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Molecular Docking Studies for the Identification of Mycobacterial Adenosine Triphosphate (ATP) Synthase Inhibitors Using Active Phytocompounds from Plants *Curcuma longa*, *Curcuma zedoaria* and *Galipea officinalis*

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Abstract

Objective: *Tuberculosis (TB), known in human history for several decades, remains the second leading infectious disease after COVID-19. Still, the occurrence of TB increases due to the bacterium's greater survival nature. They can survive in extreme conditions by being in long latency and adapting to the host. This study seeks to identify potential inhibitors from Curcuma longa, Curcuma zeodaria, and Galipea officinalis based on ancient folklore and compare them against the known mycobacterial ATP-synthase inhibitor, bedaquiline. To perform docking studies, drug-likeness, and ADMET analysis.* ***Methods:*** *The structure of mycobacterial ATP-synthase was retrieved from the PDB database. Preparation of protein and docking studies were performed using the PyRx tool for the drug-likeliness study, the SwissADME webserver was used. For ADMET analysis, the ADMETlab webserver was used.* ***Results:*** *The docking study showed potential compounds that inhibit mycobacterial ATP-synthase thereby interfering with ATP synthesis besides the known inhibitor, bedaquiline. Among these six compounds, cyclocurcumin, cusparine, and galipeine had shown a similar binding nature compared to that of bedaquiline. The known inhibitor, bedaquiline inhibits the protein by blocking the rotation of the C chain which is the catalytic headpiece. Thus, it primarily inhibits the F-ATP synthesis in the bacterium.* ***Conclusion:*** *Considering the drug-likeliness and ADMET analysis, galipeine shows better safety and toxicity profile than Cyclocurcumin. The study proposes Cyclocurcumin and galipeine as potential inhibitors of mycobacterial ATP-synthase for designing a therapeutic compound, by exploiting the phytocompounds especially known for their purpose for treating tuberculosis based on ancient folklore.*

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Keywords: Tuberculosis, mycobacterial ATP-synthase, *Curcuma longa*, *Curcuma zeodaria; Galipea officinalis*, traditional medicine

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. It is a multi-systemic disease, but most often presents as a pulmonary disease. The existence of *Mycobacterium tuberculosis* was predated for centuries by surviving through adaptation. It remains the 13th leading cause of death and the 2nd most leading infectious killer after COVID-19. This

has reversed the progress we have seen in the last decade, leading to a paradigm shift in progress. The main challenge is to tackle the increasing threat of multidrug resistance (MDR) and extensively drug-resistant (XDR) variants.

Mycobacterium tuberculosis is an acid-fast bacillus belonging to the Mycobacteriaceae family. The disease is caused by a group of genetically similar organisms called the Mycobacterium tuberculosis complex, which consists of *M. africanum, M. bovis,* and *M. microti*. Unlike other bacterial pathogens, *M. tuberculosis* does not possess the typical virulence and contains certain lipids, such as mycolic acids, cord factor, and Wax-D. It can also survive under extreme conditions, and in long-term latency, it can persist without causing damage/transmission, except in immunocompromised patients [1]. There are a few molecular mechanisms that may explain host adaptation. First, *M. tuberculosis* causes TB in immunocompromised patients. The organism exhibits a commensal-like relationship with the host during the latency phase, without any significant symptoms. Second, it establishes a symbiotic-like relationship, where it persists by suppressing adaptive and innate immunity. Third, quiescent lesions are metabolically active, thereby adapting to dynamic environments and balancing their populations in granulomas. Fourth, it can mimic or modify host signaling pathways and cellular functions. MDR-TB is caused by strains that are resistant to two standard drugs: Rifampicin or Isoniazid. XDR-TB is the most severe type, which is resistant to all four primary antituberculosis agents and is also a rare occurrence [2].

Over the past decade, efforts have been made to identify new drug targets and inhibitors for the TB drug pipeline. One such method is to target the energy metabolism of the bacterium, which is essential for optimal growth. Mycobacterial strains can downshift their metabolism to enter a dormant state, and in a dormant state, all the major activities including replication, nucleic acid, and protein synthesis are downregulated. Lipid metabolism is the primary source of energy in the dormant state. To achieve a dormant state, these strains can survive in human hosts for many years. The currently used anti-mycobacterial show little to no effect, as these dormant strains display very low susceptibility. These strains also survive in several niches that require energy adaptation to provide enough ATP. Therefore, ATP-synthase in bacteria may have features that help them adapt to and survive under low-energy conditions. Substrate-level and oxidative phosphorylation are two metabolic pathways linked to the generation of adenosine triphosphate (ATP).

