WWW.IJRPR.COM

International Journal of Research Publication and Reviews

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

Exploring Marang Seed *(Artocarpus Odoratissimus)* Extract as an Immunosuppressant: An *In Silico* Approach to Transplant Rejection and Autoimmune Disease Management

Zoe Marie B. Arquiza [a], Carmela Angela G. Lim [a], Phoemella Tricxy G. Mamaclay [a]

[a] Davao City National High School, F. Torres Street, Davao City, 8000 Philippines
DOI : https://doi.org/10.55248/gengpi.6.0425.1393

A B S T R A C T

Over the last 30 years, autoimmune diseases have significantly increased, affecting more than 10% of the global population. Some of the most prevalent conditions are multiple sclerosis (MS) and type 1 diabetes (T1D). Apart from that, 15% of kidney transplant patients develop acute rejection that causes inflammation within the first year. In response to this, healthcare providers prescribe immunosuppressants; however, they can lead to problems such as hypertension, infections, hyperlipidemia, and even cancer. This study examines Marang seed (Artocarpus odoratissimus) as a potential alternative to traditional immunosuppressants because of its high phenolic content, which includes flavonoids that are known to modulate immune responses. Using molecular docking, six phytochemicals from marang seed were docked with proteins 1A3Q, 2AZ5, and 1VLK. PyMol, Autodock, Chimera, and LigPlot were used for analysis. Among the six phytochemicals, artocarpin exhibited the lowest binding affinity, which indicates a successful binding. It is then followed by quercetin and catechin. These findings indicate that Marang seed could be a promising natural alternative to existing immunosuppressants.

Keywords: *Molecular Docking, Immunosuppressant, Transplant Rejection, Autoimmune Disease Management,Marang, Marang Seed Extract*

1. Introduction

Universal health coverage. This is one of the 17 Sustainable Development Goals the United Nations wants to achieve. They aim to reduce financial risk, provide affordable services for good basic health care, and make necessary medicines and vaccines that are not only safe, effective, high quality, and affordable but also available to all.

Over the last 30 years, autoimmune diseases have significantly increased. Out of 200 people worldwide, 1 person suffers from either multiple sclerosis (MS) or type 1 diabetes (T1D), which have autoreactive immune cells that play a critical role in pathogenesis (Kwiatkowski, Stewart, Cho, Avram, & Keselowsky, 2020). Autoimmune diseases like multiple sclerosis (MS) and type 1 diabetes (T1D) impact more than 10% of the global population. At the same time, Khush et al. (2019) stated that there are a total of 118 per year, which is 2% to 3% of all transplants in the past 10 years. Although in the Philippines, there is little information about organ transplantations, specifically kidney, even though incidences of patients treated for end-stage renal disease and kidney transplants in the US and Southeast Asia are increasing (Buere, Burgos, Lozada, Morris, Obin, Pangilinan, & Dela Cerna, 2024).

However, after a transplant, the recipient's immune system fights against the organ, recognizing it as foreign. Acute rejection, causing inflammation in the first year, is being experienced by 15% of people with kidney transplants (Arnaud, 2018). As reported by Callemeyn,, Lamarthée, Koenig, Koshy, Thaunat, & Naesens (2022), this transplant rejection is caused by the adaptive immune cells detecting mismatched human leukocyte antigens, which leads to either T-cell-mediated rejection or antibody-mediated rejection. This immune response is primarily triggered by the recognition of non-self antigens present on the donor organ, which are different from the recipient's own tissues.

In response to this, healthcare providers prescribe immunosuppressants to treat certain autoimmune diseases and prevent acute and chronic rejection (Tönshoff, 2019). Parlakpınar & Gunata (2021) stated that the use of immunosuppressants, which are particularly effective and have fewer side effects compared to other treatments, has led to reduced mortality and morbidity. However, immunosuppressive treatments still cause several side effects, such as hypertension, infection, and hyperlipidemia. Krisl & Doan (2017) agreed and noted that immunosuppression significantly contributes to cancer development by disrupting the immune surveillance process. This demonstrates the value of individualized drug use. The optimal immunosuppressive treatment post-transplant is still unknown. Therefore, discovering less toxic but more potent new agents is of great importance, and further experimental and clinical research is necessary and needed in this regard.

With this, marang seed was utilized as a potential alternative to immunosuppressive drugs. According to Hakim et al. (2007), as cited in Yulianti et al. (2022), Marang (Artocarpus odoratissimus) is rich in phenolic compounds, including flavonoids, stilbenoids, and arylbenzofurones. Alvarado (2023)

revealed that phenols and flavonoids, which are potent inhibitors of the alpha-glucosidase enzyme, are found in extracts from different parts of the Marang fruit. In addition, marang seed had a higher total flavonoid content than the flesh. Based on Hosseinzade, Sadeghi, Biregani, Soukhtehzari, Brandt, and Esmaillzadeh (2019), flavonoids can modulate immune responses; however, the exact molecular mechanisms that are part of these changes are not well understood. Martínez, Mijares, and De Sanctis (2019) concur and expound that transcriptional factors are exhibited by flavonoids and their derivatives, which modulate differentiation and proliferation as well as activation of immune cells and regulate T cell generation.

This research aims to investigate the potential of using Marang seed extract as an innovative approach to improve both the safety and effectiveness of immunosuppressive drugs in autoimmune diseases and organ transplantation. Marang's antibacterial and anticancer characteristics may not only assist in managing the negative effects of immunosuppressive therapy but also give a dual benefit of fighting infections and cancer while the immune system is subdued.

Through molecular docking analysis, the researchers' aim to assess the drug-likeness of the main phytochemical constituents of A. odoratissimus by applying the Lipinski rule using SwissADME. They will then conduct molecular docking simulations in order to investigate and understand the binding relationships between the selected phytochemicals and the three chosen proteins: NF-kappaB p52, TNF-alpha, and viral interleukin-10. The researchers will then thoroughly investigate the interactions between the chosen phytochemicals and the three proteins, determining their strength and affinity. Finally, the potential inhibitory effects of the chosen phytochemicals will be assessed based on the molecular docking results.

2. Methodology

2.1 Screening and Preparation of Phytocompounds from Marang (Artocarpus odoratissimus) Seed

The Marang seed contained a total of eleven phytochemicals. It is necessary to analyze the concepts of contents in Lipinski's rules, as well as the drug-likeness parameters that comprise the ADME (absorption, distribution, metabolism, and excretion). These characteristics consider crucial aspects of a drug's action within the human body to promote good therapy with high pharmacological potential and minimal toxicity (Truong, George, and Holien, 2021). Roskoski (2023) defines Lipinski's rule of five (Ro5) as a computational approach used in drug discovery to assess solubility, membrane permeability, and pharmacological effectiveness. It is based on four parameters: molecular weight, the number of hydrogen bond donors and acceptors, and the log of the partition coefficient. Other relevant descriptors are lipophilic efficiency, polar surface area, and the amount of rotatable bonds and aromatic rings.

Ivanović, Rančić, Arsić, & Pavlović (2020) agree with Roskoski (2023) that the Rule of Five (ROF) is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a specific pharmacological or biological activity has properties that would make it likely orally active in humans. To be considered for use as an oral medication, the biologically active molecule must meet five criteria. Poor absorption or penetration is most likely when the molar mass exceeds 500, the number of H-bond acceptors exceeds 10, the number of H-bond donors exceeds 5, and the logP exceeds 5 (or MlogP exceeds 4.15). Based on the ROF, the rating of an orally active medicine ranges from "0" to "4", indicating that a possible drug has no more than one violation of the exposure requirements.

