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Research Article

Targeted *in-silico* studies of bioactive compounds from *Lagerstroemia speciosa* against tuberculosis –TB (*Mycobacterium tuberculosis*)

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Abstract

Several cases of tuberculosis in people with COVID-19 and lung diseases have recently been reported worldwide, particularly in India. Tuberculosis treatment usually includes antifungal medications like Posaconazole, Isavuconazole, and Amphotericin B. Tuberculosis (TB) is an infection caused by bacteria that usually affect the lungs. These bacteria, called *Mycobacterium tuberculosis*, can be passed on to another person through tiny droplets spread by coughing and sneezing. This present study deals with identifying a possible alternative to the available antibiotics and steroids. Potential plant derived compounds will be the best possible alternative with negative side effects. *Lagerstroemia speciosa* is one such plant that has been proven to possess various bioactive properties. Hence based on earlier reports, one such compound, undecanoic acid was considered to be a potential agent against tuberculosis. Molecular docking analysis was carried out to evaluate the possibility to use these molecules against tuberculosis. Docking was performed for the identification of ethanolic extract from *Lagerstroemia speciosa* seeds on the binding of tuberculosis-targeted proteins (PDB ID: 7D6V and 1UH9). The molecular docking score of PDB ID: 7D6V Ethyl 11-bromoundecanoate is -3.18 kcal/mol and Isavuconazole is -4.52 kcal/mol and PDB ID: 1UH9 Ethyl 11-bromoundecanoate is -5.264 kcal/mol and Isavuconazole is -8.69 kcal/mol. All molecules of a binding score (docking score) of more than -7.0 kcal/mol with the proteins. This suggests a possible potent alternative source for tuberculosis through further research.

1. Introduction

Tuberculosis (TB) is an infectious disease usually caused by *Mycobacterium tuberculosis* (MTB). Tuberculosis generally affects the lungs, but it can also affect other parts of the body. Most infections show no symptoms, in which case it is known as latent tuberculosis. Around 10% of latent infections progress to active disease which, if left untreated, kill about half of those affected. Typical symptoms of active Tuberculosis are chronic cough with blood-containing mucus, fever, night sweats, and weight loss [1]. Active infection occurs more often in people with HIV/AIDS and in those who smoke. Diagnosis of active Tuberculosis is based on chest X-rays, as well as microscopic examination and culture of body fluids. Diagnosis of latent Tuberculosis relies on the tuberculin skin test (TST) or blood tests [2].

It involves screening those at high risk, early detection and treatment of cases, and vaccination with the *bacillus Calmette-Guérin* (BCG) vaccine [3]. Antibiotic resistance is a growing problem with increasing rates of multiple drug-resistant tuberculosis (MDR-TB) [1].

As of 2018, most Tuberculosis cases occurred in the regions of South-East Asia (44%), Africa (24%), and the Western Pacific (18%), with more than 50% of cases being diagnosed in seven countries: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (6%), Nigeria (4%), and Bangladesh (4%) [4].

It may infect any part of the body, but most commonly occurs in the lungs (known as pulmonary tuberculosis). Extra pulmonary Tuberculosis occurs when tuberculosis develops outside of the lungs, although extra pulmonary Tuberculosis may coexist with pulmonary Tuberculosis. General symptoms include fever, chills, night sweats, loss of appetite, weight loss and fatigue. Significant nail clubbing may also occur [5].

The main cause of Tuberculosis is *Mycobacterium tuberculosis* (MTB), a small, aerobic, non-motile bacillus. *Mycobacteria* have an outer membrane lipid bilayer. If a gram stain is performed, MTB either stains very weakly "Gram-positive" or does not retain dye as a result of the high lipid and mycolic acid content of its cell wall [6]. But *Mycobacterium tuberculosis* can be cultured in the laboratory [7]. The *Mycobacterium tuberculosis* complex (MTBC) includes four other Tuberculosis-causing mycobacteria: *M. bovis*, *M. africanum*, *M. canetti*, and *M. microti* [8]. Other known pathogenic *mycobacteria* include *M. leprae*, *M. avium*, and *M. kansasii*. The latter two species are classified as "nontuberculous *mycobacteria*" (NTM) or atypical *mycobacteria*. NTM causes neither TB nor leprosy, but they do cause lung diseases that resemble Tuberculosis [9].

