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Molecular Docking Study: Targeting *mycobacterium leprae* ML2640 Protein Using Active Compounds from *Coscinium fenestratum*

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Abstract

Objectives*: Leprosy is an infectious disease in skin, peripheral nerve and mucosa of upper airway. Around 70% of this infection is caused by Mycobacterium leprae, which was discovered during1873. Multi drug therapy is used for the treatment, still multiple drug resistance and reactions has been reported among patients. Hence, this work focused on identifying bioactive compounds from Coscinium fenestratum that can target mycobacterium leprae ML2640 protein by in-silico approach.* ***Method:*** *In this study, 80 phytocompounds from Coscinium fenestratum were screened against ML2640 proteins of Mycobacterium leprae from PubChem and PDB database were docked using h PyRx tool. Pharmacological studies were analysed using Swiss ADME and ADMET tools by applying Lipinski rule.* ***Results:*** *Molecular docking studies that conducted on seven selected phytocompounds among 80 phytocompound from Coscinium fenestratum, demonstrating their potential binding affinity to inhibit the activity of ML2640, thereby effectively disrupting the survival, virulence, and host infection rate of the target pathogen. Pharmacological analysis utilizing Swiss ADME and ADMET analysis confirmed the drug-like properties of these phytocompounds,* ***Conclusion:*** *This study is highlighting these seven phytocompounds are potential as valuable candidates for further drug development against Mycobacterium leprae infection.*

Keywords: Coscinium fenestratum, ML2640, ADME, ADMET, pharmacological analysis, mycobacterium leprae

INTRODUCTION

Leprosy is a chronic infection that infect skin, peripheral nerves and upper respiratory system. Despite the WHO's declaration of leprosy elimination in 2000, global new case numbers have remained constant. India, Brazil, and Indonesia account for 80.2% of cases. In 2015, the United States diagnosed 178 new cases, indicating ongoing efforts to detect and treat the disease [1]. It results in sensory loss in skin lesions, hands, or feet, along with aches, pains, and sensations like "ants running under their skin" in affected areas. Skin lesions present as hypopigmented or erythematous macules, papules, nodules, or plaques, appearing skin-coloured or slightly red [2]. The major causative agent of this infection is an acid-fast bacterium *Mycobacterium leprae* that is slightly curved, measuring 1-8 μm in length and 0.3-0.5 μm in diameter. It is a non-motile, microaerophilic that cannot be cultured in artificial medium. *M. leprae* primarily infects skin macrophages and Schwann cells [3].

Taxonomically, it belongs to the genus Mycobacterium in the family *Mycobacteriaceae*. *M. leprae* is an obligate intracellular parasite,

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multiplying slowly with a generation time of 12 to 14 days. It thrives at around 30°C and prefers cooler areas of the human body. The bacterium's gram-positive cell wall is complex and contains proteins, phenolic glycolipid, arabino glycan, peptidoglycan, and mycolic acid, which contributes to its acid-fastness. Anti-PGL-I antibody detection reflects bacterial loads and aids in assessing leprosy patients and high-risk groups [4]. *M. leprae* infection speeds up myelin breakdown, causing increased lipid droplet formation. This process involves regulating genes related to myelin maintenance, autophagy, and lipid storage [5].

In order to survive, M. leprae relies on the lipid metabolism of the host cell. Among the 24 genes encoding lipolytic enzymes in M. tuberculosis, only ML0119c (lipE), ML0314c (lipU), and ML1899 (lipG) are conserved in M. leprae [6]. M. leprae protein ML2640c is an important family of protein that are classified as a member of S-adenosylmethionine (AdoMet)-dependent methyltransferases (MTases) involved in the biosynthesis of polypeptides [7]. Inactivation of methionine biosynthesis in Mycobacterium tuberculosis (Mtb) results in an exceptionally rapid cell death, which is a highly desirable characteristic for effective chemotherapy. Methionine and S-adenosylmethionine play crucial roles in facilitating successful host infection and promoting virulence [8]. Multidrug therapy (MDT) is the standard treatment for multibacillary leprosy, involving monthly rifampicin, and daily dapsone and clofazimine for 12 months. Paucibacillary cases receive rifampicin and dapsone for 6 months. However, the emergence of drug resistance poses a significant threat to infectious disease control programs relying on chemotherapy. Although MDT has been highly effective, there have been reports of M. leprae strains resistant to one or more anti-leprosy drugs [9].

