

In silico Molecular Docking Analysis of *Stephania glabra* Phytocompounds Targeting Thymidylate Kinase for Potential Antituberculosis Activity

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Abstract

Tuberculosis (TB) is a contagious illness caused by a type of bacteria known as Mycobacterium tuberculosis. It mainly affects the lungs and spreads through the air when a person with active TB in their lungs' coughs, sneezes, or spits. This study investigates several bioactive compounds derived from plants to forecast how effective plant-based ligands will be at preventing TB. The purpose of the study was to use computational techniques to assess the effectiveness of several phytochemicals against Mycobacterium. Molecular docking was systematically carried out using the virtual screening software PyRx. The top five phytocompounds from Stephania glabra were chosen among them to test their compatibility with the thymidylate protein 5NR7, using the ADMET filters, the ligands pharmacological evaluation was performed. The phytocompounds roemerine, stepholidine, tetrahydropalmatine, rotundine, and corydalmine from the plant S. glabra were discovered to be the most potent antagonist for the protein thymidylate kinase. All the bioactive chemicals could be considered as deserving candidates for the suppression of TB due to their strong affinity for the protein. Among the top ligands the phytoconstituent roemerine demonstrated better binding affinity with the target.

Keywords: Adenosine triphosphate (ATP), blood–brain barrier (BBB), C-type lectin receptors (CLRs), gastrointestinal absorption (GI), Mycobacterium tuberculosis (MTB), molecular docking (MD), nod-like receptors (NLRs), Stephania glabra, toll-like receptors (TLRs), thymidylate kinase (TMK), thymidine monophosphate (dTMP), thymidine diphosphate (dTDP), thymidine 5'-triphosphate (dTTP), tuberculosis (TB)

INTRODUCTION

Tuberculosis (TB) is a long-known disease in humans, caused by the bacterium *Mycobacterium tuberculosis* (MTB). TB has been around for thousands of years, with signs of infection found as far back as 5,800 BC. What makes TB unique is that it can stay inactive, or latent, in a person's body for decades without causing symptoms [1]. TB is mostly a pulmonary pathogen; MTB can infect nearly any area of the body. MTB infection may progress from containment within the host, with the bacteria

isolated in granulomas (latent TB infection), to an infectious state, when the patient will exhibit signs that may include cough, fever, night sweats, and weight loss. Only active pulmonary TB is infectious [2]. The primary route of entry of the causative agent is the respiratory route; the important cell types involved are the alveolar macrophages, which fight the pathogen. Different dimensions of macrophage–mycobacterium interactions and the function of macrophage in host response, such as MTB to macrophages through surface receptors, phagosome–lysosome fusion, inhibition/killing of mycobacteria through

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free radical-mediated mechanisms like reactive oxygen and nitrogen intermediates; cytokine-dependent mechanisms; accessory immune cell recruitment for local inflammation; and antigen presentation to T cells for acquired immunity development [3]. The body's first line of defense, known as the innate immune response, along with certain special immune cells and inflammatory molecules, plays a key role in protecting against MTB infection. Several types of pattern recognition receptors help the body detect MTB, including toll-like receptors (TLRs), C-type lectin receptors (CLRs), and nod-like receptors (NLRs), which are involved in activating the inflammasome. Among the TLRs, TLR1, TLR2, TLR4, and TLR9, along with their associated signaling proteins, play a critical role in triggering the immune response during the development of TB. The inflammasome pathway helps trigger the release of cytokines like IL-1 β and IL-18, which are involved in the body's immune response and contribute to the development of TB [4].

Bacterial thymidylate kinase (TMK), a type of enzyme involved in nucleotide metabolism, has recently gained attention as a potential target for developing new antibacterial drugs. Several TMK inhibitors have demonstrated potent antibacterial effects, especially against MTB [5]. TMK is an important enzyme that helps convert thymidine monophosphate (dTMP) into thymidine diphosphate (dTDP) by adding a phosphate group from adenosine triphosphate (ATP) [6]. Improvements in computational capacity and computational chemistry software are largely engaged in identifying the probable molecules acting on the different MTB proteins. Additionally, various in-silico investigations on MTB TMK using pharmacophore [7], virtual screening [8], and molecular docking [9] have been documented.

To narrow down the range of potential compounds, several key methods were used, including multiple molecular docking studies, analysis of pharmacokinetics and toxicity, and machine learning-based predictions of binding energy. Additionally, molecular dynamics (MD) simulations involving all atoms were used to assess how stable the selected molecules were when bound to TMK over time [10].

