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# Structure-Based Study of Piperine Phytochemical Against InhA Protein of Tuberculosis

**Rani Sathe[1], Syeda Afifa[2]**

[1]Student, [2]Assistant Professor
[1,2]Department Of Pharmacy, Sayali Charitable Trust's College Of Pharmacy Chhatrapati Sambhajinagar

**Abstract:**
The docking analysis demonstrated that piperine exhibited favorable binding affinity toward the InhA protein, indicating the formation of a stable ligand–protein complex. The interaction study revealed significant hydrophobic interactions along with hydrogen bond formation between piperine and important amino acid residues present in the active site region of the protein. The negative docking score suggested strong intermolecular interactions and good binding stability, thereby supporting the inhibitory potential of piperine against the selected target protein. Visualization of 2D and 3D ligand interaction profiles further confirmed the effective accommodation of piperine within the active binding cavity of InhA. conclusion, the present molecular docking study suggests that piperine possesses promising inhibitory activity against the InhA protein of Mycobacterium tuberculosis. The observed binding affinity and interaction profile indicate that piperine may serve as a potential lead compound for the development of novel anti-tubercular agents. However, further in vitro and in vivo studies are required to validate its biological efficacy, safety, and therapeutic potential in tuberculosis treatment.

**Keywords:** piperine phytochemical, molecular docking, targe protein (InhA), Anti- tubercular activity

## Introduction

### 1.1 Tuberculosis:

Tuberculosis (TB) is a chronic infectious disease caused by the bacterium Mycobacterium tuberculosis. It primarily affects the lungs (pulmonary TB) but can also involve other organs such as the lymph nodes, bones, and central nervous system (extrapulmonary TB). Despite being preventable and treatable, TB remains one of the leading causes of mortality [1] worldwide, particularly in developing countries. According to the World Health Organization, TB is responsible for millions of new infections and deaths annually, posing a significant global health burden. tuberculosis (TB) remains one of the most persistent and devastating infectious diseases worldwide, caused primarily by Mycobacterium tuberculosis, an aerobic, acid-fast bacillus that primarily affects the lungs but can also involve extrapulmonary sites. Despite significant advancements in medical science, TB continues to pose a serious global health challenge, particularly in developing countries. According to global health reports, millions of new TB cases are reported annually, with a substantial number of deaths attributed to complications arising from delayed diagnosis, poor treatment adherence, and the emergence of drug-resistant strains.[2] **Multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB)** have further complicated treatment strategies, rendering many conventional antibiotics ineffective. These limitations highlight the urgent need for the discovery and development of novel therapeutic agents that are more effective, less toxic, and capable of overcoming resistance mechanisms.

Traditional anti-tubercular therapy involves prolonged treatment regimens using first-line drugs such as isoniazid, rifampicin, ethambutol, and pyrazinamide.[3] While these drugs have been effective historically, they are associated with several drawbacks, including hepatotoxicity, adverse drug reactions,

long treatment durations, and poor patient compliance. Moreover, the emergence of resistant strains of M. [4] tuberculosis has significantly reduced the efficacy of these drugs. As a result, there is increasing interest in exploring alternative sources of anti-tubercular agents, particularly from natural products and phytochemicals, which have been used for centuries in traditional medicine systems and are known for their diverse biological activities.[5]

**Limitations and Disadvantages of TB Drugs**

Tuberculosis (TB) drugs often face challenges including long intensive treatment regimens (6– 20+ months), **severe adverse side effects** (hepatotoxicity, hearing loss, neuropathy), and low bioavailability, which can lead to poor patient adherence and drug-resistant strains. These limitations are worsened by the need for complex, daily, multi-drug regimens

**Long Duration & Poor Compliance:** Treatment is notoriously long, spanning months or even years for Multi-Drug-Resistant TB (MDR-TB), which leads to patients stopping medication early.

**Severe Side Effects:** Common adverse reactions include drug-induced hepatitis, gastrointestinal issues (nausea, vomiting), peripheral neuropathy, skin rashes, and vision changes. Second-line drugs for MDR-TB can cause severe toxicity, such as kidney damage, psychosis, and hearing loss.

**Low Bioavailability & Formulation Issues:** Some drugs, particularly rifampicin within Fixed-Dose Combinations (FDCs), can have reduced effectiveness due to poor absorption or inadequate manufacturing, causing low bioavailability.

**Drug Resistance:** Inadequate treatment or poor adherence fuels the development of multi-drug resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains. **High Burden of Treatment:** Regimens can require up to 20+ pills daily, leading to psychological and physical burdens.

**Drug Interactions:** TB medication, especially rifampicin, has significant interactions with other drugs (e.g., HIV antiretrovirals)

**1.2 Need for Novel Therapeutic Approaches:**

The current anti-tubercular therapy involves a combination of drugs such as isoniazid, rifampicin, ethambutol, and pyrazinamide. Although effective, these drugs are associated with several limitations including long treatment duration, adverse side effects, poor patient compliance, and increasing drug resistance. Therefore, there is an urgent need to explore alternative and complementary therapeutic agents, particularly from natural sources. Natural products, especially phytochemicals derived from medicinal plants, have gained attention due to their diverse biological activities, lower toxicity, and potential synergistic effects. These compounds can act as lead molecules for **drug discovery and development against TB**.

**1.3 Piperine: A Natural Therapeutic Lead**

Piperine is a major alkaloid found in black pepper (Piper nigrum) and long pepper (Piper longum). It is responsible for the pungency of pepper and exhibits a wide range of pharmacological activities, including anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory effects. piperine possesses significant antimicrobial activity against various pathogenic microorganisms, including M. tuberculosis. Its ability to enhance drug bioavailability and inhibit bacterial growth makes it a promising candidate for anti-TB drug development. Piperine has also been reported to interfere with key metabolic pathways and enzymes essential for bacterial survival.[6]

Piperine has demonstrated significant potential as an antimicrobial agent against a wide range of pathogens, including bacteria, fungi, and parasites. Its ability to inhibit the growth of Mycobacterium tuberculosis has been a subject of growing research interest. Studies suggest that piperine may interfere with bacterial cell wall synthesis, inhibit enzymatic pathways essential for bacterial survival, and enhance the efficacy of existing anti-tubercular drugs by improving their bioavailability. Furthermore, piperine has been shown to modulate immune responses, which may contribute to its therapeutic effects in TB

treatment. These properties make piperine a promising candidate for further investigation as a potential anti-TB agent.

**Biological Activities of Piperine:**

Piperine, a naturally occurring alkaloid predominantly found in Piper nigrum (black pepper) and Piper longum (long pepper), has attracted significant scientific attention due to its diverse pharmacological and biological activities. Traditionally used in Ayurvedic and Chinese medicine, piperine has been extensively investigated in modern pharmacology for its potential therapeutic applications. Its wide spectrum of biological effects includes **antimicrobial, antiinflammatory, antioxidant, anticancer, antitubercular, immunomodulatory, and bioavailability-enhancing properties**. These multifaceted biological activities make piperine an important phytochemical in drug discovery and development, particularly in the context of infectious diseases such as tuberculosis.[7]

One of the most well-documented biological activities of piperine is its **antimicrobial activity**, which encompasses antibacterial, antifungal, and antiviral effects. Piperine has demonstrated inhibitory activity against a wide range of pathogenic microorganisms, including both Grampositive and Gram-negative bacteria. Studies have shown that piperine can disrupt bacterial cell membranes, interfere with protein synthesis, and inhibit enzymatic pathways essential for microbial survival. In the context of tuberculosis, piperine has been reported to exhibit inhibitory effects against Mycobacterium tuberculosis, suggesting its potential as a lead compound for antitubercular drug development. Additionally, its antifungal activity against species such as Candida albicans further highlights its broad-spectrum antimicrobial potential [7,8]

Another significant biological property of piperine is its anti-inflammatory activity, which plays a crucial role in managing chronic inflammatory conditions. Piperine exerts its antiinflammatory effects by modulating key signalling pathways, including the inhibition of proinflammatory cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL1β), and interleukin-6 (IL-6). It also suppresses the activation of nuclear factor-kappa B (NFκB), a transcription factor that regulates inflammatory responses. Furthermore, piperine inhibits cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (ion's), thereby reducing the production of inflammatory mediators such as prostaglandins and nitric oxide. These mechanisms collectively contribute to its potential use in inflammatory diseases, including arthritis and inflammatory lung disorders [8]

