"In Silico Discovery of Promising JAK1 Inhibitors for Vitiligo from Plant-Derived Phytochemicals: A Combined ADMET and Molecular Docking Study"

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CANDIDATE'S DECLARATION

I Firoz Tyagi Roll Number: 2K21/MSCBIO/15, student of M.Sc. Biotechnology, hereby declare that the work which is presented in the Major Project entitled — "In Silico Discovery of Promising JAK1 Inhibitors for Vitiligo from Plant-Derived Phytochemicals: A Combined ADMET and Molecular Docking Study" in the fulfilment of the requirement for the award of the degree of Master of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi, is an authentic record of my own carried out during the period from January- May 2023, under the supervision of Prof. Yasha Hasija.

The matter presented in this report has not been submitted by me for the award for any other degree of this or any other Institute/University. The work has been accepted in SCI/SCI expanded /SSCI/Scopus Indexed Journal OR peer reviewed Scopus Index Conference with the following details:

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ABSTRACT

The non-receptor tyrosine-protein kinase family member Janus Kinase 1 (JAK1) is essential for several biological processes, including cell survival, cell-cell adhesion, cell differentiation, and cytoskeleton remodelling. JAK1, which is present in high concentrations in autoimmune illnesses such as Vitiligo, Rheumatoid Arthritis as well as tumours such oesophageal, lung, and bladder cancers, offers itself as a prospective target for therapeutic approaches. A virtual screening method was used in this study to find possible JAK1 inhibitors in the IMPPAT database. Following the Lipinski rule of five, substances were first filtered according to their physicochemical characteristics. To find promising hits that were both non-toxic and had advantageous properties, binding affinity calculations, PAINS filter application, ADMET analysis, and PASS analysis were then carried out. Two particular substances from the plant-based database (IMMPAT) are berberine and dehydroaporheine, showed notable affinity and specific interaction with JAK1. It will be suggested that berberine and dehydroaporheine further investigated in in vitro and in vivo settings to determine their potential as anticancer and antiviral treatments based on the findings of this study. Their particular interaction with JAK1 emphasises their potential as targeted treatments for autoimmune and cancerous conditions.

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LIST OF ABBREVIATIONS

JAK1	Janus Kinase 1
STAT	Signal transducer and activator of transcription
TYK2	Tyrosine kinase 2
CSK	Colony-stimulating factors
ILs	Interleukins
IFNs	Interferons
FGF	Fibroblast growth factor
EGF	Epidermal growth factor
PIAS	Proteins inhibiting activated STATs
SOCS	Proteins suppressing cytokine signaling
PAINS	Pan-assay interference compounds
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
PASS	Prediction of Activity Spectra for Substances
IMMPAT	Indian Medicinal Plants, Phytochemistry and Therapeutics
NCBI	National Center for Biotechnology Information
PDB	Protein Data Bank
GI	Gastrointestinal
LD50	Lethal Dose 50
RAM	Random Access Memory
vHTS	Virtual High-Throughput Screening
BLAST	Basic Local Alignment Search Tool
NMR	Nuclear Magnetic Resonance

CHAPTER 1

INTRODUCTION

The Janus Kinase (JAKs) is considered to act as a key role in causing Auto-immune disease and various type of cancer, thus is majorly focused to be as a drug target. Lots of researchers being done on JAK1 to check its cancerous activity and drug inhibitor are designed against JAK1 protein which has shown their useful therapeutics activities. JAK1 is involved in the STAT Signalling Pathway and has connect to a number of serious illnesses, consists inflammatory bowel disease (IBD), cancer, vitiligo, rheumatoid arthritis, psoriasis, and auto-immune diseases [1].The Janus kinases family (JAKs), a subclass of non-receptor protein tyrosine kinases. The JAK family of enzymes consists of TYK2, JAK1, JAK2, and JAK3. JAK play crucial roles in the growth, development, survival, and differentiation of diverse cell types, with particular significance for hematopoietic and immunological cells [2]. The JAK-signal transducer and activator of transcription (STAT) pathway, which is used by numerous cytokines, growth factors, and hormones, is one of the mechanisms by which they contribute to the transfer of extracellular signals to the nucleus. The regulation of gene expression by outside influences is made possible through this mechanism [3]. Disturbances or dysregulation in JAK-STAT functionality can contribute to Auto-immune diseases and cancer development. The tyrosine kinase JAK1 has numerous functional domains and is a member of the family. It has a catalytic kinase domain responsible for its enzymatic activity, which phosphorylates target proteins. Additionally, JAK1 has a C-terminal region crucial for receptor binding and N-terminal FERM domain play role in protein-protein interactions. JAK1 is primarily located in the cytoplasm of cells. Janus kinase 1 (JAK1) belongs to the Janus kinase family and is a protein with a critical function in cell signalling. JAK1 is crucial for the signalling pathways of several cytokines involved in immune responses, such as (CSFs), (ILs), and (IFNs). Through JAK1-mediated phosphorylation of STATs, these cytokines regulates the expression of genes related to immune cell activation, differentiation, and inflammation. JAK1 signalling controls cell growth, survival, and differentiation in a variety of cell types [4] . It participates in the signalling a number of growth factors, including (FGF) and (EGF), have different routes, influencing processes such as tissue development, wound healing, and organ homeostasis. The

activity of JAK1 is tightly regulated to maintain proper cellular signalling. JAK1 activity is influenced by Among other unfavourable regulators, PIAS and SOCS (proteins suppressing cytokine signalling) proteins are also present. They can inhibit JAK1's kinase function or promote its degradation, ensuring the balance and accuracy of signal transduction. Activated JAK1 creates docking sites for signal transducer and activator of transcription (STAT) proteins on the receptor by phosphorylating certain tyrosine residues on the receptor itself. STAT genes are then recruited to the receptor complex, where they become phosphorylated by JAK1 [5]. Phosphorylated STATs form Dimers separate from the receptor and go into the nucleus, where they control gene transcription.

JAK1 primarily interacts with STAT1, STAT2, STAT3, and STAT6, although it can also interact with other signalling molecules. These interactions are mediated through specific Protein domains that recognise and bind to phosphorylated tyrosine residues on receptors, like the SH2 (Src Homology 2) domain found in STATs, and other signalling proteins [6] . A number of cytokines, such as interleukins, interferons, and growth factors, use the JAK-STAT pathway, which is primarily composed of JAK1. Upon receptors activation, JAK1 phosphorylates STAT proteins, leading to their dimerization, nuclear translocation, and subsequent gene transcription. This pathway plays a significant role in immune responses, haematopoiesis, inflammation etc. Dysregulation of Janus kinase 1 (JAK1) protein can contribute to the development of various diseases, including autoimmune disorders and certain forms of cancer. diseases such inflammatory bowel disease, rheumatoid arthritis, and immune dysregulation, Eosinophilia and Vitiligo Caused by activation of JAK1 [7]. It is important to note that the dysregulation of JAK1 alone is not the sole cause of these diseases. Multiple factors, including genetic predisposition, environmental triggers, and interactions with other signalling pathways, contribute to the overall disease development and progression [8].

Human JAK1 protein consists of 1154 amino acids. The binding and active sites of JAK1 are primarily located within its domains, including the regulatory domain, tyrosine kinase domain, FERM domain, and SH2 domain. These domains play crucial roles in protein-protein interactions, substrate recognition, and enzymatic activity [9]. The FERM domain (amino acids 34 - 420) is responsible for protein folding, stability, and interaction with membrane-associated proteins. The SH2 domain (amino acids 439-544) is involved in binding to phosphorylated tyrosine residues on receptor proteins, enabling JAK1 to be recruited to activated cytokine receptors. The tyrosine kinase domain (amino acids 583-855) contains the catalytic site is in charge of phosphorylating JAK1's tyrosine residues itself and its substrate

proteins, including the STAT transcription factors [10]. The regulatory domain (Protein Kinase) (amino acids 875-1154) plays a role in modulating JAK1 activity and interaction with other proteins. The protein kinase domain which comprises the amino acids 875- 1154 which has binding site at Lys908 and active site at Asp1003 of JAK1 Protein. Since the other first two domains do not have active sites. The kinase domain has ATP-binding region This structure can offer crucial information for the development of highly effective, selective JAK1 ATP-competitive inhibitors [11].

Today, computer-aided drug design includes virtual screening-based research as a critical component. Finding ligands that could accurately and functionally bind the target receptor was made simple with the use of molecular docking-assisted virtual screening [12] .This technology, known as virtual screening by molecular docking, is very useful for finding ligands that bind target receptor proteins with high affinity. It is a major step in the computer-aided drug design process, allowing for the identification of possible drug-like molecules by screening a large number of chemical compounds that are available in different chemical databases.

In virtual screening by molecular docking, the binding interactions between a target receptor protein and tiny chemical compounds are predicted using computer simulations and algorithms. The potential binding mechanisms and affinities of these chemicals to the target protein can be investigated by researchers using molecular docking methods [13]. Additional filters and analytics are frequently incorporated during the virtual screening process to improve the accuracy and calibre of the outcomes. Compounds with desirable drug-like qualities can be found using filters like Lipinski's Rule of Five (RO5), which assesses physicochemical features. Other filters, such the PAINS (Pan Assay Interference Compounds) filter, assist in removing substances that might interfere with the assay or display unwanted properties. In addition, other analyses are used to evaluate crucial pharmacological features. These consist of assessing the (ADMET) qualities, which shed light on the compound's bioavailability and potential toxicity. To find substances that could be dangerous for human health, assessments of toxicity and carcinogenicity are made. In addition, the examination of PASS Analysis aids in predicting the biological activity profile of the substance.