Stephania is a medicinal plant that belongs to the Menispermaceae family. China is home to 39 species of the *Stephania* genus, with most of them found in areas south of the Yangtze River. Most plants in the *Stephania* genus are herbaceous climbing vines with large, swollen tubers, which are commonly used for their medicinal properties. The large tuber of a mature *Stephania* plant can reach around 23 cm in diameter and weigh up to 30 kg, making up a significant portion of the whole plant. *Stephania* plants are especially rich in alkaloids and have been widely used in traditional Chinese medicine to treat a range of conditions, including inflammation, asthma, TB, high blood sugar, cancer, and neurodegenerative disorders [11]. Recent studies have shown that even a small amount of stephanine can effectively block the replication of COVID-19, suggesting that this compound holds great potential for helping to prevent the spread of the virus. From a pharmacological perspective, compounds extracted from *Stephania* tubers have shown pain-relieving, anti-inflammatory, and bacteria-inhibiting effects. Alkaloids are the typical active pharmaceutical ingredients extracted from *Stephania* plants. It has been found that *Stephania* tubers yield about 3–4% alkaloids. Various alkaloids, like palmatine, sinomenine, stephanine, and tetrahydropalmatine have been separated from plants belonging to this genus, and most of these compounds were tested for biological activity. Nevertheless, the applications of *Stephania* resources are restricted by the superficial concept of the genetic diversity of *Stephania* species [12].

METHODS

Retrieval of Ligands

The Dr Dukes phytochemical and ethnobotanical (biological activity, phytochemistry, chemical compound, and therapeutic use) database (<https://phytochem.nal.usda.gov/>) was used to look for potential ligands from the medicinal plant *S. glabra* [12]. The canonical smiles of all ligands selected for the current experiment were recorded. The top 24 ligands from medicinal plant *S. glabra* were retrieved from the PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) in SDF format [13].

Retrieval of Proteins

The crystal structure of MTB TMK complex compound 43 with PDB ID 5NR7 was downloaded from the PDB databank (<https://doi.org/10.2210/pdb5NR7/pdb>). The protein was obtained in PDB format. TMK, an essential enzyme for bacterial DNA synthesis, has become a target for developing new antibacterial drugs to combat MTB, the bacterium responsible for the widespread infectious disease TB. The resolution of protein is 2.35 Å, and the method of protein retrieval is X-ray diffraction [14].

Protein Purification

Before molecular docking, proteins undergo a purification process to ensure accurate and reliable docking results. The crystallographic structure obtained from experimental studies or databases often contains water molecules that do not match the free energy of the system. Since these water molecules can influence docking score and impact ligand binding, they were removed completely before docking. Additionally, many protein structures include prebound ligands that could interfere with the study's chosen ligands. To ensure unbiased binding interactions, the ligands that were originally bound to the crystal structure were removed before analysis. Furthermore, the protein structures can consist of multiple chains, which may not always be necessary for docking analysis. To simplify the structure and reduce the computational complexity, extra chains were removed and while retaining only chain A for further study. Since hydrogen atoms, particularly polar hydrogens, are crucial for protein–ligand interactions but are often absent in crystallographic data, they were explicitly added to refine and optimize the protein structure. The entire purification process, including water removal, ligand elimination, structural simplification, and removal of hetero atoms and hydrogen addition, was performed using DS BIOVIA Discovery Studio, a widely used molecular modeling and docking tool [15].

Pharmacological Studies

The drug-like features of the ligands were predicted with a SWISSADME (<http://www.swissadme.ch/>) by analyzing their physiochemical parameters, such as lipophilicity, instauration, insolubility, flexibility, molecular size, and polarity [16]. Afterward ligands were filtered based on Lipinski's rule of five. In addition, ADMET lab 2.0 (<https://admetmesh.scbdd.com/>) was used to predict the toxicity parameters of ligands [17, 18].

Molecular Docking

The purified protein 5NR7 was uploaded into PyRx as a macromolecule, whereas the plant-derived phytocompounds were uploaded as ligands. The ligands were converted from SDF format to PDB format by using Open Babel [19]. The active site grid dimensions were defined with center coordinates at X: -2.2748, Y: 20.1905, and Z: -13. 9394. Each ligand was docked separately against 5NR7 through the PyRx web server, and then energy minimization was done. Based on docking results and their binding affinity with a target protein, the most promising compounds – remerine, stepholidine, tetrahydropalmatine, rotundine, and corydalmine were selected for further research.

Visualization

The 2D and 3D structures of the top five docked ligands with the best binding scores were created and obtained using Dassault systems with BIOVIA Discovery studio visualizer [20].

RESULTS

Protein Structure and Analysis

The Ramachandran plot is a tool for visualization of energetically favorable amino acid torsion angles in protein structure. The Ramachandran plot of purified 5NR7 (Figures 1 and 2) was created using PROCHEK, and its secondary structure was analyzed using PROSITE (Figure 3). The red regions on the graph represent sterically allowed regions that favor stable peptide conformations. For computational analysis, amino acids must be mostly in the allowed regions, more than 88%. Here, the most favorable regions include 88.5% of the amino acid residues, representing 115 residues. The

disallowed regions are zero residues, and the remaining 10.8% (1 residue) is in subregions. A total of 162 residues, out of which 130 are non-glycine and non-proline, 7 are glycine, 11 are proline, and 14 residues are in the end regions. The secondary structure of purified protein 5NR7 that was predicted that contains a single sheet, two beta-alpha-beta-motifs, a single beta hairpin, a single psi loop, six strands, eight helices, three helix-helix contacts, and 10 beta turns, as indicated by PDBsum data.