Piperine is also known for its potent **antioxidant activity**, which helps in combating oxidative stress and preventing cellular damage. Oxidative stress is a major contributor to the pathogenesis of various chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. Piperine acts as a free radical scavenger and enhances the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. By reducing lipid peroxidation and maintaining redox balance, piperine protects cells from oxidative damage. This antioxidant property is particularly important in tuberculosis, where oxidative stress plays a role in disease progression and tissue damage [9]

The **anticancer activity** of piperine has been extensively studied in recent years, revealing its potential as a chemo preventive and therapeutic agent. Piperine has been shown to inhibit the proliferation of various cancer cell lines, including breast, colon, prostate, and lung cancer cells. It induces apoptosis (programmed cell death) through the activation of caspases and modulation of mitochondrial pathways. Additionally, piperine inhibits angiogenesis, the process by which tumour's develop new blood vessels, thereby restricting tumour growth and metastasis. It also interferes with cell cycle progression and downregulates oncogenic signalling pathways such as PI3K/Akt and MAPK. These multifaceted mechanisms highlight the potential of piperine as a promising anticancer agent.[10]

A particularly important biological activity of piperine in the context of infectious diseases is its **antitubercular activity**. Tuberculosis remains a global health challenge, and the emergence of multidrug-resistant strains of Mycobacterium tuberculosis necessitates the for new therapeutic agents. Piperine has

shown inhibitory activity against M. tuberculosis by targeting key enzymes involved in mycolic acid synthesis, such as enoyl-acyl carrier protein reductase (InhA). Molecular docking studies have demonstrated that piperine can bind effectively to the active site of InhA, suggesting its potential role as an inhibitor. Additionally, piperine enhances the efficacy of conventional antitubercular drugs by improving their bioavailability, making it a valuable adjunct in TB therapy.

One of the most unique and clinically significant properties of piperine is its role as a **bioavailability enhancer**. Piperine has been shown to increase the absorption and bioavailability of various drugs and nutrients, including curcumin, rifampicin, and betacarotene. It achieves this by inhibiting drug-metabolizing enzymes such as cytochrome P450 (CYP3A4) and P-glycoprotein (P-gp), which are responsible for drug efflux and metabolism. By reducing first-pass metabolism and enhancing intestinal absorption, piperine significantly increases the plasma concentration of co-administered drugs. This property is particularly beneficial in tuberculosis treatment, where prolonged drug therapy often leads to reduced efficacy due to poor bioavailability [11]

Piperine also exhibits significant **immunomodulatory activity,** which enhances the body's immune response against infections. It has been reported to stimulate the proliferation of lymphocytes, enhance macrophage activity, and increase the production of antibodies. Piperine modulates both innate and adaptive immune responses, making it a valuable agent in managing infectious diseases and immune-related disorders.[12] In tuberculosis, where the immune response plays a critical role in disease piperine may help in strengthening host mechanisms against M. tuberculosis.

The **neuroprotective activity** of piperine is another area of growing interest. Piperine has been shown to improve cognitive function and protect against neurodegenerative diseases such as Alzheimer's and Parkinson's disease. It exerts its neuroprotective effects by reducing oxidative stress, inhibiting neuroinflammation, and modulating neurotransmitter levels. Piperine also enhances the bioavailability of neuroprotective compounds, thereby amplifying their therapeutic effects. These properties suggest its potential application in the treatment of neurological disorders.[12]

In addition to these activities, piperine also demonstrates antidiabetic and antihyperlipidemic properties. It has been reported to regulate blood glucose levels by improving insulin sensitivity and enhancing glucose uptake in peripheral tissues. Piperine also reduces lipid accumulation and lowers cholesterol levels, thereby contributing to cardiovascular health. These metabolic effects further expand its therapeutic potential in the management of lifestyle-related disorders.

Moreover, piperine exhibits **hepatoprotective activity**, protecting the liver from toxic insults and oxidative damage. It enhances liver function by improving antioxidant defence mechanisms and reducing inflammation. This property is particularly relevant in tuberculosis treatment, where long-term use of antitubercular drugs can cause hepatotoxicity. Piperine may help mitigate such adverse effects, improving patient compliance and treatment outcomes.[13]

**Mechanism of Piperine Against Tuberculosis**

One of the primary mechanisms by which piperine exerts anti-tubercular activity is through the **inhibition of the InhA enzyme (enoyl-acyl carrier protein reductase),** a critical component in the fatty acid synthesis II (FAS-II) pathway of M. tuberculosis. This enzyme is responsible for the biosynthesis of mycolic acids, which are essential constituents of the mycobacterial cell wall. Molecular docking studies have demonstrated that **piperine binds effectively to the active site of InhA,** forming stable ligand–protein interactions via hydrophobic contacts and hydrogen bonding. This interaction reduces enzyme activity, thereby inhibiting mycolic acid synthesis, ultimately compromising the integrity of the bacterial cell wall and leading to bacterial death. [15]In addition to targeting InhA, piperine has also been reported to interfere with the efflux pump systems of M. tuberculosis. Efflux pumps are membrane proteins that expel antibiotics from bacterial cells, contributing to multidrug resistance (MDR). **Piperine acts as an efflux pump inhibitor,** enhancing the intracellular concentration of anti-TB drugs such as Rifampicin and Isoniazid. This synergistic effect improves drug efficacy and helps overcome resistance mechanisms

Another important mechanism is the disruption of the mycobacterial cell membrane. Piperine, being a lipophilic compound, integrates into the lipid bilayer of the bacterial membrane, altering membrane permeability and fluidity. This disruption leads to leakage of intracellular components and eventual cell lysis.[16] The lipid-rich nature of the M. tuberculosis cell wall makes it particularly susceptible to such lipophilic agents. piperine has been shown to possess anti-inflammatory and antioxidant properties, which help in reducing tissue damage during TB infection. By modulating oxidative stress pathways, it balances the host immune response and prevents excessive inflammation, thereby aiding in better disease management. At the molecular level, computational studies such as molecular docking and molecular dynamics simulations have confirmed the stable binding affinity of piperine with several TB-related protein targets, including InhA, KasA, and DNA gyrase. [17] These findings suggest that piperine may act as a multi-target inhibitor, which is advantageous in combating drug-resistant strains of M. tuberculosis.

**1.4 Reasons for Using Piperine Against Tuberculosis**

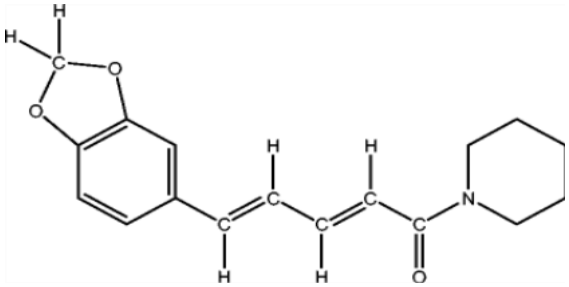
1. **Effective Against Drug-Resistant** TB Drug resistance (MDR-TB and XDR-TB) is a major problem in TB treatment. Piperine offers advantages because: It may bind differently than standard drugs, helping overcome resistance. Its multi-target action makes it harder for bacteria to adapt.
2. **Bioavailability Enhancer (Very Important Advantage) Piperine** is widely known as a natural bioenhancer. It increases the absorption of drugs by inhibiting enzymes like cytochrome P450. It enhances the effectiveness of anti-TB drugs like rifampicin and isoniazid. This can lead to: Lower drug doses Reduced side effects better patient compliance.
3. **immunomodulatory Effect: TB** is an intracellular infection that survives inside macrophages. Piperine helps by: Enhancing immune response Increasing macrophage activity Regulating cytokines (immune signalling molecules) This dual action (direct antibacterial + immune support) makes it highly valuable.
4. **Anti-inflammatory and Antioxidant Properties** TB infection causes inflammation and oxidative stress in lung tissues. Piperine: Reduces inflammation Neutralizes free radicals Protects tissues from damage

**5. Natural and Safer Source**

Piperine is derived from commonly used spices (black pepper).
It is relatively safe and well-tolerated compared to synthetic drugs.
Suitable for long-term use (important in TB therapy).[14]

**Efflux Pump Inhibition (EPI):**

One of the main mechanisms M. tuberculosis uses to develop drug resistance is the use of efflux pumps, which actively push antibiotics out of the bacterial cell before they can take effect.
**Action:** Piperine has shown the ability to inhibit these bacterial efflux pumps.