The process of finding new drugs depends heavily on these thorough techniques and analysis. Researchers can quickly screen a large number of compounds and identify those with the best chance of being developed by integrating virtual screening with molecular docking, filters, and

assessments. The drug discovery pipeline is optimised by this method, which greatly speeds up the identification of new therapeutic candidates.

In this research, we have used the 2000 phytochemical compounds from the IMMPAT (https://cb.imsc.res.in/imppat/basicsearch/phytochemical) once Lipinski RO5 violation value zero has been applied to the database. The IMMPAT is the free resource that has been carefully curated on the phytochemicals of Indian medicinal plants. From the Alpha fold database, we were able to extract the three-dimensional structure of JAK1. Following that, we used InstaDock to perform virtual screening of these compounds against JAK1 to identify its high-affinity binding partners [14] . We chose the top hits based on binding affinities and the scoring function, and then we ran SwissADME to filter out substances without PAINS patterns. The pkCSM server was used to determine ADMET characteristics after the PAINS filter. Last but not least, we have chosen chemicals that specifically attach to the JAK1 binding site based on the unique interactions [15].

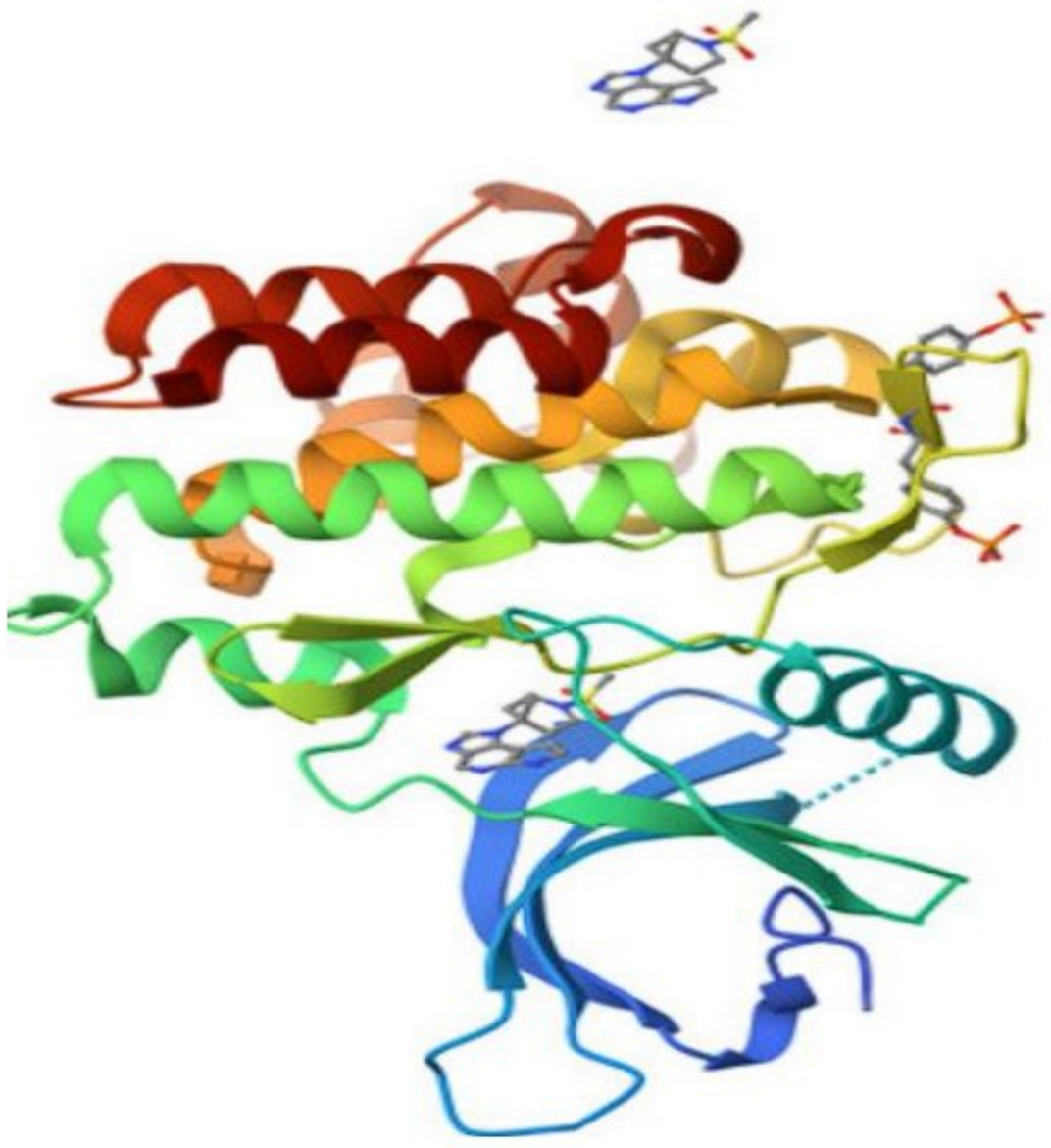

Fig 1 : (Crystal structure of JAK1 drawn from Protein Data Bank ID: 4E4L.)

CHAPTER 2

2. REVIEW OF LITERATURE

2.1 Computer aided drug design includes

2.1.1 Computational molecule model construction

When two or more homo/hetero atoms that are Molecules are held collectively by chemical bonds, that found both in living and non-living system. Drugs are basically small form of molecule having properties of interaction, binding and controlling the function of receptor that has the capacity to control and cure any disease. Receptors are proteins showing interaction with ligands, molecules, compounds and maintain cellular function in living system. Likewise, some of the major receptors in our body are cell signalling receptors, enzymes, hormone receptors, neurotransmitter receptors. Drug design is a technique to structural designing of small molecules that can act suitable for binding and inhibiting the behaviour of particular protein receptors, which is responsible for causing diseases [16].

2.1.2 Calculation of dihedral angle of molecule

Dihedral angle or torsion angle is the rotation around the angle shared between 1st and 4th atoms any chemical structure. It has two major forms eclipsed and staggered form. The torsion angle is positive for clockwise rotational conformation and negative for anticlockwise rotational conformation. Bond length, bond angle and bond order are the key points that are involved to decide the structure of molecule. Bond order is determined by VSEPR theory to predict the shape and structure of the molecule. which is found to be in the order as single bond > double bond > triple bond. The amino acid's dihedral angle is a crucial biological factor that aids in predicting the protein's structure [17].

2.1.3 "Exploring Molecular Stability: The Significance of Energy Minimization"

Nuclear motion is the basis for determining a molecule's energy and structure and in molecular mechanics the main objectives are the energy minimization is the process of reducing a molecule's energy and calculating the lowest energy configuration. In order to get lowest

molecule's energy configuration it requires to calculate bond length and bond angle that shows lowest steric energy. Molecule steric energy is decided by combining the effect of atoms that are bound and unbonded [18]. Molecular mechanics has concentrated on three primary parameters factors that are: force fields, parameter set, minimizing algorithm. The force field concept is responsible for determining the molecules' potential energy in molecular mechanics. A force field is a system of constants and functions that use to explain the energy potential of a molecule. The second is a set of parameters that includes bond angle and atomic mass, bond length, dihedral angle, van der Waals radii.

The force-field equation, expressed as:

$$Epot = \sum Ebon + \sum Eang + \sum Etor + \sum Eoop + \sum Enb + \sum Eel,$$

describes the total potential energy (Epot) of a molecular system in terms of various energy components. Each component contributes to the overall energy and represents specific interactions within the system.

The term $\sum Ebon$ represents the energy resulting from changes in bond length, calculated using Hooke's equation, which describes the deformation of a spring. It depends on the force constant (kb), the difference between the current bond length (b) and the equilibrium bond length (b0).

The term $\sum Eang$ accounts for the energy associate with deviations from the original bond angles in the molecule.

$\sum Etor$ represents the energy arising from the deformation of dihedral or torsion angles within the molecule [19].

$\sum Eoop$ captures the steric energy related to out-of-plane bending components.

$\sum Enb$ corresponds to the energy arising from non-bonded interactions between atoms, such as van der Waals forces.

$\sum Eel$ accounts for the energy resulting from coulombic forces or electrostatic interactions between charged particles.

By summing up these energy components, the force-field equation allows us to evaluate the overall potential energy of a molecular system, providing insights into its stability and structural properties.

2.1.4 Homology Modelling: Predicting Protein Structure

It's a method for generating a structure from a sequence that should correspond to the outcomes of experiments. The sequences of amino acids in a protein determines its shape. Protein structures can be predicted using X-ray crystallography, NMR spectroscopy, theoretical models based on experimental data, or homology modelling. Most proteins of determined from real experiment, NMR or X-ray crystallography do not show high resolution clear image [20].

Here in homology modelling the 3D structure is constructed based on alignment of chosen protein to a template sequence that is already known. We can fetch the protein sequence either from UniProt or NCBI database. Depending upon the similarity index between the database having maximum similarity aligned and the target sequence, determines the quality of the protein structure. If the index range more than 30% then is safe zone, homology modelling less than 30% is not used to predict the structure (Takeda-Shitake et al., 2004). The index range between 10% to 30% is known as twilight zone and those less than 10% is midnight zone. Sometimes in the rare case less than 20% is considered [21]. The homology modelling follows multistep as well as database searches, energy minimization, sequence alignment, structure evaluation, and structure prediction.