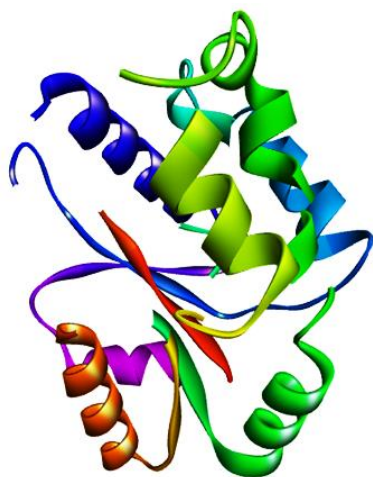


Figure 1. Purified structure of thymidylate kinase protein (PDB ID 5NR7).

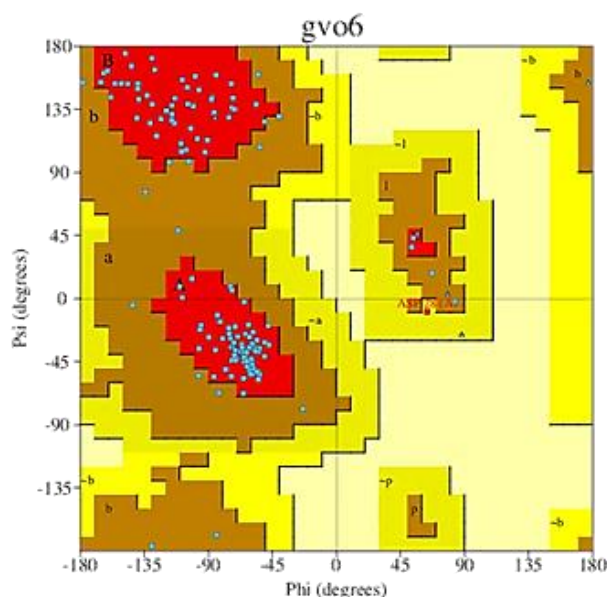


Figure 2. Ramachandran plot of 5NR7 protein using PDBsum.

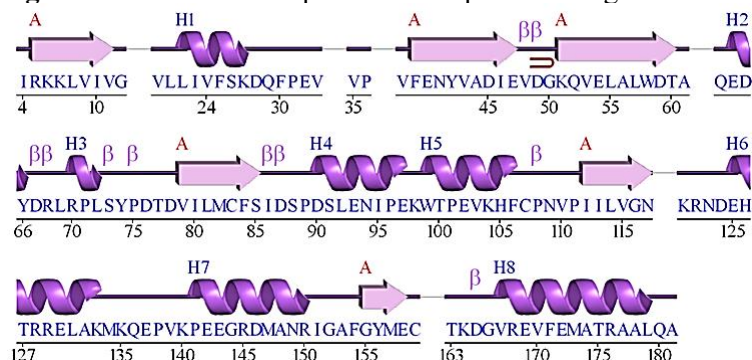


Figure 3. Secondary structure of protein 5NR7.

Drug Likeness Analysis

The requirements for a compound to be used in drug products are its physicochemical characteristics, ADMET characteristics, and Lipinski's rule of 5, in the development of medicine with minimum side effects, toxicity prediction, and cumulative data. Hence, the phytocompounds isolated from *S. glabra* were subjected to pharmacological tests to establish their drug-likeness characteristics.

Pharmacological Studies

The phytoconstituents from *S. glabra* were subjected to physicochemical evaluation the ligands were screened based on the parameters like lipophilicity (xLogP: -0.7 to +5.0), heavy atoms, molecular weight (150–500 g/mol), molar refractivity (40–130), polarity (topological surface area: 20–130Å²), and solubility. The top five ligands fulfil all the physicochemical parameters (Table 1).

Lipinski's Filter Analysis

Lipinski's rule of 5 is stated as the golden rule for a pharmacological screening of candidates of compound molecules. According to Lipinski's rule, the compound should have a molecular weight (150–500 Daltons), lipophilicity (MlogP < -4.15), the hydrogen bond donors should be <5, the hydrogen bond acceptors should be <10, and the molar refractivity should be between 40 and 130Å. The top five ligands were subjected to Lipinski's evaluation, and the ligands fulfilled Lipinski's parameters without any violations (Table 2).

ADME Analysis

An ADME analysis has four main features. The Blood–Brain Barrier (BBB) acts as a protective filter that limits which substances can pass from the bloodstream into the brain. Understanding the BBB is crucial when developing drugs, especially those intended to treat brain-related conditions. For a drug to work well, it must be easily absorbed in the gastrointestinal (GI) tract. In addition, it should be highly soluble, as poor solubility can reduce its overall effectiveness. A lower value of negative solubility is acceptable. According to the findings listed in Table 3, all the ligands show high GI absorption. Additionally, they are well soluble and show good availability. These factors increase the likelihood of the drug being effective for therapeutic use.