Mycobacterium tuberculosis requires high basal energy to generate ATP [3]. Mycobacterial F_1F_0-ATP-synthase contains ATP-synthesizing(F_1) and proton trans locating (F_0) parts that use transmembrane electrochemical ion gradient to generate ATP by a rotary mechanism [4, 5]. Bedaquiline is a mycobacterial-specific ATP-synthase inhibitor that belongs to a class of diarylquinolines. In a whole-cell-based screening against *M. smegmatis,* ATP synthesis was blocked, and ATP was depleted, resulting in a pH imbalance in mycobacterial species [6]. Bedaquiline is an important medication for the treatment of MDR-TB in combination with other antituberculosis medications. However, they are also susceptible to resistance mutations and off-target effects. It increases the abnormalities in transient liver tests as well as in some cases of apparent liver injury. Bedaquiline is discontinued if there is a constant increase in liver tests, such as ALT, bilirubin, and any mild to moderate signs of liver injury; therefore, new inhibitors are needed [7].

Jamu (Javanese in origin) is an herbal medicine that is a concoction made from various parts of plants, such as roots, bark, flowers, seeds, leaves, and fruits. Indigenous to Indonesia, Jamu was prepared according to different prescriptions but was not written down but passed on between generations. The rhizomes of the plant family *Zingiberaceae* are common ingredients in all prescriptions and are safe and tolerated by people [8]. Along with these plants, *Galipea officinalis* indigenous to South America and commonly referred to as Angostura, has proven antimycobacterial activity [9]. The major phytocompounds in *Curcuma longa* and *Curcuma zedoaria* contain phenolics and terpenoids, which include diarylheptanoids called curcuminoids, diarylheptanoids, alkaloids, and monoterpenes, and the

alkaloids of *Galipea officinalis* also known as Hancock alkaloids, including cusparine, galipinine, angusterine, galipeine and N-methyl-2-quinolone [10]. Weighing the proven antimycobacterial activity of these plants against TB. Polyphenolics, phenolics, and alkaloids exert antimycobacterial activity whereas polyphenolic compounds show ATP-synthase inhibition [11]. This study focuses on the virtual screening of phytocompounds from *Curcuma longa*, *Curcuma zeodaria*, and *Galipea officinalis* which possess inhibitory action against mycobacterial ATP-synthase. We prepared a protein and ligand library for molecular docking assessed the level of inhibitory action in the docking simulation utilizing binding affinity and performed a drug-likeliness and toxicity study to identify compounds that are stable inhibitors against mycobacterial ATP-synthase enzyme.

METHODOLOGY

Preparation of Protein

Using Research Collaboratory for Structural Bioinformatics(RCSB) Protein Data Bank(PDB) (www.rcsb.org), The three-dimensional crystal structure of the target enzyme mycobacterial ATP-synthase with Protein Data Bank-Identity Document(PDB-ID) 4V1G was retrieved using the Research Collaboratory for Structural Bioinformatics Protein Data Bank (www.rcsb.org) [5]. It has three chains A, B, and C with a sequence length of 86 and a resolution of about 1.55Å. For better pose generation and docking simulation, the protein has to be prepared by removing unnecessary bonds and adding essential bonds thereby optimizing them.

Preparation of Ligands

A library of 69 phytocompounds from *Curcuma longa*, *Cucruma zedoaria*, and *Galipea officinalis* was retrieved from the literature. Their corresponding structures were downloaded from the PubChem database in a three-dimensional Structured Data Format (SDF) (https://pubchem.ncbi.nlm.nih.gov/).

Molecular Docking

PyRx v0.8 (https://pyrx.sourceforge.io/home/35-introduction/53-index) [10] is a structure-based virtual screening tool integrated with modules, such as Open Babel [12] for processing ligands and AutoDock Vina 1.12(https://vina.scripps.edu/) [13, 14] for molecular docking. Molecular docking simulates the interaction between proteins and small molecules at the atomic level to understand the biochemical actions of the complex formed. The refined 3D structures of the proteins and ligands were imported into the PyRx tool. The grid box was set to a maximum to check all the probable binding sites of the ligand. Exhaustiveness was set to eight, and docking was initialized. The results are displayed as a table containing the binding affinity and Root Mean Square Deviation (RMSD) values.

Molecular Visualization

Discovery Studio Visualizer is a leading tool for visualizing and analyzing proteins and modeling data. The protein-ligand interactions obtained after docking were visualized using Discovery Studio Visualizer 21.1. The interactions are viewed in 2D plots for the type of bonds formed between individual atoms, and in 3D plots, the extent of hydrophobicity in the protein-ligand complex was visualized, which helped us to understand the specificity of binding of the ligand to the protein and the stability of the complex once formed.

