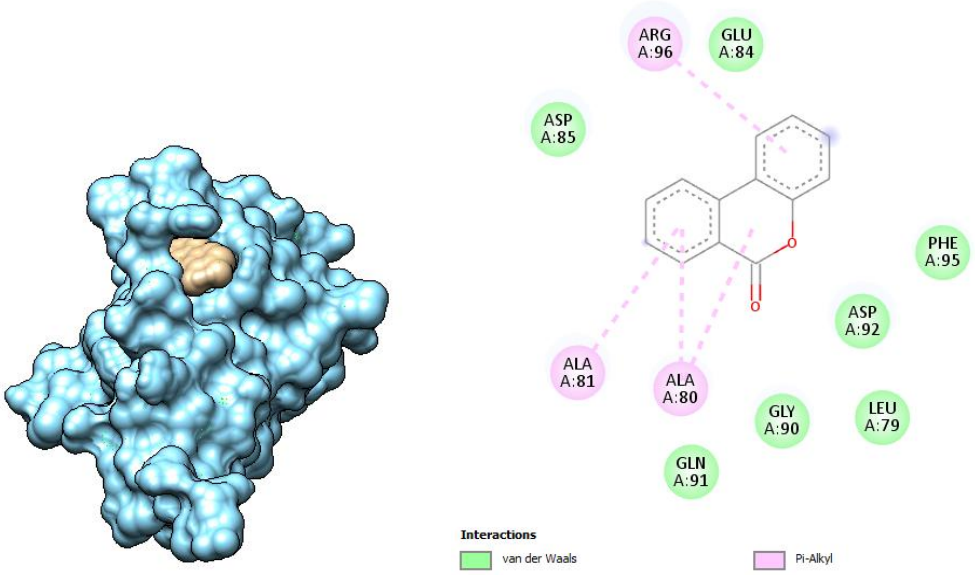

Figure 7. *Urolithin D - PD-1 Chimera 3D visualization (left); Receptor-Ligand interaction Biovia 2D visualization (right)*

Conclusion and Recommendations

The results from the in silico docking analysis showed promising potential for phytochemicals derived from jackfruit (Artocarpus heterophyllus) in inhibiting PD-1, a key protein involved in cancer immune evasion. Among the 16 tested compounds, Artocarpesin had the strongest binding affinity to PD-1, standing out as a top candidate. While all 16 compounds exhibited promising interactions with PD-1 in the computational analysis, this is just the first step.

To build on these findings, lab-based experiments, specifically in vitro studies using cancer cell lines, are needed to test whether these compounds can effectively block PD-1 in a biological setting. This will provide essential data on their inhibitory strength and potential therapeutic uses. Moreover, performing dose-response studies would help to identify the optimal concentrations of these compounds to ensure both safety and effectiveness.

More in-depth molecular research could also reveal how these compounds interact with PD-1 at a structural level, improving our understanding of their mechanisms. Tests like biochemical assays or molecular dynamics simulations could help fine-tune these findings and assess the compounds' pharmacological properties. Before considering clinical development, it's also crucial to evaluate their safety profile by assessing off-target effects, cytotoxicity, and potential side effects.

Eventually, animal model studies would offer insights into how these compounds behave in a living organism, providing crucial data on their distribution in the body, metabolism, and overall effectiveness. Additionally, exploring how the chemical structure of these phytochemicals relates to their biological activity may lead to the development of more potent derivatives with improved properties.

In conclusion, while these in silico results are promising, experimental validation and further safety evaluations are critical to advancing these compounds as potential anticancer therapies targeting PD-1.

Acknowledgements

The researchers extend their heartfelt gratitude to Sir Edward Brian M. Ranjo for his invaluable guidance and patience in assisting them through each step of the docking procedures in this research. They also wish to acknowledge their research adviser, Dr. Sherwin Fortugaliza, for his continuous support and encouragement, which kept the team motivated throughout the study. Special thanks are extended to their seniors, Joi Issachar B. Aguelo, Yarra Marie B. Amancio, and Elizabeth Arwen Pasco, for introducing the concept of molecular docking and inspiring the researchers to pursue this significant study. Lastly, the researchers express their sincere gratitude to their parents for their unwavering support throughout the entire process.