Diagnosing active tuberculosis based only on symptoms is difficult as is diagnosing the diseases in those who have a weakened immune system. A diagnosis of Tuberculosis should be considered in those with signs of lung disease or constitutional symptoms lasting longer than two weeks [10]. A chest X-ray and multiple sputum cultures for acid-fast bacilli are typically part of the initial evaluation [10]. A definitive diagnosis of Tuberculosis is made by identifying *Mycobacterium tuberculosis* in a clinical sample (e.g., sputum, pus, or a tissue biopsy). However, the difficult culture process for this slow-growing organism can take two to six weeks for blood or sputum culture [11]. Thus, treatment is often begun before cultures are confirmed [12].

The Mantoux Tuberculin Skin Test is often used to screen people at high risk for Tuberculosis [13]. The test may be falsely negative in those with sarcoidosis, Hodgkin's lymphoma, malnutrition and most notably, active tuberculosis. Interferon gamma release assays, on a blood sample, are recommended in those who are positive to the Mantoux Test. The most environmental mycobacteria, so they are affected by *M. szulgai*, *M. marinum*, and *M. kansasii* [14].

The US Preventive Services Task Force (USPSTF) has recommended screening people who are at high risk for latent tuberculosis with either tuberculin skin tests or interferon-gamma release assays [15].

Tuberculosis prevention and control efforts rely primarily on the vaccination of infants and the detection and appropriate treatment of active cases. The World Health Organization (WHO) has achieved some success with improved treatment regimens, and a small decrease in case numbers.

Available vaccines as of 2021 and 2022 are Bacillus *Calmette-Guérin* (BCG) [16]. Bacilli *Calmette-Gué*rin (BCG) is a vaccine for tuberculosis (TB) disease. This vaccine is not widely used in the United States. However, it is often given to infants and small children in other countries where Tuberculosis is common. BCG does not always protect people from getting Tuberculosis. Intradermal MVA85A vaccine in addition to BCG injection is not effective in preventing tuberculosis [17].

Treatment of Tuberculosis uses antibiotics to kill the bacteria. Effective Tuberculosis treatment is difficult, due to the unusual structure and chemical composition of the mycobacterial cell wall, which hinders the entry of drugs and makes many antibiotics ineffective [18]. Active Tuberculosis is best treated with combinations of several antibiotics to reduce the risk of the bacteria developing antibiotic resistance.

The traditional use of plant products for the treatment of infectious diseases is a significant source of antimycotic drugs. There are several diseases caused by fungal infections, including Dermatophytosis, Candidiasis, tuberculosis and Mucormycosis. Included antimicrobial, antioxidant, anticancer, ant diabetic, hypolipidemic, antiobesity, anti-inflammatory, analgesic, gastrointestinal, diuretic, thrombolytic, cardiovascular, central nervous, inhibition of TNFα production, xanthine oxidase inhibition, hepatoprotective, lung diseases and nephroprotective effects.

Lagerstroemia speciosa (giant crepe-myrtle, queen's crepe-myrtle, banabá plant or pride of India) is a species of *Lagerstroemia* native to tropical southern Asia. It is a deciduous tree with bright pink to light purple flowers [19]. The genus *Lagerstroemia* was first described by