Chemical Structure

**Source: black and white pepper,**

**Chemical name: 1-piperoylpiperidine (Piper nigrum)**
**Molecular formula: $C_{17}H_{19}NO_3$ Phytochemical Class: An alkaloid Molecular weight: ~285.34 g/mol**

**1.5 Molecular Docking in Drug Discovery**

Molecular Docking is a computational technique used to predict the interaction between a ligand (such as piperine) and a target protein. It plays a crucial role in structure-based drug design by estimating the binding affinity and orientation of a molecule within the active site of a protein. The primary goal of molecular docking is to determine the most favorable binding orientation and estimate the binding affinity between the ligand and the target macromolecule. This approach significantly reduces the time, cost, and effort required in traditional experimental drug discovery processes. In drug discovery, the interaction between a drug molecule and its target protein is crucial for therapeutic activity. Molecular docking helps in understanding these interactions at the atomic level by simulating the binding process. The technique evaluates various conformations of the ligand within the active site of the protein and calculates the binding energy using scoring functions. Lower binding energy indicates a more stable and favourable interaction, suggesting higher potential as a drug candidate.[14]

Molecular docking technology is a kind of technology to recognize and predict the structure of receptor-ligand complex through the simulation of molecular geometry and intermolecular forces supported by stoichiometry and other disciplines. The mechanism of molecular docking can be explained by the "lock & key principle" of the interaction between ligands and receptors. The interaction between ligand and receptor is a process of molecular recognition, including electrostatic interaction, hydrogen bonding, hydrophobic interaction, van der Waals interaction and so on. The binding pattern and affinity between the two can be predicted by calculation.

**Steps of Molecular Docking:**

Molecular ligand is a relatively mature method for direct drug design in recent years. The general research steps of molecular docking are as follows:[18]

1. Preparation of receptor structure: download or construct three-dimensional structure of receptor molecule through database;
2. Preparation of drug molecules: 3d structure of drug molecules can be constructed by downloading from crystal database or molecular simulation;
3. Pre-docking processing: processing ligand and receptor and selecting target;
4. Task submission: Select appropriate docking software and parameters for different systems;
5. Analyse and graph results.

**Types of Molecular Docking**

1. **Rigid body docking**: conformation of the research system does not change during docking. It is suitable for studying relatively large systems, such as the docking between proteins and nucleic acids.
2. **Semi-flexible docking**: In the docking process, the conformation of the research system, especially the ligand, is allowed to change within a certain range. Suitable for handling docking between large and small molecules. In the docking process, the conformation of small molecules is generally changeable, but most are rigid and cannot be changed.
3. **Flexible docking**: In the docking process, the conformation of the research system can change freely. Generally, it is used to accurately consider the recognition between molecules. Because the conformation of the system can be changed during the calculation, the amount of calculation is the largest.[18]

**1.6 Application of Molecular Docking**

Molecular docking can study the detailed interaction between ligand and receptor, predict its binding mode and affinity, and be used to discover and optimize drug lead molecules, thus realizing structure-based drug design. The essence of molecular docking is a process of mutual recognition between two or more molecules, which involves spatial matching and energy matching between molecules. According to the energy matching level, the initial optimal intermolecular structure and binding mode are obtained. The application of molecular docking in drug research includes:

1. Explore specific modes of action and binding configurations of small and large molecule receptors for drugs;
2. Screening lead drugs that can bind to the target;
3. Explain the reasons for the activity of drug molecules;
4. Guide rational optimization of drug molecular structure.

**1.7 Importances of molecular docking:**

molecular docking is its ability to estimate the binding affinity between the ligand and the receptor. Docking software calculates binding energy values, where a lower binding energy indicates a more stable and stronger interaction. This helps in ranking different compounds based on their potential effectiveness, thereby enabling researchers to select the most promising candidates for further study.

In addition, molecular docking significantly reduces the cost and time associated with traditional drug discovery processes. Experimental screening of compounds in laboratories is often expensive and time-consuming, whereas docking allows for rapid virtual screening of thousands of molecules in a relatively short period.

Molecular docking also provides detailed information about the types of interactions involved in ligand–protein binding, such as hydrogen bonds, hydrophobic interactions, and van der Waals forces.

Understanding these interactions is essential for rational drug design, as it enables researchers to modify the chemical structure of compounds to enhance their binding efficiency and specificity.
Furthermore, docking plays an important role in addressing the challenge of drug resistance, particularly in diseases like tuberculosis. By analysing how mutations in target proteins affect drug binding, researchers can design new compounds that retain efficacy against resistant strains.

In addition to these advantages, molecular docking supports the screening of large compound libraries and facilitates multi-target drug discovery, where a single compound can be evaluated against multiple proteins. This is particularly useful in complex diseases such as tuberculosis, which involve multiple biological pathways.

Moreover, molecular docking helps reduce the reliance on animal testing in the early stages of research by providing preliminary data on drug efficacy and interaction. Overall, molecular docking is an essential and powerful tool in pharmaceutical research, as it enhances the efficiency, accuracy, and success rate of drug discovery and development processes.[14]

Molecular docking is used because it predicts ligand–protein interactions, estimates binding affinity, saves time and cost, enables virtual screening, and supports drug design and optimization. It is an essential tool in modern drug discovery, especially for identifying new treatments against diseases like tuberculosis.

**1.8 Molecular Docking: Purpose and Scientific Rationale:**

Molecular docking is a widely employed computational technique in the field of Computational Chemistry and Drug Discovery, primarily used to predict the interaction between a small molecule (ligand) and a target macromolecule (usually a protein).

The fundamental objective of molecular docking is to determine the optimal binding orientation and conformation of a ligand within the active site of a target protein, along with estimating the binding affinity.

This approach helps researchers understand how a drug candidate may interact at the molecular level with its biological target.[19] One of the main reasons molecular dockings is performed is to facilitate structure-based drug design (SBDD). By analysing the three-dimensional structure of target proteins. Molecular docking is also used to predict binding mechanisms and interactions.

It provides detailed insights into hydrogen bonding, hydrophobic interactions, van der Waals forces, and electrostatic interactions between ligand and receptor. This information is crucial for understanding the stability and specificity of the ligand–protein complex.[20] Additionally, docking helps in identifying key amino acid residues involved in binding, which can be targeted for drug optimization.

Furthermore, molecular docking plays a vital role in lead optimization and drug repurposing. After identifying a lead compound, docking studies can be used to modify its chemical structure to improve binding affinity and pharmacological properties. [21] It is also useful in repurposing existing drugs by testing their binding potential against new targets.

**Application of molecular modelling in modern drug development:**

The application of molecular modelling has become a cornerstone of modern drug development, enabling researchers to design, optimize, and evaluate therapeutic compounds with greater efficiency and precision.

Molecular modelling refers to a collection of computational techniques used to represent and simulate the behaviour of biological molecules such as proteins, nucleic acids, and small druglike compounds. In contemporary pharmaceutical research, these methods are widely integrated into the early stages of drug discovery, significantly reducing the time, cost, and experimental burden associated with traditional trial-and-error approaches.

One of the most important applications is structure-based drug design (SBDD), where the three-dimensional structure of a biological target—often obtained through techniques like Xray crystallography or Nuclear Magnetic Resonance spectroscopy—is used to identify and optimize compounds that can bind effectively to the target site.

In this context, molecular docking plays a crucial role by predicting the preferred orientation and binding affinity of a ligand within a protein's active site, helping researchers screen thousands of compounds virtually before laboratory validation.

Another key application is ligand-based drug design (LBDD), which is employed when the structure of the target protein is unknown.

