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Alkaloids Lead to Potential Inhibition of the Acyl Carrier Protein Reductase to Attenuate Tuberculosis; an *in-silico* Analysis

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Abstract

Tuberculosis (TB) is a contagious infection that mostly affects the lungs. *Mycobacterium tuberculosis* causes tuberculosis infection, leading to granulomatous lesions in affected lung tissue. It is one of the most prevalent and deadly infectious diseases among the under developed countries. This study aims to investigate the possible inhibition of the acyl carrier protein reductase for preventing tuberculosis by well-known alkaloids, thereby reducing *Mycobacterium tuberculosis* growth in the lungs and thereby reducing the incidence of latent and active TB. About five natural alkaloids were subjected to the molecular docking analysis, which produced favorable findings in terms of best pose and binding energies of these compounds towards the active residues of mycobacterial ACP reductase, with values ranging from -10 kcal/mol to -9.1 kcal/mol. The molecular dynamics simulation produced similar encouraging results. All of the prospective alkaloid compounds were subjected to an *in-silico* toxicity investigation, which determined that every compound was safe and non-toxic. Further studies may be necessary for effective formulation development employing these compounds as part of the process of drug discovery and development. The findings from this study may be helpful in the development of the novel nanoformulations using natural products for pharmacotherapy of tuberculosis infection.

Keywords: Tuberculosis; alkaloids; ACP reductase; molecular docking analysis; *Mycobacterium tuberculosis*.

Abbreviations: MTB: *Mycobacterium Tuberculosis*, COVID-19: Corona Virus Disease 19, HIV: Human Immunodeficiency Viruses, AIDS: Acquired immunodeficiency syndrome (AIDS), ACP: Acyl carrier protein, PDB: Protein Data Bank.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by the bacteria *Mycobacterium tuberculosis.* Globally, tuberculosis is the 13th leading cause of death after COVID-19 (ahead of HIV and AIDS) and the second infectious disease killer. In 2021, approximately more than 10 million people worldwide were infected with tuberculosis. This infection affects the people of all age groups in many countries (WHO, 2023).

Mycobacterium tuberculosis (MTB) has a unique cell envelope that is linked to its pathogenicity. The presence of trehalose dimycolate (TDM), a free glycolipid that accumulates in a cord-like pattern on the surface of the cells and protects the tubercle bacillus from harmful agents and the host's immune system, is another feature of the cell envelope that is very noticeable. TDM is covalently linked to the cell wall peptidoglycan via an inner leaflet of the outer membrane by a phosphodiester bond. The primary components of this protective layer are mycolic acids. More precisely, the cyclopropane rings in mycolic acid layer help maintain the complex's structural integrity and shield the bacillus from oxidative stress (Takayama et al, 2005). The development of an immune response is necessary for the control of MTB infection in individuals who are resistant to the infection. This type of response entails the involvement of resident alveolar macrophages, dendritic cells, T lymphocytes (TCD4+, TCD8+ cells) and the release of pro-inflammatory cytokines such as interferon-gamma (IFN-γ), interleukin-2 (IL-2), IL-12, IL-18, and tumor necrosis factor –alpha (TNF- α). All of these are crucial for attracting more immune cells to the infection site to create a granuloma that traps and destroys tuberculosis bacilli while also providing the long-term niche required for latent tuberculosis infection (Druszczyńska, Kowalewicz-Kulbat, Fol, Włodarczyk, & Rudnicka, 2012). If the ongoing bacterial reproduction is not stopped, the bacilli and expanding tubercle may invade the nearby draining lymph nodes. As a result, lymphadenopathy develops, which is a typical sign of primary TB. If the lesion caused by the tubercle's expansion progresses to the lymph node and lung parenchyma, a Ghon complex may form. The Ghon focus, which describes the main TB infection, is often seen in the center of the lungs and is a hallmark of TB

infection. Latent TB reactivates into active tuberculosis in the presence of immunosuppression in the host (Ruiru & Isamu, 2013).

Changes in the host's physiological and immunological condition brought on by aging, malnutrition, diabetes or the emergence of other illnesses, including HIV/AIDS, as well as latent infections, might trigger their activation. The use of many antibiotics given over the course of six months is required for chemotherapy for active TB caused by drug-sensitive bacteria. Patient compliance is typically low as a result of this difficult and potentially hazardous treatment regimen. As a result, antibiotic-resistant bacteria have developed, necessitating lengthier treatment regimens, the use of less effective and more toxic medications, and increasing failure rates (Reddy et al., 2008). The aim of this study is to analyze the anti-TB effects of several natural alkaloids and their potential to weaken the pathogenesis of tuberculosis using *in-silico* tools. In order predict the potential activity of alkaloids against the active TB infection, this study utilized molecular docking and molecular dynamics simulation as methodological techniques.