REFERENCES

Ali, M. H., Anwar, S., Kumar Roy, P., & Ashrafuzzaman, M. (2018). Virtual Screening for Identification of Small Lead Compound Inhibitors of Nipah Virus Attachment Glycoprotein. *Journal of Pharmacogenomics & Pharmacoproteomics*, *09*(02). https://doi.org/10.4172/2153-0645.1000180

Anastasia Wheni Indrianingsih, Putri Styaningrum, None Suratno, Anjar Windarsih, Suryani, R., Eka Noviana, & Itoh, K. (2024). The effect of extraction method on biological activity and phytochemical content of Artocarpus heterophyllus (jackfruit) leaves extract concurrent with its principal component analysis. *Process Biochemistry*, *143*. https://doi.org/10.1016/j.procbio.2024.04.034

Banerjee, P., Eckert, A. O., Schrey, A. K., & Preissner, R. (2018). ProTox-II: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Research*, *46*(W1), W257–W263. https://doi.org/10.1093/nar/gky318

Benet, L. Z., Hosey, C. M., Ursu, O., & Oprea, T. I. (2016). BDDCS, the Rule of 5 and Drugability. *Advanced Drug Delivery Reviews*, *101*, 89–98. https://doi.org/10.1016/j.addr.2016.05.007

Cardoso, F., Paluch-Shimon, S., Senkus, E., Curigliano, G., Aapro, M. S., André, F., Barrios, C. H., Bergh, J., Bhattacharyya, G. S., Biganzoli, L., Boyle, F., Cardoso, M.-J. ., Carey, L. A., Cortés, J., El Saghir, N. S., Elzayat, M., Eniu, A., Fallowfield, L., Francis, P. A., & Gelmon, K. (2020). 5th ESO-ESMO international consensus guidelines for advanced breast cancer (ABC 5). *Annals of Oncology*, *31*(12), 1623–1649. https://doi.org/10.1016/j.annonc.2020.09.010

Chen, D. S., & Mellman, I. (2013). Oncology meets immunology: the cancer-immunity cycle. *Immunity*, 39(1), 1-10. https://doi.org/10.1016/j.immuni.2013.07.012

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a Free web Tool to Evaluate pharmacokinetics, drug-likeness and Medicinal Chemistry Friendliness of Small Molecules. *Scientific Reports*, *7*(1), 1–13. https://doi.org/10.1038/srep42717

Gupta, D., Mann, S., Sood, A., & Gupta, R. (2011). *International Journal of Pharma and Bio Sciences PHYTOCHEMICAL, NUTRITIONAL AND ANTIOXIDANT ACTIVITY EVALUATION OF SEEDS OF JACKFRUIT (ARTOCARPOUS HETEROLPHYLLUS LAM.)*. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=fa5236acf16be9aa230144f7cf503ff2ca4ec0db

Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E., & Hutchison, G. R. (2012). Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Journal of Cheminformatics*, *4*(1). https://doi.org/10.1186/1758-2946-4-17

Hudait, A., Qiu, Y., Odendahl, N., & Molinero, V. (2019). Hydrogen-Bonding and Hydrophobic Groups Contribute Equally to the Binding of Hyperactive Antifreeze and Ice-Nucleating Proteins to Ice. *Journal of the American Chemical Society*, *141*(19), 7887–7898. https://doi.org/10.1021/jacs.9b02248

Jiang, Y., Chen, M., Nie, H., & Yuan, Y. (2019). PD-1 and PD-L1 in cancer immunotherapy: clinical implications and future considerations. *Human Vaccines & Immunotherapeutics*, *15*(5), 1111–1122. https://doi.org/10.1080/21645515.2019.1571892i.org/10.1093/ajcn/78.3.517s