Molecular Docking

The binding affinity of each of the selected ligands for the 5NR7 protein, as determined by PyRx, is listed in Table 4. For deeper analysis, the best docking conformations of the highest binding energy was used. Of the listed phytocompounds, those having a binding energy of greater than -7.4 were designated as best. In particular, the following compounds had the greatest binding energy: remerine, stepholidine, tetrahydropalmatine, rotundine, and corydalmine. Due to this, the five compounds were chosen for further investigation.

Table 1. Physicochemical properties of *Stephania glabra* compounds.

Ligand Name	Molecular Weight (g/mol)	Heavy Atoms	MR	TPSA(Å ²)	Solubility	Lipophilicity
Remerine	338.38	25	97.33	51.80	-4.37	1.78
Stepholidine	279.33	21	85.04	21.70	-4.07	3.07
Tetrahydropalmatine	327.37	24	95.05	62.16	-3.74	1.75
Rotundine	355.43	26	103.99	40.16	-4.16	2.20
Corydalmine	355.43	26	103.99	40.16	-4.16	2.20

Visualization

The ligand remerine had the least binding energy against the target protein. Therefore, this docked complex was visualized with Bio via software. The pattern of interaction was studied through 3D and 2D interaction diagrams in Figures 4–9. These diagrams explicitly reveal that remerine tends to bind at certain interacting residues are LEU 34, LEU 93 and LEU 130 are Alkyl and Pi–Alkyl interaction,

TYR 96 has Pi-Pi T-shaped interaction, ASP 73 has Pi-anion interaction and ALA 71 and ASP 94 are carbon-hydrogen bond. Other ligands exhibit binding affinity less than roemerine like stepholidine, tetrahydropalmatine, rotundine, and corydalmine are shown in Figures 4–8. The binding interaction reveals information regarding the stability and probable effectiveness of the ligands.

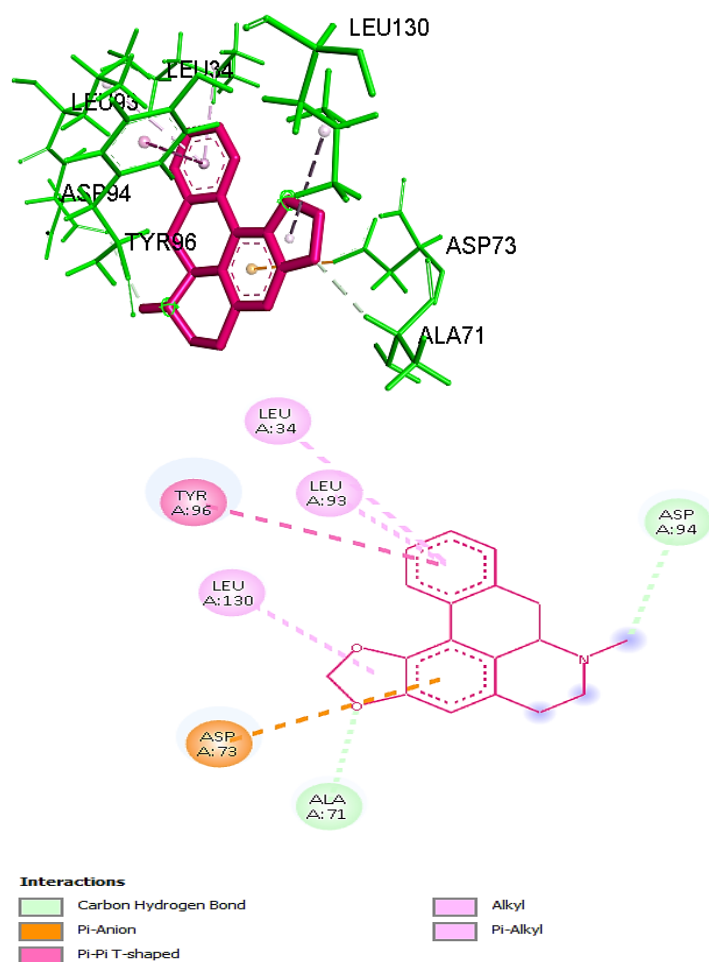
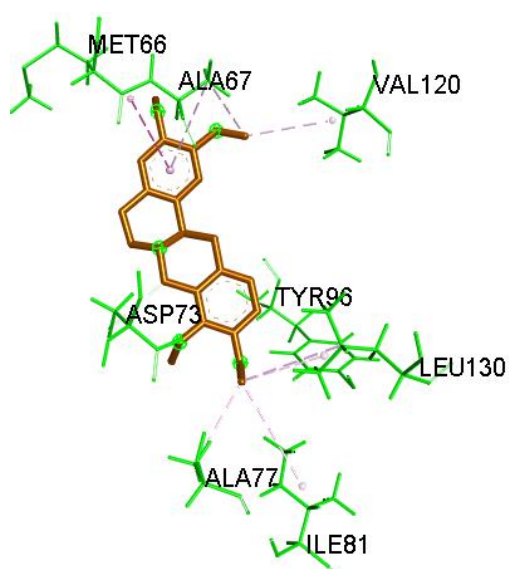


Figure 4. 3D and 2D structure of roemerine ligand.