Plants have served as a primary source of drugs and alternative medicine in the battle against diseases since ancient times. In recent years, there has been a notable surge in research interest regarding medicinal plants, as evidenced by the growing number of publications focusing on plant-based pharmacological interactions and synergistic principles [10]. The results indicate that both angiosperms and gymnosperms possess potential antimicrobial compounds against multidrug-resistant (MDR) pathogens. However, angiosperms have received more extensive research attention in the context of studying their effectiveness against MDR pathogens [11]. *Zanthoxylum leprieurii*, *Lantana camara*, and *Cryptolepis Sanguinolenta* showed antimycobacterial activity against drug-resistant strains of *M. tuberculosis* [12].

Coscinium fenestratum, a member of the *Menispermaceae* family, has been widely utilized as a medicinal plant across numerous Asian countries. *C. fenestratum* holds a prominent position as one of the most valuable medicinal plants due to its renowned reputation for exhibiting high and broad therapeutic efficacy. The Thai Pharmacopoeia includes the prescription of crude drugs derived from this medicinal plant [13]. phytochemical screening of *C. fenestratum* fruit extracts revealed its significant anthelmintic and antioxidant activities [14]. In the study of stem extract of *Coscinium fenestratum* (Gaertn.) Colebr. demonstrated significant activity against *Neisseria gonorrhoeae* ATCC 49226, with berberine identified as the active compound. Furthermore, no acute toxicity was observed at a dose of 5 g of *Coscinium fenestratum* crude extract per kilogram [15]. Numerous studies have provided evidence supporting the antiproliferative effect of *C. fenestratum* stem extract against various types of cancers [16].

In the field of drug discovery, researchers extensively rely on bioinformatics tools and computational methods to identify and analyse the efficacy of novel drugs. In-silico analysis holds significant potential to replace or complement conventional on-silica bioassay-guided fractionation methods, offering the advantage of reducing the number of required bioassays, as well as saving time and costs associated with drug discovery processes [17]. Molecular docking, structure-based virtual screening (SBVS), and molecular dynamics (MD) are extensively employed strategies in structure-based drug design (SBDD), offering versatile applications in the examination of molecular recognition phenomena, including binding energetics, molecular interactions, and conformational

changes induced by ligand-protein interactions [18]. Identification of plant based active compounds enables the determination of the three-dimensional complex structure, facilitating the analysis of molecular recognition through intermolecular features, binding conformations, interactions, binding sites, mechanisms, and ligand-induced conformational changes are the features of molecular docking analysis [19].

Based on molecular docking studies results, the water-extracted *C. fenestratum* exhibited the highest efficacy in downregulating PCSK9, a formula comprising three parts *C. fenestratum* as the primary herb, two parts *Z. officinale* as the assistance herb, and one part *C. tinctorius* as the servant herb, which establishes a rational herbal ratio for the intervention and prevention of PCSK9-related disorders [20]. In this study, our aim was to identify novel active compounds against the *mycobacterium leprae* ML2640 Protein. To achieve this, we screened a total of 80 different active phytoconstituents derived from *C. fenestratum*.