2.1.5. The relationship between the drug and the receptor

The interaction between a pharmacological molecule and a receptor is examined using docking studies. Drug design is the process of creating a therapeutic molecule that can bind to a target and interact with it. Receptors are molecules that can be seen on the surface of cells. When a small molecule connects to them, it causes the receptor to relay signals and activate the biological activity.

When the receptor is in an unbounded state with the receptor, the receptor's functions are silenced. By the definition it seems, Despite the fact that a receptor only interacts with one ligand at a time and the opposite is true, in some cases, ligands with high concentrations can bind to many receptor sites. Normal drug receptor binding by endogenous ligands is not the case. Receptors for these pharmacological molecules can be found in proteins, ion channels, nucleic acids, and enzymes. The drug's molecule can attach to DNA cross-links and prevent DNA replication from occurring. It is useful to treat malignant tumours (that are growing faster & showing metastasis) that are going to more dangerous stage after being cancerous. The autacoids, cytokines, growth factors, hormones, neurotransmitters, and Additional endogenous regulatory ligands have receptors. Therefore, these receptors' function is to recognise ligands and initiate reaction [22].

By utilising a complementary molecule needed for a biological function, a pharmacological molecule makes signal transmission easier. For the evolution of receptor function, distinct sites that are intended to bind drug molecules must first emerge. The ability of the drug molecule to bind to biological molecules is essential for the control of biological function. Drug molecules' interactions with receptors change the state and functionality of the receptor.

Computational approaches used to model a molecule are referred to as molecular modelling. Utilising these modelling tools to create medications is known as computer-aided drug design. The computer-assisted drug design method is practical, automatic, rapid, valuable, affordable, and virtual. In contrast to structure-based drug design, which is based on the three-dimensional structure of the target molecule, ligand-based drug design is primarily concerned with the model that will bind to the target. Any target that is still not available there can be designed using homology modelling.

When two molecules are joined to form a stable complex, a procedure called docking is used to forecast how they will interact. Molecular docking can be described using the "Lock and Key" paradigm. The ligand serves as the key and the protein as the lock in the system. It specifies the proper ligand orientation for binding to a specific protein. Protein molecules must be used for docking. The inputs for docking are the protein structure and ligands.

There are two different platforms on which docking can occur:

2.1.5.1Algorithm for search

The search algorithm aims to generate a set of optimal configurations that primarily consist of confirmed binding modes. These configurations are evaluated using scoring formulas to distinguish them from other modes. Several commonly used search algorithms in this context are:

I) Monte Carlo methods: These algorithms use random sampling and probabilistic transitions to explore the search space and find energetically favorable binding configurations.

II) Genetic algorithms: Inspired by biological evolution, genetic algorithms involve populations of configurations that undergo selection, crossover, and mutation operations to iteratively improve and converge towards favorable binding modes.

III) Fragment-based methods: These methods break the ligand and receptor into smaller fragments and explore combinations of fragments to identify binding modes. The aim is to efficiently sample a reduced search space while maintaining accuracy.

IV) Point Complimentary methods: These algorithms search for complementary points on the ligand and receptor surfaces to identify potential binding modes. The search is driven by the geometric and chemical complementarity between the interacting molecules.

V) Tabu searches: Tabu searches utilize a memory mechanism to avoid revisiting previously explored configurations. This helps to diversify the search and prevent getting trapped in local optima.

VI) Systematic searches: Systematic searches systematically explore the search space by iterating through various combinations of ligand orientations, conformations, and receptor configurations. They aim to cover a broad range of possibilities to identify potential binding modes.

VII) Molecular dynamics: Molecular dynamics simulations simulate the motion of atoms over time using physics-based force fields. By exploring the conformational space and accounting for molecular flexibility, these simulations can capture binding events and provide insights into binding modes.

These search algorithms play crucial roles in computational chemistry and drug discovery, enabling the exploration of complex molecular interactions and the identification of promising binding modes for further analysis and experimental validation.

2.1.5.2.Scoring Function

To detect interactions between proteins as well as between proteins and DNA, scoring functions have been built. Scoring techniques are mathematical approaches for predicting the strength of two-molecule interaction.

Steps involved in Docking Process:

I) Choose or select molecule:

Consider receptor and ligand molecules. The receptor molecule is rigid, the ligand molecule is flexible in contrast.

II) Dock the molecules:

Dock the ligand molecule into the binding pocket of the receptor. Create as many different orientations as you can.

III) Model evaluation:

Based on the resultant energy, evaluate the model/docking results.

2.1.5.3.Docking Types

Two type of docking method:

Rigid Docking:

rigid docking, the ligand and the target molecule are assumed to be rigid structures. The ligand is placed within the binding site of the target molecule without allowing any flexibility or movement of the atoms. This type of docking is typically used when the conformational changes upon binding are expected to be minimal.

Flexible Docking:

flexible docking, the ligand and the target molecule are allowed to be flexible. It takes into account the conformational changes that may occur upon binding. The flexibility of the molecules is taken into consideration during the docking process, allowing the ligand and the target to adjust their conformations to optimize the binding interaction [23].

2.1.6 Pharmacokinetics: The Analysis of Drug ADMET Properties:

Successful therapeutic molecules must withstand numerous tests, including those for absorption, distribution, metabolism, elimination/excretion, and toxicity (ADMET) properties. An adsorption test's goal is to quantify how much medication has been effectively absorbed. Following absorption, it should be transported inside the body via blood and lymphatic systems in appropriate amount to reach the target organ. The ability of drug molecules to reach their target should also be analysed, which is performed by metabolism and elimination procedures. Metabolism is the process by which the body breaks down smaller molecules in the liver using enzymes. The other method by which drugs are removed is elimination, which occurs through the kidneys or feces. Thus, pharmacokinetic features are critical in the production of a drug molecule The study of pharmacokinetics examines how the body reacts to drugs. Pharmacokinetics measures the rate of GI absorption, BBB permeation , OCT substrate, and elimination/excretion [24].

Fig 2. Pharmacokinetic properties Identifier (https://biosig.lab.uq.edu.au/pkcsm/prediction)

2.1.6.1.Absorption

A chemical is transported through absorption from the site of delivery to the intended destination. Smaller drug molecules are more swiftly and easily able to diffuse across membranes than larger ones. Most medications are absorbed passively, but some need to be carried by a carrier. Because basic medications are better absorbed in the intestine and acidic pharmaceuticals are better absorbed in the stomach, the pH level of drugs may alter depending on where they are absorbed. It's probable that mostly drug absorption takes place in the small intestine as its surface area is substantially greater than that of the stomach. Amphipathic medications absorb without any issues. Some medications that float as globules in the colon because they are insoluble in water are emulsified into smaller, finer molecules by bile salts. Small holes in the capillary walls allow drugs to pass through after being injected into the subcutaneous layer or a muscle and enter the circulatory system. The solubility of substances in the aqueous content of the gastrointestinal (GI) tract, which is eventually mediated via GI tract barriers to reach blood, is a prerequisite for the oral medications' absorption.

Factors that impact absorption include:

i.) The drugs physiochemical properties,

ii.) Form of administration

iii.) the physiological and pathological aspects, such as blood flow, pH, and the impact of meals and other medications. A medicine's bioavailability is 100% when administered intravenously. It varies from patient to patient, but when a medication is administered in another form (for instance, orally), its bioavailability frequently decreases (due to inadequate absorption and first pass metabolism) [25].

2.1.6.2.Distribution

Blood flow through the tissues can be used to determine drug transport throughout the body. The hepatic portal system is important in drug distribution when it is absorbed through the GI tract. The majority of drugs absorbed via the hepatic system, where they are stored and may be

metabolised before they are distributed throughout the body. Due to the high hepatic first pass, medications that are metabolised by liver enzymes when taken orally provide a significant danger. Using plasma-bound binding proteins and medicines with lipophilic characteristics that can pass the blood-brain barrier, drug distribution is made possible.

2.1.6.3.Binding of plasma proteins

When insoluble substances attach to structurally amphipathic proteins, the circulation is able to transport the substances. The lipophilic group of proteins are only weakly joined to the lipophilic substances. Drugs occur in two states bound and unbound in plasma because they are partially bound to protein and partially in solution. With the free drug present, the protein-bound state is in equilibrium. When a drug's free unbound level reaches a certain point, the bound component of the drug is released from plasma protein to maintain homeostasis.

2.1.6.4.The BBB Permeation

Only small molecules can get through the blood brain barrier because connective tissue closes the pores in the capillaries of the central nervous system. These tiny molecules should be particularly lipophilic in order to cross the blood-brain barrier. This barrier, the only primary protective layer of the CNS, keeps some medications from penetrating the CNS and having negative effects. Due to the fact that most drugs fail in clinical trials, researchers and pharmaceutical corporations have created an effective method for predicting drug similarity by ADME-tox/ADMET test for a certain unique molecule [26].