Techniques such as Quantitative Structure-Activity Relationship analyse the relationship between chemical structure and biological activity, allowing scientists to predict the efficacy of new compounds based on known data.

Molecular modelling also supports pharmacophore modelling, which identifies the essential structural features required for a drug to interact with its target, thereby guiding the design of novel molecules with improved potency and selectivity.

Furthermore, molecular dynamics simulation is widely used to study the stability and conformational flexibility of biomolecular complexes over time, providing insights into dynamic interactions that cannot be captured by static models alone.[14]In addition to drug design, molecular modelling plays a significant

role in lead optimization, where promising compounds are refined to enhance their pharmacokinetic and pharmacodynamic properties. Computational tools are used to predict absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles, helping to eliminate compounds with undesirable characteristics early in the development pipeline. This predictive capability minimizes the risk of late-stage drug failure, which is a major challenge in pharmaceutical research.

Moreover, molecular modelling contributes to target identification and validation, enabling researchers to understand disease mechanisms at the molecular level and identify novel therapeutic targets.
For example, in diseases such as tuberculosis, modelling techniques help in identifying key enzymes and proteins involved in pathogen survival, facilitating the design of inhibitors against them.[14] Another emerging application is in personalized medicine, where molecular modelling is used to predict how individual genetic variations affect drug response.
By simulating drug interactions with variant proteins, researchers can tailor treatments to specific patient populations, improving therapeutic outcomes and reducing adverse effects.

Additionally, molecular modelling is instrumental in biologics development, including the design of antibodies and peptide-based drugs, where understanding protein-protein interactions is critical.

**Aim & Objective**

**Aim**

To evaluate the anti-tubercular potential of Piperine by performing molecular docking studies against selected target proteins (InhA) of Tuberculosis and to analyse the binding interactions, docking score, and affinity of the phytoconstituent with the target proteins (InhA).

**Objective**

1. Selection of Target Protein

The first step of the molecular docking study involves the selection and retrieval of the target protein associated with Tuberculosis. The three-dimensional (3D) crystal structure of the InhA protein was retrieved from the RCSB Protein Data Bank using the Protein Data Bank code (PDB ID: 2H7M).
This analysis is essential for identifying the active site region and preparing the protein for further molecular docking studies with the phytoconstituent.

2. Protein Preparation and Refinement

To prepare and refine the InhA protein structure using PyMOL by removing water molecules, optimizing the protein structure, and generating a clean receptor model suitable for molecular docking studies.

3. Ligand Selection and Optimization

To obtain the 3D structure of the phytochemical Piperine (from PubChem website) and perform energy minimization to achieve its most stable conformation. Energy minimization of the ligand was performed to remove steric hindrance, reduce molecular strain, and obtain the most stable conformational state of the compound. This process helped in optimizing bond angles, bond lengths, and torsional parameters, thereby improving the accuracy of ligand–protein interaction studies.

4. Active Site Identification (Grid Generation)

To identify the catalytic binding pocket of InhA and define the grid box coordinates (X, Y, Z) covering the active site residues where the natural cofactor (NADH) or inhibitors bind. The generated grid box served as the docking search space during molecular docking studies, ensuring accurate positioning and interaction analysis of the phytochemical Piperine within the active site of the InhA protein.

5. Molecular Docking Simulation

Molecular docking simulation was performed to evaluate the binding interaction between the phytochemical Piperine and the target protein InhA using the PyRx platform integrated with the Auto Dock Vina docking engine.

The docking simulation was then executed to predict the most favourable binding conformations of Piperine within the catalytic pocket of InhA. The binding affinity of the ligand–protein complex was calculated in terms of Gibbs free energy of binding kcal/mol), where lower negative values indicated stronger binding.

6. Interaction Analysis and Visualization

To visualize and analyse the protein-ligand complex in 2D and 3D to identify key interactions like Hydrogen bonding, Hydrophobic contacts, and Pi-stacking using BIOVIA Discovery Studio.

Both 2D and 3D visualization techniques were employed to identify the important amino acid residues involved in ligand binding within the active site of the protein. Various interactions such as hydrogen bonding, hydrophobic interactions.

7. Comparative Analysis

To compare the docking score and binding pose of Piperine with a standard anti-TB drug (like Isoniazid) to evaluate its relative inhibitory potential. The comparative study helped in assessing whether Piperine exhibited binding characteristics like or the standard drug, thereby providing insight into its possible role as a potential anti-tubercular agent.

**Literature Review**

Tuberculosis (TB), caused by Mycobacterium tuberculosis, remains one of the most significant global infectious diseases, posing a major challenge to public health systems worldwide. The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains has further complicated TB management and highlighted the urgent need for novel therapeutic agents. Conventional anti-tubercular drugs such as isoniazid and rifampicin are associated with prolonged treatment regimens, adverse effects, and increasing resistance, thereby necessitating the exploration of alternative drug candidates derived from natural sources.

Phytochemicals have gained considerable attention in recent years due to their diverse biological activities, structural complexity, and relatively low toxicity. Among these, piperine, an alkaloid derived from Piper nigrum (black pepper) and Piper longum, has been extensively studied for its pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, and bioavailability-enhancing effects. Several studies have demonstrated that piperine exhibits inhibitory activity against various bacterial pathogens, including Mycobacterium tuberculosis, making it a promising candidate for anti-TB drug development.

Molecular docking has emerged as a powerful computational tool in modern drug discovery, enabling the prediction of ligand–protein interactions at the molecular level. It allows researchers to evaluate the binding affinity and interaction patterns between small molecules and target proteins, thereby facilitating the identification of potential drug candidates. In the context of tuberculosis, key target proteins such as InhA (enoyl-acyl carrier protein reductase), KasA (β-ketoacyl-ACP synthase), and DNA gyrase play crucial roles in bacterial survival and replication, making them attractive targets for docking studies.

Several researchers have employed molecular docking techniques to investigate the interaction of piperine with TB-associated protein targets. For instance, docking studies targeting the InhA enzyme have revealed that piperine exhibits significant binding affinity within the active site, comparable to standard drugs like isoniazid.

The binding interactions are primarily stabilized through hydrophobic interactions, π–π stacking, and hydrogen bonding with key amino acid residues such as Tyr158, Lys165, and Met199. These interactions suggest that piperine may inhibit the fatty acid biosynthesis pathway, which is essential for the formation of mycolic acids in the bacterial cell wall.

In addition to InhA, docking analyses involving other TB targets such as KasA and arabinosyltransferase have also demonstrated favourable binding energies for piperine. Studies indicate that piperine can occupy the catalytic pocket of these enzymes, thereby interfering with cell wall biosynthesis and bacterial growth. Furthermore, comparative docking studies have shown that piperine derivatives and analogues may exhibit even stronger binding affinities, highlighting the potential for structural optimization in drug design.

The integration of molecular docking with molecular dynamics simulations has further strengthened the understanding of piperine–protein interactions.

Molecular dynamics studies provide insights into the stability and conformational behaviour of ligand–protein complexes over time, confirming the reliability of docking predictions. Research findings suggest that piperine forms stable complexes with TB target proteins, maintaining consistent interactions throughout the simulation period, which supports its potential as a lead compound.

Despite promising in silico results, it is important to note that molecular docking studies represent only an initial step in drug discovery. Experimental validation through in vitro and in vivo studies is essential to confirm the biological activity, pharmacokinetics, and safety profile of piperine. Some experimental studies have reported that piperine enhances the efficacy of existing anti-TB drugs by improving their bioavailability and reducing drug resistance, further supporting its therapeutic potential.

Recent advancements in computational biology and structure-based drug design have accelerated the identification of novel anti-TB agents. The application of molecular docking in screening natural compounds like piperine provides a cost-effective and time-efficient approach to drug discovery. Moreover, the combination of piperine with conventional drugs may offer synergistic effects, thereby improve treatment outcomes and reduce the risk of resistance.

In conclusion, the literature strongly supports the potential of piperine as a promising phytochemical for anti-tuberculosis therapy. Molecular docking studies have demonstrated its ability to effectively bind with key TB target proteins, particularly InhA, suggesting a possible mechanism of action through inhibition of mycolic acid synthesis. While further experimental validation is required, the integration of computational and experimental approaches may pave the way for the development of novel, safe, and effective anti-TB agents based on piperine.