MATERIALS & METHODS

Preparation of Protein

The three- dimensional crystal structure of ML2640c (PDB ID: 2CKD) of Mycobacterium leprae was downloaded from RCSB Protein Data Bank (https://www.rcsb.org/) [7]. The protein consists of A chain with 310 amino acids and exhibits a crystallographic resolution of 2.80 Å. In preparation for docking and optimization of hydrogen bonds, protein crystal structures undergo a series of steps. Using Discovery Studio Visualizer 21.1, a standard protocol is followed for protein preparation, which includes the removal of water molecules and heteroatoms from the proteins. Additionally, polar hydrogen atoms are added, and active site prediction is performed on the prepared protein (Figure 1).

Figure 1. Purified ML2640 protein from Discovery Studio Visualizer 21.1.

Selection of Ligand

To study the best interacting ligand on ML2640 protein of *Mycobacterium leprae*, a diverse array of 80 bioactive phytochemicals sourced from *Coscinium fenestratum* were compiled from numerous literary references. The structures of the phytocompounds were recovered from the PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/) in the 3D SDF (Three-Dimensional Structure Data File) format [21]. Utilizing the PyRx software, modifications to the ligands were performed through a series of distinct stages, encompassing ligand optimization, energy minimization, and the transformation of ligands into the three-dimensional PDB format [22].

Molecular Docking

Molecular docking technique play a crucial role in drug design and the understanding of biochemical pathways by predicting the structure of ligand-protein complexes. These methods can be tested and validated by comparing predicted complexes with structures obtained from protein databases [23]. All the 80 active phytocompounds of *Coscinium fenestratum* were docked with using PyRx-virtual screening tool software ML2640 (PDB ID: 2CKD). The protein was loaded into the Open Babel tool and underwent a transformation to convert it into a macromolecule optimized for docking purposes. Following that, the ligands were imported and prepared in accordance with the required specifications. Using open babel tool protein was loaded and transformed into a macromolecule suitable for docking. Subsequently, ligands were imported and prepared accordingly [24]. After setting the grid parameters docking was performed, result was obtained in a table which was downloaded in csv format. Based on better binding affinity values that are obtained in the Table, 7 phytocompounds were selected. The top 7 compounds were saved in the PDB file format. To perform a 2D-3D interactive visualization study, Discovery Studio Visualizer 21.1 was utilized as a tool for analysis and visualization.

Pharmacokinetics Analysis

A drug should be a potent molecule that must adequately reach its target and remain in a bioactive form long enough for desired biological effects. Drug development now involves early assessment of absorption, distribution, metabolism, and excretion (ADME) using computer models [25]. The drug-likeness properties and ADMET analysis of the top 6 phytocompounds from *Coscinium fenestratum* were examined using the SwissADME web tool. Files should be uploaded in SDF format which is then converted to canonical smiles. ADME tool provides free access to a diverse selection of dependable predictive models encompassing physicochemical properties, pharmacokinetics, drug-likeness, and suitability in medicinal chemistry. These include both established methods like Lipinski's rule and in-house approaches such as BOILED-Egg, iLOGP, and Bioavailability Radar [26]. Important Lipinski parameters involves, Molecular mass < 500 Dalton, logP < 4.15, H-bond donor < 5, H-bond acceptor < 10 and 40 < molar refractivity < 130.

RESULT

Molecular Docking

Through molecular docking analysis, it was discovered that several active phytocompounds derived from *Coscinium fenestratum* exhibit notable binding affinity with the ML2640 protein of *Mycobacterium leprae*. Table 1 represents the top phytocompounds from *Coscinium fenestratum* that demonstrate the highest binding affinity with ML2640 protein.

Table 1. Top 7 phytocompounds from *Coscinium fenestratum* having highest binding affinity with ML2640 protein.

S.N.	PubChem compound ID	Name of phytocompound	Binding energy (Kcal/mol)
1	CID 23345	1-Benzylisoquinoline	-7.6
2	CID 969516	Curcumin(synthetic)	-7.2
3	CID 72704	Berberrubine	-7.2
4	CID 114911	Aporphine	-6.9
5	CID 124886	Glutathione	-6.9
6	CID 72703	Berberrubine chloride	-6.8
7	CID2353	Berberine	-6.6

Based on the results obtained from molecular docking studies conducted with PyRx, it was observed that out of the 80 compounds from *Coscinium fenestratum*, 7 compounds exhibited significant binding affinity (greater than 6.5 kcal/mol) with ML2640 protein of *Mycobacterium leprae*. Among these docking results, the top 7 compounds (Table 1) were chosen for further analysis of drug-likeness prediction and ADME (absorption, distribution, metabolism, and excretion).