2.1.7 Molecule toxicity prediction

Using in silico-based techniques, researchers and pharmaceutical companies have recently developed ADME and toxicity tests. These methods can be used to predict a medicinal molecule's toxicity even before it is developed. Even if in-silico techniques are simpler, there are certain challenges to overcome.

i.)Toxicity can refer to a wide range of concerns such as carcinogenicity, cytotoxicity, etc.

ii.)There is lack of data, particularly for humans related samples.

iii.)The in-silico techniques are class specific, and identifying whether toxicity is on or off is the least reliable.

Drug molecules can produce toxicity in many ways . For example, it is possible that the toxicity is caused by the metabolite as well as by the drug itself. For certain situations, the drug

molecules cytotoxic and mutagenic qualities are intended to kill disease or cancerous cells, but it is highly possible that it will also impact normal cells. Cytotoxicity is the most basic type of toxicity, in which the drug molecule or its metabolite causes severe cell damage.

2.1.7.1.Carcinogenicity

Some drugs can induce DNA mutations or damage, which affects cell metabolism and can lead to cancers. These drugs may also trigger oncogenes, causing normal cells to become malignant.

Mutagenicity: Some drug molecules are potent to cause DNA alterations in germ cells, resulting in mutations that inherit in the progeny.

Drug Allergy*:* Drug Allergy refers to an allergy produced by a drug. Antibodies formed within the body in response to a drug may overreact and cause allergies such as itching, rashes, and so on.

2.1.7.2.Toxicity measurement

Toxicity is a measurable quantity; the LD50 is a basic measure of toxicity. It is a drug dosage that kills 50% of treated animals in an amount of time. The therapeutic window is the dose range between the lowest effective therapeutic concentration and the lowest toxic concentration [27].

CHAPTER 3

3. MATERIALS AND METHOD

3.1. Computer programmes, web servers, and infrastructure required for vHTS

We combined computational tools, databases, and online internet servers for our investigation. To do this operation quickly and accurately, a high-performance computer support system was also required. With the aid of the HP 14s-f1000 11th Gen AMD Ryzen 5 5500U with Radeon Graphics, 8GB installed RAM, Windows 11' Home single Language operating system version 21H2, a 64-bit operating system, an x64-based processor, and a reliable power and internet supply are also required. Molecular docking and vHTS were performed using bioinformatics tools including InstaDock, AutoDock Vina, MGL Tools, and Discovery Studio. VMD, QtGrace, and PyMol were used to visualise and analyse structural data. GROMACS was used to conduct MD simulation investigations. Numerous internet servers and resources were utilised for data extraction, evaluation, and analysis, including the NCBI, RCSB PDB, IMPPAT data base(https://cb.imsc.res.in/imppat/), SwissADME, PreADMET, pkCSM, CarcinoPred-EL, and Way2drug for PASS Analysis, among others [28] .

3.2. Structure Refinement

Extracted the 3D Structure of protein JAK1 from RCSB PDB (https://www.rcsb.org/structure/4E4L). The protein's X-ray crystal structure, which has a resolution of 2.00 Å, with a total structural weight of 140.32 Da (PDB ID: 4E4L), was chosen for processing. The structure is improved with PyMol by removing non-essential regions from the JAK 1 protein, leaving only the kinase sequence region of interest. Exporting the protein structure as a PDB version file will allow it to be further analysed and used for analysis. This protein model is validated using the SAVES Server (https://saves.mbi.ucla.edu/), and the updated JAK1 model is used for further research.

3.3. Compound filtration

Using the SwissADME web tools/server, compounds were screened according to their

phytochemical and ADMET characteristics. The Lipinski rule of five and the ADMET filter were used in the selection process. The PAINS filter was also used to eliminate compounds having patterns that bind to numerous targets. All substances were then screened for carcinogenicity, and only those found to be non-carcinogenic were given consideration for additional research.

3.4. Database for screening

In this study, The Indian Medicinal Plants Phytochemistry and Therapeutics (IMPPAT) database was used, which contains a wide range of organic compounds. This database is a crucial tool for our research because it contains a sizable quantity of natural substances. For virtual screening, we specially selected 2000 natural chemicals from the IMPPAT database. This database is well-known for its thorough curation and accessibility of chemicals with commercial availability. IMPPAT serves as a centralized platform that makes it easier to use cheminformatics methods to improve the identification of natural product-based medicines.

3.5. Receptor bases virtual screening

Molecular docking has a significant impact on drug discovery as it enables the study of molecular interactions that are vital for biological processes. To initiate ligand-receptor interaction, virtual screening of substances is carried out to find potential ligand-protein interactions. Virtual screening employs computational methods to search large databases for compounds that have a high chance of attaching to a particular receptor protein. Receptor-based the optimal binding must be found for virtual screening ligands that exhibit optimal molecular interactions with the receptor protein in its known three-dimensional structure. The core component of virtual screening is molecular docking, which predicts the spatial arrangement and orientation of a ligand when it binds to a protein. Given the large number of ligands to be docked with the receptor protein, a fast and efficient docking process is necessary.

To find medications with a high affinity for the JAK1 protein, we used molecular docking-based virtual high-throughput screening (vHTS) in our investigation. The scoring function and docking algorithm's precision is critical in predicting the structural positioning and orientation of ligands during the docking process. All protein and ligand structures were processed and filtered in their three-dimensional orientations for receptor-based virtual screening.[29]

3.6. Required Input files and Directories

For my research, I used InstaDock an easy-to-use GUI application for molecular docking and virtual screening . I found InstaDock to be a useful tool because it made docking possible with only one click. I required the numerous ligand files and the receptor protein structure in order to start the docking process. InstaDock supported a number of file types, as well as.pdb, sdf, mol, and. mol2. I kept the receptor and ligands files in the same folder as the InstaDock.exe file to guarantee smooth operation. InstaDock automatically created configuration files for each of the ligands and the protein in order to get everything ready for docking. These configuration files made it easier to convert the files to the InstaDock-compatible. pdbqt format. For the docking to be effective, this conversion process was required. I simply selected the start button in InstaDock to start the docking procedure after creating the configuration files and organizing all the files properly. With this simple strategy, I was able to complete the docking procedure quickly.[14]

3.7. vHTS and molecular docking

In our computer-aided drug design study, we recognized the importance of virtual high-throughput screening molecular docking and (vHTS). To find a substance with a high binding affinity for JAK1, we specifically carried out molecular docking-based vHTS. Molecular docking involves arranging molecules in a favorable configuration by matching complementary features. It is a crucial step in assessing how well a compound interacts with its target protein. We utilized InstaDock, a powerful software tool, to carry out the docking process efficiently. Through this approach, we aimed to identify a compound that exhibits strong binding affinity for JAK1. This selection is valuable for further exploration and optimization as we strive to develop effective drug candidates. In my research, I conducted blind docking for all the natural compounds selected from the IMPPAT database. I employed grid sizes of 62, 64, and 70, along with corresponding spacing values of -10.791, 33.407, and -9.812 for the X, Y, and Z axis, respectively. After the molecular docking process, I generated docked compounds based on their binding affinities. To assess potential interactions with JAK1, I examined the conformers' interactions using Discovery Studio software. Following this analysis, I carefully selected specific compounds that exhibited favorable interactions with JAK1 for further investigation. These compounds hold promise for potential therapeutic applications and will be subjected to additional studies and optimization in our drug development efforts.

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1 Molecular Docking for Virtual Screening

During our research, we employed InstaDock to conduct molecular docking experiments involving the JAK1 receptor protein found in the Protein Data Bank (PDB) and organic phytochemicals sourced from the IMPPAT database. To ensure a focused analysis, we applied filters to refine the library and selected 2000 phytochemical compounds for docking using InstaDock. By executing the docking process, we obtained docked conformations and corresponding binding affinities for each of the 2000 compounds. To prioritize the most promising candidates, we further filtered the docked compounds based on their binding affinities, specifically considering those with values greater than -8.0 kcal/mol. Subsequently, we identified the top 14 hits from the initial set of 2000 compounds, leveraging their impressive binding affinities as indicators of a strong affinity for JAK1. To provide a comprehensive overview, we present these top 14 hits in Table 1, along with details of their respective binding affinities with JAK1. The selection of these compounds exhibiting notable binding affinities opens up exciting avenues for in-depth investigation and optimization. These findings suggest their potential as promising candidates for future drug development endeavors, with a specific focus on targeting JAK1.[30]

Table 1: Top hits selected based on the binding affinity towards JAK1

S.No	Compound Name	Binding Energy *(kcal/mol)*	*pKi*	Ligand Efficiency (kcal/mol)
1.	IMPHY006549_3D	-9.5	6.97	0.3519
2.	IMPHY005471_3D	-9	6.6	0.3913
3.	IMPHY004619_3D	-8.9	6.53	0.4045
4.	IMPHY005665_3D	-9.4	6.43	0.296
5.	IMPHY004192_3D	-8.5	6.23	0.4048
6.	IMPHY005342_3D	-10.9	7.99	0.519
7.	IMPHY008993_3D	-10.4	7.63	0.4522
8.	IMPHY005339_3D	-10.2	7.48	0.4857
9.	IMPHY006072_3D	-9.9	7.26	0.3536
10.	IMPHY004661_3D	-9.2	6.75	0.46
11.	IMPHY004660_3D	-9.2	6.75	0.4381
12.	IMPHY004643_3D	-9.1	6.67	0.455
13.	IMPHY006055_3D	-9	6.6	0.45
14.	IMPHY006585_3D	-8.9	6.53	0.3708

4.2 PAINS Filter and ADMET properties analysis:

In order to ensure the identification of viable compounds, a rigorous filtration process was applied to the initial pool of 14 hits. The primary step involved subjecting these hits to the PAINS filter, which helped identify and eliminate compounds displaying particular patterns that can bind to many targets. As a result, only 8 out of the initial 14 hits passed this filter and progressed to the next stage. Subsequently, the ADMET properties of these 8 hits were assessed using the SwissADME web tool to evaluate their drug likeness features. The evaluation criteria followed the Lipinski Rule of 5, This establishes the following parameters: molecular mass< (500), H-bond acceptor <(10), H-bond donor <(5), and log P value <(5). Only those hits that complied with these parameters and exhibited favorable ADMET properties were considered for further analysis.