The pathophysiology of tuberculosis infection is depicted in figure 1.

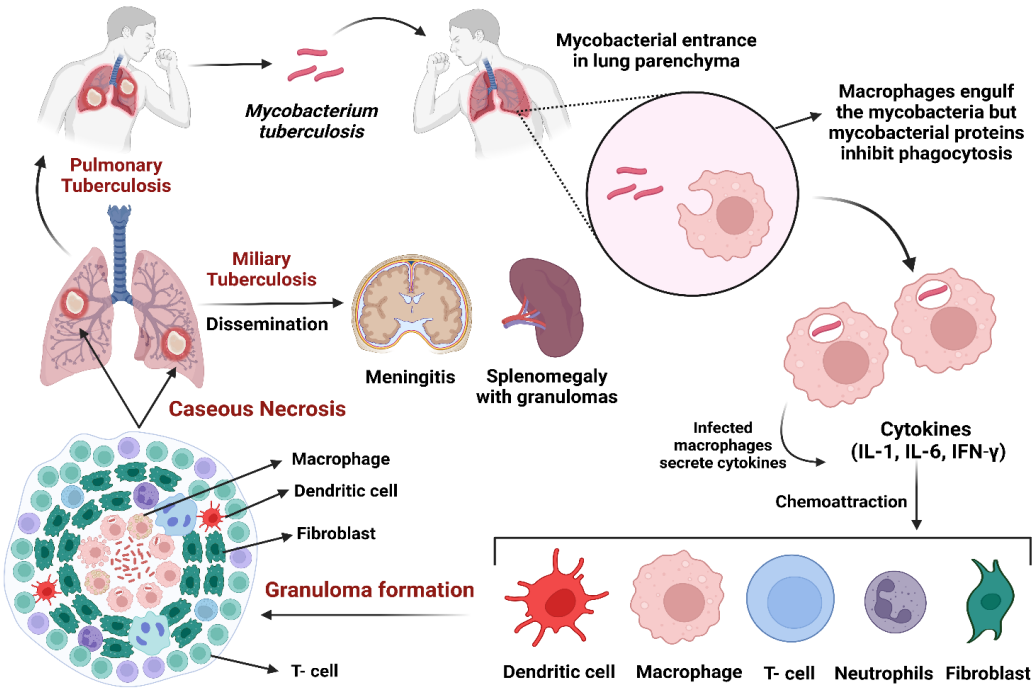


Figure 1. The figure illustrates the pathogenesis of tuberculosis infection caused by Mycobacterium tuberculosis.

MATERIAL AND METHODS

Macromolecule Preparation

RCSB (Protein Data Bank) was used to retrieve the 3D Structure of mycobacterial ACP (acyl carrier protein) reductase enzyme (PDB ID: 4R9S) in protein data bank (PDB) format. PDB contains the structure elucidation data of proteins obtained by X-ray crystallography and nuclear magnetic resonance (NMR) spectrometry submitted by biologists and biochemists from all over the world. Macromolecule was prepared by removing water molecules, heteroatoms, ligands, extra protein chains, and metal ions that could interact with compounds during visualization of docking between compounds and target proteins using BIOVIA Discovery Studio Visualizer v17.2.0.16349 (Sharma et al, 2021).

Ligand Preparation

The compounds used as ligands for the antimicrobial activity were from the alkaloidal class of phytochemicals. The structures of these compounds were downloaded from the PubChem database and Chem3D 16.0 was used to obtain the PDB format of these 3D-structured compounds. C1 (Protopine), C2 (Apropine), C3 (Avicine), C4 (Penduline), and C5 (Chelerythrine) were used to study the potential inhibition of mycobacterial acyl carrier protein reductase for preventing tuberculosis infection.

These five compounds were used against the target protein to check the interaction of the target protein with these compounds (C1- C5). Figure 2 shows the graphical representation of the complete methodology of the research study.

The chemical structures and botanical sources of compounds (C1 - C5) are provided in table 1 while figure 3 depicts the IUPAC names of the compounds C1 – C5.