Carl Linnaeus. The name *Lagerstroemia* recognizes Magnus von Lagerstroem, a Swedish naturalist who provided specimens from the East for Linnaeus. The flowers in this plant bloom only once in a year at the peak of summer. It is grown in South East Asia, India, Bangladesh and the Philippines. It is also widely cultivated as an ornamental plant in tropical and subtropical areas. The leaves of the banabá and other parts are used widely in the Philippines, Taiwan, and Japan as a tea preparation. Banabá herb is one of the 69 herbal plants promoted by the Philippine Department of Health (DOH) [20]. In Vietnam the plant's young leaves are consumed as vegetables, and its old leaves and mature fruit are used in traditional medicine for reducing glucose in blood [21]. Queen's crape myrtle will grow in full sun on a wide range of well-drained soils but is not salt-tolerant. Where there are no overhead restrictions, this makes a nice large street tree due to the upright-spreading habit of growth. This reduces the regular pruning needed to remove lower drooping branches on some other trees. However, when the trees are young, some lower branches will need to be removed for street tree planting to create clearance for passage of pedestrians and vehicles. The tree should tolerate storms well having hard wood with flexible branches, as long as they are well spaced along the trunk and not clumped together growing from one point on the trunk. Plants should be watered faithfully and protected from frost. Not a tree to plant and forget, Queen's crape myrtle appreciates regular fertilization or leaves become chlorotic. It will tolerate alkaline soil. Propagation is by cuttings, division of root suckers, or by seed which germinate readily. Plants will flower the second year from seed [22]. There are other species of tropical *Lagerstroemia*, some available in selected nurseries. Banaba (*Lagerstroemia speciosa* L.) extracts have been used as traditional medicines and are effective in controlling diabetes and obesity. The aim of this study was to evaluate the anti-HIV property of the extracts prepared from the leaves and stems of banaba, and further purification and characterization of the active components [23]. It is popular for its values in Ayurvedic and folklore medicines [24]. It is widely used in the treatment of diabetics, obesity, kidney diseases, and other inflammatory disorders [25]. These phytochemical compositions differ with respect to the part of the plant and the solvent of extraction. The therapeutic effects of *Lagerstroemia speciosa* are often related to the presence of phytochemicals such as corosolic acid, lagerstroemin, and ellagitannins [26]. The most commonly isolated phytochemicals are of the leaf extracts of *Lagerstroemia speciosa*, which are investigated for their anti-diabetic, anti-obesity, antimicrobial and anti-inflammatory activities [27]. The *Lagerstroemia speciosa* seeds have been previously studied for the estimation of total phenolic content, keto-fatty acids, and their antioxidant activity [28]. The phytochemical profile of the seed extract has not been well explored. The extracts are rich in organic compounds such as long -chain fatty acids, hydrocarbons, esters, vitamins, and phytosterols which are responsible for their superior medicinal significance.

2. Materials and Methods

2.1. Plant selection

Plants have the ability to modify the microclimate, especially temperature and humidity factors. Microclimate created in an environment is the result of interaction between plants with other landscape elements. Hence, the selection of appropriate plants is a very important factor considering the ecological and aesthetic functions (MOE version–2015.10–Chemical Computing Group: Montreal, QC, Canada) [29]. Several tree species that grow well in microclimate conditions of the cities in India namely *Alstonia scholaris*, *Azadirachta indica*, *Delonix regia*, *Lagestroemias*, *Magnolia champaca*, *Polyalthia longifolia*, *Plumeria* sp. etc. *Lagerstroemia* is a genus of some 80 species in the family Lythraceae. Crape Myrtle are almost insect and disease free although they may see aphids from time to time.

2.2. Primary database preparation

The selected plants were again subjected to literature analysis for enumeration of phytochemicals present, mainly through GCMS (Gas Chromatography-Mass Spectrometry) reports published in reputed peer review journals [30, 31, 32-42]. A primary database of bioactive phytochemicals was prepared using Molecular operating Environment software [32].

2.3. Virtual screening of ligands

A virtual screening of ligands was carried out to scrutinize the database for the identification of compounds with higher bioactivity and low toxicity. The ADME properties of the compounds were predicted using the Swiss ADME (Adsorption, Distribution, Metabolism, and Excretion) web server and Lipinski rule of five along with **"drug likeness"** predictions that were used to filter out the best compounds with required activity [40].

2.4. Structure prediction

Undecanoic acid is a white crystalline solid. Insoluble in water. Specific gravity 0.85. Hence floats on water. Undecanoic acid is a straight-chain, eleven-carbon saturated medium-chain fatty acid found in body fluids; the most toxic of fatty acid series. It has a role as a human metabolite and an antifungal agent. It is a straight-chain saturated fatty acid and a medium-chain fatty acid. It is a conjugate acid of an undecanoate. It derives from a hydride of an undecane. Undecanoic acid is a natural product found in *Staphisagria macrosperma*, *Erucaria microcarpa*, and other organisms with data available. Undecanoic acid is suitable for chemical modification and might be useful in synergic therapies. This research highlights the use of undecanoic acid in treatment against tuberculosis, reinforcing its known activity against dermatophytes. The structure of protein is predicted from Pubchem for molecular docking based on their ligands and the *in silico* structure is formed from Ligplot Software.