Following that, only compounds that passed the Lipinski test were employed for docking simulations with Autodock Vina. The SDF files were converted to PDB format with PyMOL and the compounds were ready for docking with MGL AutodockTools and the Autodock Vina system. The Gasteiger charges and torsional degrees of freedom for each ligand were automatically set in AutodockTools using Autodock Vina. Following optimization, all compounds were stored as PDBQT files for docking.

2.2 Preparation of human NF-kappaB p52 bound to DNA, TNF-alpha with a small molecule inhibitor, and Viral Interleukin-10

The researchers chose three proteins for this study. NFkB was chosen as one of the receptors due to its critical role in regulating immune and inflammatory responses, which are directly related to the development of autoimmune diseases and transplant rejection. NFkB is used as one of the receptors in this study because of its important role in immune system regulation, particularly in controlling immune and inflammatory responses, which are critical in autoimmune diseases and transplant rejection. NFkB is a transcription factor that activates genes that regulate immune system processes such as cytokine, chemokine, and inflammatory mediator synthesis. It regulates immunological responses to aid in infection defense, but when dysregulated, it can contribute to immune system malfunctions, resulting in chronic inflammation and autoimmune illnesses (Serasanambati and Chilakapati, 2016).

According to Serasanambati and Chilakapati (2016), NFkB is required in the immune system to activate both innate and adaptive immune responses. It controls the survival, proliferation, and development of immune systems such as T cells, B cells, and macrophages. In autoimmune illnesses, this regulation becomes problematic when NFkB increases the survival of auto-reactive T and B cells, which mistakenly attack the body's own tissues. By sustaining the inflammatory response and delaying apoptosis (programmed cell death) of these damaging cells, NFkB contributes to the persistence of autoimmune diseases. Its constant activity contributes to the breakdown of immunological tolerance, a vital process that keeps the immune system from targeting the body's own tissues.

Furthermore, Serasanambati and Chilakapati (2016) report that NFkB has a role in transplant organ rejection. During transplant rejection, NFkB is activated in response to the stress of the transplant procedure (such as ischemia/reperfusion injury), amplifying the immune system's attack on the donated tissue, resulting in inflammation and organ rejection.

Due to its multifunctional nature as a cytokine, tumor necrosis factor alpha (TNF-α) is also the receptor of choice for this study. It has been discovered to play a significant role in the progression of inflammatory and autoimmune disorders. TNF-α is a protein with three identical components and 157 amino acids. It is mostly produced by activated immune cells such as macrophages, T lymphocytes, and natural killer cells. TNF-α increases the production of other inflammatory chemicals, such as cytokines and chemokines. TNF-α exists in two forms: transmembrane (tmTNF-α) and soluble (sTNF-α). TNF-α-converting enzyme (TACE) converts the transmembrane form, the first to be generated, into soluble form. Soluble TNF-α interacts with two receptors: TNFR1 and TNFR2. Although both tmTNF-α and sTNF-α can bind to these receptors, tmTNF-α primarily signals through TNFR2, which is predominantly present in immune cells. TNFR1, present in all human organs, is the primary receptor that signals TNF-α's actions (Jang, Lee, Shin, Song, Park, Kang, & Yang, 2021).

Jang et al (2021) further explained that TNF-α is crucial for modulating immune responses and reducing inflammation. Overproduction of TNF-α can injure the body and cause illnesses. Excessive TNF-α production has been associated with illnesses such as RA, IBD, PS, and NIU, demonstrating its key role in disease pathogenesis. Developing therapeutics that target TNF-α in inflammatory and autoimmune illnesses requires a thorough understanding of its processes and interactions with various receptors.

The Epstein-Barr virus (EBV) is a widespread virus that affects over 90% of adults at some point in their lives. It is a member of the gamma herpesvirus family and has been associated with a variety of malignancies, including nasopharyngeal carcinoma, Burkitt's lymphoma, and Hodgkin disease. In addition to malignancy, EBV is linked to autoimmune illnesses such as systemic lupus erythematosus (SLE) and multiple sclerosis. After initial infection, EBV can hide in certain immune cells known as B cells and reactivate on occasion, which can be detected by looking for specific antibodies (IgG and IgA) to EBV proteins in the blood (Dunmire, Verghese, & Balfour, 2018).

Houen & Trier (2021) stated that EBV can elude detection by the immune system by generating proteins that resemble human cytokines, which are signaling molecules that help regulate immune responses. One of these proteins is called viral interleukin-10 (vIL-10), which is related to the human cytokine interleukin-10 (hIL-10). vIL-10 is produced at the late stages of viral replication and is approximately 80% identical to hIL-10. While hIL-10 is important in lowering inflammation and boosting the growth of certain immune cells, vIL-10 can accomplish similar things, such as reducing inflammation and encouraging B cell growth and antibody production. However, there are some peculiarities; for example, vIL-10 does not excite other immune cells in the same manner as hIL-10 does.

Higdon, Tan, & Maltzman, (2023) explained that Epstein-Barr virus (EBV) infection is a major issue for transplant patients because it is associated with posttransplant lymphoproliferative disease (PTLD), a syndrome characterized by aberrant proliferation of EBV-infected B cells. EBV infects B cells and expresses the viral genes LMP1 and 2A, which mimic survival and growth signals. This can sometimes result in malignant lymphomas.

Higdon et. al (2023) added that EBV's role in immune regulation entails decreasing T-cell function, which is essential for virus management and inhibiting the spread of infected B cells. EBV-infected patients, particularly those with poor T-cell responses, have a higher risk of developing PTLD. One of EBV's methods is the development of viral interleukin-10 (vIL-10), which allows the virus to elude the immune response. vIL-10 inhibits T-cell activation and cytokine production, reducing the immune system's ability to combat the virus and control infected cells. The balance between preventing rejection and avoiding EBV-related problems such as PTLD is dependent on the careful control of immunosuppressive medication.

To prepare the protein for docking, the 3D structure of NF-KAPPA-B P52 (RCSB PDB ID: 1A3Q), TNF-α (RCSB PDB ID: 2az5), and viral interleukin-10 (vIL-10) (RCSB PDB ID: 1vlk) were downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/) as a 3D model. The protein was then imported into MGL Autodock Tools for preparation. During this process, water molecules (HOH1363.A-HOH1572.A), other heteromolecules (IOD1.A-IOD19.A), and naturally bound ligands (NAG1210.A, NAG1.B-NAG2.B, NAG1.C-NAG2.C, NAG1.D-NAG2.D, BMA3.D-BMA4.D) were removed. Additionally, polar hydrogens and Kollman charges were added to the protein structure. The modified file was then saved in the PDBQT format for use in docking simulations.

2.3 Receptor Grid Box Manual Generation

In this study, the researchers used three proteins wherein each required a different receptor grid area. The XYZ values of the coordinates of the center of the grid box were determined using CASTp, as well as Autodock Vina, by highlighting the amino acids on a specific protein needed chain. For NF-KAPPA-B P52, the XYZ coordinates were 18, 65, and 1 while 68, 6.7, and 53 for viral interleukin-10 and TNF-α's coordinates were -17, 72, and 33. Through this, the researchers were able to predict the protein's active binding residues.