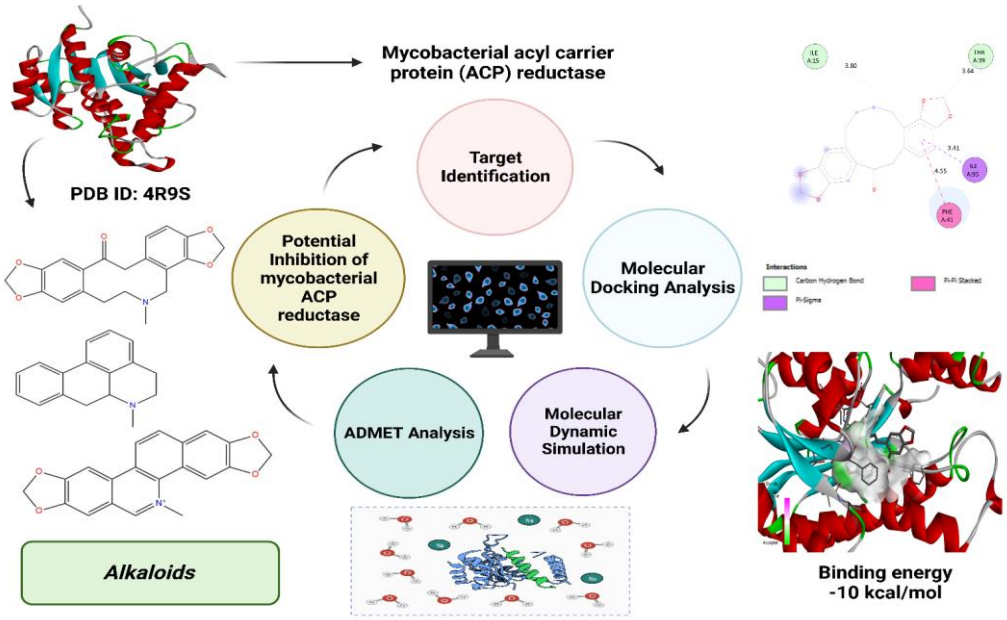
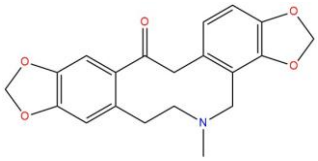
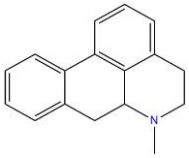
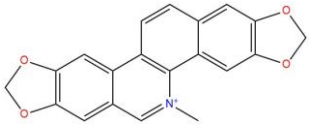
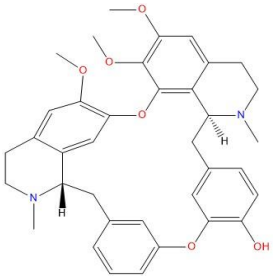
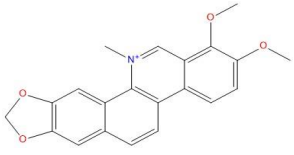


Figure 2. The figure shows the step by step methodology of the research study.

Table 1. The table shows the names of alkaloids employed in this study, their PubChem ID, chemical structures and botanical sources.

Compound	Compound Name	PubChem ID	Structure	Botanical Source	Literature Source
C1	Protopine	4970		*Corydalis heterocarpa var. japonica*	PubChem
C2	Aporphine	114911		*Antizoma angustifolia*	PubChem
C3	Avicine	356657		*Zanthoxylum asiaticum*	PubChem
C4	Penduline	179472		*Leptopus cordifolius*	PubChem
C5	Chelerythrine	2703		*Zanthoxylum fagara*	PubChem

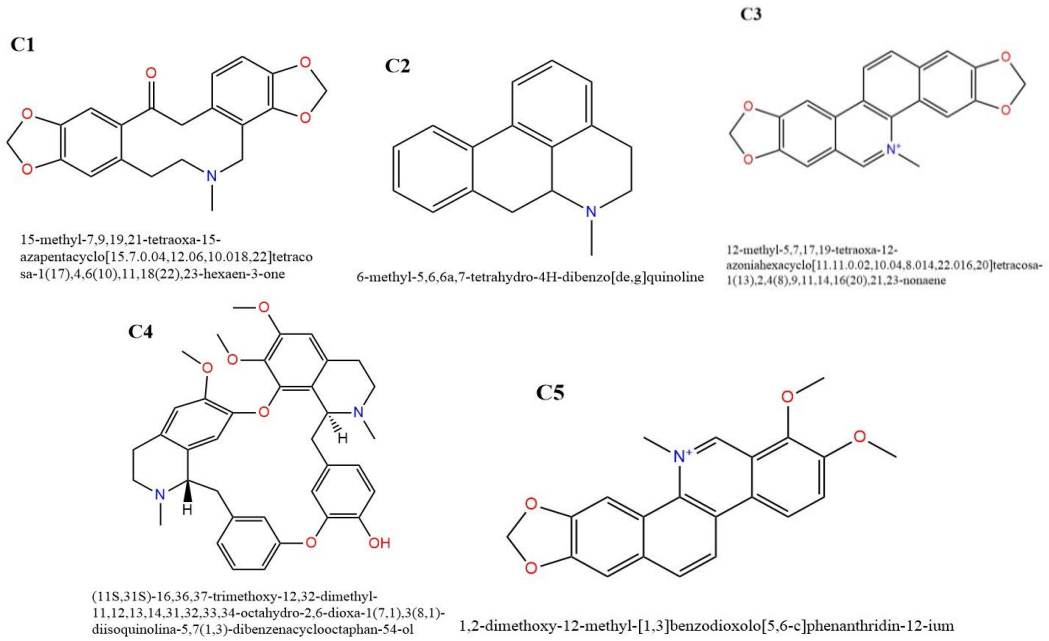


Figure 3. The figure demonstrates the chemical structures of compounds C1 to C5, along with their IUPAC names obtained from PubChem.