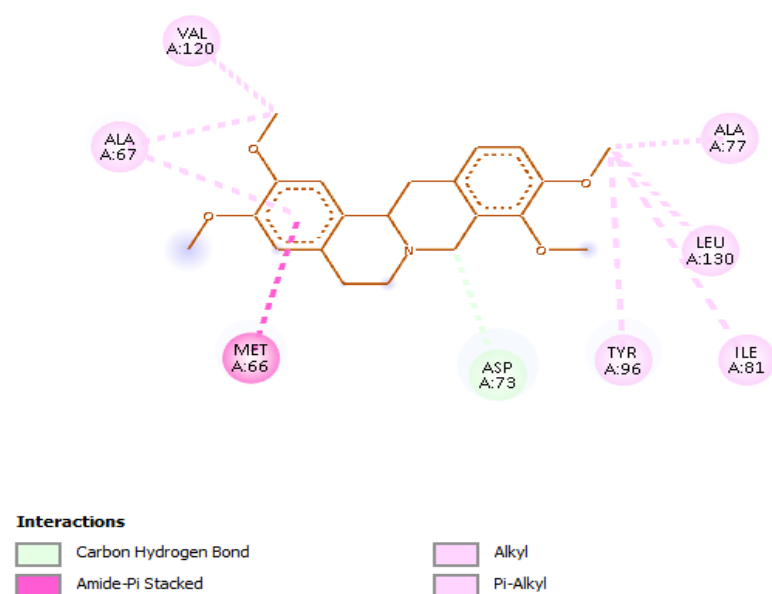


Figure 5. 3D and 2D structure of stepholidine ligand.

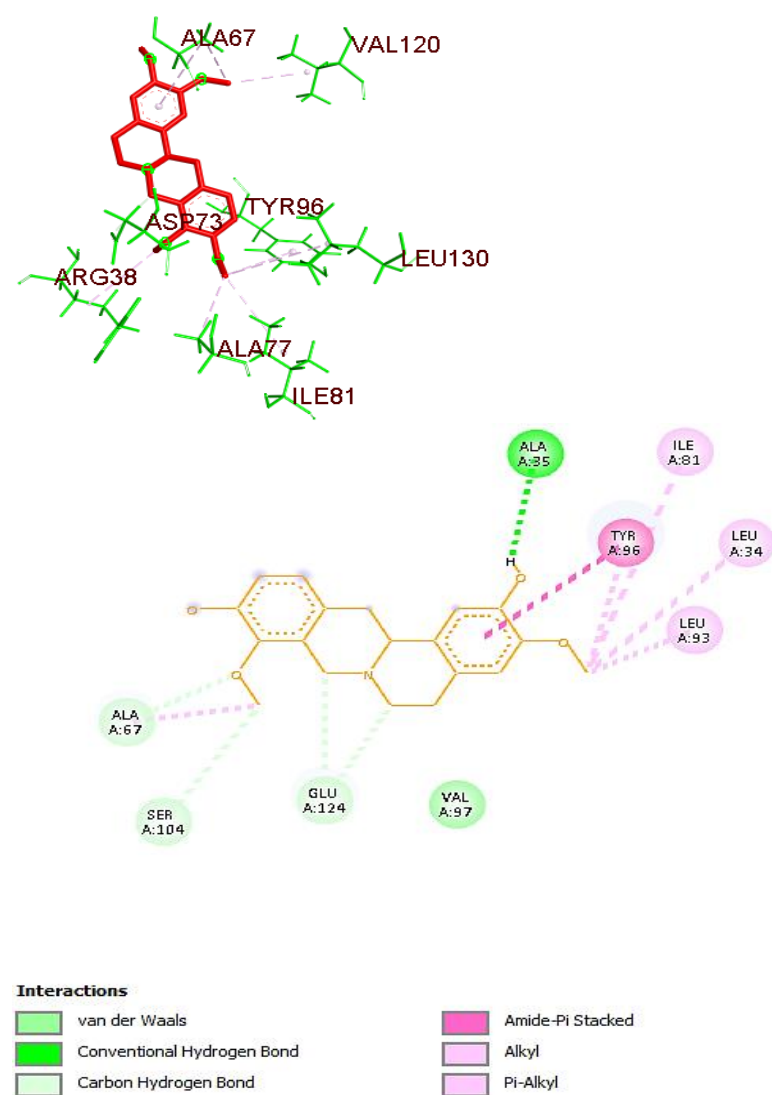


Figure 6. 3D and 2D structure of tetrahydropalmatine ligand.