**Methodology**

**Materials**

1. Hardware Requirements:
   Computer/Laptop
2. Operating System: Windows / Linux / macOS
3. Software Requirement

- PyRx (main docking platform)
- PyMOL – for 3D structure visualization & protein preparation
- Discovery Studio Visualizer – for interaction analysis
- Open Babel – for format conversion (SDF to PDB, etc.)

4. Online Database
   PubChem – to download piperine structure
   Protein Data Bank (RCSB PDB) – to obtain 3D structure of InhA protein

**Molecular Docking Procedure**

**1.Ligand Download**

Open PubChem website by searching the keyword “Piperine” or using its PubChem CID 638024. After accessing the compound page, the structure can be downloaded from the download section in SDF format. This downloaded ligand file is then further processed and converted into appropriate formats (such as PDB and PDBQT) for molecular docking analysis.

**2. Protein Download**

Open Protein Data Bank website the three-dimensional structure of this protein is obtained from the Protein Data Bank. The retrieval process involves accessing the PDB website and searching for “InhA Mycobacterium tuberculosis,” which provides multiple structural entries. Among these, well-characterized and widely used structures such as **2H7M,** are recommended due to their good resolution and reliability for docking studies.

protein structure is downloaded in PDB format using the download option provided on the website. This downloaded file serves as the primary input for subsequent protein preparation and molecular docking analysis in the study.

**3. Preparation of Protein Protein Preparation Procedure Using PyMOL**

The preparation of the target protein for molecular docking studies was performed using PyMOL to obtain a clean and optimized protein structure suitable for docking analysis. Initially, the crystal structure of the target protein InhA was downloaded from the RCSB Protein Data Bank in PDB format and extracted from the compressed downloaded file.

The extracted protein file was then opened in the PyMOL software by selecting the “File” menu followed by the “Open” option and importing the required PDB file into the workspace.

After loading the protein structure, all structural components such as water molecules ($H_2O$), co-crystallized ligands, and hetero atoms present within the protein complex were visualized in the object panel located on the right side of the PyMOL interface.

To prepare a clean receptor structure, the “A” (Action) tab corresponding to the protein object was selected. From the available options, unnecessary molecules including water molecules were removed by choosing the appropriate delete or remove selection commands. Water molecules were specifically eliminated because they may interfere with docking interactions and affect binding accuracy. Similarly, unwanted ligands, ions, and hetero atoms not involved in the active binding process were also removed from the structure to avoid computational interference during docking simulation.

Polar hydrogen atoms were then added to stabilize the protein structure and improve interaction analysis during docking studies. The prepared protein was finally saved in PDB format for further conversion into PDBQT format using docking software such as PyRx .

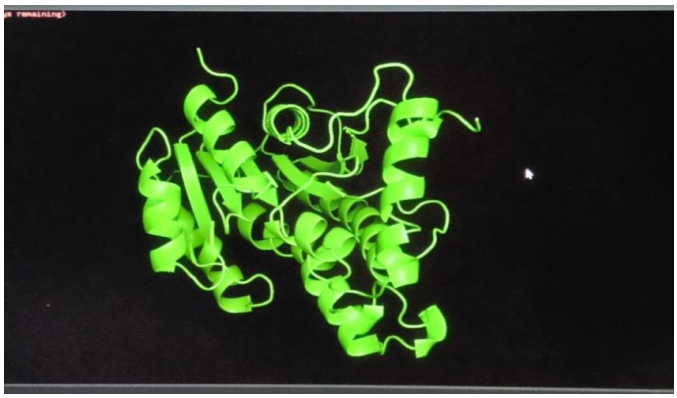


**Figure 1: prepared protein by pymol**

**4. Molecular Docking Procedure Using PyRx**

The prepared **protein structure was imported** into PyRx by opening the software homepage and loading the refined protein file in PDB format. The protein was then converted into Auto Dock-compatible macromolecule format (PDBQT) using the "Auto Dock" option available in the software. During this preparation step, the macromolecule was selected, and PyRx automatically converted the protein structure from **PDB format to PDBQT format**, which is essential for molecular docking analysis.

Subsequently, the **ligand molecule Piperine** was imported into the software using the "Open Babel" option located at the bottom panel of PyRx. The ligand structure in SDF format was loaded into the workspace and subjected to energy minimization using the **"Minimize All"** function to obtain a stable conformational structure. After minimization, the ligand was converted from SDF format to PDB format and finally transformed into Auto Dock ligand PDBQT format for docking studies.

After successful preparation of both protein and ligand files, the docking procedure was initiated through the "Vina Wizard" module of PyRx. The "Start" option was selected, followed by the selection of the prepared ligand and macromolecule files. The docking setup window displayed a grid box over the target protein structure. The grid box dimensions were adjusted and enlarged appropriately to completely cover the active binding region of the protein, ensuring accurate interaction analysis between the ligand and receptor.

Once the grid box parameters were finalized, the docking process was started by clicking the forward option, and the molecular docking simulation was executed using the Auto Dock Vina engine integrated within the software.

Upon completion of the docking process, multiple binding conformations with different binding affinity values were generated. Among these, the docking pose **showing a binding affinity of −8.5 kcal/mol was selected for further analysis because it demonstrated strong ligand–protein interaction and better binding stability with the target protein**. The selected docked complex was then exported and converted into PDB format for detailed visualization and interaction analysis using BIOVIA Discovery Studio sof

**5. Analysis of Results**

The prepared protein and ligand structures were imported into BIOVIA Discovery Studio for visualization and interaction analysis. Initially, the software was opened and the target protein file in PDB format was loaded through the File menu. Subsequently, the docked ligand file containing the phytochemical Piperine was imported into the workspace. In cases where the protein and ligand structures appeared in separate

windows or tabs, the ligand molecule was copied and merged into the protein structure window to generate a complete protein–ligand complex within a single visualization environment. This allowed proper observation of the binding orientation and molecular interactions between the ligand and the receptor protein. After successfully loading both structures, receptor and ligand definitions were assigned within the software for accurate interaction analysis. The protein molecule was selected from the hierarchy explorer and defined as the receptor, while the docked Piperine molecule was selected and assigned as the ligand. This step enabled the software to recognize the biomolecular components correctly and facilitated the generation of interaction profiles such as hydrogen bonding, hydrophobic interactions, binding affinity, amino acid residue mapping, and 2D/3D ligand interaction diagrams.

**1. Hydrogen Bonding**

The interaction analysis indicated that Piperine formed conventional hydrogen bonding interactions with key active site residues, particularly with **Tyr158,** which contributed significantly to the stabilization of the ligand–protein complex. **Hydrogen bonds are important non-covalent interactions** that enhance binding specificity and improve the stability of the docked complex within the receptor cavity.

**2. Hydrophobic interactions**

In addition to hydrogen bonding, several important hydrophobic interactions were observed between Piperine and the active site residues of the InhA protein. The ligand showed strong Pi–Alkyl interactions with amino acid residues including MET147, ILE215, and
TYR158 residues, along with Alkyl interaction with PHE149. Additionally, residues such as LYS165, ASP148, MET199, ALA157, PRO156, LEU218, MET155, PRO193, ILE194, and ILE21 These hydrophobic interactions enhance the stability of the docked complex through van der Waals forces and non-polar interactions within the hydrophobic pocket of the protein.

**3. Binding affinity**

Binding affinity score of **−8.5 kcal/mol** against the target protein (InhA), indicating the formation of a stable ligand–protein complex within the active site region. The highly negative docking energy reflects strong intermolecular interactions, including hydrophobic and van der Waals interactions, which contribute to the stability and favorable binding conformation of the complex.

**4. Amino acid residue**

The ligand exhibited significant hydrophobic interactions, including Pi–Alkyl interactions with MET147, ILE215, and TYR158 residues, along with Alkyl interaction with PHE149. Additionally, residues such as LYS165, ASP148, MET199, ALA157, PRO156, LEU218, MET155, PRO193, ILE194, and ILE21 contributed through van der Waals interactions, stabilizing the ligand–protein complex.