Upon completion of this comprehensive filtration process, the final selection comprised the top 8 hits that fulfilled the requirements of both the PAINS filter and possessed desirable ADMET properties. These compounds were deemed drug-like, indicating their potential as viable candidates without any observed toxic behavior. The observed value of the PAINS filter can be found in Table 2, while Table 3 presents the ADMET properties in conjunction with their association to JAK1.

Table 2: PAINS and RO5 filter of top 9 hits of natural compounds.

Compound ID	***Molar Mass***	***H-bond acceptors***	**<H-bond donors>**	**<TPSA>**	**<log P>**	**PAINS alerts**
IMPHY005665	337.37	4	0	40.16	3.50	0
IMPHY005342	277.32	2	0	21.70	3.07	0
IMPHY008993	312.27	6	3	100.13	2.47	0
IMPHY005339	281.31	4	2	50.72	2.45	0
IMPHY006072	386.35	8	2	95.84	2.59	0
IMPHY004660	286.24	6	4	111.13	2.43	0
IMPHY004643	270.24	5	3	90.90	2.57	0
IMPHY006055	266.33	2	1	40.46	2.83	0

Table 3: ADMET properties of top 9 hits of natural compound

Compound Name	**<ABSORPTION>**	**<DISTRIBUTION>**	**<METABOLISM>**	**<EXCRETION>**	**<TOXICITY>**
	GI Absorption	BBB permeation	CYP2D6 Inhibitor	OCT2 substrate	AMES/ Hepatotoxicity
IMPHY005665	Highest	Y	Y	Nil	Nil/Nil
IMPHY005342	Highest	Y	Nil	Nil	Nil/Nil
IMPHY008993	Highest	Nil	Y	Nil	Y/Nil
IMPHY005339	Highest	Y	Y	Nil	Y/Nil
IMPHY006072	Highest	Nil	Y	Nil	Nil/Nil
IMPHY004660	Highest	Nil	Y	Nil	Nil/Y
IMPHY004643	Highest	Nil	Y	Nil	Y/Nil
IMPHY006055	Highest	Y	Y	Nil	Y/Nil

%YES=Y, No=Nil

4.3 By Utilized PASS Analysis to predict Biological Activity

To assess the biological activity of the hits, a comprehensive analysis was conducted using the Web PASS webserver (http://www.way2drug.com/) . This analysis aimed to evaluate the effectiveness of the hits and investigate the range of biological properties they exhibited. The PASS analysis focused on identifying hits that demonstrated significant biological potential, indicated by values between 0.410 and 0.916 when Pa (probability of activity) exceeded Pi (probability of inactivity). This approach allowed for the identification of hits that displayed favorable biological characteristics and were likely to possess desired activities. Upon completion of the analysis, it was determined that out of the initial pool of eight hits, three demonstrated this desirable behavior. These three hits exhibited strong probabilities of activity and showed promising potential for further investigation and development. In PASS analysis the natural compounds IMPHY005342, IMPHY006072, IMPHY005665 shows Neurotransmitter uptake inhibitor, Antineoplastic alkaloid, Caspase 3 stimulant and Muscle relaxant. IMPHY005342*(Nelumbo nucifera*) and IMPHY006072 *(Gmelina arborea)* Both are effective in Neurotransmitter uptake inhibitor, Antiparkinsonian, rigidity relieving, MAP kinase stimulant, Antineoplastic alkaloid, and antineurotic properties.

Table 4: PASS analysis.

S. No.	Compound Name	<Pa>	<Pi>	Activity
1.	**IMPHY005342** (Nelumbo nucifera) *Dehydroaporheine*	0,882	0,002	Neurotransmitter uptake inhibitor
		0,811	0,002	Antiparkinsonian, rigidity relieving
		0,718	0,036	Antineurotic
		0,670	0,008	MAP kinase stimulant
		0,606	0,003	Antineoplastic alkaloid
2.	**IMPHY006072** (Gmelina arborea)	0,945	0,004	Membrane integrity agonist
		0,789	0,004	Neurotransmitter uptake inhibitor
		0,729	0,010	Caspase 3 stimulant
		0,660	0,000	Protein phosphatase 2A inhibitor
		0,570	0,004	Antineoplastic alkaloid
3.	**IMPHY005665** (Argemone Mexicana)	0,891	0,002	MAP kinase stimulant
		0,632	0,005	Muscle relaxant
		0,564	0,004	Antineoplastic alkaloid
		0,516	0,010	Skeletal muscle relaxant

	Berberine	0,522	0,048	JAK2 expression inhibitor

A. ***B.***

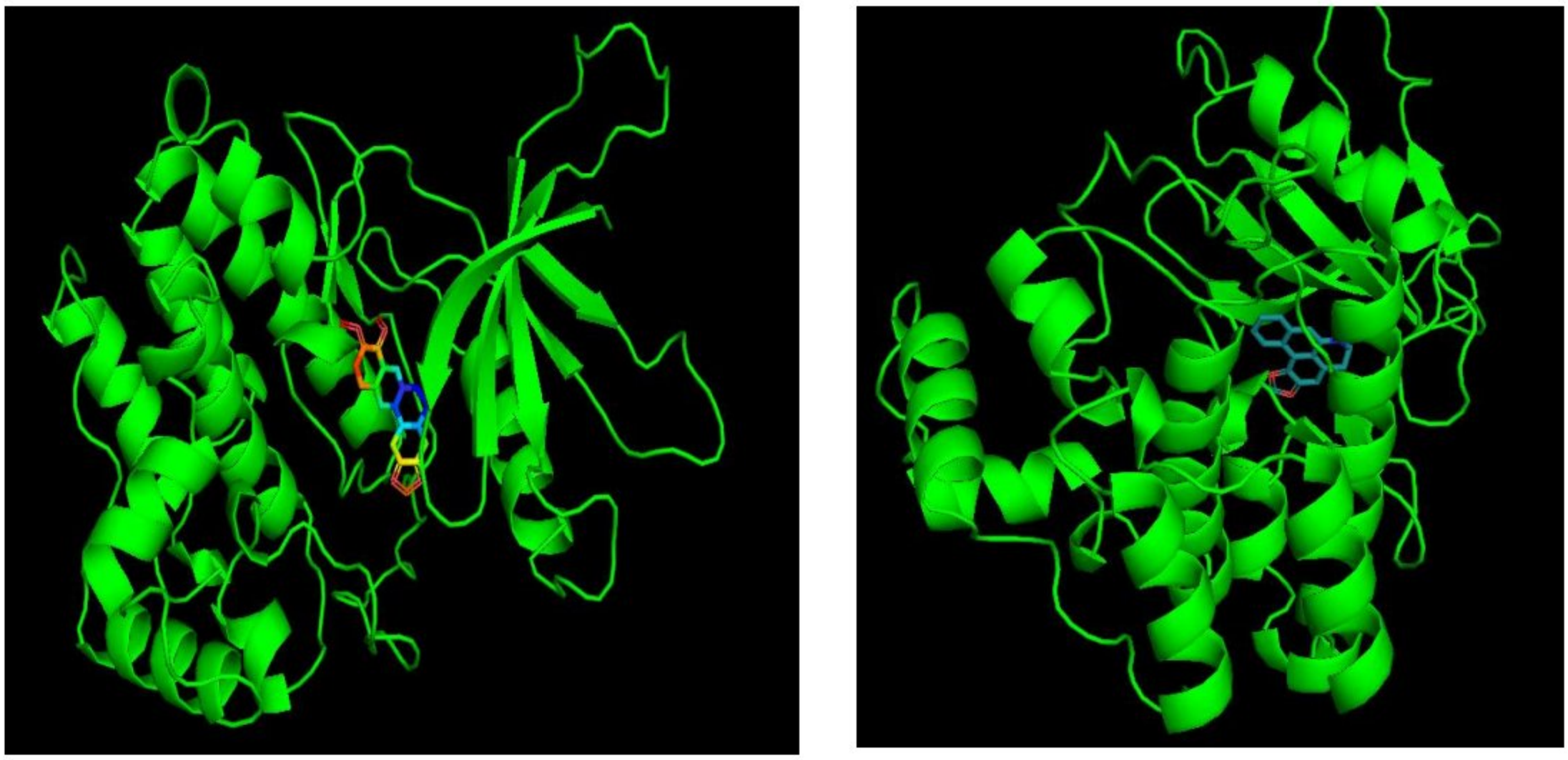

Figure 3. Interaction of Janus kinase 1 (JAK1) with (A) *Berberine* & (B) *Dehydroaporheine*