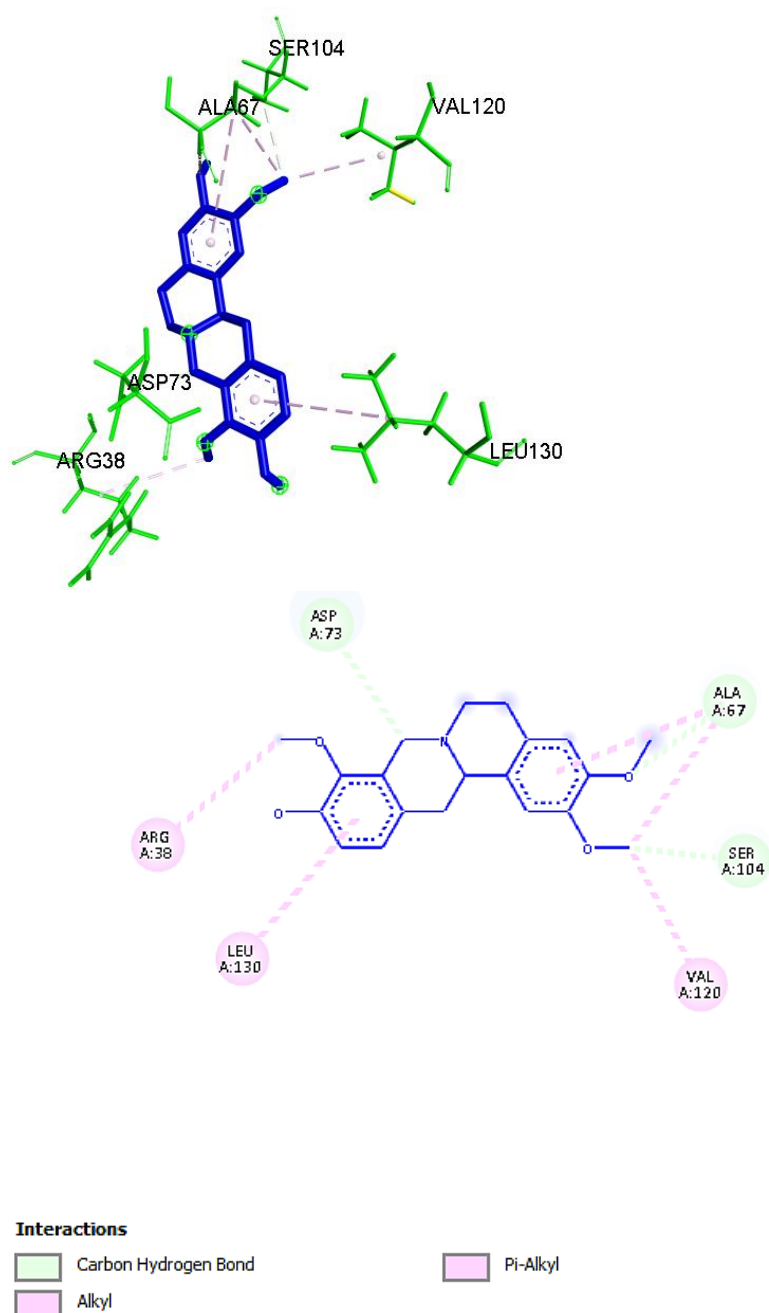
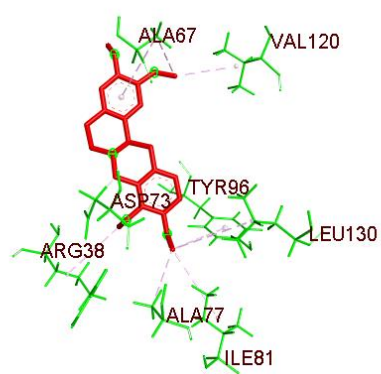


Figure 7. 3D and 2D structure of corydalmine ligand.



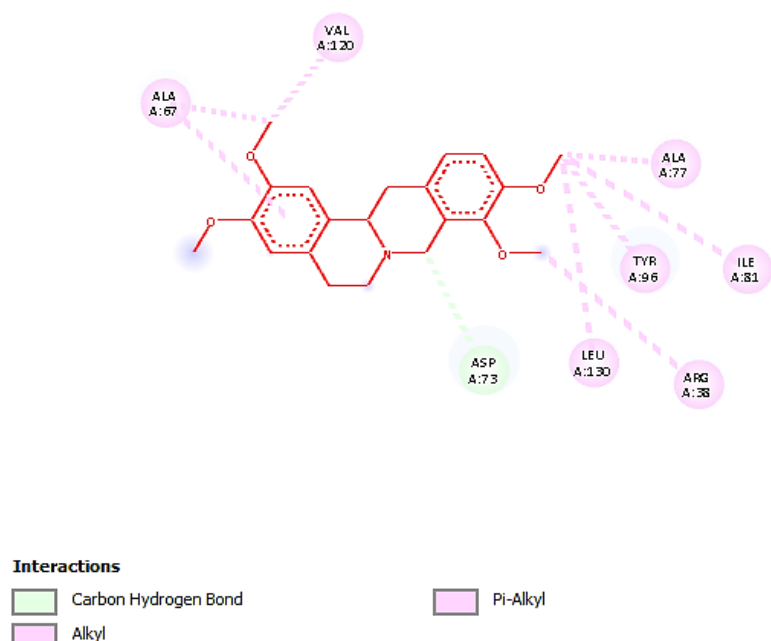


Figure 8. 3D and 2D structure of rotundine ligand.