2.5. Ligand preparation and target protein

The three-dimensional structure of the target protein *Mycobacterium smegmatis Sdh1* was retrieved from the protein data bank using its PDB ID and it was visualized using discovery studio visualizer (DSV) software. Water molecules, ions, and standard inhibitors if present were also removed using the discovery studio visualization tool [43]. Marvin sketch software was used to construct the three-dimensional structures of the ligands to be studied. Avogadro (molecular modelling tool) was used for energy minimization of ligand molecules to obtain the best pose with low energy (Most Table 3D form). Both local charge and torsion (rotational motion) was provided to three-dimensional ligand structures for obtaining an effective binding complex with target protein. Similarly, Kollmann charge and polar hydrogen bonds were added to the protein [44]. Three dimensional PDBQT files of both the ligand and the protein were generated using MGL tools.

2.6. Molecular docking

3D structures of the phytosterols studied were drawn using draw by Pubchem Sketcher V2.4 (https://pubchem.ncbi.nlm.nih.gov/edit3/index.html) in mol file format and later converted into the.pdb format using Open Babel GUI software [45]. The X-ray crystal structure of 17 beta-1, 3-glucanosyltransferase and 1, 3-beta-glucanase were retrieved as a PDB file from the Protein Data Bank for docking. A grid box with the sizes 60, 60, and 60 along the X-, Y-, and Z-axes respectively were set during docking. The docking analysis was carried out using AutoDockVina software [46]. The 3D docked protein–ligand complex poses were visualized using PyMol molecular visualization software program [47]. The 2d view of protein-inhibitor interactions was generated using Ligplot Software version LIGPLOT v.4.5.3 [48] (https://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/).

2.7. Drug likeness

Phytochemical compounds which showed nil violation for Lipinski rule of five and moderate "drug likeness" were selected for the process of molecular docking. Docking was carried out to evaluate the real time interaction between the phytochemical compounds with the target protein of interest *Mycobacterium smegmatis Sdh1* and Rhizopuspepsin by measuring the binding energy of the complex (drug with target protein). Auto Dock 4.0 (AD) and Auto Dock Vina 1.5.6 (ADV) (Centre for Computational Structural Biology, La Jolla, CA, USA) were used for this process and the results were visualized using the Chimera UCSF visualization tool (Resource for Biocomputing, visualization, and Informatics (RBVI), San Francisco, CA, USA) [49].

2.8. Overall steps of molecular docking

Get the complex (CPLX) coordinates (from the PDB). Clean the complex (delete all the water, solvent molecules, and non- interacting ions). Add the missing hydrogen's/side chains atoms and minimize the complex (AMBER Program). Clean the minimized complex (delete all the water, solvent molecules, and non- interacting ions). Separate the minimized CPLX in macromolecule (LOCK) and ligand (key). Prepare the docking suitable files for LOCK and KEY (PDBQT files). Prepare all the needed files for docking (grid parameter file, map files, docking parameter files). Run the docking. Analyse the docking results.

3. Results and Discussion

3.1. Plant selection and database preparation

The phytochemical compounds are recognized from GC -MS reports of existing literature and collected from various databases were used to prepare a primary database for the study. The two dimensional structures of the compounds were downloaded from Pubchem and were compiled into a single structural database. Phytochemical compounds recognized from GCMS reports of existing literature and collected from various

databases were used to prepare a primary database for the study. Initially, a total of 168 potential chemical entities (data not shown) were identified as bioactive phyto ingredients present in 11 indigenous medicinal plants. Two dimensional structures of the compounds were downloaded from Pubchem and were compiled into a single structural database.