RESULTS AND DISCUSSION

Molecular Docking Analysis & Results

In structural molecular biology, medicinal chemistry and computer-assisted drug development, molecular docking is a useful technique. Predicting the prevailing binding modes and poses of a compound that interacts with a protein having a known three-dimensional structure is the major aim of compound-protein docking. Successful docking methods employ a scoring system that accurately ranks the drug candidate dockings and efficiently explores binding affinities and best poses. Lead optimization greatly benefits from the use of docking to do virtual screening to provide insights for how the ligands interfere with the target (Fan, Fu, & Zhang, 2019). PyRx was utilized for this experiment. PyRx includes a docking wizard with an easy-to-use user interface, which makes it a valuable tool for Computer-Aided Drug Design (CADD). PyRx also includes chemical spreadsheet-like functionality that helps in novel drug molecule designing (Dallakyan & Olson, 2015). Software version 17.2.0.16349 of BIOVIA Discovery Studio Visualizer was used. It offered a clear depiction of the 2D and 3D interactions as well as bond lengths, aromatic, hydrophobic, and other interactions. Results from the molecular docking studies of the mycobacterial ACP protein with Compounds C1–C5 were valuable, as shown in Table 2.

Table 2. The table shows the findings and results of the molecular docking analysis done using compounds C1 to C5.

Compound-Protein Interaction	Binding Energy (kcal/mol)	Bonding Residues	Type of bond	Bond Length (Å)	Other Residues
C1	-10	Phe 41	Pi Pi	4.55	Ile 15
		Ile 95	Pi Sigma	3.41	
		Thr 39	Carbon hydrogen	3.64	
C2	-9.8	Phe 41	Pi Pi Stacked	3.74	Val 65, Ile 122.
		Ile 95	Pi Alkyl	5.23	
C3	-9.7	Phe 41	Pi Pi Stacked	4.26	Ile 122, Ile 16
		Ile 95	Pi Sigma	3.86	
		Asp 64	Carbon hydrogen	3.27	
		Ser 20	Conventional Hydrogen bond	2.87	
C4	-9.6	Phe 97	Pi Pi T shaped	5.92	Leu 207, Met 103, Ile 21, Leu 207
		Ile 16	Pi Sigma	4.00	
C5	-9.1	Phe 41	Pi Pi Stacked	4.30	Gly 14, Ile 122 , Phe 97
		Ile 16	Pi Sigma	3.78	
		Asp 64	Carbon Hydrogen Bond	3.44	
		Ser 20	Conventional Hydrogen bond	3.26	

All the compounds C1 to C5, exhibited their firm interactions with the binding site residues of mycobacterial ACP reductase enzyme receptor i.e., Phe 41, Ile 95, Ile 16 and Ser 20 with a binding pose ranging from – 10 to -9.1 kcal/mol. The best binding pose with binding energy of – 10 kcal/mol was expressed by Protopine (**C1**), while the binding energies exhibited by other compounds in this study were -9.8 kcal/mol for Aporphine (**C2**), -9.7 kcal/mol for Avicine (**C3**),-9.6 kcal/mol for Penduline (**C4**) and -9.1 kcal/mol for Chelerythrine (**C5**).All the compounds showed absolute interactions with the active site residues of mycobacterial ACP reductase enzyme. Protopine (**C1**), Aporphine (**C2**), and Avicine (**C3**) were bound to Phe 41 and Ile 95 residues with lower binding energies. Penduline (**C4**) and Chelerythrine (**C5**) manifested binding with Phe 97, Ile 16, Phe 41, Ile 16, Asp 64, Ser 20 residues with reasonable bond lengths. Compounds Protopine (C1) and Aporphine (C2) also exhibited firm interactions with other residues including Ile 15, Val 65, and Ile 122, that may also be the active site amino acid residues of mycobacterial ACP reductase enzyme. Avicine (C3), Penduline(C4) and Chelerythrine (C5) demonstrated robust binding affinities with other active residues Ile 122, Ile 16, Leu 207, Met 103, Ile 21, Leu 207 and Gly 14, Ile 122, Phe 97 with substantially shorter bond lengths. Figure 4 demonstrates the interactions of all compounds with mycobacterial ACP reductase enzyme.