For the protein TNF-α, the active residues are ASP 10, LYS 11, ALA 14, ASN 19, PRO 20, GLN 21, ALA 22, GLN 25, ASN 30, LEU 36, LEU 37, ALA 38, ASN 39, GLY 40, VAL 41, PRO 51, SER 52, GLY 54, LEU 55, TYR 59, GLY 66, GLN 67, GLY 68, LEU 75, ARG 82, ILE 83, VAL 85, SER 86, LYS 90, VAL 91, ASN 92, LEU 93, SER 95, ILE 97, LYS 98, SER 99, PRO 100, GLN 102, PRO 113, TYR 115, GLU 116, ILE 118, TYR 119, LEU 120, GLU 127, PRO 139, ASP 140, TYR 141, GLU 146, SER 147, GLY 148, PHE 152, ILE 155, and LEU 157. While the proteins of viral interleukin-10 are LEU23, LEU26, ARG27, PHE30, VAL33, LYS34, PHE37, GLN38, LYS40, ASP41, GLN42, LEU43, ASN45, LEU46, LEU47, LEU48, LYS49, GLU50, LEU52, LEU53, PHE56, LEU65, MET68, ILE69, PHE71, TYR72, GLU75, VAL76, MET77, GLN79, ALA80, GLN83, VAL91, LEU94, LEU98, LEU101, LEU105 (Rajalakshmi, Ramu, Chinnappan, Velmurugan, Pathak, Pashameah, Oyouni, Al-Amer, Alasseiri, Hamadi, Alanazi, Sathiamoorthi, 2023). On the other hand, the active residues of viral interleukin-10 were based on the study of Ranjanamala, Krishanan, Shreemaya, Rajeswari, Sangeetha, Ghidan, and Ghidan (2021) are ASP 94, GLY 95, ARG 103, ALA 104, GLY 105, LYS 107, SER 108, ALA 109, LEU 117, GLU 118, ILE 119, LYS 153, GLN 154, ARG 156, GLU 157, ARG 160, ARG 193.

The binding site size of each of the proteins were then estimated on the area covered by the specific amino acids highlighted which were 80x98x114 for 1A3Q, 62x54x82 for 1VLK, and 126x122x64 for 2AZ5 as shown below in figure 1. These values were then written in a text document for the configuration file with additional measurements that are default which are energy range of 3 and exhaustiveness level of 8.

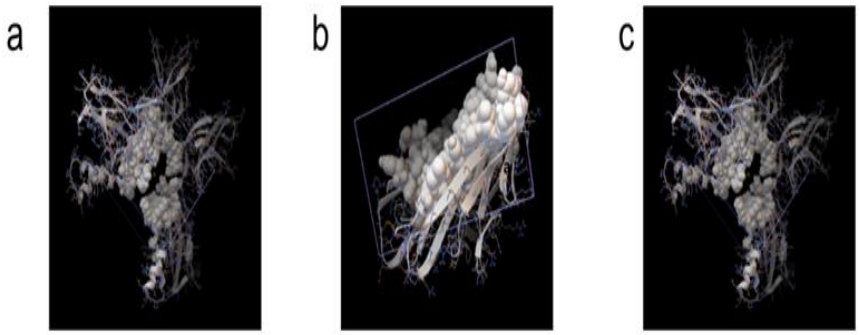


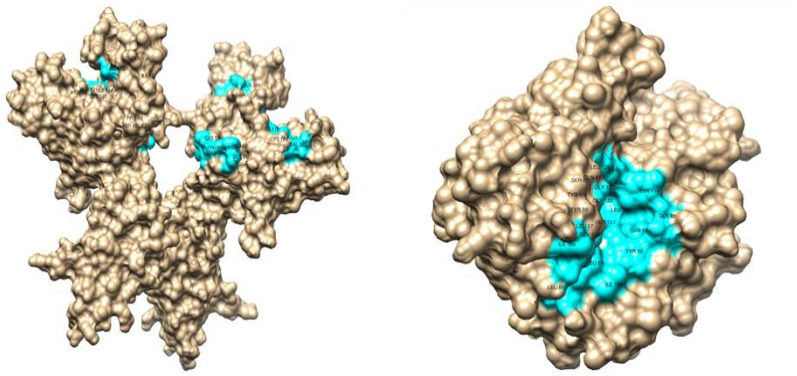
Fig. 1. – (a) NF-KAPPA-B P52 (RCSB PDB ID: 1A3Q) receptor grid box visualization through MGL AutodockTools v. 1.5.7. Amino acids of the main binding site predicted by CastP were set to appear as spheres; (b) TNF-α (RCSB PDB ID: 2az5) receptor grid box visualization through MGL AutodockTools v. 1.5.7. Amino acids of the main binding site predicted by CastP were set to appear as spheres; (c) viral interleukin-10 (vIL-10) (RCSB PDB ID: 1vlk) receptor grid box visualization through MGL AutodockTools v. 1.5.7. Amino acids of the main binding site predicted by CastP were set to appear as spheres

2.4 Molecular Docking Analysis and Simulation

In molecular docking analysis and simulation, the researchers used the Command Prompt of the Windows 10 system after it was made sure that the ligand, proteins, and configuration files were saved in one folder. Then the location was changed to where the ligand, protein, and configuration files were stored, which is in the folder's directory. In the command prompt, "C:\Program Files (x86)\The Scripps ResearchInstitute\Vina\vina.exe" --receptor protein.pdbqt --ligand [ligand.pdbqt] --config [config.txt] --log [log.txt] --out [output.pdbqt]" was written on in the computer. After the docking was done, the binding affinities of the proteins were given. Next, the output and protein files all in PDBQT format were exported to PyMol for 3D stimulation.

Scoring and Analysis. When the docking computation results went out, it was saved in a text file wherein the other files were located. The binding affinity levels of different phytochemicals to the proteins were displayed in the text file. A scoring function assesses how well the ligand's shape and positioning fits within the protein's binding site. It also quantifies binding affinity, which represents the strength of the interaction between the ligand and the protein. In a study by Gu, Zhang, Xu, Chen, Liu, Wu, Mo, Hu, Liu, and Luo (2022), lower values were seen as favorable, indicating a stronger binding between the protein and ligand in the complex. Thus, if a binding affinity is more negative, it means that the ligand and the protein have a stronger interaction, which indicates that it is successful binding. The data found inside the protein's main pocket were the only ones considered to be competent. Using this data, the output that showed the lowest binding affinity value in the docking attempts was chosen to be the representative data for docking interactions for each ligand.

The structure-based virtual screening method (Ali, Anwar, Roy, & Ashrafuzzaman, 2018) was used for visual analysis, and PyMol was used to see the 3D outputs of the protein-ligand docking. The hydrogen bonds and hydrophobic interactions were viewed using Ligplot+. These interactions play a crucial role in ligand-protein docking as they determine the specificity and binding affinity of the protein-ligand complex (Hudait, Qiu, Odendahl, & Molinero, 2019). The interacting residues are also evaluated after docking through Ligplot+.



a b

Fig. 2 - (a) Interacting residues of Human NF-KAPPA-B P52 bound to DNA (PDB ID: 1A3Q) (cyan); (b) Interacting residues of TNF-alpha with a small molecule inhibitor (PDB ID: 2AZ5) (cyan).