Keir, M. E., Butte, M. J., Freeman, G. J., & Sharpe, A. H. (2008). PD-1 and its ligands in tolerance and immunity. *Annual Review of Immunology*, 26, 677-704. https://doi.org/10.1146/annurev.immunol.26.021607.090331

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, *23*(1-3), 3–25. https://doi.org/10.1016/s0169-409x(96)00423-1

Liu, R. H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *The American Journal of Clinical Nutrition*, *78*(3), 517S520S. https://doi.org/10.1093/ajcn/78.3.517s

Pantsar, T., & Poso, A. (2018). Binding Affinity via Docking: Fact and Fiction. *Molecules*, *23*(8), 1899. https://doi.org/10.3390/molecules23081899

Pardoll, D. M. (2012). The blockade of immune checkpoints in cancer immunotherapy. *Nature Reviews Cancer*, 12(4), 252-264. https://doi.org/10.1038/nrc3239

ProTox. (2015, July 13). *ProTox-3.0 - Prediction of TOXicity of chemicals*. Tox.charite.de. https://tox.charite.de/protox3/

PubChem. (2024). *PubChem*. Nih.gov; National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/

Ranasinghe, R. A. S. N., Maduwanthi, S. D. T., & Marapana, R. A. U. J. (2019). Nutritional and Health Benefits of Jackfruit (Artocarpus heterophyllus Lam.): A Review. *International Journal of Food Science*, *2019*. https://doi.org/10.1155/2019/4327183

Sharma, P., Hu-Lieskovan, S., Wargo, J. A., & Ribas, A. (2017). Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell*, 168(4), 707-723. https://doi.org/10.1016/j.cell.2017.01.017

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

SwissADME. (2017). *SwissADME*. Swissadme.ch. http://www.swissadme.ch/

Topalian, S. L., Hodi, F. S., Brahmer, J. R., Gettinger, S. N., Smith, D. C., McDermott, D. F., Powderly, J. D., Carvajal, R. D., Sosman, J. A., Atkins, M. B., Leming, P. D., Spigel, D. R., Antonia, S. J., Horn, L., Drake, C. G., Pardoll, D. M., Chen, L., Sharfman, W. H., Anders, R. A., & Taube, J. M. (2012). Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer. *New England Journal of Medicine*, *366*(26), 2443–2454. https://doi.org/10.1056/nejmoa1200690

UCSF Computer Graphics Laboratory. (2020). *Minimize Structure*. Ucsf.edu. https://www.cgl.ucsf.edu/chimera/current/docs/ContributedSoftware/minimize/minimize.html

World Health Organization. (2024, February 1). *Global Cancer Burden growing, Amidst Mounting Need for Services*. Www.who.int. https://www.who.int/news/item/01-02-2024-global-cancer-burden-growing--amidst-mounting-need-for-services

Zhang, L., Tu, Z., Xie, X., Wang, H., Wang, Z.-X., Sha, X.-M., & Lu, Y. (2017, May 1). *Jackfruit (Artocarpus heterophyllus Lam.) peel: A better source of antioxidants and a-glucosidase inhibitors than pulp, flake and seed, and phytochemical profile by HPLC-QTOF-MS/MS.* ScienceDirect. https://doi.org/10.1016/j.foodchem.2017.05.003

Ranasinghe, R. A. S. N., Maduwanthi, S. D. T., & Marapana, R. A. U. J. (2019). Nutritional and Health Benefits of Jackfruit (Artocarpus heterophyllus Lam.): A Review. *International Journal of Food Science*, *2019*. https://doi.org/10.1155/2019/4327183

Ribas, A., & Wolchok, J. D. (2018). Cancer immunotherapy using checkpoint blockade. *Science*, 359(6382), 1350-1355. https://doi.org/10.1126/science.aar4060