Table 2. Data for the properties of Lipinski rule obtained using Swiss ADME.

Ligand Name	Molecular Weight(G/Mol)	HBA	HBD	Lipophilicity
Remerine	338.38	4	1	1.78
Stepholidine	279.33	3	0	3.07
Tetrahydropalmatine	327.37	5	2	1.75
Rotundine	355.43	5	0	2.20
Corydalmine	355.43	5	0	2.20

Table 3. ADME data obtained using Swiss ADME.

Ligand Name	GI	BBB	B. A	Pains	Brenk	Toxicity
Remerine	High	yes	0.55	0	0	4
Stepholidine	High	yes	0.55	0	0	4
Tetrahydropalmatine	High	yes	0.55	0	0	4
Rotundine	High	yes	0.55	0	0	4
Corydalmine	High	yes	0.55	0	0	4

Table 4. Docking score of 5NR7 protein with selected ligands.

Ligand Name	Binding Affinity
Remerine (235224)	-9
Stepholidine (6917970)	-7.8
Tetrahydropalmatine (72301)	-7.6
Rotundine (5417)	-7.5
Corydalmine (161665)	-7.4

DISCUSSIONS

TB of the lungs, but also possibly spread to other organs. TB bacteria use evasion of the immune system by living inside macrophages and granuloma formation. Symptoms are chronic cough, fever, night sweats, and weight loss. Sputum microscopy, culture, and molecular tests diagnose TB, while treatment is by long courses of antibiotics. Partial protection comes from vaccination using BCG, but new drugs are essential to control drug-resistant TB. The global TB monitoring started in 1995.

Today, there are about 10.6 million people living with active TB. Of these, 5.8 million are men, 3.5 million are women, and 1.3 million are children. Also, about 25% of the global population has TB infections [21]. According to the 2024 WHO report, 5–10% of infected individuals eventually get active TB. Humans are the sole known reservoirs of MTB, but other animals can be infected [22].