Drug-likeliness and Toxicity Prediction

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) property prediction during the early phase of drug discovery plays a significant role, as it accounts for the failure of more than 50 percent of drug molecules during the drug development process. This eventually indicates the safety and efficacy of a drug candidate. The selected molecules were subjected to ADMET analysis using SwissADME (http://www.swissadme.ch/) [15] to account for drug-likeliness. Lipinski's rule of five was used as a filter to identify drugs with optimal oral bioavailability [16]. Boiled-egg analysis was performed using SwissADME to predict the extent of absorption in the gastric area and access to the blood-brain barrier [17]. Further, toxicity prediction was done using Webserver ADMETLAB 2.0

(https://admetmesh.scbdd.com/) [18] for finding ligands establishing carcinogenic properties and filtering them out.

RESULTS

Preparation of Protein

The binding site of the enzyme was identified and refined by removing atomic clashes and adding polar hydrogens. The optimization of hydrogen bonds was also performed before docking using Discovery Studio Visualizer 21.1.

Preparation of Ligands

Ligand preparation was performed by optimization, energy minimization, and conversion of ligands to the Protein Data Bank, Partial Charge(Q), and Atom type (T) format (PDBQT) using the PyRx tool v0.8. [10].

Molecular Docking

Docking studies were carried out between the target enzyme mycobacterial ATP-synthase and 69 ligands were selected to predict the best active compounds. A total of seven ligands were selected based on the highest binding affinity for further studies, including bedaquiline, for a better comparison of results. These docked compounds were saved in the PDB file format. The top six ligands docked with the target enzyme with the highest binding affinities are listed in (Table 1).

Binding affinity: Totally six ligands with higher binding affinities were selected to perform further analysis in comparison with bedaquiline; the higher the negative binding energy, the higher the stability of the protein-ligand complex.

Molecular Visualization

Using Discovery Studio Visualizer 21.1, receptor-ligand interactions were studied for the seven docked compounds with the highest binding affinities. The 2D and 3D interactions of each docked compound were visualized to understand the intermolecular energy interactions and the residues involved in the interactions of these six compounds. The various observed interactions included van der Waals interactions, π-alkyl stacked bonds, π-alkyl bonds, π-anion bonds, and several unfavorable bonds. These bonds are represented in the 2D plot of the protein-ligand complexes in the following Figures (Figures 1–7). The 3D representations show the hydrophobic residues(brown) and hydrophilic residues(blue) present on the surface of the protein-ligand complex.

The 3D interaction represents the hydrophobicity of the protein–bedaquiline complex. The 2D plot represents the van der Waals force of the π-alkyl and π-stacked bonds. The ligand forms a bond with residues, such as Ala38, Leu53, Arg52, and Pro56 in chain C.

The 3D structure depicts the hydrophobic surface of the protein-beta-sitosterol complex. The 2D plot represents the bonds formed between the residues in chains A and B.

Table 1. Phytocompounds having the highest binding affinity with the target enzyme, mycobacterial ATP-synthase.

S.N.	PubChem ID	Phytocompound	Source	Binding affinity (Kcal/mol)
1	CID_222284	Beta-sitosterol	*Galipea officinalis*	-7.0
2	CID_5280794	Stigmasterol	*Curcuma zeodoaria*	-7.0
3	CID_442893	Cusparine	*Galipea officinalis*	-6.9
4	CID_5388906	Bedaquiline	*Diarylquinolines*	-6.8
5	CID_173183	Campesterol	*Curcuma zeodoaria*	-6.6
6	CID_69879809	Cyclocurcumin	*Curcuma longa*	-6.5
7.	CID_163184282	Galipeine	*Galipea officinalis*	-6.4