ADMET analysis

Absorption, distribution, metabolism, elimination and toxicity (ADMET) analysis is essential for navigating the pharmacodynamics and pharmacokinetics of a potential drug candidate. *In-silico* ADME parameters were studied using Swiss ADME. The druglikeness score and toxicity profile of the potential drug candidates was probed using DataWarrior V5.5.0. Table 3 shows the pharmacokinetic and toxicity parameters of the potential drug candidates from C1 to C5. Figure 5 manifests the bioavailability radar of best docked ligand, compound C1, obtained from SwissADME tool.

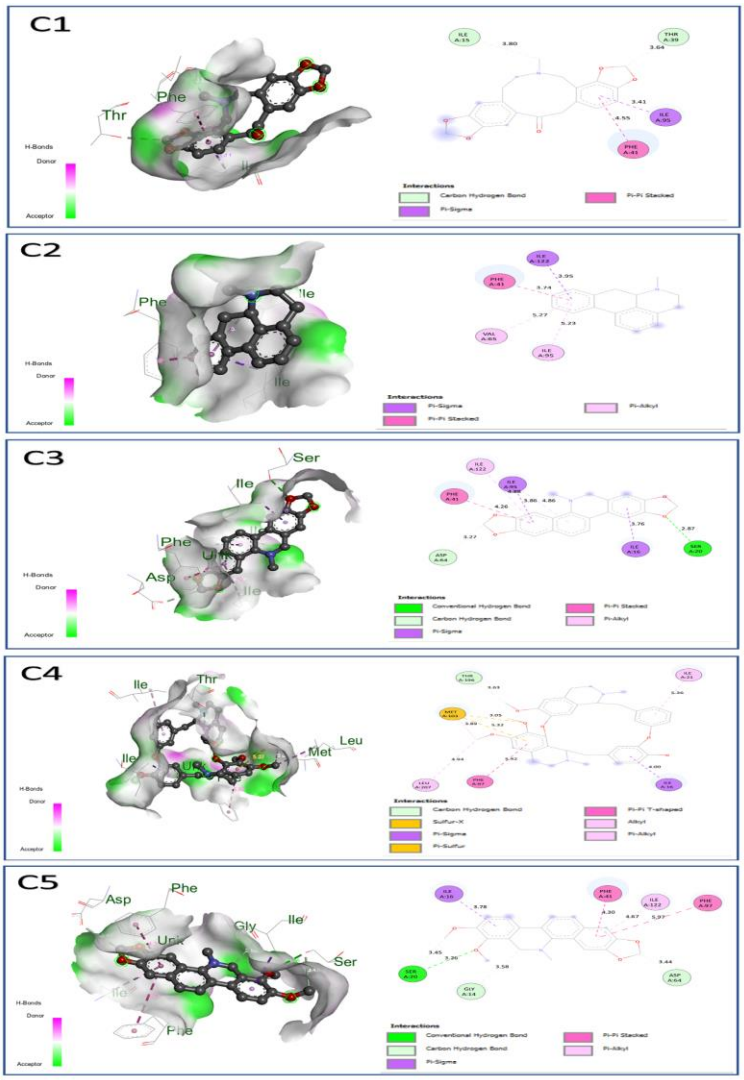


Figure 4. The figure displays the 3D and 2D images of the interactions between active amino acid residues of mycobacterial acyl carrier protein reductase (PDB ID: 4R9S) and compounds (C1, C2, C3, C4 and C5).

Table 3. The table provides the pharmacokinetic and toxicity profile of compounds (C1 – C5) using SwissADME and DataWarrior tools.

ADMET PROFILING OF THE POTENTIAL DRUG CANDIDATES (C1 – C5)					
Potential Drug Candidates	**C1**	**C2**	**C3**	**C4**	**C5**
Physicochemical Properties					
Molecular Weight (g/mol)	353.37 g/mol	235.32 g/mol	332.33 g/mol	608.72 g/mol	348.37 g/mol
Log P	2.67	3.23	2.84	5.17	3.02
H-Acceptor	6	1	4	8	4
H-Donor	0	0	0	1	0
N.R.B	0	0	0	3	2
TPSA (Å²)	57.23 Å²	3.24 Å²	40.80 Å²	72.86 Å²	40.80 Å²
Bioavailability score	0.55	0.55	0.55	0.55	0.55
Drug-likeness parameters					
Lipinski's rule violations	0 violations	0 violations	0 violations	1 violation	0 violation
Drug-likeness score	4.7237	4.9313	- 2.4707	4.6261	- 2.1842
Metabolic profiling					
p-glycoprotein substrate	Yes	Yes	Yes	No	Yes
GI Absorption	High	High	High	High	High
CYP 3A4	Yes	No	No	No	Yes
CYP 2D6	Yes	Yes	No	No	No
CYP 1A2	Yes	No	Yes	No	Yes
CYP 2C9	Yes	No	No	No	Yes
CYP 2C19	No		No	Yes	No Yes
Toxicity profiling					
Tumorgenicity	No		No	No	No No
Mutagenesis	No		No	No	No No
Irritant	No	No	No	No	No
Reproductive effects	No	No	No	No	No

Mol. W (g/mol) Molecular Weight, Log P prediction of octanol/water partition coefficient, N.R.B Number of Rotable Bonds, TPSA (Å²) Topological Polar Surface Area, CYP Cytochrome P-450 enzyme.