3.2. Virtual screening of ligands

The virtual screening of ligands was carried out using the Swiss ADME server and parameters like Lipinski's rule of five and **"drug likeness"** were used as a filter for narrowing down the number of compounds to be taken up for docking. Initially, among 168 compounds present in the database, a total of 51 compounds with less than three violations were filtered out. Then these compounds were subjected to the same Lipinski filter

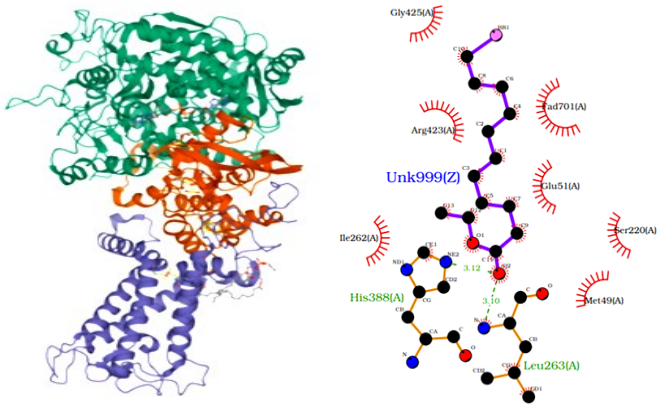

Figure 1. *Mycobacterium smegmatis sdh1* (pdb id: 7d6v) ethyl 11-bromoundecanoate.

(without any violation) along with "drug likeness" and finally 25 compounds were found to have nil violations in Lipinski rule and also zero to one violation for "drug likeness", proving it as a lead target molecule for docking studies. The compounds selected through virtual screening.

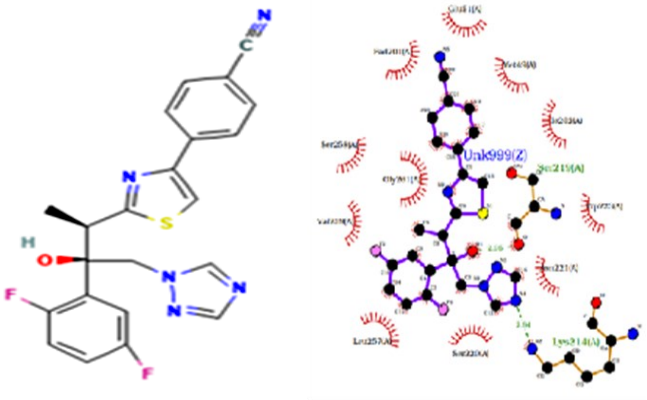

Figure 2. Isavuconazole (Drug) 2d – Molecular Docking.

3.3. Molecular docking of compounds

Molecular docking analysis was carried out to evaluate the possibility to use these molecules against black fungus. Docking was performed for identification from the ethanolic extract of *Lagerstroemia speciosa* seeds on the binding of tuberculosis targeted proteins (PDB ID: 7D6V and 1UH9) (Fig. 1). The molecular docking score of PDB ID: 7D6V Ethyl 11-bromoundecanoate is -3.18 kcal/mol and Isavuconazole is -4.52 and PDB ID: 1UH9 Ethyl 11-bromoundecanoate is -5.264 kcal/mol and Isavuconazole is -8.69 kcal/mol (Fig. 2). The proteins which are all the hydrogen bonding falls under the electrostatic force interactions. The number of residues taking part in hydrogen bond interactions varies from 6

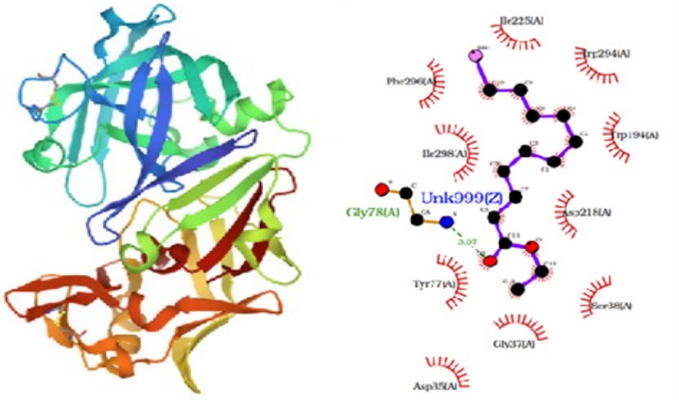

Figure 3. Rhizopuspepsin (Pdb Id: 1uh9) Ethyl 11-Bromo-undecanoate.