Ligplot+ is utilized to analyze the interacting residues after docking. The residues of the binding sites of the protein that interact with the ligand will be compared to the residues of the protein that naturally interact with the residues of 1A3Q: TYR 55, CYS 57, GLU 58, GLY 59, PRO 60, SER 61, ILE 119, CYS 120, ALA 121, VAL 122, SER 123, ILE 151, GLN155, GLN 157 and the residues of 2AZ5: LEU 55, LEU 57, TYR 59 SER 60 GLN 61 ,TYR 119, LEU 120, GLY 121, GLY 122, TYR 151, ILE 155, LEU 157.

The binding affinity levels were used as a base to rank the ligands according to their interaction with the 1A3Q, 2AZ5, 1VLK proteins. By binding to these proteins, the ligands could potentially inhibit the activation of immune responses involved in autoimmune diseases and transplant rejection, thus preventing the overactivation of the immune system. A descriptive analysis will evaluate the phytocompounds according to their binding affinity levels. Azathioprine is also assessed on its docking performance with the three proteins (1A3Q, 2AZ5, 1VLK) for a more objective discussion. Azathioprine functioned as the positive control for this study. Due to its outstanding efficacy and safety profile, physicians have widely used Azathioprine as an immunosuppressive medication to treat a wide range of SIDs, including SLE, rheumatoid arthritis, dermatomyositis, polymyositis, systemic sclerosis, and systemic vasculitis. In addition to cardio immunology, AZA may also be used to treat inflammatory bowel illnesses and prevent organ transplant rejection (Grzechocińska, Tymińska, Giordani, Wysińska, Ostrowska, Baritussio, & Ozierański, 2023).

2.5 Methodological Framework

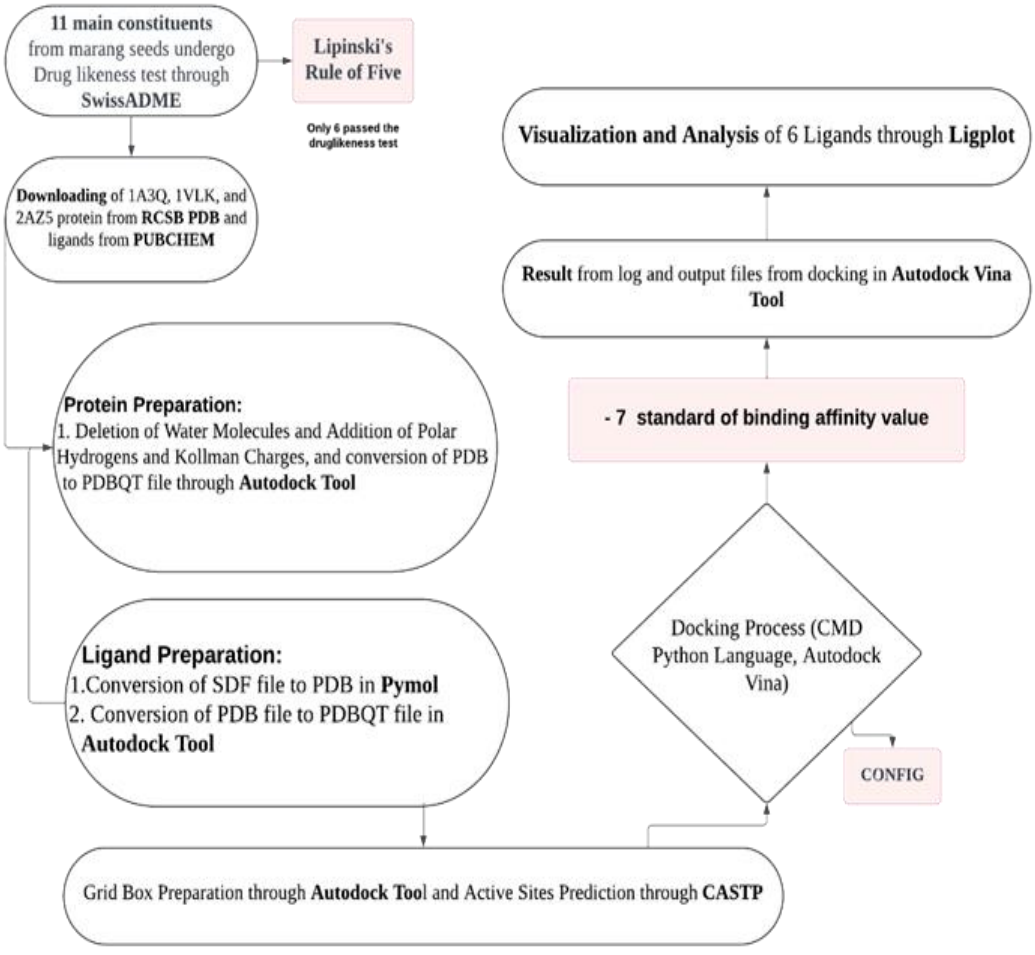


Fig. 3. Methodological Framework of the study

3. Results and Discussion

In order to target the active binding sites of viral interleukin-10, TNF-alpha, and human NF-KAPPA-B P52, eleven major phytochemical components have been determined from Atrocarpus odoratissimus seeds and subjected to analysis. The eleven phytochemicals were put through to SwissADME utilizing the Lipinski rule, which examined the physicochemical properties of the phytochemical ligands prior to the molecular docking method. Out of eleven, only six passed Lipinski's fifth rule as specified in table 1 below.

Table 1: *Phytochemicals found in Artocarpus Odoratissimus Seed*

a	b	c	d	e	f
Catechin	290.27 g/mol	5	6	0.24	0
Quercetin	302.24 g/mol	5	7	-0.56	0
Artocarpin	436.50 g/mol	3	6	2.30	0
Ferulic Acid	194.18 g/mol	2	4	1.00	0
p-Coumaric Acid	164.16 g/mol	2	3	1.28	0
Gallic Acid	170.12 g/mol	4	5	-0.16	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)*

The six phytochemicals were docked on PDB 1VLK vIL-10, PDB 2AZ5 TNF-A, and PDB 1A3Q NF-KAPPA-B P52 as control variables. To guarantee accuracy, ten runs through were done. Following the docking procedure, ten distinguishable outputs and reports were generated, with the output displaying the lowest binding affinity recorded for every repetition.

3.1. Scoring Functions

Using Pymol, the positions of six docking attempts within the binding pocket of the Human NF-KAPPA-B P52 protein were visualized and analyzed. A binding affinity threshold of -7 was set to identify phytochemicals with a higher likelihood of interaction with this protein, which helped reduce the candidate list to three. The first conformations of each ligand were then saved as PDB files, resulting in three ligand complexes that were subjected to visual analysis. Among the tested phytochemicals, Artocarpin showed the strongest negative binding affinity score, followed by Quercetin and Catechin as indicated in Table 2.

Table 2: *Binding affinity scores of Marang (Artocarpus odoratissimus) seed phytochemical on Human NF-KAPPA-B P52 bound to DNA*

a	*b*	*c*	*d*
Catechin	9064	-7.0	Output 8
Quercetin	5280343	-7.2	Output 7
Artocarpin	5458461	-7.5	Output 1
Ferulic acid	445858	-5.0	Output 0
p-Coumaric Acid	637542	-5.0	Output 1
Gallic Acid	370	-5.6	Output 0

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

In this analysis, Pymol was utilized to visualize the positions of six docking attempts in the binding pocket of the TNF-alpha protein. A standardized affinity value of -7 was established to highlight phytochemicals likely to interact with this protein, allowing the selection to be narrowed down to three candidates. The first conformations of these ligands were compiled into PDB files, creating three ligand complexes for subsequent visual analysis. Based on table 3, the phytochemical with the most significant negative binding affinity score was Artocarpin, followed by Catechin and Quercetin.