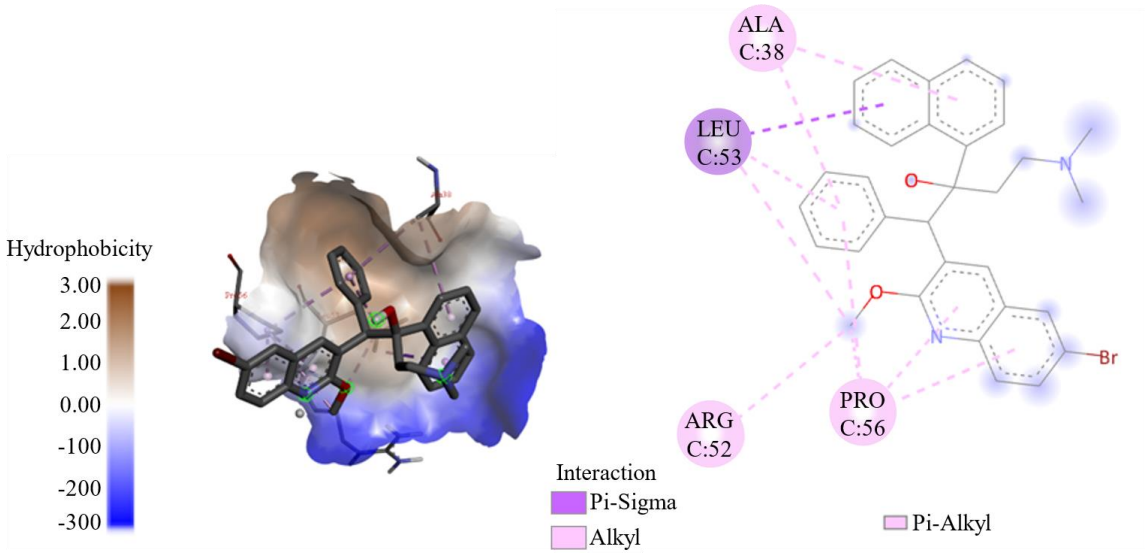

Figure 1. 3D and 2D interactions between bedaquiline with mycobacterial ATP-synthase.

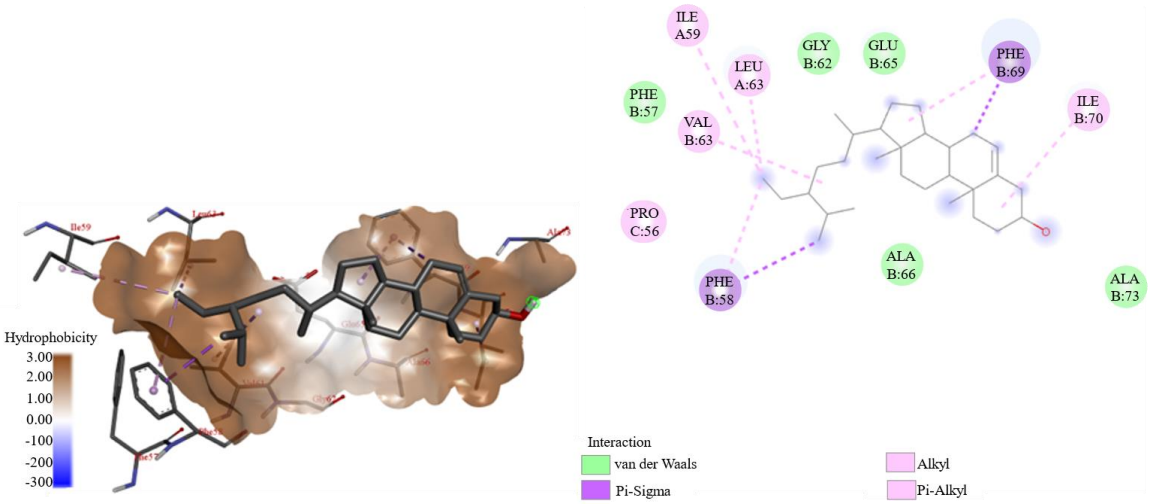

Figure 2. 3D and 2D interactions between beta-sitosterol and mycobacterial ATP-synthase.

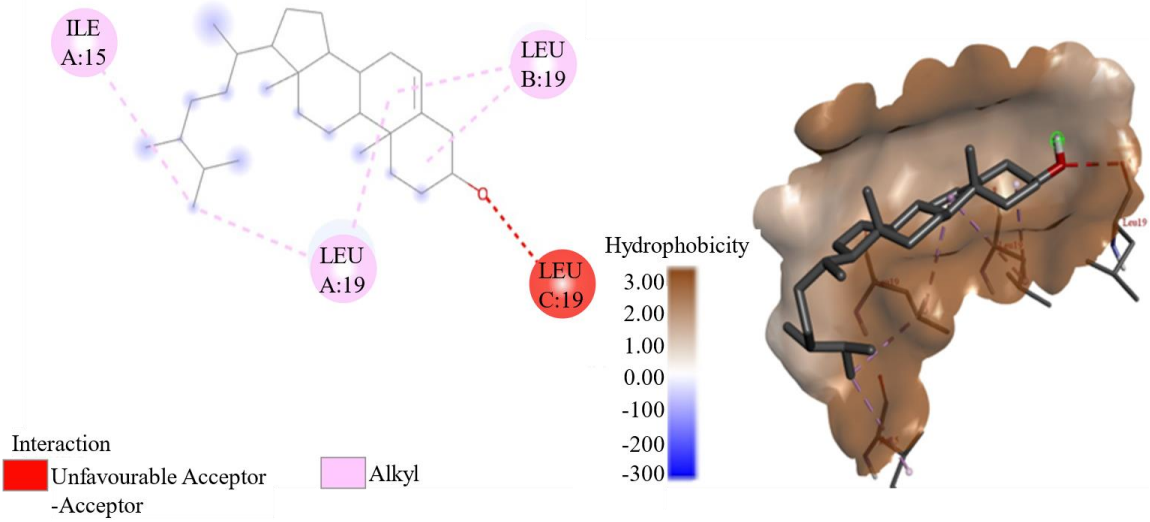

Figure 3. 2D and 3D interactions of campesterol with mycobacterial ATP-synthase.