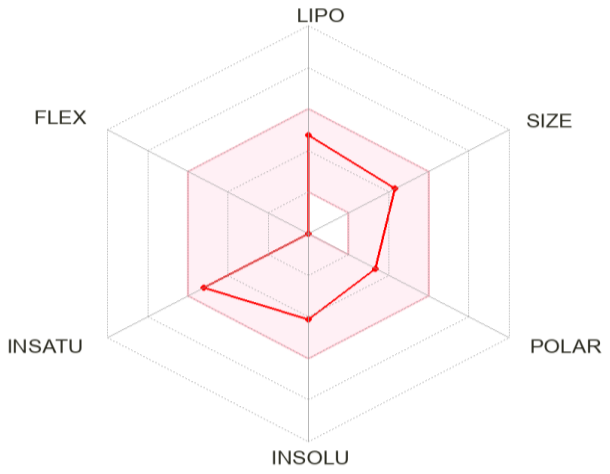


Figure 5. The figure the bioavailability radar of ligand C1 (having the most favorable binding energies as per the findings of molecular docking).

Molecular Dynamic Simulation

Molecular dynamic (MD) simulations depict the dynamics of a macromolecule bound by a ligand, interactions between the amino acid residues and navigating the configurations of a protein molecule (Y. Wang, Ribeiro, & Tiwary, 2020). MD simulations unveil the dynamics of a protein and assist in discovering the cryptic sites in a protein macromolecule that may have affinity towards a ligand, thereby facilitating its binding to the hydrophobic pocket (Kuzmanic, Bowman, Juarez-Jimenez, Michel, & Gervasio, 2020). MD simulations were done using the online MD simulation tool, iMODS server (López-Blanco, Aliaga, Quintana-Ortí, & Chacón, 2014; Lopéz-Blanco, Garzón, & Chacón, 2011). The ligand and macromolecule docked complex C1 (Protopine) was loaded on iMODS server and MD simulation was performed on Normal Mode Analysis (NMA). The B-factor yielded information regarding the flexible nature of the macromolecule. It illustrates the deforming tendency of the protein residues in order to interact with protopine molecule. The arrow field shows the orientation of the macromolecule in the virtual experiment. The figures 6,7 and 8 provide the graphical presentation of the finding of molecular dynamic simulation analysis.

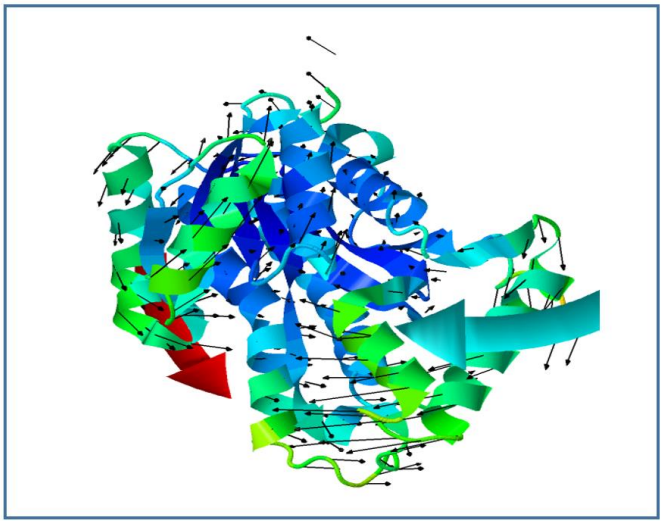


Figure 6. The figure illustrates the orientation of the docked complex (C1 and macromolecule) obtained through molecular dynamic simulations using iMODS.

The eigenvalue for the complex in this virtual experiment was measured as 5.340225e-04. The eigenvalue manifests the energy required to deform a particular amino acid residue while interacting with other residues in a real-life environment created virtually. Variance is inversely proportional to eigenvalue and the comparison between the two is shown in the figure.