Molecular Visualization

For visualizing the interactions between the ligands and the receptor, the top 7 phytocompounds with the strongest binding affinity, Discovery Studio Visualizer 21.1 was employed. The docked ligands, saved in PDB format using PyRx, were then loaded and examined in conjunction with the purified ML2640 protein from *Mycobacterium leprae*. Various 2D and 3D interactions between the phytocompounds and ML2640 were observed. Notably, the 2D interaction diagram revealed a diverse range of interactions, including van der Waals forces, conventional and carbon hydrogen bonds, π-sulfur interactions, alkyl and π-alkyl interactions, π-π T-shaped interactions, as well as unfavourable interactions.

1-Benzylisoquinoline

1-Benzylisoquinoline forms different 2D-3D interactions with ML2640. It involves in conventional hydrogen bonding with Glu 303, π- anion interaction with Asp 299, π- donor hydrogen bonding with Val 262, alkyl interaction with Val 261 and π- sigma bond between Arg 259 and Leu 221 residues as shown in Figure 2.

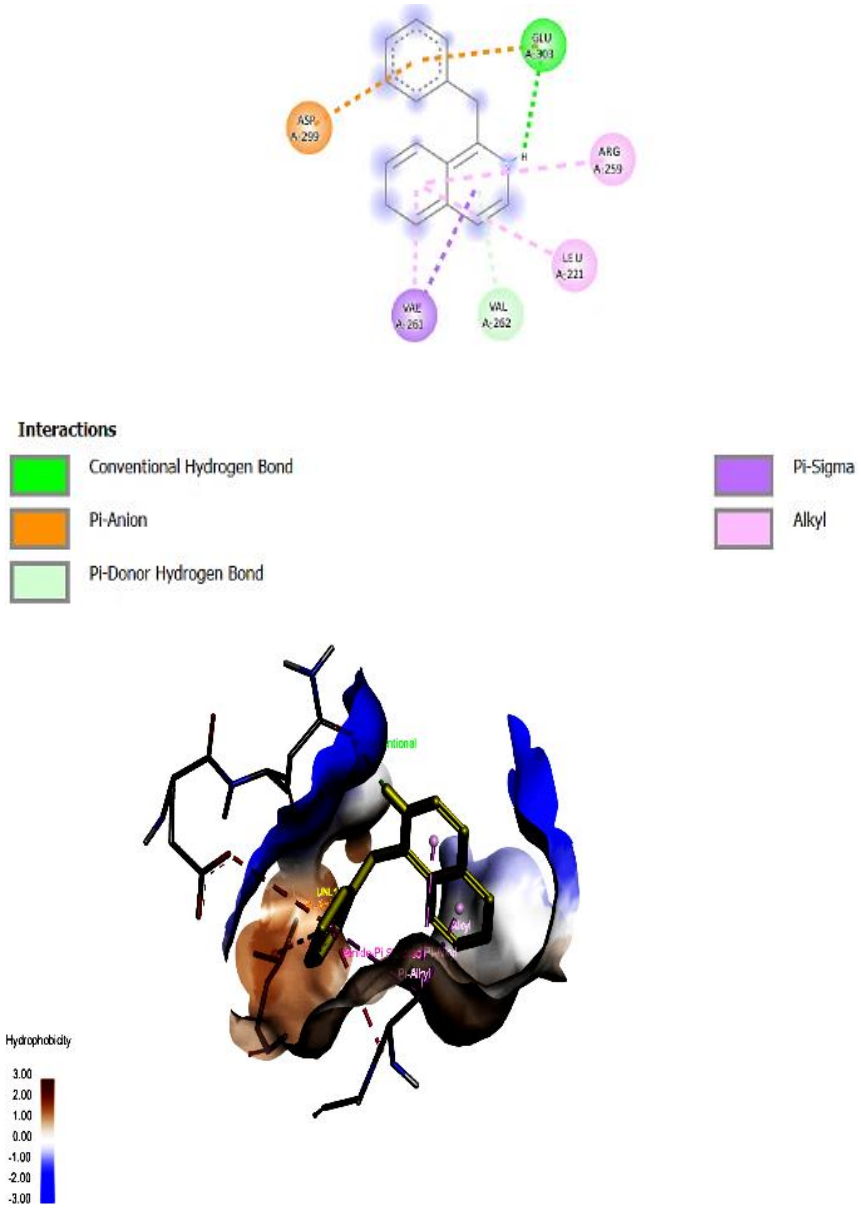

Figure 2. 2D and 3D diagram of interactions of 1-Benzylisoquinoline with ML2640.

Curcumin (Synthetic)

Curcumin (synthetic) forms 2Dand 3D interaction with ML2640. It involved in conventional hydrogen bonding with Glu 186 and Arg 163, carbon hydrogen bond with Ala 18 and Tyr 191, Alkyl and π- alkyl interaction with Ala 22, Leu 113, Val 21, Tyr 254 and Ile 253. It is also forming an unfavourable donor- donor bonding with Arg 87 residue as shown in Figure 3.