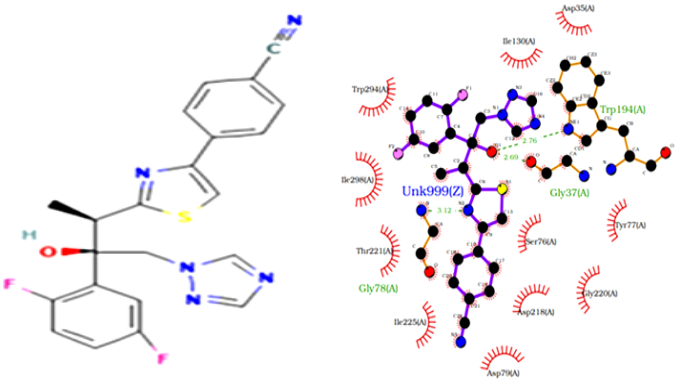

Figure 4. Isavuconazole (Drug) 2d – Molecular Docking.

Table 1. *Mycobacterium smegmatis Sdh1* (PDB ID: 7D6V).

Sl. No	Com-pounds	Docking score (kcal/mol)	Glide energy	Hydrogen bond interac-tions	Distances (Å)
1.	Ethyl 11-bro-moundeca noate	-3.18	-31.54	(HIS388)NE2-H...O2 (LEU263)N-H...O2	3.12 3.10
2.	Isavucona-zole (Drug)	-4.52	-50.00	O1-H...O (SER219) (LYS214)NZ-H...N4	2.95 2.94

Table 2. Rhizopuspepsin (PDB ID: 1UH9)

Sl. No	Com-pounds	Docking score (kcal/mol)	Glide energy	Hydrogen bond inter-actions	Distances (Å)
1.	Ethyl 11 - bro-mounde canoate	-5.264	-37.00	(GLY78)N-H...O2	3.07
2.	Isavuco nazole (Drug)	-8.69	-54.10	(TRP194) NE1-H...O1 (GLY78)N-H...N2 O1-H...O (GLY37)	2.76 3.12 2.69

to 15. The hydrogen bond interactions with amino acids residues, (HIS 388), (LEU 263), (SER 219), (LYS 214) in *Mycobacterium smegmatis Sdh1* and amino acids of (GLY 78), (TRP 194), (GLY 78), (GLY 37) in Rhizopuspepsin (Figs. 3 and 4) (Table 1 and 2). Upon considering the various parameters of docking such as binding energy, the number of hydrogen bonds, and inhibition constant, it was clearly evident that the selected phytochemicals have greater specificity towards the undecanoic acid binding site and could serve as potent mycobacterium inhibitors. The three-dimensional and two-dimensional conformations of the ligand having the highest binding score (with all the three proteins studied).

4. Conclusions

The *L. speciosa* is the identification of ethanolic extract using molecular docking against tuberculosis by the proteins called *Mycobacterium smegmatis Sdh1* and Rhizopuspepsin through *in silico* targeted proteins. The protein identified from the extract was predicted using *in silico* approaches for their inhibiting activities against tuberculosis. One of the proteins studied violates the "rule of five"which is common in natural products. All molecules are shown to have positive BBB and Caco-2 values indicating their permeability through membranes. The molecular docking analysis revealed the high binding nature of the *Mycobacterium smegmatis Sdh1* and Rhizopuspepsin with the Tuberculosis (*Mycobacterium tuberculosis*) is studied. A derivative of diosgenin, showed the highest inhibitory effect against all the three proteins as evident from its binding scores. The results support that proteins obtained from the ethanolic seed extract of *Lagerstroemia speciosa* could act as potential therapeutics against tuberculosis. The current predictions over these protein derivatives will be needed to further investigate in silico conditions to identify the optimum therapeutic efficacy and least toxicity.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Y.D; P.A and S.D. The first draft of the manuscript was written by S.S and G.R and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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