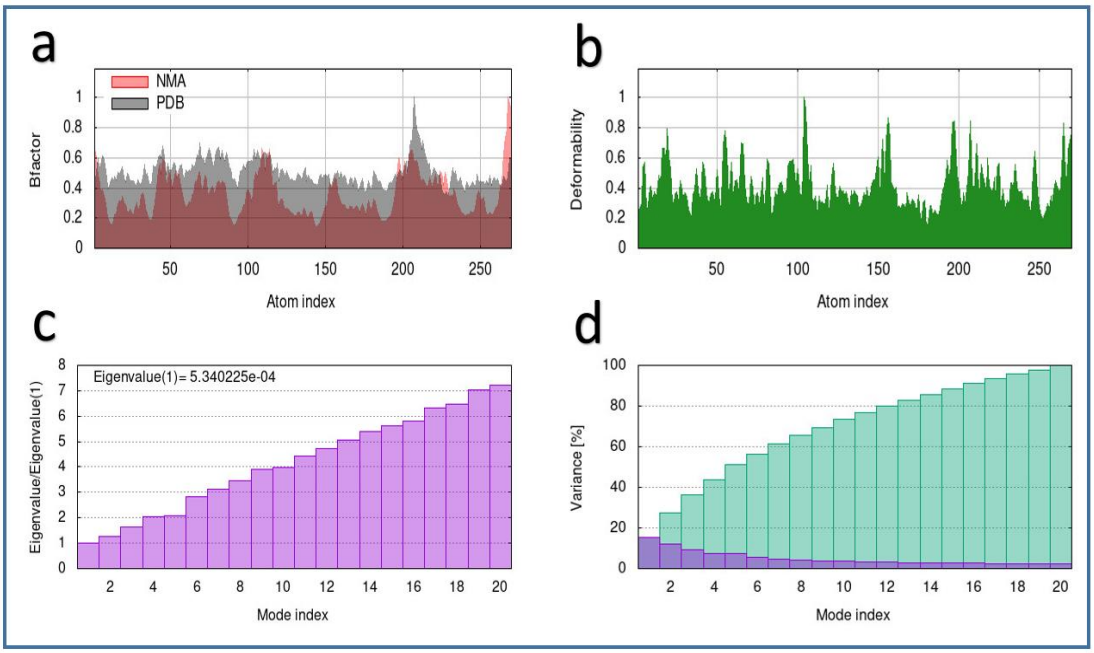


Figure 7. The figure shows the findings of molecular dynamic simulation i.e. Image (a) shows the deformability of the docked complex. Image (b) provides the graphical view of the B- factor. Image (c) provides the eigenvalue for the complex while the Image (d) demonstrates the percentage variance.

The covariance map displays the interactions between the residues of the dynamic protein molecule, with red areas showing the correlating or interacting residues, white areas demonstrating the residues with no interactions, and blue areas showing residues with repulsive behaviour within the protein molecule. The elastic network provides an estimate regarding the ease of deformation of the target amino acid residues within the macromolecule. The dark greyish areas show residues with a higher degree of stiffness, while the lighter areas depict ease of deformation of the target residues.

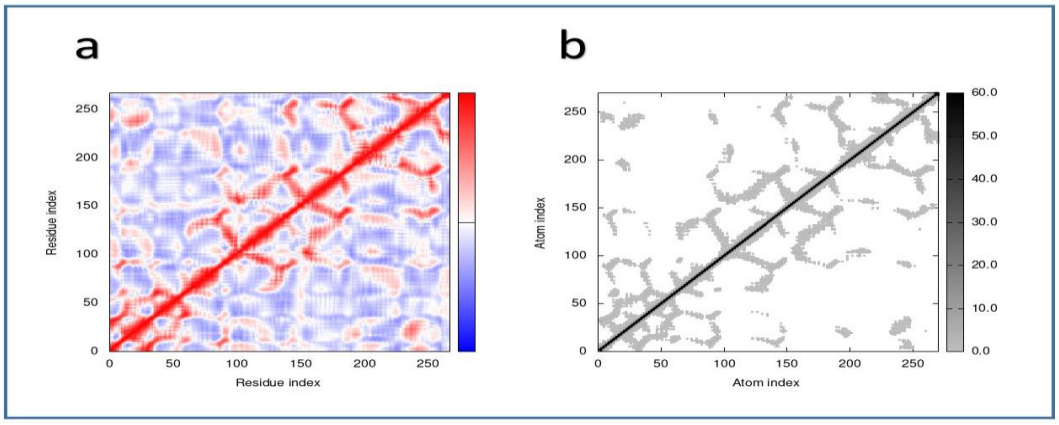


Figure 8. The Image (a) displays the covariance graph of the complex (C1 and macromolecule) while Image (b) represents elastic network map of the complex.