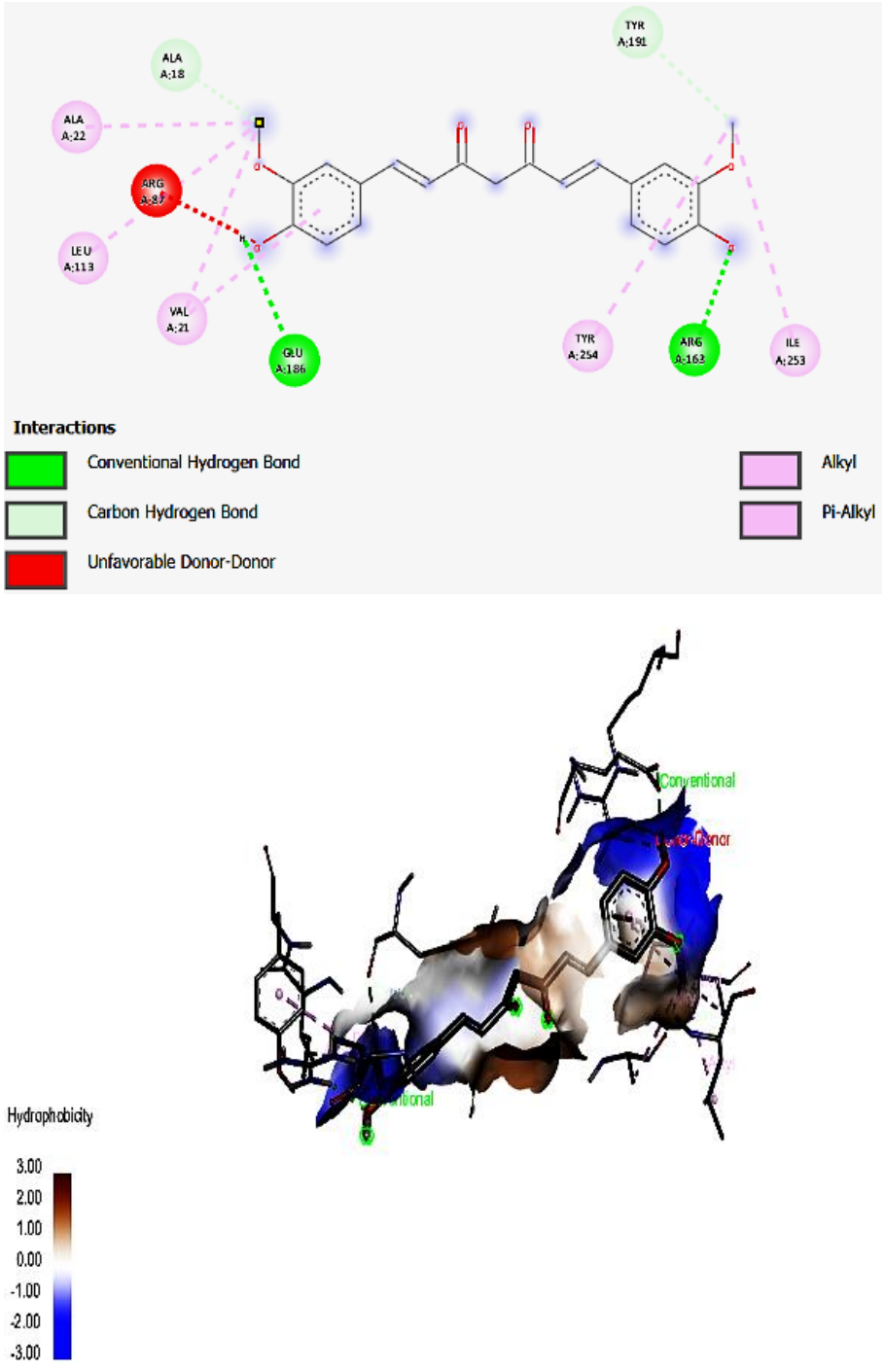

Figure 3. 2D and 3D diagram of interactions of Curcumin (synthetic) with ML2640.

Berberrubine

Berberrubine only showing 3D interaction with ML2640. It is also forming π- alkyl and alkyl interactions, π- anion bonding with different residues is shown in Figure 4.