4.4 Interaction Analysis:

For the interaction analysis, the top 3 chemicals were chosen using Discovery Visual Studio. Outflies from the top 3 chosen compounds produced a total of 27 docked conformers. Each of these substances was examined based on how it interacted with JAK1. When each chemical was thoroughly examined, it was discovered that several of them interacted with the JAK1 binding pocket. Two natural compounds were found to interact with JAK1 as a functionally significant residue out of the three substances that were examined for interaction analysis. The interaction of these two organic compounds (IMPHY005342 and IMPHY005665) with JAK1 revealed that they may serve as this receptor protein's inhibitors. While IMPHY005342 displays interaction via H-bond with Lys 908, Gly1023,Lys888,Glu883,Gly882,Asp1003, and other interaction with Leu1024,Asp1021 and Val889, whereas IMPHY005665 displays interaction via H-bond with Lys 908, Asp1003, Gly1023,Val889, Arg1007,Gly882,Glu966, and others interaction with ,Asp1021,Leu1024, and Asn1008

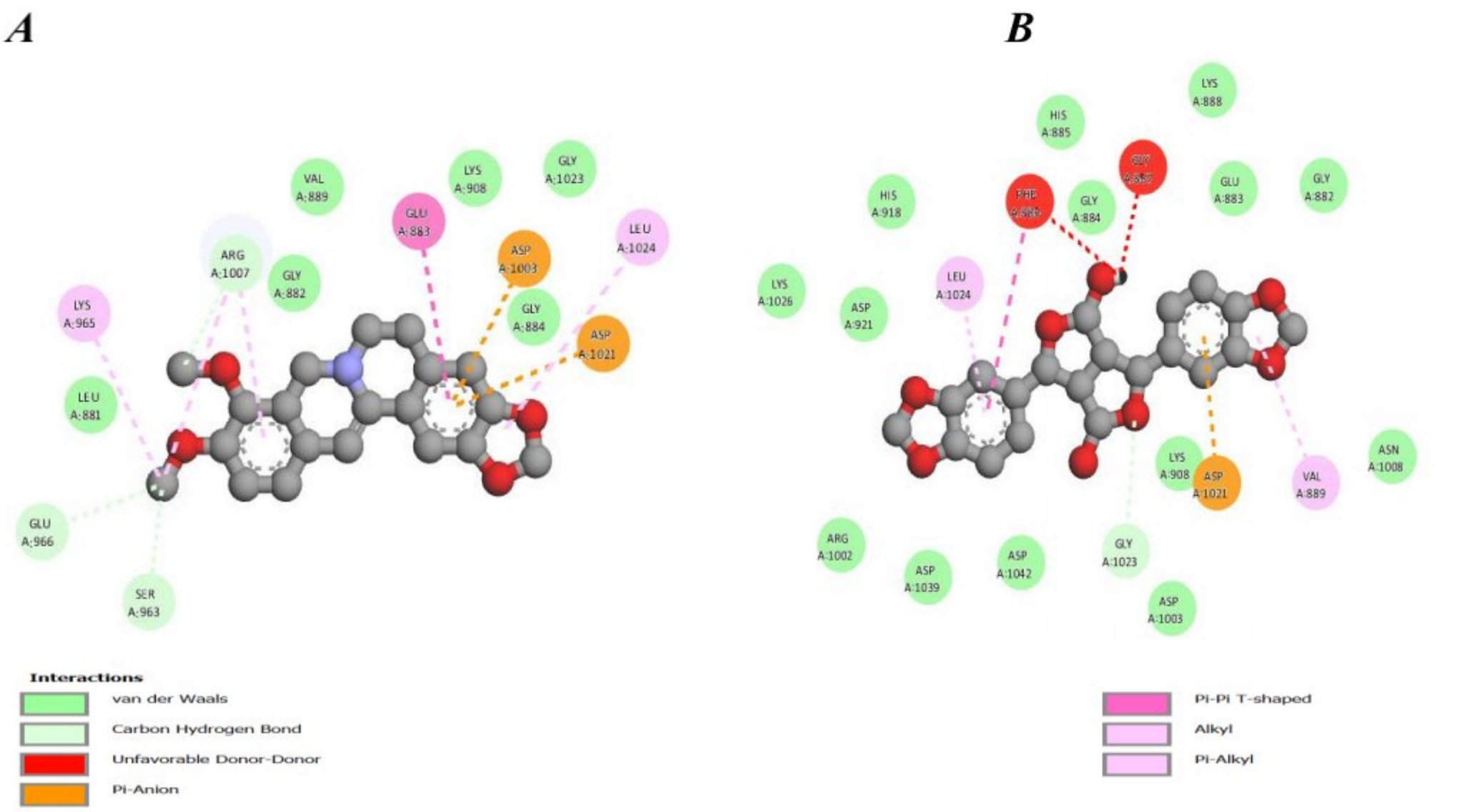

Figure 4: Representation of the 2D interaction of receptor protein JAK1 with (A) *Berberine* & (B) *Dehydroaporheine*

CONCLUSION

JAK1 can be targeted with plant-based natural chemicals to alter pathways that have anti-vitiligo and anti-cancer properties. A multi-tiered virtual screening method was used on the IMPPAT database to identify potential phytoconstituents for the dysregulated JAK1. Due to its function as a beneficial regulator of cancer growth and auto-immune diseases, JAK1 was viewed in this study as a crucial pharmaceutical target. Based on their binding affinity, specific interactions, physicochemical characteristics, and drug-like qualities, two substances, *berberine* and *dehydroaporheine*, were chosen. After this we have to propose these compounds for MD Simulation to investigate the dynamic behaviour and structural features of protein-ligand complexes. At lastly we propose to further investigate *Berberine* and *Dehydroaporheine* in vivo and in vitro to develop anti-vitiligo and anticancer treatments.

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In-Silico medication of vitiligo by targeting 6AAH protein and riboflavin Ligand

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Abstract—

Depigmentation of the skin is a primary symptom of the vitiligo disorder. By reducing their self-esteem and causing them psychological distress, it lowers patients' quality of life. The study made use of a number of computational tools, including Cyto Hubba, BioVia Discovery Studio through, Open babel, Drug bank, Avogadro, Auto dock, and Protein-Interaction Ligand profiler. The interaction between 6AAH and (Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6-Dimethyl-7-octene-2,3,6-triol) has been examined in this study using Cyto Hubba and PILP clustering interactions, followed by Molecular Docking of Protein and Ligand. Due to its polygenic nature, vitiligo is frequently associated with a number of autoimmune or autoinflammatory disorders, including thyroid disease, psoriasis, atopic dermatitis, diabetes mellitus, and pernicious anaemia. Hence, it is conceivable to think about riboflavin as a possible drug for Vitiligo treatment. The findings imply that riboflavin laboratory tests reveal its inhibitory potential on skin depigmentation.

Keywords— gene expression, vitiligo, reversal gene, treatment, depigmentation, drugs

I. INTRODUCTION

Depigmentation of the skin is brought on by the primary pigmentary disorder known as vitiligo. By reducing their self-esteem and causing them psychological distress, it decreases patients' quality of life. The prevalence varies widely by location and is between 0.5% and 2%. Due to a persistent loss of melanin, certain areas of the skin become depigmented. Melanocytes generate the pigment known as melanin. Melanocytes may stop producing melanin in vitiligo or they may even die.[1] An acquired autoimmune disorder is vitiligo. Celsus used the term "vitiligo" for the first time in his well-known Latin work De Medicina in the second century BCE. The Atharvaveda, an ancient work of Indian literature, also makes reference to this illness and describes the horrifying consequences of son-daughter marriage on those who have vitiligo.[2] Hindu writings also claim that stealing garments in a previous life is a potential cause of vitiligo.

Skin consists of three layers: the epidermis, dermis, and subcutaneous tissue. A type of cell found in the dermis is called a melanocyte. [3]These melanocytes create the pigment melanin. Keratinocytes take up melanin in their bodies. Whenever the immune system is activated, melanocytes are decreased or eliminated (autoimmune melanocyte death). Distal extremity tips, segmental, periorificial, and sites of friction are among the body parts where depigmented patches can be found. [4]Occasionally, asymmetrical depigmented skin may also exist patches that develop on one axil after the other. Macule or patchy skin is described as having no pigment. Compared to a macule, which has a diameter of 10mm, a patch is a flat skin lesion that is larger. The five different kinds of vitiligo are focal, segmental, acro facial, generalised, and universal. Since normal skin and skin with pigmentation can be easily distinguished from one another, these might be easy to spot. Although the exact cause of vitiligo is unknown, there are various theories about how it arises . [5]Here are a few potential explanations: The first is genetics, where genes involved encode a part of the molecular network that controls the immune system and encourage melanocyte death. Second, the immune system of the body targets and destroys melanocytes as a result of an autoimmune response. The immune responses that occur after vitiligo are crucially triggered by oxidative damage. Stressed melanocytes produce DAMPs or autoantigens, which then activate innate immunity and adaptive immunity, leading to melanocyte malfunction and death via an inflammatory cascade Cellular proteins and membrane lipids are impacted by oxidative stress, which is brought on by an increase in reactive oxygen species (ROS) levels and subsequent decrease in antioxidant enzymes, in both lesioned and unlesioned skin.[6] The antioxidant system is therefore less active. Due to its polygenic nature, vitiligo is frequently associated with a number of autoimmune or autoinflammatory disorders, including thyroid disease, psoriasis, atopic dermatitis, diabetes mellitus, and pernicious anaemia. Risk factors include a long family history of vitiligo and genetic vulnerability to depigmentation.