Table 3: *Binding affinity scores of Marang (Artocarpus odoratissimus) seed phytochemical on TNF-alpha with a small molecule inhibitor*

a	*b*	*c*	*d*
Catechin	9064	-7.2	Output 1
Quercetin	5280343	-7.2	Output 0
Artocarpin	5458461	-8.0	Output 0
Ferulic acid	445858	-5.9	Output 8

p-Coumaric Acid	637542	-5.7	Output 6
Gallic Acid	370	-5.5	Output 0

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

For the viral interleukin-10 protein, Pymol was also employed to visualize and identify the positions of six docking attempts within its binding pocket. A binding affinity threshold of -7 was set to pinpoint phytochemicals with a greater chance of interaction with this protein, ultimately narrowing the options down to three. Each ligand's first conformation was converted into PDB files, resulting in three ligand complexes that were analyzed visually. Among these phytochemicals, Artocarpin displayed the most negative binding affinity score, followed by Quercetin and Catechin as illustrated in table 4 below.

Table 4: *Binding affinity scores of Marang (Artocarpus odoratissimus) seed phytochemical on Viral Interleukin-10*

a	***b***	***c***	***d***
Catechin	9064	-7.1	Output 0
Quercetin	5280343	-7.3	Output 0
Artocarpin	5458461	-8.0	Output 0
Ferulic acid	445858	-5.0	Output 2
p-Coumaric Acid	637542	-5.2	Output 2
Gallic Acid	370	-4.9	Output 0

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

3.2. Protein and Ligand Interactions between interacting residues.

Transplant rejection and autoimmune diseases pose significant health challenges, affecting millions globally. Autoimmune diseases, such as rheumatoid arthritis and lupus, occur when the immune system mistakenly attacks the body's own tissues, causing inflammation and damage. According to MedlinePlus, transplant rejection occurs when a recipient's immune system recognizes the transplanted organ as foreign due to mismatched antigens, triggering an immune response that can damage or destroy the organ. Current treatments for both conditions often involve broad immunosuppressive drugs that effectively prevent rejection or control autoimmune activity but carry risks like increased infection susceptibility and long-term organ damage. By conducting in silico docking studies with key proteins involved in immune responses, this research aims to identify new treatment options that could improve outcomes for individuals facing transplant rejection and autoimmune diseases. This research looks at three phytochemicals: Artocarpin, catechin, and quercetin. Among these, Artocarpin showed the best results in molecular docking studies with three target proteins: Human NF-KAPPA-B P52 bound to DNA, TNF-alpha with a small molecule inhibitor, and Viral Interleukin-10, which are involved in immune responses.

To understand how Artocarpin works, we looked at its interactions with important residues in these proteins. For protein Human NF-KAPPA-B P52, some key residues include LEU 55, LEU 57, ILE 58, TYR 59, SER 60, GLN 61, TYR 119, LEU 120, GLY 121, GLY 122, VAL 123, TYR 151, ILE 155, and LEU 157. While the key residues for TNF-alpha are LEU 55, LEU 57, TYR 59, SER 60, GLN 61, TYR 119, LEU 120, GLY 121, GLY 122, TYR 151, ILE 155, and LEU 157. On the other hand, the active residues of Viral Interleukin-10 include LEU23 to LEU105 (inclusive), with important residues like ARG27 and PHE30. All three phytochemicals had a binding affinity of less than -7 kcal/mol during the docking studies as shown in table 5, 6, and 7 below.

Table 5: *Ligplot Analysis of the 3 Phytochemicals on Human NF-KAPPA-B P52 bound to DNA*

a	***b***	***c***
Catechin	LYS90 (A)	GLU92 (A)
	[3.16]	ARG103 (A)
	GLU92 (A)	ALA104 (A)
	[3.06]	ILE110 (A)
	ARG103 (A)	SER115 (A)
	[3.14,3.15]	LYS153 (A)
	SER115 (A)	ARG 156 (A)
	[3.19]	**GLN157 (A)**

	ARG 193 (A)	ARG 160 (A)
	[3.15, 3.01]	
Quercetin	ASP94 (B)	ASP94 (B)
	[2.71]	ALA104 (B)
	LEU117 (B)	LEU117 (B)
	[2.83]	**ILE119 (B)**
	LYS153 (B)	LYS153 (B)
	[3.21, 3.32]	**GLN157 (B)**
	GLN157 (B)	ARG161 (B)
	[3.03]	ARG193 (B)
	SER161 (B)	
	[3.20]	
Artocarpin	LYS 90 (A)	LYS90 (A)
	[3.17]	GLU92 (A)
	ARG103 (A)	ASP94 (A)
	[3.05]	ARG103 (A)
	ARG156 (A)	ALA104 (A)
	[3.11]	SER115 (A)
		LEU117 (A)
		ILE119 (A)
		ALA 121 (A)
		LYS153 (A)
		ARG156 (A)
		ARG160 (A)
		ARG193 (A)

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

Table 6: *Ligplot Analysis of the 3 Phytochemicals on TNF-alpha with a small molecule inhibitor*

a	***b***	***c***
Catechin	SER60 (B)	TYR50 (A)
	[2.70]	**LEU57 (A)**
	LEU120 (B)	**TYR59 (B)**
	[2.84]	**SER60 (B)**
	TYR151 (B)	**GLN61 (B)**
	[2.97]	**TYR119 (B)**
		LEU120 (B)

		GLY121 (A)
		TYR151 (B)
Quercetin	TYR151 (A)	TYR50 (A)
	[3.09, 3.19]	**TYR59 (B)**
	TYR151 (B)	**SER60 (B)**
	[2.75]	**TYR119 (A)**
		LEU120 (A)
		GLY121 (A)
		TYR151 (A)
		TYR151 (B)
		ILE155 (A)
Artocarpin	SER60 (B)	**LEU57 (A)**
	[2.76]	**TYR59 (A)**
	LEU120 (B)	**TYR59 (B)**
	[3.05]	**SER60 (B)**
		TYR119 (A)
		TYR119 (B)
		LEU120 (B)
		GLY121 (A)
		TYR151 (A)
		ILE155 (A)

Table 7: *Ligplot Analysis of the 3 Phytochemicals on Viral Interleukin-10*

a	***b***	***c***
Catechin	LYS34 (A)	**PHE30 (A)**
	[3.18, 3.26]	**VAL33 (A)**
	LYS40 (A)	**LYS34 (A)**
	[2.91, 2.97]	**PHE37 (A)**
		THR39 (A)
		LYS40 (A)
		ASP41 (A)
		VAL76 (A)
		ALA80 (A)
		VAL91 (A)
		LEU94 (A)
Quercetin	LYS34 (A)	**PHE30 (A)**

	[3.16]	**VAL33 (A)**
	LYS40 (A)	**LYS34 (A)**
	[2.70, 3.15]	**PHE37 (A)**
		THR39 (A)
		LYS40 (A)
		ASP41 (A)
		ALA80 (A)
		LEU94 (A)
		VAL191 (A)
Artocarpin	GLU42 (A)	**PHE30 (A)**
	[3.06]	**PHE37 (A)**
		GLN38 (A)
		ASP41 (A)
		GLU42 (A)
		TYR72 (A)
		VAL76 (A)
		MET77 (A)
		ALA80 (A)
		LEU94 (A)
		LEU98 (A)

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

According to this ranking, the top three ligands were artocarpin, quercetin, and catechin. The ligands demonstrated a wide range of impressive binding affinities, with Atrocarpin consistently exhibiting the highest value of -7.5 kcal/mol for NFkB p52, -8.0 kcal/mol for TNF-alpha, and -8.0 kcal/mol for Viral Interleukin-10 (vIL-10). This is followed by Quercetein, which had a value of -7.2 for NFkB and Tnf-a and -7.3 for vIL-10. Catechin is the third leading factor, with a value of -7.0 for NF-kappaB, -7.3 for Tnf-a, and -7.1 for vIL10. The remaining ligands, which included Ferulic Acid, p-Courmic Acid, and Gallic Acid, had binding affinities ranging from -5.9 to -4.9 kcal/mol in all three proteins.