**5. 2D/3D ligand interaction**

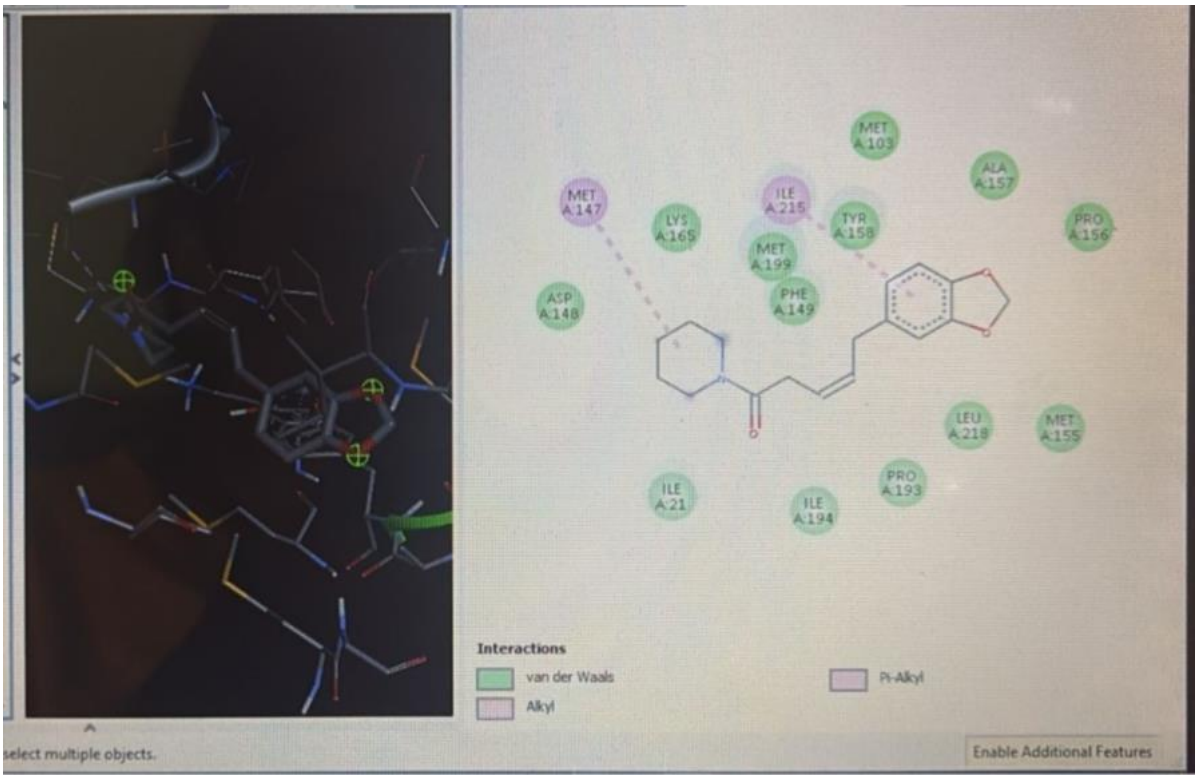



**Figure 2: 2D and 3D Interaction Map of Piperine within the Binding Pocket of InhA Protein.**

**Pharmacokinetic and Toxicological (ADMET) Profiling**

While strong binding affinity within the active site of the InhA protein is considered an essential requirement for anti-tubercular activity, an effective lead compound must also exhibit favorable pharmacokinetic and toxicological characteristics to qualify as a promising therapeutic candidate. A significant number of potential drug molecules fail during advanced stages of pharmaceutical development due to poor absorption, inadequate oral bioavailability, unfavourable metabolic behaviour, or toxic side effects. Therefore, to evaluate the druglikeness and pharmacological suitability of Piperine, a comprehensive in-silico ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling study was performed.

To maintain computational reliability and ensure accurate pharmacokinetic prediction, the ADMET profiling of Piperine was conducted using the online cheminformatics platform **swissadme.** computational tool employed for predicting physicochemical properties, pharmacokinetic behaviour, drug-likeness parameters, and medicinal chemistry characteristics of bioactive compounds. The platform utilizes validated predictive algorithms to estimate the biological behaviour of small molecules and assists in identifying compounds with suitable therapeutic potential prior to experimental investigations.

**Computational Protocol for ADMET Profiling**:

1. **Retrieval of Piperine Structure**

The chemical structure of Piperine was retrieved from the pubchem.ncbi.nlm.nih. The compound information page was opened, and the canonical SMILES notation of Piperine was copied for further pharmacokinetic analysis.

Canonical SMILES of Piperine:

COC1=CC=CC=C1/C=C/C(=O)N2CCCCC2=C/C=C/C3=CC=CC=C3

**2. Accessing the SwissADME Web Server**
The ADMET profiling was carried out using the online tool swissadme.which is widely used for predicting physicochemical, pharmacokinetic, drug-likeness, and medicinal chemistry properties of bioactive compounds.

3. **Input of Compound Data**
a. Open the SwissADME homepage.
b. Paste the copied SMILES notation of Piperine into the input box labelled "Paste your molecules here."
c. Enter the compound name as "Piperine."
d. Click on the "Run" button to initiate the analysis.

**4. ADMET Parameters Evaluated**
The SwissADME server automatically generated the ADMET profile of Piperine based on various computational models.

5. **Interpretation of Results**
The obtained ADMET results indicated the pharmacokinetic behaviour and drug-likeness characteristics of Piperine. The compound showed acceptable oral bioavailability, favorable gastrointestinal absorption, and compliance with Lipinski's Rule of Five, suggesting its potential as a promising drug candidate against the InhA target protein.

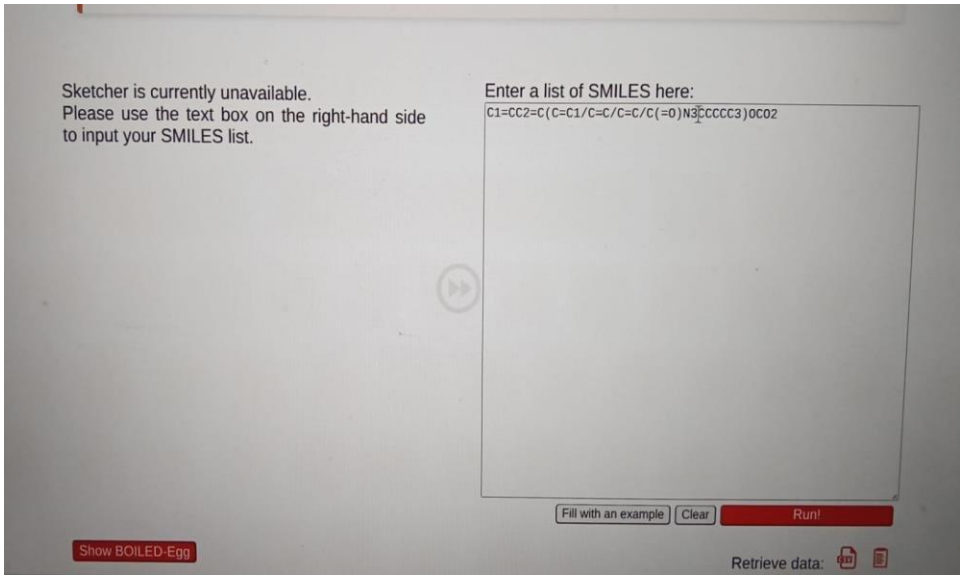



**Figure 3: Preparation of Phytochemical SMILES for Pharmacokinetic Analysis Plan of work**

**Introduction**
Molecular docking is a computational approach used to predict the interaction between bioactive compounds and target proteins for drug discovery studies.
The study involves protein and ligand preparation, active site identification, molecular docking analysis, and interaction visualization using computational software to determine the binding affinity and stability of the Piperine–InhA complex. The findings of this study may contribute to the development of natural anti-tubercular agents for future therapeutic applications.