Discussion

In this study, five alkaloids were screened computationally for their potential to inhibit the mycobacterial acyl carrier protein reductase and attenuate tuberculosis. Molecular docking and scoring is a reasonable method to analyse various drug candidates for their potential for particular biological activity before entering the wet lab (Torres, Sodero, & Jofily, 2019). The results of the molecular docking analysis illustrated optimistic outcomes. All five compounds exhibited strong interactions with the target protein. The binding energies of the best poses of these potential drug candidates were in a range of -10 to -9.1 kcal/mol. Protopine (C1) provided most suitable binding energies and poses. Strong bonds with catalytic amino acid residues having considerably shorter bond lengths were observed with all five compounds. The compounds showed firm interactions with Phe 41, Ile 95, and Ser 20 residues. The compounds (C1– C5) were alkaloids with multiple botanical sources and a vast range of biological activities. Protopine (C1) is obtained from *Papaver somniferum* (Pubchem, 2023). The compound has also been tested for its potential biological activity against cancer, Alzheimer's disease, inflammation and other ailments (Son et al., 2019; Sreenivasmurthy et al., 2022; Zhang et al., 2019). In this study, the protopine has been tested *in-silico* for its potential anti-mycobacterial effect and auspicious results have been furnished in this regard. The second drug candidate, aporphine (C2) has been widely tested for its anti-inflammatory, analgesic, anti-parasitic and anti-oxidant effects in various *in-silico*, *in-vitro* and *in-vivo* models (Pieper, McHugh, Amaral, Tempone, & Anderson, 2020; B. Wang et al., 2019; R. Wang, Zhou, Shi, Liu, & Yu, 2020). The exploration of anti-tuberculosis activity was done in this study and promising outcomes were witnessed. The biological activity of avicine (C3) in Alzheimer's disease is well established through animal models (Cahlíková et al., 2021; Plazas, 2020). Avicine has also displayed hopeful anti-mycobacterial activity predictions in this study. Penduline (C4) has been studied for its anti-cancer and anti-inflammatory activities while chelerythrine (C5), a potent inhibitor of protein kinase C, has revealed to be active against cancer, bacterial infections and COVID 19 (Gong et al., 2019; Grabarska et al., 2021; Lin et al., 2017; Valipour et al. 2021). Both of these alkaloids have furnished significant results in the molecular docking analysis in this study. The molecular dynamics simulations have further explored the dynamic behavior of the targeted protein.

Isoniazid is a well-known inhibitor of mycobacterial enoyl-ACP reductase (Unissa et al, 2016). Isoniazid inhibits the synthesis of mycolic acid in mycobacteria and halts the progression of cell wall synthesis, thereby providing bactericidal effects (Vilchèze & Jacobs, 2019). Isoniazid is one of the anti-tubercular drugs that have indicated notable efficacy through the decades. (Charan et al., 2018; Huang et al., 2018; Pease et al., 2017). Keeping in view these facts, severe toxicities and adverse drug reactions are also associated with the use of isoniazid in the treatment regimen for tuberculosis (Bliven-Sizemore et al., 2015). The hepatotoxicity and peripheral neuritis are the most significant toxicities associated with isoniazid (Lamb & Mauermann, 2021; Mafukidze, Calnan, & Furin, 2016; M Russom et al., 2019; Mulugeta Russom et al., 2018). These considerable toxicities demand a safer treatment option with similar efficacy. In this study, potential drug candidates, i.e., natural compounds (alkaloids) with no reported toxicity and strong affinity for target proteins, could attenuate tuberculosis infection after the development of a stable formulation. This study recommends the formulation scientists to employ novel drug formulation techniques for formulating natural products and secondary metabolites such as alkaloids as nanocarriers in order to achieve their delivery to the targeted sites, maintain efficacy and avoid any undesirable or noxious effect. Natural products and phytochemicals have caught the attention of formulation scientists and pharmacologists to a greater extent. The coupling of natural products and

nanomedicine can make major breakthroughs in the field of therapeutics. This study also puts emphasis on this idea.

CONCLUSION

Tuberculosis is a public health concern that claims millions of lives annually. The current therapy for tuberculosis has many challenges, with toxicity at the top of the list. Natural products have been used traditionally owing to their minimal toxicities and decent efficacy. Many studies have established that phytochemicals can attenuate bacterial infections such as tuberculosis. Through *in-silico* analysis, this study has concluded that the alkaloids such as protopine, aporphine, avicine etc. have potential inhibitory activity for the mycobacterial acyl carrier protein reductase enzyme, hence disrupting mycobacterial survival and pathogenesis. The molecular docking and molecular dynamics simulation provided optimistic results regarding the interactions of these alkaloids with the active amino acid residues of mycobacterial acyl carrier protein reductase. This study recommends the conduct of *in vitro* and *in vivo* studies in order to validate the findings of this research study and the development of novel formulations for better delivery of these alkaloids for attenuating tuberculosis.

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Authors' Contributions: Ahsan Ibrahim designed the study. Pernia Kamran and Ahsan Ibrahim carried out the computational work. Pernia Kamran & Ahsan Ibrahim wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

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