As noted by Wang et al. (2021), binding affinity gives important information about the strength of protein-ligand interactions, which is commonly characterized as inhibition constant (Ki), dissociation constant (Kd), or half-maximal inhibitory concentration (IC50). Identifying binding affinities is critical to the virtual screening process for drug discovery and repurposing of existing medicines. The finding of high-affinity ligand-binding proteins is a key focus in early-stage drug research.

According to Bulusu and Desiraju (2020), hydrogen bonds are known as the "master key of molecular recognition," because they are weaker than covalent bonds but stronger than van der Waals forces. Their ubiquity and flexibility make them indispensable in biomolecular systems, particularly in watery ones. Hydrogen bonding is essential for chemical and biological processes that need specificity and reversibility, such as ligand binding and enzyme catalysis. Weaker interactions, such as hydrogen bonds, are useful in biological processes because they can form and dissociate more quickly than stronger connections.

Tan, Singh, Hazra, and Madhusudhan (2021) agreed with Bulusu and Desiraju (2020) that hydrogen bonding is an important non-covalent interaction between an electronegative acceptor atom and a hydrogen atom, which is covalently linked to an electronegative donor atom. It is important for its directed and atom-specific nature, often working over a distance of 2.5-3.5 Å between donor and acceptor atoms, with an energy range of around 1.3-9 kcal/mol. While hydrogen bonding is predominantly electrostatic in nature, it also has certain covalent properties, which adds to its complexity.

Hydrogen bonds in proteins are essential for preserving structural integrity and stability, since they contribute to secondary structure creation, appropriate folding, and molecular recognition processes. These bonds also alter the pKas of ionizable amino acid residues and can indirectly stabilize protein structures by increasing packing density in the protein's interior, which improves van der Waals interactions (Tan et al., 2021).

Tan et al. (2021) concluded in their paper that to qualify as a hydrogen bond, it requires a distance between the donor and acceptor atoms of 3.5 Å or fewer and an antecedent angle (Ĥ) higher than 100°. These geometric values of hydrogen bonding are determined from statistical investigations of known protein structures, which provide valuable insights into protein stability and function.

3.3. Phytochemical with the most interacting residues

Artocarpin was the most effective among the three phytochemicals. Its interactions with the residues of the three proteins showed strong binding. In the protein Human NF-KAPPA-B P52 bound to DNA, it had hydrophobic interactions in LYS90 (A), GLU92 (A), ASP94 (A), ARG103 (A), ALA104 (A), SER115 (A), LEU117 (A), ILE119 (A), ALA 121 (A), LYS153 (A), ARG156 (A), ARG160 (A), and ARG193 (A). It specifically interacted with binding sites ILE119 (A) and ALA121 (A) and established two moderate hydrogen bonds with LYS 90 (A), ARG103 (A), ARG156 (A) at distances of 3.17 Å, 3.05 Å, and 3.11 Å respectively.

Moreover, the protein TNF-alpha with a small molecule inhibitor interacted with the hydrophobic LEU57 (A), TYR59 (A), TYR59 (B), SER60 (B), TYR119 (A), TYR119 (B), LEU120 (B), GLY121 (A), TYR151 (A), and ILE155 (A) in which all of them are in the binding sites. There were also hydrogen bonds at 2.76 in SER60 (B) and 3.05 in LEU120 (B). While in the protein Viral Interleukin-10, Artocarpin had a hydrophobic interaction in PHE30 (A), PHE37 (A), GLN38 (A), ASP41 (A), GLU42 (A), TYR72 (A), VAL76 (A), MET77 (A), ALA80 (A), LEU94 (A), and LEU98 (A) and all of these are present in the binding sites. Its hydrogen bond is at 3.06 in GLU42 (A).

The many interactions of Artocarpin with important residues in all three proteins suggest that it could be a strong candidate for immunosuppression. The presence of positively charged residues like arginine and lysine in the interaction sites indicates that these interactions might help stabilize Artocarpin's binding. This stability is essential for effectively controlling immune responses. Additionally, Artocarpin's ability to connect with multiple key residues across different proteins shows its potential as a useful treatment for transplant rejection and autoimmune diseases. This highlights the need for more research into how Artocarpin works in the body and its role in immunosuppression. In conclusion, Artocarpin's strong binding ability and interaction with critical protein residues make it a promising candidate for further studies aimed at developing new immunosuppressive therapies from Marang seed extracts.

3.4. Other Phytochemicals with notable interacting residues

Several other phytochemicals demonstrated noteworthy interactions with key residues in the target proteins. Quercetin with a binding affinity score of (-7.3 kcal/mol) on the three proteins, Quercetin formed stable interactions with Human NF-KAPPA-B's hydrophobic interactions in ASP94 (B), ALA104 (B), LEU117 (B), ILE119 (B), LYS153 (B), GLN157 (B), ARG161 (B), and ARG193 (B) wherein ILE119 (B) and GLN157 (B) are in binding sites. There were also hydrogen bonds in ASP94 (B) at 2.71, LEU117 at 2.83, LYS153 (B) at 3.21 and 3.32, GLN157 (B) at 3.03, and SER161 (B) at 3.20, suggesting its potential to modulate viral-induced immune responses. On the other hand, it also had hydrophobic interaction with TYR50 (A), TYR59 (B), SER60 (B), TYR119 (A), LEU120 (A), GLY121 (A), TYR151 (A), TYR151 (B), and ILE155 (A) in protein TNF-alpha. All of these were binding sites except TYR50 (A) and had hydrogen bonds at 3.09 and 3.19 in TYR151 (A) and at 2.75 in TYR151 (B). While in protein Viral Interleukin-10, PHE30 (A), VAL33 (A), LYS34 (A), PHE37 (A), THR39 (A), LYS40 (A), ASP41 (A), ALA80 (A), LEU94 (A), and VAL191 (A) were the hydrophobic interactions wherein all were binding sites except VAL191 (A). Its hydrogen bonds occur in LYS34 (A) at 3.16 and LYS40 (A) at 2.70 and 3.15.