**Molecular Docking procedure**

The molecular docking procedure involves several steps:

1. Selection of Target Protein (download structure from the Protein Data Bank (PDB)
2. Ligand Selection and Preparation (download phytochemicals form the PubChem)
3. Grid Box Generation (Setting the docking area around the active site for ligand binding analysis.)
4. Molecular Docking Simulation (Performing docking using software)
5. Interaction Analysis
6. Visualization of Docked Complex (Visualization of 2D and 3D protein–ligand interactions)
7. Result Interpretation

**Analysis of Phytochemical Active Binding Site**

The molecular docking analysed for phytochemical Active site of Target Protein:

The following points are analysed in active site:

1. Binding Affinity / Docking Score (The docking score (kcal/mol) indicates the strength of interaction between the phytochemical and the target protein. A more negative value represents stronger and more stable binding.)
2. Hydrogen Bond Interactions (Hydrogen bonds formed between the ligand and amino acid residues of the protein are analysed)
3. Hydrophobic Interactions
4. Amino Acid Residues Involved
5. RMSD Value
6. Visualization of 2D and 3D Interactions

**Molecular Docking Perform**

Macular bocking performs importance:

1. Prediction of Ligand–Protein Interaction

Molecular docking helps in predicting the interaction between a ligand and the target protein at the molecular level, which is essential for understanding the mechanism of drug action.

2. Identification of Binding Affinity evaluates the binding affinity and stability of the ligand–protein complex through docking scores and binding energy values.

3. Active Site Analysis

Docking assists in identifying the active binding pocket and important amino acid residues involved in molecular interactions.

4. Drug Discovery and Development

Molecular docking plays a significant role in computer-aided drug design by screening potential compounds before experimental studies.

5. Reduction of Experimental Cost and Time silico docking studies reduce laboratory workload, experimental cost, and time by selecting the most promising compounds for further investigation.

6. Prediction of Drug Efficacy

Docking studies help in predicting the biological activity and effectiveness of compounds against specific targets.

7. Structure-Based Drug Design
The optimization and modification of lead compounds for improved binding and pharmacological activity.

8. Visualization of Ligand Orientation helps visualize the orientation and conformation of the ligand within the protein binding cavity.

9. Improvement of Research Accuracy
Molecular docking increases the reliability and scientific accuracy of pharmaceutical and biochemical research studies.

**Molecular Docking Perform Using the Following Software's:**

1. PyRx (main docking platform)
2. PyMOL – for 3D structure visualization & protein preparation
3. Discovery Studio Visualizer – for interaction analysis
4. Open Babel – for format conversion (SDF to PDB, etc.)
5. PubChem – to download piperine structure
6. Protein Data Bank (RCSB PDB) – to obtain 3D structure of InhA protein

**Graphical Flow Chart Docking:**

[ Target Protein Selection]

Identification of Mycobacterium tuberculosis InhA Protein (PDB ID: 2H7M, 4DRE)

↓

[ Protein Preparation]

Removal of Water Molecules and Heteroatoms, Addition of Polar Hydrogens and Kollman Charges

↓

[ Ligand Preparation]

Retrieval of Piperine Structure from PubChem and Energy Minimization

↓

[ Conversion to PDBQT Format]

Preparation of Protein and Ligand Files for Docking Study

↓

[ Grid Box Configuration]
Selection and Optimization of Active Binding Site Coordinates

↓

[ Molecular Docking Process]

Docking Simulation Performed Using Auto Dock Vina in PyRx Software

↓

[ Docking Result Analysis]

Evaluation of Binding Affinity, RMSD Values, and Binding Pose

↓

[ Protein–Ligand Interaction Analysis]

Identification of Hydrogen Bonds, Hydrophobic Interactions, and Pi–Pi Interactions

↓

[ Visualization of Docked Complex]

2D and 3D Interaction Visualization Using PyMOL and Discovery Studio

↓

[ Interpretation and Conclusion]

Assessment of Piperine as a Potential InhA Protein Inhibitor

**Result And Discussion**

| Sr. No. | Parameter Evaluated | Observation / Result |
|---|---|---|
| 1 | Ligand name | piperine |
| 2 | Target Protein | InhA (Mycobacterium tuberculosis) |
| 3 | Docking Software Used | Auto Dock Vina (PyRx) |
| 4 | Binding Affinity | −8.5kcal/mol |
| 5 | Hydrogen Bond Interaction | Hydrogen bond observed with amino acid residue Tyr158 |
| 6 | Hydrophobic Interactions | Pi–Alkyl and hydrophobic interactions observed |
| 7 | Amino Acid Residues Involved | MET147, ILE215, and TYR158 LYS165, ASP148, MET199, ALA157, PRO156, LEU218, MET155, PRO193, ILE194, and ILE21 |

| | | |
|---|---|---|
| 8 | RMSD Value | Within acceptable docking range (0) |
| 9 | 2D Ligand Interaction Analysis | 2D interaction map showed hydrogen bonding and hydrophobic contacts between Piperine and active site residues |
| 10 | 3D Ligand Interaction Analysis | Stable binding orientation of Piperine observed inside the active binding pocket of InhA protein |
| 11 | Type of Molecular Interactions | Hydrogen bonding, Van der Waals interaction, Pi–Pi interaction, and Pi–Alkyl interaction |

**ADMET Profile Result**

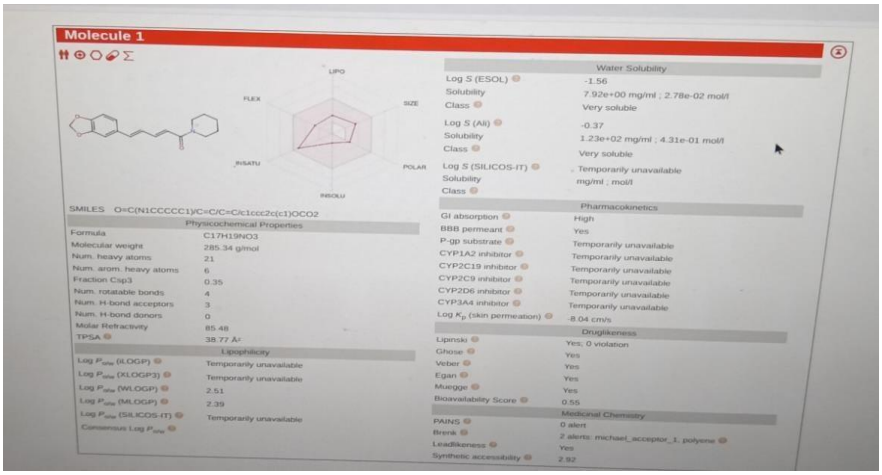



Figure 4: In Silico ADME Screening Results

| **Parameter Category** | **Specific Property** | **Calculated Value** | **Standard Threshold** |
|---|---|---|---|
| Physicochemical Properties | Molecular Weight | 285.34 g/mol | < 500 g/mol |
| | Molecular Formula | C17H19NO3 | – |
| | Lipophilicity (cLogP) | 2.80 | < 5.0 |
| | Hydrogen Bond Donors | 0 | ≤ 5 |
| | Hydrogen Bond Acceptors | 0 | ≤ 10 |
| | Topological Polar Surface Area (TPSA) | 38.77 Å² | < 140 Å² |

| | | | |
|---|---|---|---|
| | Rotatable Bonds | 4 | ≤ 10 |
| Drug-Likeness | Lipinski's Rule of Five | Accepted | 0 Violations |
| | Bioavailability Score | 0.55 | Acceptable |
| Pharmacokinetics | Gastrointestinal (GI) Absorption | High | Favorable |
| | Blood–Brain Barrier (BBB) Permeation | Yes | Predicted |
| | P-gp Substrate | No | Favorable |
| Toxicity Profile | Mutagenic Risk | None | – |
| | Irritant Risk | None | – |
| | Reproductive Effect Risk | None | – |