In addition to other techniques, skin biopsies and blood tests can be used to diagnose vitiligo. The production of pigment can be induced by combining herbs Psoralean in pure form with UVA light, often known as PUVA treatment. A tiny area is treated with topical medications. Ultra- or potent topical steroids are used for lesions, whilst mid-potency steroids are used for children.[7] Systemic medications, which also include oral corticosteroid mini-pulses, converted steroid sparing agents, and antioxidants, are a different type of therapy. Another option for phototherapy is using a blacklight, also known as a Wood's lamp, which exposes skin to UV radiation. Similar to this, localised illnesses are treated using excimer laser therapy, which also uses another laser and additional lighting equipment. Surgical treatment is another option for grafting skin and tissue. [8]

I. MATERIAL AND METHOD

A. *Integration of Protein 6AAH-Ligand (Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6-Dimethyl-7-octene-2,3,6-triol)*

The FASTA format of protein 6AAH and Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6-Dimethyl-7- octene-2,3,6-triol were extracted from RCSB PDB (Protein

Data

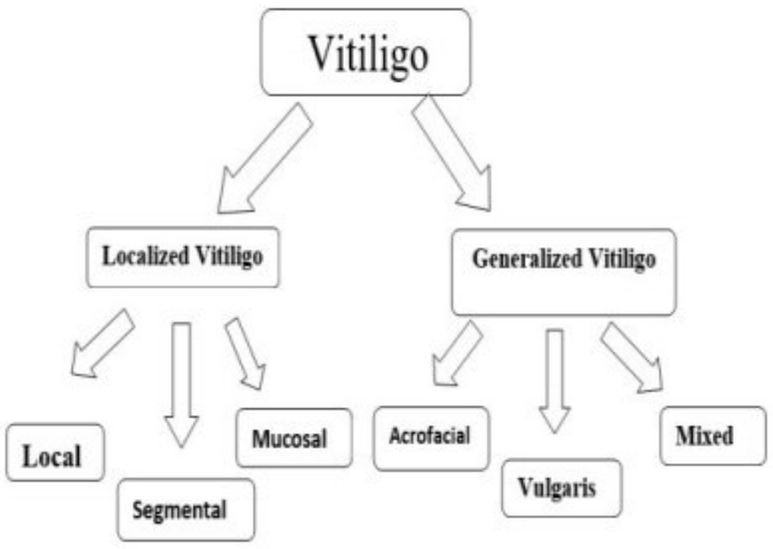

Figure 1. Types of Vitiligo

Bank)RCSB PDB: Homepage. The PLIP (Protein – Ligand interaction Profiler) web-tool was then used to shows the PLIaggregation. All the ligands sucessfullly binds with the proteinshowing residual values as 879A, 881B, 889B, 101B,959B respectively. Also find out the amino acids by which they binds are ARG, LEU, VAL, LEU, LEU respectively as abovesequence.[9]

B. *Docking procedure for proteins or ligands.*

The heteroatoms, polar hydrogen, and water molecules were carefully taken out. The KOLLMANN charges were received by the ligand and both receptors. The PDBQT file forthe agonist or the PDB folder for the receptor were both storedfor auto docking. A PDBQT file for agonist is required by autodock, and this file was converted online using open babel.[10]

C. Used the Auto dock to bind proteins and ligands.

For pharmaceuticals and proteins, there is molecular docking software service . The web server was given the PDBfiles for the target and the medication, and docking was doneby making a grid mapping. Launch the auto-grid file first, thenthe auto-docking.

D. Uses software called Bio via to do a structural evaluationof the docked protein-drug complex.

The result downloads from the auto dock itself were analysed for structural interaction between protein and drug from BioVia Discovery Studio. Submit the complex of proteinand ligand to BioVia Discovery Studio and see the interaction with the help of a 2D image. It also helps to analyse by whichamino acid ligands bind to the receptor.

E. SWISS ADME examination of the Pharmacodynamicfor the ligand.

Absorption, Dispersion, Metabolic activity, and Excretion are combined known as ADME. These variables are examined by adding canonical smiles to study the water solubility, Pharma kinetics, Physiochemical property, lipophilicity, and medical chemistry. These analyses provide evidence for the potency and efficacy of the drug. SWISS ADME (SwissADME). This online tool evaluates the agonist (drug) molecule using these parameters. For the evaluation, the canonical smiles add to sever and run. We can also find out whether the drug crosses the blood-brain barrier or not by seeing egg boiled figure whether the resides inside the yolk (BBB+) or in egg white (BBB-).[11]

II. RESULT AND DISCUSSION

A. *Interaction between 6AAH and Ligand (Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6-Dimethyl-7-octene-2,3,6-triol)*

Significant relations between protein 6AAH and Ligand ([redacted], [redacted] Riboflavin, Propanol, 2,6-Dimethyl-7-octene-2,3,6-triol) hae been observed. All the ligands sucessfullly binds with the protein showing residual values as 879A, 881B, 889B, 101B,959B respectively. Also find out the amino acids by which they binds are ARG, LEU, VAL, LEU, LEU respectively as above sequence. Having hydrophic interaction distances as 2.93 3.83, 4.00, 3.65, 2.37.[12]