On the other hand, Catechin revealed a binding affinity score of (-7.2 kcal/mol), with hydrophobic interactions occurring at Human NF-KAPPA-B's GLU92 (A), ARG103 (A), ALA104 (A), ILE110 (A), SER115 (A), LYS153 (A), ARG 156 (A), GLN157 (A), and ARG 160 (A), indicating its role in modulating inflammatory pathways. The binding site GLN157 (A) was also present, along with the hydrogen bonds LYS90 (A) at 3.16, GLU92 (A) at 3.06, ARG103 (A) at 3.14 and 3.15, SER115 (A) at 3.19, and ARG 193 (A) at 3.15 and 3.01. In the next protein TNF-alpha, it has hydrophobic interactions in TYR50 (A), LEU57 (A), TYR59 (B), SER60 (B). GLN61 (B), TYR119 (B), LEU120 (B). GLY121 (A), and TYR151 (B) wherein all except TYR50 (A) are binding sites. It also had hydrogen bonds at 2.70 in SER60 (B), 2.84 in LEU120 (B), and 2.97 in TYR151 (B). And in the last protein Viral Interleukin-10, the hydrophobic interactions were PHE30 (A), VAL33 (A), LYS34 (A), PHE37 (A), THR39 (A), LYS40 (A), ASP41 (A), VAL76 (A), ALA80 (A). VAL91 (A), and LEU94 (A) in which all of them except THR39 (A) is a binding site. Its hydrogen bonds occur in LYS 90 (A) at 3.17, ARG103 (A) at 3.05, and ARG156 (A) at 3.11. Overall, the interactions of these phytochemicals showed that it also has a potential to be an immunosuppressant.

3.5 Comparison of Artocarpin with Azathioprine as a Positive Control

In this study, Azathioprine was used as the positive control to evaluate the potential of Artocarpin as an immunosuppressant. Mohammadi and Kassim (2023) state that Azathioprine (AZA) is commonly used for managing active rheumatoid arthritis (RA) and preventing kidney transplant rejection. In the molecular docking results, it showed that Artocarpin exhibited a binding affinity lower than -7 kcal/mol, indicating a strong interaction with the target receptor. In comparison, Azathioprine, despite being a known immunosuppressant, showed a weaker binding affinity, suggesting reduced stability in receptor binding. These results can be seen in the table 8 below. While Azathioprine interacted with key active site residues, LigPlot analysis revealed no hydrogen bon0ds, further indicating a less stable interaction. These findings suggest that Artocarpin may offer stronger and more stable receptor binding, positioning it as a promising alternative for immunosuppression.

Table 8: *Azathioprine's activity on different protein*

a	***b***	***c***	***d***
NF-KAPPA-B P52	-6.0	LYS252 (A)	GLY50 (A)
		[3.19]	LYS221 (A)
		SER220 (A)	SER222 (A)
		[3.12]	PRE223 (A)
			SER226 (A)
			LYS283 (A)
			TYR285 (A)
TNF-alpha	-6.4	TYR151 (B)	**TYR59 (B)**
		[3.08]	GLU61 (B)
			TYR119 (A)
			TYR119 (B)
			LEU120 (A)
			LEU120 (B)
			GLY121 (B)
Viral Interleukin-10	-5.5	GLN63 (A)	**PHE56 (A)**
		[3.16]	TYR59 (A)
		GLY61 (A)	CYS62 (A)
		[3.04]	**LEU65 (A)**
		PHE111 (A)	ARG110 (A)
		[3.31]	PRO113 (A)
NF-KAPPA-B P52	-6.0	LYS252 (A)	GLY50 (A)
		[3.19]	LYS221 (A)
		SER220 (A)	SER222 (A)
		[3.12]	PRE223 (A)
			SER226 (A)
			LYS283 (A)
			TYR285 (A)

a *= protein ;* ***b*** *= binding affinity;* ***c*** *= hydrogen bonds with interacting residues;* ***d*** *= Hydrophobic interactions with interacting residues;* ***bold*** *= binding sites*

3.6 Visualization of the top Phytochemical with the most interacting residues.

Artocarpin is displayed through both 3D and 2D visualizations using Chimera and Ligplot+. These tools allow for the clear representation of the interactions between this phytochemical and target proteins—NF-kappaB P52, TNF-alpha, and Viral Interleukin-10. The 3D visualizations illustrate how the ligand fits within the binding pocket of the protein, while the 2D visualizations detail the hydrogen bonds and hydrophobic interactions between the phytochemicals and specific amino acid residues. This visualization provides a comprehensive view of how this compound may modulate immune responses by targeting key proteins involved in inflammatory pathways. By examining these visual representations, researchers can better understand the molecular mechanisms behind the immunosuppressive potential of this phytochemical, supporting its potential use in autoimmune disease treatment and transplant rejection management.

NF-KAPPA-B P52

a b

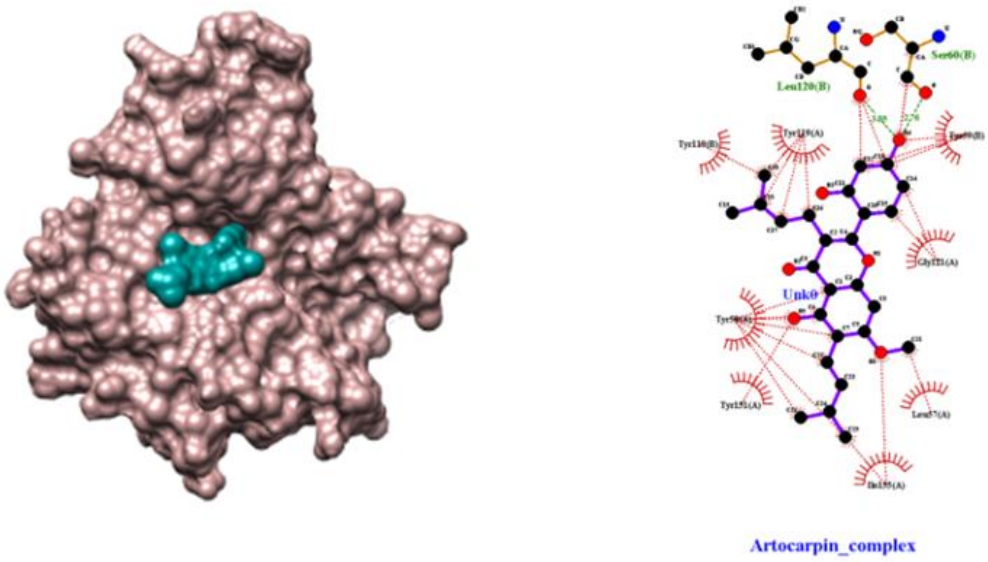


Fig. 4 - ***(a) Artocarpin. Chimera 3D visualization ; (b) Ligplot+ hydrogen bonds and hydrophobic interactions 2D visualization.***