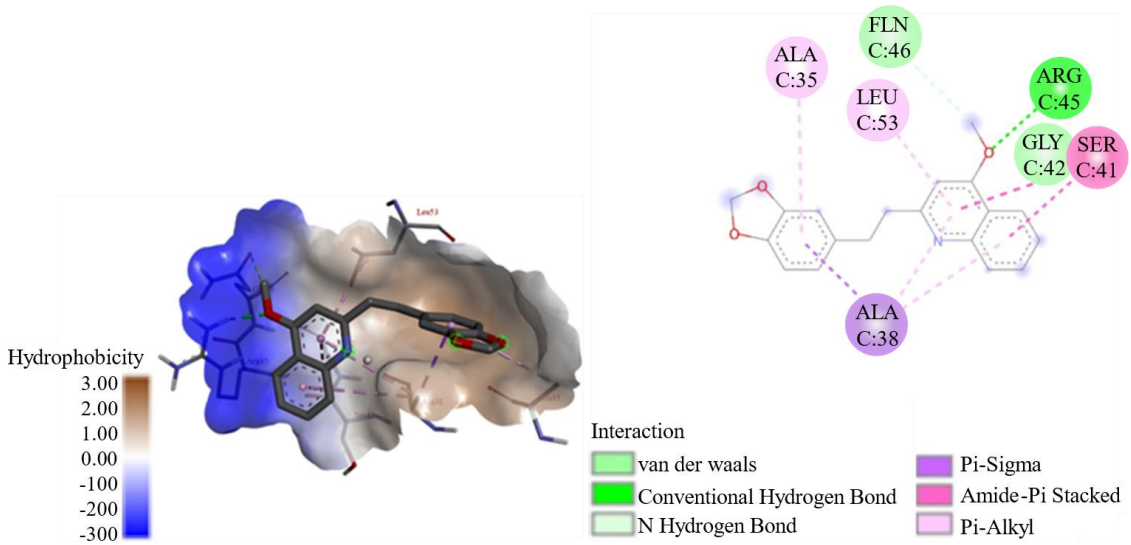

Figure 4. 3D and 2D interactions of cusparine with mycobacterial ATP-synthase.

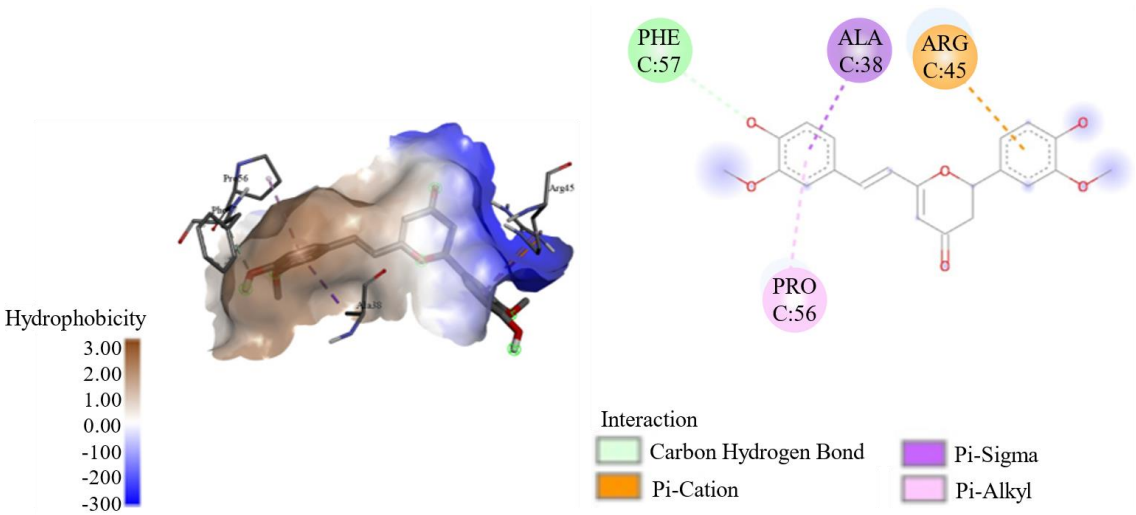

Figure 5. 3D and 2D interactions between cyclocurcumin with mycobacterial ATP-synthase.