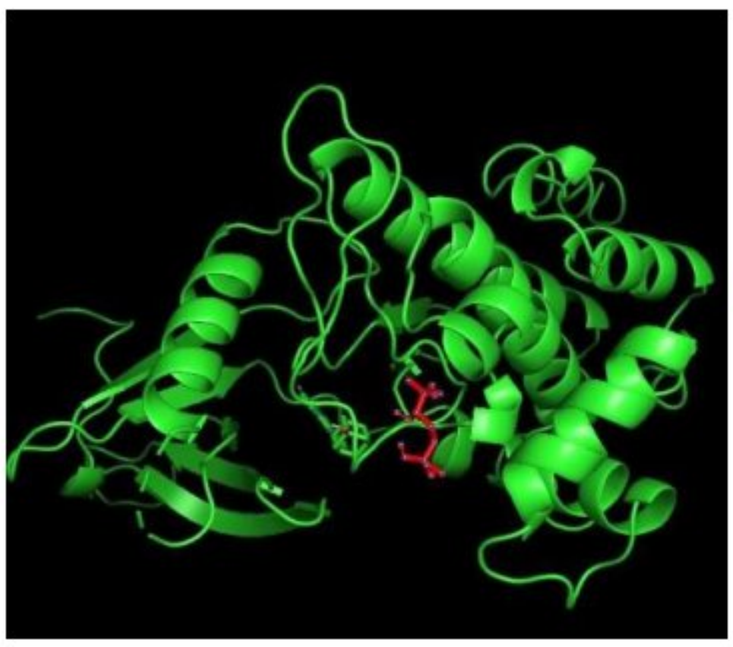

Figure 2.Interaction Of 6AAH and 2,6-Dimethyl-7-octene-2,3,6-triol

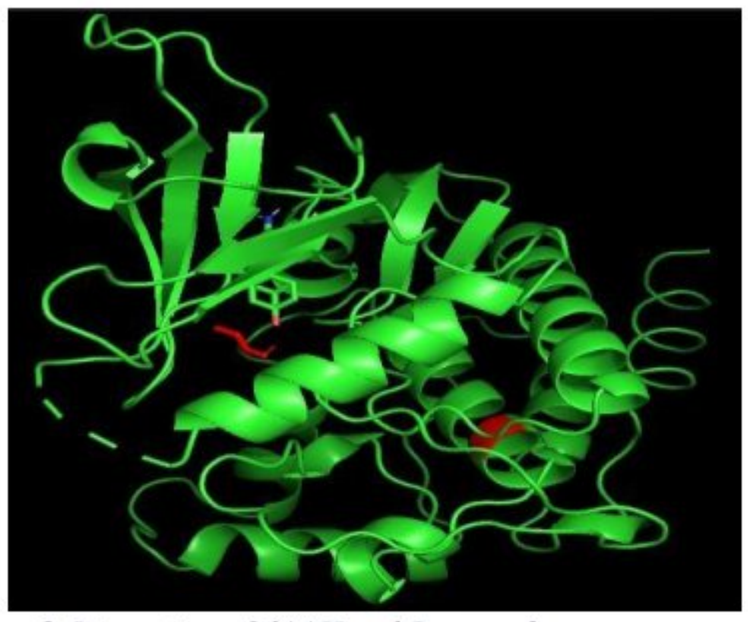

Figure 3. Interaction of 6AAH and Propanol

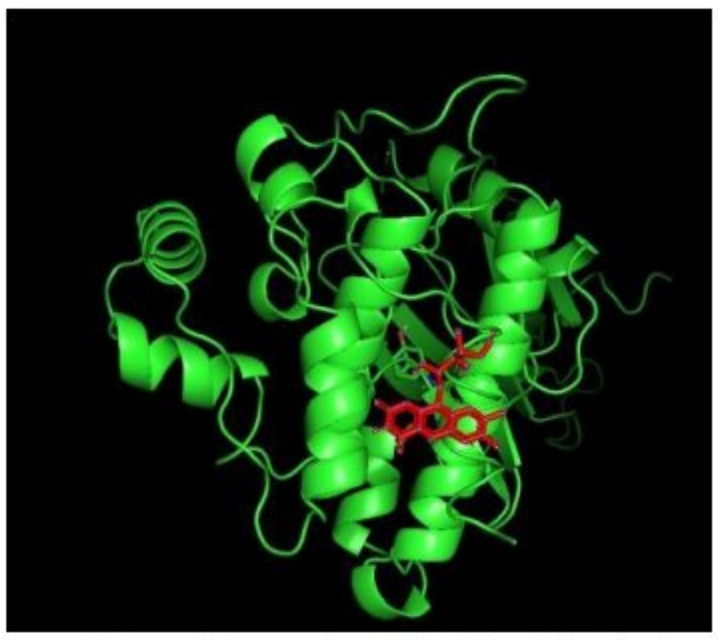

Figure 4. Interaction of 6AAH and Riboflavin

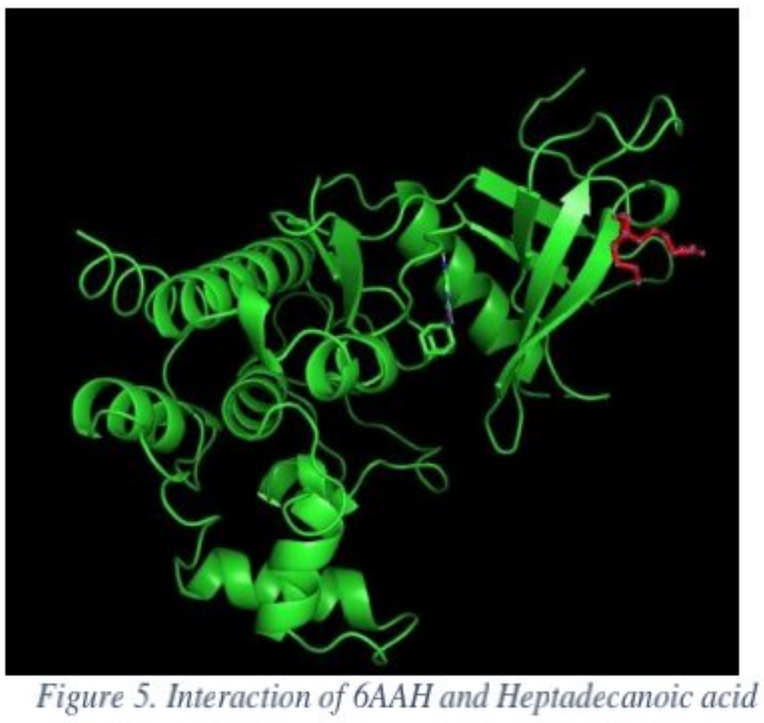

Figure 5. Interaction of 6AAH and Heptadecanoic acid

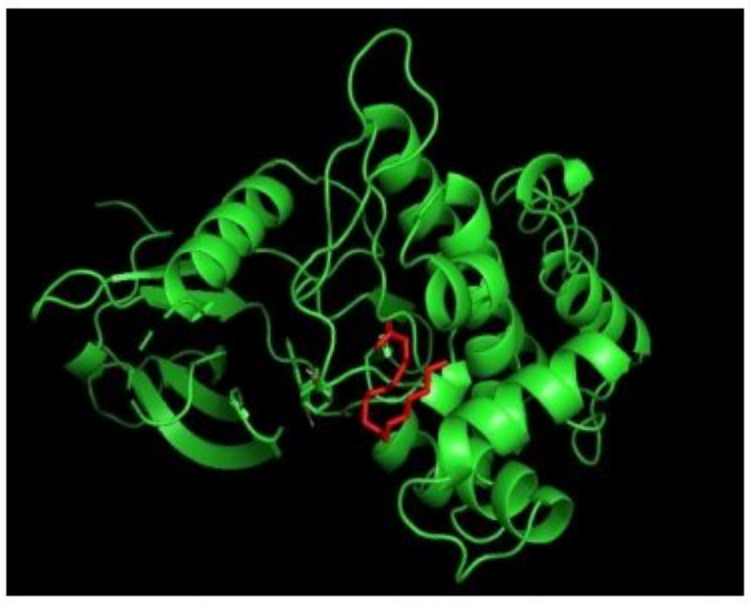

Figure 6. Interaction of 6AAH and Myristic acid

A. *Interaction between 6AAH and Ligand (Myristic acid,Heptadecanoic acid, Riboflavin, Propanol, 2,6- Dimethyl-7-octene-2,3,6-triol)*

After docking was performed using Auto dock, The outcomes were positive, demonstrating a high level of interaction between 6AAH and Ligand (Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6-Dimethyl- 7-octene-2,3,6-triol). The docking scores show that Ligand (Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6-Dimethyl-7-octene-2,3,6-triol) binds efficiently to the 6AAH protein complex. The binding energy is calculated as –4.00kcal/mol, -2.4kcal/mol, -6.6kcal/mol, -3.6kcal/mol and -4.00kcal/mol respectivelyand the Cluster RMSD value is 0. This had been analyzedvia docking results. Docking was used to examine the topology of the molecules' interaction. The structure shows the target protein helical as a green in color consisting of A chain and B chain whereas the potential ligand is represented by the spheroid and the linear structure between the ribbons i.e., ligand. The geometry depicts the interactions of the ligand with the target protein's receptor site. [Fig.2, Fig,3, Fig.4, Fig.5, Fig6].

Table2. Result of Protein interaction from Swiss ADME byusing boiled – egg.

Index	Ranks	Ligands
1	Ist	Myristic aci
2	IInd	Heptadecanc acid
3	IIIrd	2,6-Dimethyl octene-2,3,6-t
4	IVth	Propanol
5	Vth	Riboflavin

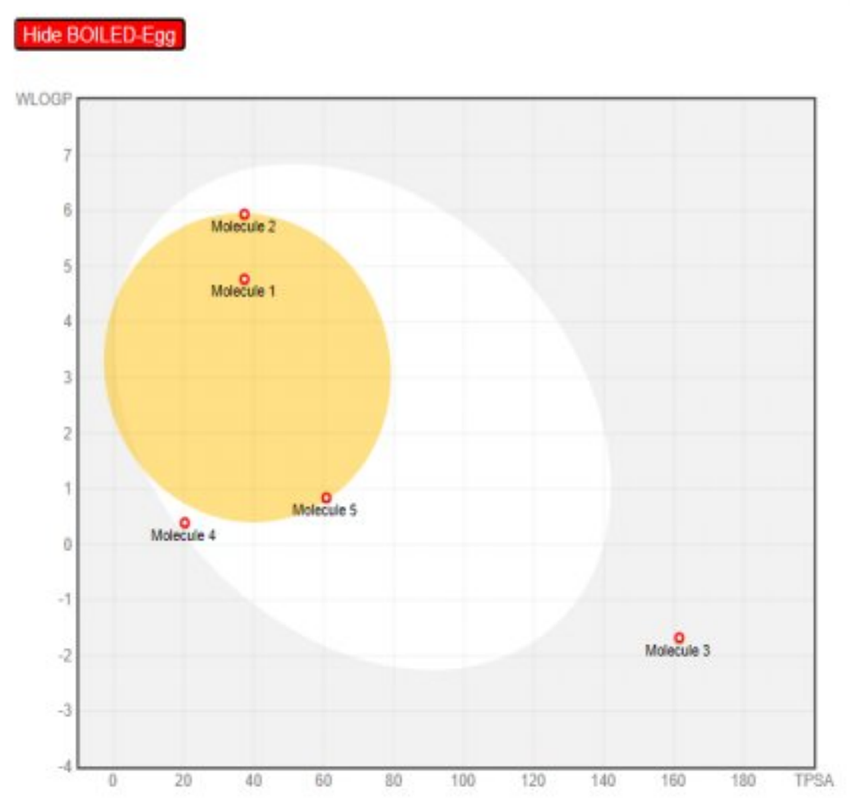

Figure 7 BBB (Blood brain barrier) crossing identified by the helpof boiled egg.

A. ADME analysis of the ligand.

The skin permeation value (log Kp) is low, with values of -3.35 cm/sec, -2.49 cm/sec, -9.63 cm/sec, -6.49 cm/sec, and -7.14 cm/sec, respectively. (With Lipinski's value as required and has proven water solubility under logs (ESOL); logs (Ali), and logs (SILICOS-IT) categories with a value of -4.31, -5.37, -1.31, -0.30, -0.95; -6.67, -8.31, -1.43, -0.24, -1.28; -4.51, -5.71, -2.62, -0.33, -0.72 respectively. The pharma kinetics score of 6.11, 7.69, -1.46, 0.25, and 0.44 and cast log P o/w (XLOGP3) were used to determine the drug's lipophilicity.[13]

I. CONCLUSION AND FUTURE PROSPECTS:

The aim of this research was to ascertain how ligands and proteins interact. Protein-Ligand Interaction Profiler was utilized in the research. Find out precisely where the ligand attaches to the target protein by using bio to determine which amino acid is involved. With an estimated G value of -4.0, -2.4, -6.6, -3.6, -4.0kcal/mol, the docking research also demonstrated a notable relationship between protein and ligand value. As a consequence of its high binding values, this finding supports the notion that riboflavin is the best choice for the uses. Although myristic acid has average binding values, it successfully penetrates the blood–brain barrier.
Therefore, according to the binding energy, we are able to determine that riboflavin is the greatest option out of all five ligands.
We may consider this as a benefit for us and use it further as an experiment in the future since there is no best medication for this disorder

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