TNF-alpha

a b

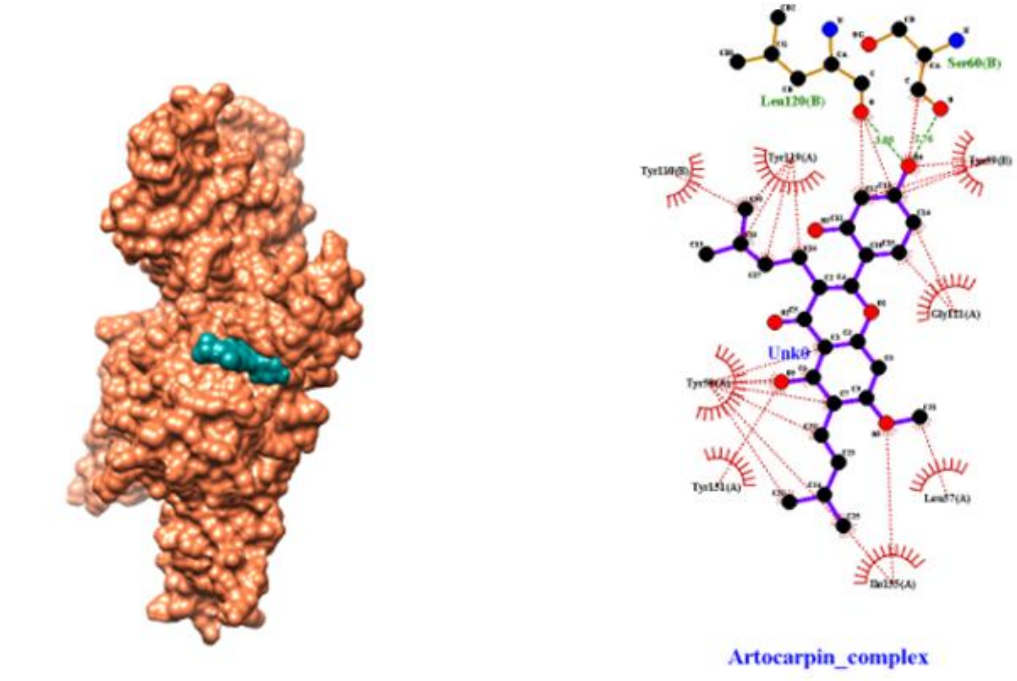


Fig. 5 - ***(a) Artocarpin. Chimera 3D visualization ; (b) Ligplot+ hydrogen bonds and hydrophobic interactions 2D visualization.***

Viral Interleukin-10

a b

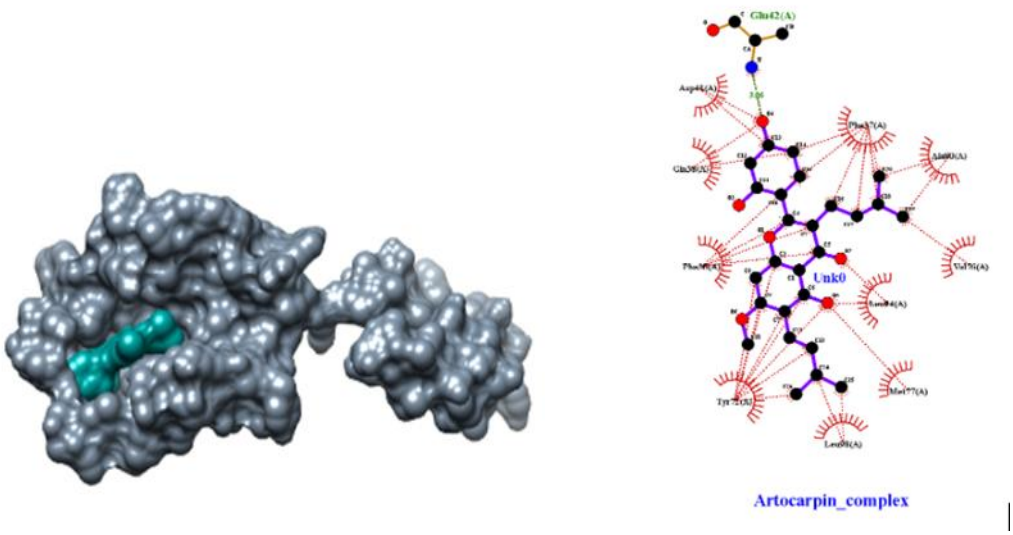


Fig. 6 - ***(a) Artocarpin. Chimera 3D visualization; (b) Ligplot+ hydrogen bonds and hydrophobic interactions 2D visualization.***

4. Conclusion and Recommendations

Marang seed extract as an alternative to immunosuppressive drugs presents significant implications for the treatment of autoimmune diseases and organ transplantation. The marang extract is rich in phenolic compounds and has shown the ability to modulate immune responses effectively while minimizing the side effects typically associated with conventional treatments. This approach aligns with global health objectives, providing safer, more effective options for managing autoimmune conditions and improving patient outcomes.

The molecular docking analysis revealed that Artocarpin, Quercetin, and Catechin had the highest binding affinities for key immune proteins, such as NF-kappaB p52, TNF-alpha, and Viral Interleukin-10, with it's significant interactions at critical binding sites. In the docking analysis, Artocarpin demonstrated the highest binding affinity among the tested phytochemicals, which are Catechin and Quercetin. Artocarpin consistently demonstrated the highest binding affinity across all three target proteins; also, Artocarpin is indeed a flavonoid specifically derived from Artocarpus and classified as an isoprenyl flavone, which also proves that Marang extract, which is rich in flavonoids, can help with our focus of the study because it can help with its anti-inflammatory, antioxidant, and anticancer properties. With a binding score of -7.5 kcal/mol for NF-kappaB p52, -8.0 kcal/mol for TNF-alpha, and -8.0 kcal/mol for viral interleukin-10. This negative value indicates a stronger interaction between artocarpin and the active sites of these immune regulatory proteins, suggesting that artocarpin could provide more stable and effective immune modulation compared to its counterparts. Though quercetin and catechin also exhibited notable binding affinities, their interactions were not as strong as artocarpin. This negative value indicates a stronger interaction between artocarpin and the active sites of these immune regulatory proteins, suggesting that artocarpin could provide more stable and effective immune modulation compared to other proteins. Though quercetin and catechin also exhibited notable binding affinities, their interactions were not as strong as artocarpin. This study reveals artocarpin as the primary phytochemical candidate for immunosuppression due to its better binding performance and potential to modulate immune responses. These interactions indicate that these phytochemicals may effectively reduce immune system overactivation and inflammatory reactions, which are critical in preventing transplant rejection and treating autoimmune diseases.

Despite these promising outcomes, more in vivo study must be conducted in order to confirm the pharmacodynamics and pharmacokinetics of these phytochemicals. Studies should look at the ideal dosages, potential side effects, and long-term effects of these compounds on immune response and infection resistance. Considering Marang's occurrence in research, its use in medical applications holds enormous potential for drug development. In addition, exploring the complementary impacts of Marang phytochemicals and existing immunosuppressive treatments could improve the effectiveness of therapy while reducing side effects.

In summary, the findings of this study underscore the potential of Marang seed-derived phytochemicals as alternative immunosuppressive agents. Their ability to target critical proteins involved in immune responses highlights their potential in managing autoimmune diseases and reducing transplant rejection rates. However, further experimental validation and clinical trials are necessary to fully establish their therapeutic value.

Acknowledgements

This study would not have been possible without the help and advice of several individuals. We would like to extend our heartfelt gratitude to Dr. Sherwin Fortugaliza, our research adviser, for his invaluable assistance and guidance throughout our research.

We are grateful to our parents for their continuous encouragement and support, which helped us pursue success. Their confidence in our talents and consistent motivation gave us the courage to take on challenges and persevere in our studies. We are deeply grateful for their efforts and the love they showed us, which pushed us to strive for excellence.

Furthermore, we are grateful to our research colleagues, whose shared ideas significantly increased our understanding of the molecular docking process. We would also like to thank the authors of the fundamental studies and literature that influenced our study, allowing us to understand our research in the context of existing knowledge.

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