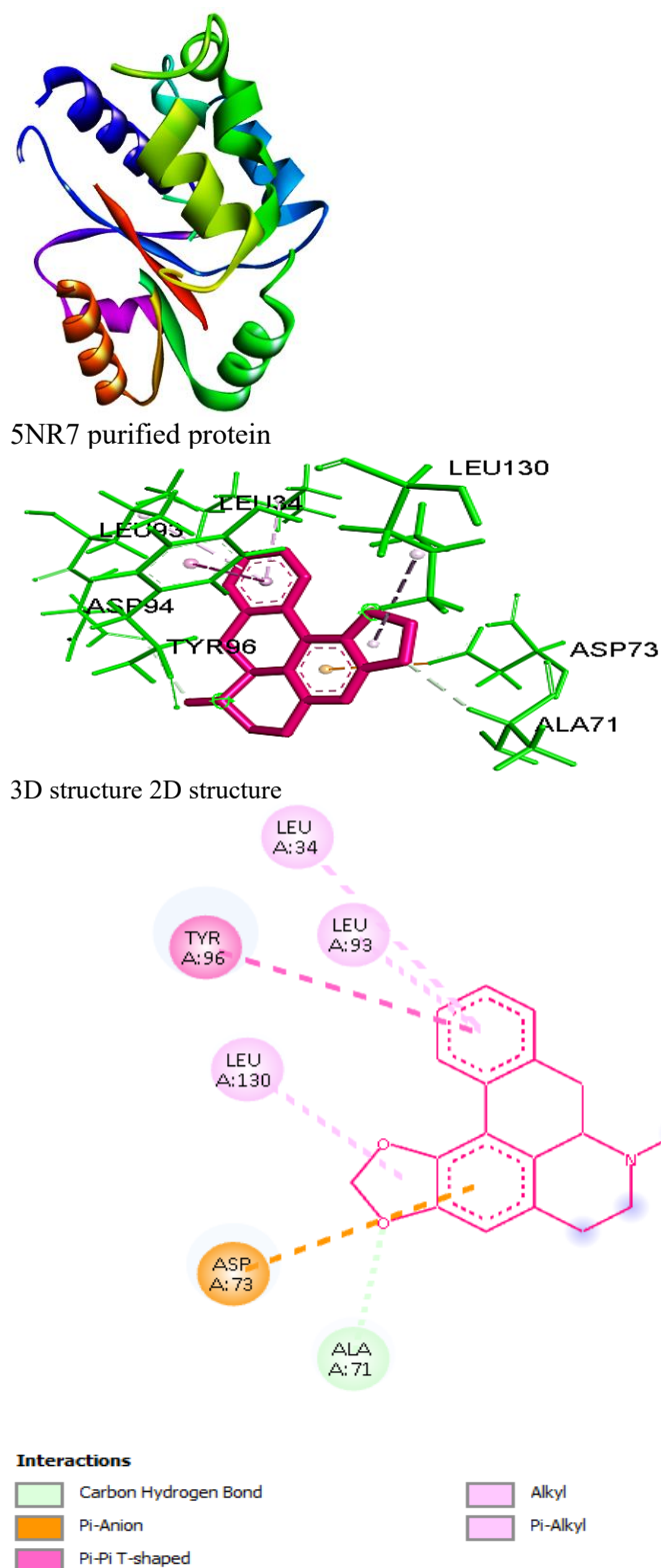


Figure 9. Visualization of molecular interactions of 5NR7 with roemerine ligand.

There are about 30 drugs belonging to various lines of defense that are employed in the present TB regimen for drug-susceptible and resistant TB [23]. The greatest challenge in the development of novel antituberculosis agents is due to the complexity and uniqueness of MTB's physiology [24]. To start with, the lipid-abundant, intricate cell envelope guards MTB against the detrimental milieu of human macrophages and lethal drugs. Secondly, the huge number of genes committed to metabolism is of vital importance for the viability and chronic lifestyle of MTB. Due to these properties of MTB, most existing drugs for TB, like isoniazid, the most active and least toxic first line medication, is focused against cell wall biosynthesis [25]. Other than rifampicin inhibiting RNA synthesis [26], very few drugs against TB target elsewhere other than cell wall biosynthesis. With continuously growing reports of drug-resistant TB, there is a huge need to design candidate drugs with new action mechanisms.

TMK is a member of the nucleoside mono phosphate (NMP) kinase family and is also known as thymidylate kinase or thymidine monophosphate kinase or ATP:dTMP phosphotransferase (EC: 2.7.4.9.). It catalyzes the reversible phosphorylation of thymidine 5'-mono phosphate (dTMP) to thymidine 5'-diphosphate (dTDP) in the presence of Magnesium and ATP. The dTDP is then converted to thymidine 5'-triphosphate (dTTP) by nucleoside diphosphate kinase. The TMK function was first established in *Saccharomyces cerevisiae*, as *cdc8* gene [27, 28]. Inhibition of TMK activity impacts on cell viability and, therefore, its unyielding success as antiviral and anticancer drug targets [29, 30].

In *Stephania* plants, alkaloids are the main and widely present active medicinal compounds. Pharmacological studies have shown that palmatine has a range of benefits, including neuroprotective, anticancer, antibacterial, antiviral, antioxidant, anti-inflammatory, and blood lipid-lowering effects. Stephanine has demonstrated strong antimalarial and anticancer properties. Tetrahydropalmatine is known for its pain-relieving, calming, and antiarrhythmic effects. Sinomenine has been found to have powerful immunosuppressive, anti-inflammatory, and antiarthritic activities [31]. Recently, stephanine has been shown to effectively block the replication of the COVID-19 virus. In our study, the roemerine compound showed the strongest binding affinity to the target protein. Roemerine is an aporphine alkaloid isolated from *S. glabra*, it exhibits notable antimicrobial activity, including against MTB. Roemerine's antimycobacterial effects are attributed to its ability to disrupt cell wall integrity and interfere with DNA functions. Additionally, its anti-inflammatory effects could help lessen lung damage caused by TB. However, more pharmacological and clinical studies are necessary to improve its effectiveness and use it as a treatment for TB. The findings of this study indicate that the phytocompounds roemerine, stepholidine, tetrahydropalmatine, rotundine, and corydalmine showed the strongest binding with the protein target 5NR7 TMK. That is, the phytocompound roemerine was a potent inhibitor of the 5NR7 protein.

CONCLUSIONS

Over the past few years, TB has become more prevalent, and it usually strikes humans. Several vaccines are currently being made to eliminate this condition. mortality. In our study, we implemented computational techniques to evaluate the impact of several phytochemicals on the A. peroxiredoxin protein PyRx. A virtual screening tool was employed to carry out molecular docking in a systematic manner. Based on the findings, the ligand roemerine has the most favorable binding affinity toward 5NR7 protein, while corydalmine ligand has the least binding affinity toward the target protein. Equally important as the vaccines are medicinal plants that can be utilized to produce medicines for the treatment of TB. An example of a plant with various therapeutic attributes is *S. glabra*. In this method, better prevention and cure can be realized with the assistance of various studies. Due to increased utilization of medicinal plants in medication development, medicine will undergo a huge transformation.

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