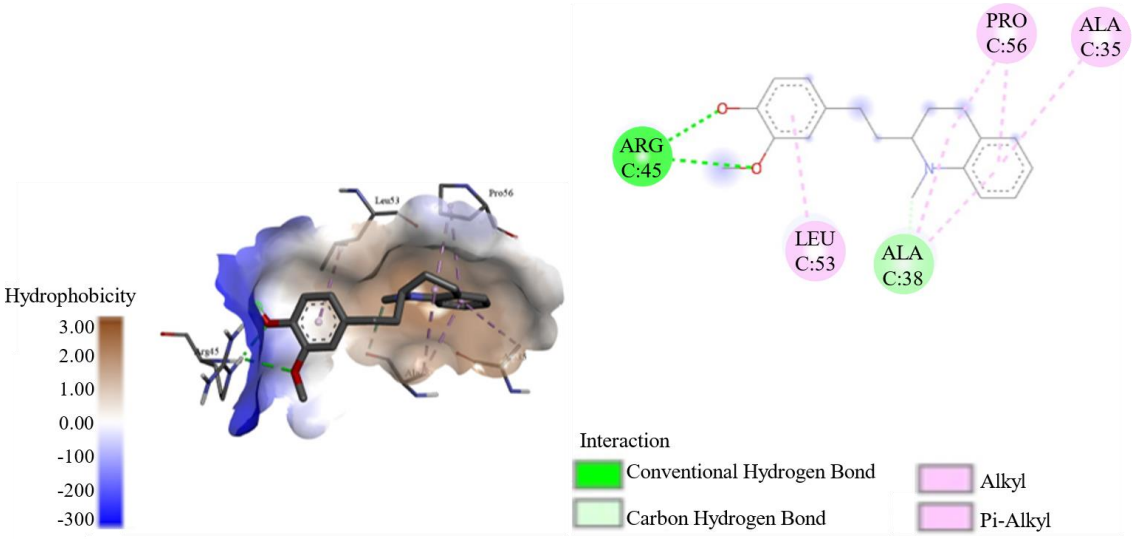

Figure 6. 3D and 2D interactions of galipeine with mycobacterial ATP-synthase.

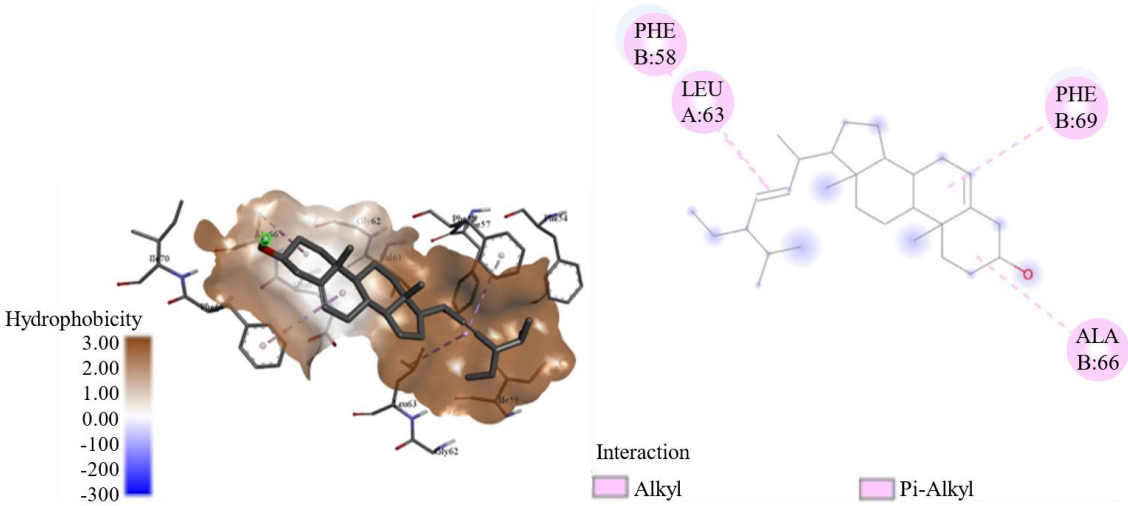

Figure 7. 3D and 2D interactions between Stigmasterol with mycobacterial ATP-synthase.

The 2D plot shows the interaction of the ligand with protein residues IleA15, LeuA19, and LeuB19 in chains A and B. The 3D plot represents the nature of the hydrophobic surface in the protein-campesterol complex.

The 3D structure depicts the hydrophobic surface of the protein-cusparine complex, and the 2D plot represents the bonds formed with residues in chain C. Interaction between the amino acid residues is fairly similar to that of the bedaquiline-sharing residues Ala38 and Leu53 forming pi-alkyl bonds.