**DOCKING RESULT IMAGE:**

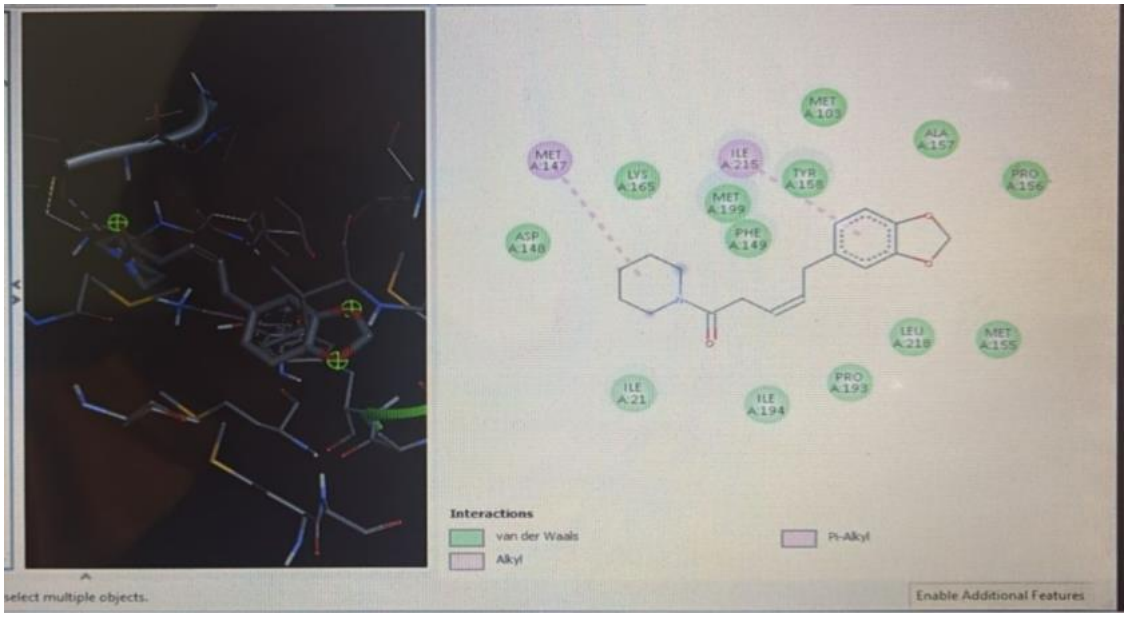



Figure 5: 2D and 3D Interaction visualization

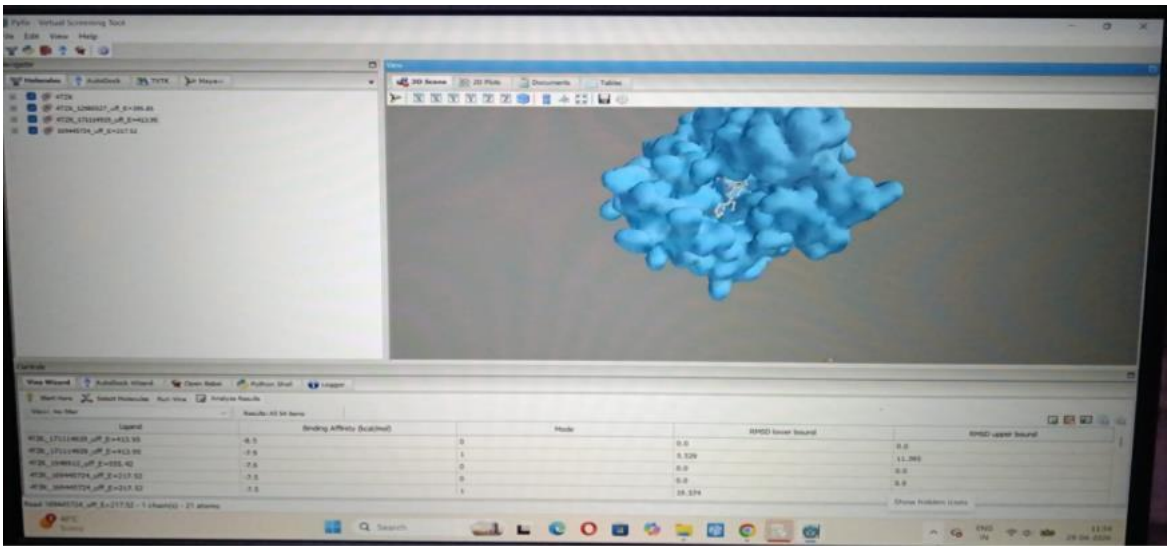


**Figure 6: RMSD Value**

**Summary**

The present study was conducted to investigate the molecular docking interaction of the phytochemical Piperine against the InhA target protein of Mycobacterium tuberculosis to evaluate its potential anti-tubercular activity. Tuberculosis remains one of the major global health challenges, and the emergence of multidrug-resistant strains has increased the need for the development of new and effective therapeutic agents. pharmacological activities and comparatively lower toxicity.

Piperine, a bioactive alkaloid isolated from black pepper (Piper nigrum), was selected for this study because of its reported in recent years, phytochemicals have attracted considerable attention in drug discovery due to their di antimicrobial and medicinal properties.

The three-dimensional crystal structure of the InhA target protein was obtained from the Protein Data Bank (PDB), while the chemical structure of Piperine was retrieved from the PubChem database. The ligand and protein structures were prepared and optimized prior to molecular docking analysis using appropriate computational tools and docking software. The docking study was performed to analyse binding affinity, hydrogen bonding, hydrophobic interactions, amino acid residue interactions, and ligand–protein complex stability.

The molecular docking results revealed that Piperine exhibited favourable binding affinity toward the active binding pocket of the InhA protein, suggesting the formation of a stable ligand–protein complex.

Interaction analysis demonstrated the involvement of significant amino acid residues through hydrogen bonding and hydrophobic interactions, which contributed to the stabilization of the docked complex. Furthermore, the 2D and 3D interaction studies confirmed the effective orientation and proper accommodation of Piperine within the active site region of the InhA protein.

Piperine possesses promising inhibitory potential against the InhA target protein of Mycobacterium tuberculosis and may serve as a potential lead molecule for anti-tubercular drug development.

The study also highlights the importance of molecular docking as a reliable computational approach for predicting ligand–protein interactions during the early stages of drug discovery. However, further in vitro

and in vivo studies are required to validate the biological activity, safety, and therapeutic efficacy of Piperine.

**Conclusion**

The primary objective of the present computational investigation was to evaluate the inhibitory potential of **Piperine,** a major bioactive phytochemical of black pepper, against the InhA protein target enzyme associated with the survival and pathogenicity of Tuberculosis. Molecular docking studies were successfully performed using AutoDock Vina and pyrx to investigate the thermodynamic stability and structural interaction profile of the Piperine–InhA complex.

The docking results demonstrated that Piperine exhibited a favorable and spontaneous binding interaction affinity **−8.5kcal/mol** for the InhA active site. Furthermore, 2D and 3D interaction analysis performed using BIOVIA Discovery Studio revealed that Piperine effectively occupied the hydrophobic cavity of the InhA protein and established multiple stabilizing intermolecular interactions. The complex formation was supported by conventional hydrogen bonding, hydrophobic interactions, van der Waals forces, and π-alkyl interactions with important active site amino acid residues present within the catalytic domain of the protein.

Detailed interaction profiling indicated that the stability of the Piperine–InhA complex was maintained through strong ligand–protein association, suggesting efficient inhibition of enzymatic activity involved in mycolic acid biosynthesis. Since the InhA enzyme plays a crucial role in the formation of the mycobacterial cell wall, inhibition of this target may contribute significantly toward suppression of bacterial growth and survival. The molecular docking findings therefore support the therapeutic relevance of Piperine as a potential antitubercular phytochemical compound.

Comparative assessment with previously reported anti-tubercular ligands and standard inhibitors suggested that Piperine possesses promising binding characteristics and may act as a competitive natural scaffold for future drug discovery applications. In addition, the interaction profile demonstrated good structural stability and favourable molecular orientation within the receptor binding site, thereby strengthening its potential as a lead compound for antituberculosis therapy.

Overall, the present in-silico molecular docking investigation provides strong computational evidence that Piperine may serve as a promising naturally derived inhibitor of the InhA protein. The study highlights the importance of phytochemical-based drug discovery approaches and supports the traditional medicinal significance of black pepper–derived bioactive compounds.

Furthermore, the findings emphasize the usefulness of computational docking techniques for rapid screening, interaction analysis, and identification of novel therapeutic candidates against tuberculosis-associated molecular targets.

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