The 3D structure depicts the hydrophobic surface of the protein–cyclocurcumin complex. The 2D plot represents the bonds formed with residues in chain C. It is also fairly similar to that of the bedaquiline-sharing residues Ala38 and Pro56 forming pi-sigma and alkyl bonds.

The hydrophobic surface of the protein-galipeine complex is represented in the 3D plot. The 2D plot depicts the interactions that also share common amino acid residues with bedaquiline, namely Leu53, Ala38, and Pro56, in chain C.

The 3D structure depicts the hydrophobic surface of the protein-stigmasterol complex. The 2D plot represents the bonds formed between the residues in chains A and B.

Drug-likeness and Toxicity Prediction

The drug-likeliness of the screened compounds was evaluated using Lipinski's rule. All six compounds complied with this rule (Table 2). ADMET analysis using ADMETLAB and SwissADME predicted the physiochemical properties and also evaluated gastrointestinal absorption (HIA) and passive blood-brain barrier access of the six compounds. Along with those criteria for their solubility nature (LogS) [19], Permeability glycoprotein (PgP-Sub) [20] accounting for the elimination of drugs from cells and blood-brain barrier and carcinogenicity for ruling out potential carcinogens (Table 3).

DISCUSSION

Tuberculosis has a long-standing and challenging history given its unique etiological features and complications. To combat the rising cases of drug-resistant TB, new lines of drugs are essential; one such method is to make use of chemical libraries to virtually screen them for target and wipe out unfavorable drug candidates, resolving the failure rate of drugs at the end of drug pipelines due to unfavorable ADMET and physiochemical properties; mycobacterial ATP-synthase is an enzyme responsible for oxidative phosphorylation and was found to be a novel target with a diarylquinoline, bedaquiline, which has greater potential to block ATP synthesis against M. smegmatis model.

Table 2. Evaluating docked compounds using Lipinski's rule of five.

S.N.	Ligand	Molecular Weight(g/mol)	H-bond donors	H-bond acceptors	LogP	Molar Refractivity
1	Cusparine	307.34	0	4	3.79	88.56
2	Cyclocurcumin	368.38	2	6	2.82	100.78
3	Campesterol	400.68	1	1	6.90	128.42
4	Beta-sitosterol	414.71	1	1	7.19	133.23
5	Galipeine	297.39	1	2	3.60	93.86
6	Stigmasterol	412.69	1	1	6.97	132.75
7	Bedaquiline	555.50	1	4	6.25	155.36

Criteria for Lipinski's rule: Orally active drug with no more than one violation in the following criteria: H-bond acceptors ≤10; H-bond donors ≤5; molecular weight ≤500; octanol-water partition coefficient (logP) ≤5.

Table 3. ADMET analysis using ADMETLAB 2.0 Webserver.

S.N.	Ligand	LogS	HIA	Pgp-sub	BBB	Carcinogenicity	Lipinski's violations
1	Cusparine	-5.679	0.002	0.007	0.368	0.888	0 violation
2	Cyclocurcumin	-4.222	0.038	0.006	0.083	0.677	0 violation
3	Campesterol	-7.006	0.004	0.001	0.854	0.067	1 violation
4	Beta-sitosterol	-7.052	0.004	0.001	0.84	0.047	1 violation
5	Galipeine	-7.052	0.004	0.001	0.84	0.318	0 violation
6	Stigmasterol	-6.978	0.005	0.001	0.691	0.054	1 violation
7	Bedaquiline	-6.907	0.006	0.001	0.871	0.032	2 violations

ADMET analysis: Drug-likeliness, safety, and toxicity were determined based on biochemical properties. Galipeine and Cyclocurcumin showed good results among the six compounds in safety and toxicity.

Mycobacterial ATP-synthase is essential for optimizing energy conditions for survival. Inhibiting this enzyme depletes ATP synthesis, and thereby, the survival of the cell. People who consume bedaquiline are at a fold higher risk of death than placebo controls due to the possible action of human ATP-synthase. Therefore, there is a need to identify alternative small molecules for the treatment of TB.

Cyclocurcumin extracted from *Curcuma longa* being a derivative of curcumin, exhibits anti-inflammatory, anticancer, and immune-modulating properties. Cusparine is one of the five quinoline compound alkaloids derived from the plant *Galipea officinalis*, which has clinically shown actions against *M. tuberculosis* predominantly by the basic alkaloidal fractions with campesterol, beta-sitosterol, and stigmasterol, which are essential biomolecules with known anti-inflammatory, anti-anti-diabetic, and anticancer properties. The hindrance is they are found in low concentrations in plants as well as they are not economically lucrative. However recent developments allow us to exploit the use of recombinant DNA technology to increase the existing level of phytosterols in plants. However, all the chemically active constituents in these plants are not fully extracted or studied about their true actions, and structure therefore still being an uncharted realm in research. The three plants *Curcuma longa*, *Curcuma zedoaria*, and *Galipea officinalis* whose parts had been used for treating TB for many generations.

The results of docking were sorted out by observing the RMSD values and binding affinity: RMSD accounts for the difference between the initial structural conformation and the final docked conformation in the backbones of the protein. RMSD signifies the stability of the protein-ligand complex by determining the deviations produced before and after simulation; smaller deviations give more stable complexes; almost all the complexes were below 2Å, and the binding energy is an efficiency parameter for protein-ligand interactions. The binding energy signifies the strength of the protein and ligand interaction, and this docking study revealed that out of 69 docked compounds,6 of them showed greater binding efficiency ranging from -6.4 to -7.0 kcal/mol. The more negative the binding energy,

the greater the strength of the interactions; the seven docked compounds are cusparine, cyclocurcumin, campesterol, beta-sitosterol, galipeine, bedaquiline, and stigmasterol.

The docked compounds were subjected to further visualization of interactions. The 2D plot is used to identify the no. of residues of enzymes that formed bonds with ligands. The 3D representation used here determines the hydrophobicity of the protein-ligand surface. Usually, stronger hydrophobic clusters are found in the binding site of the protein. Surface hydrophobicity identifies regions of a protein favorable for interacting with a ligand for a possible complex. The positive number depicts higher hydrophobicity shown by the residues in the binding pockets. This helps to visualize the extent of hydrophobicity displayed by the surface of the complex.

The known inhibitor, bedaquiline, interacts with chain C in the enzyme, thereby blocking the rotation of chain C and inhibiting ATP synthesis in the enzyme's catalytic headpiece. Compounds that inhibit the C chain include cyclocurcumin, galipeine, and cusparine. It is suggested that by binding to the C chain, these compounds can inhibit the enzyme's F-ATP activity.

All the compounds were tested to comply with Lipinski's rule for an orally active biomolecule. In ADMET analysis, LogS accounts for solubility value, an essential and early measurement property to check whether the molecule has low solubility and complete oral absorption. PgP-sub attribute has pharmacokinetic aspects in modulation of the P-glycoprotein mediated transport. P-glycoprotein is involved mainly in drug transport to many organs. If drugs act as substrates, there will be reduced bioavailability, it also limits penetration to the blood-brain barrier and sensitive tissues. HIA accounts for human intestinal absorption and is proven to have a closer relationship with oral bioavailability. Both Pgp-sub and HIA account for the absorptive characteristics of the drug. For the blood-brain barrier, Drugs with action in the periphery, need little to no blood-brain barrier penetration to avoid any other effects. Carcinogenicity is used to determine whether a given molecule is a potential carcinogen. After analyzing these properties, molecules galipeine and cyclocurcumin showed good binding affinity, safety, and efficacy through ADMET analysis. To determine the best of all, the toxicity results showed better results for galipeine based on the ADMET parameters.

CONCLUSION

This study aims to bring out the phytocompounds from the traditional folklore medicinal plants and identify the ingredients responsible for their actions against the enzyme. Tuberculosis remains an active infection without causing any significant symptoms in healthy hosts, but it triggers the disease in immunocompromised hosts. As discussed earlier, tuberculosis forms a commensal then a symbiotic relationship with the host thereby leading to host immune ignorance. The adaptability, survival, and evolution of *Mycobacterium tuberculosis* a substantial factors in bringing out new drugs. Not only novel drugs but also potential drug targets that are well-studied structures which provides insights on how the drug works dynamically in our body. The still ongoing research for an archaic disease is to find perennial, fail-safe drugs that have greater therapeutic potential but also minimize the risks in our body and probably can shorten the course of the drug regimen. Phytocompounds are exploited in various arenas of research, but their usage is limited only to certain diseases due to their natural existence or due to synthesizing difficulties. Alternative medicine has greater probabilities since it was used for many decades before the evolution of modern medicine. Most medicines prepared are commonly found in nature but they are altered by adding additional constituents to further increase their therapeutic potential. The discussed compounds, stigmasterol and cyclocurcumin may be optimized to a good inhibitor and can be used in treating tuberculosis in alternative medicine.

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Authors Contribution

The authors confirm their contribution to the paper as follows: study conception and design: Shubham Wanarase and Priyanka Raja; data collection and analysis: Priyanka Raja; interpretation of results: Shubham Wanarase and Priyanka Raja; and draft manuscript preparation: Priyanka Raja. All authors have reviewed the results and approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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