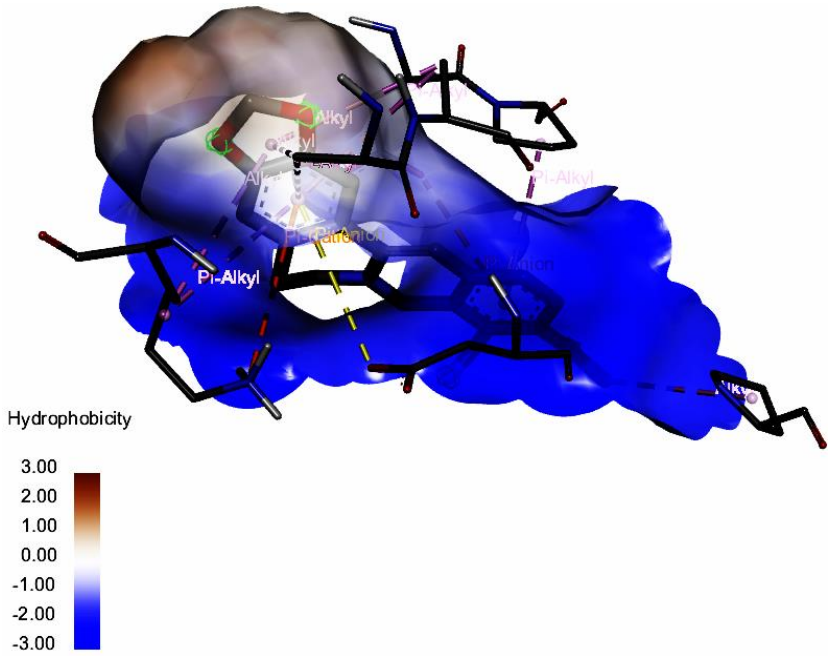

Figure 4. 3D diagram of interactions of Berberrubine with ML2640.

Aporphine

Aporphine forms 2Dand 3D interaction with ML2640. It also involved in π- alkyl interactions with some of the residues as shown in the Figure 5.

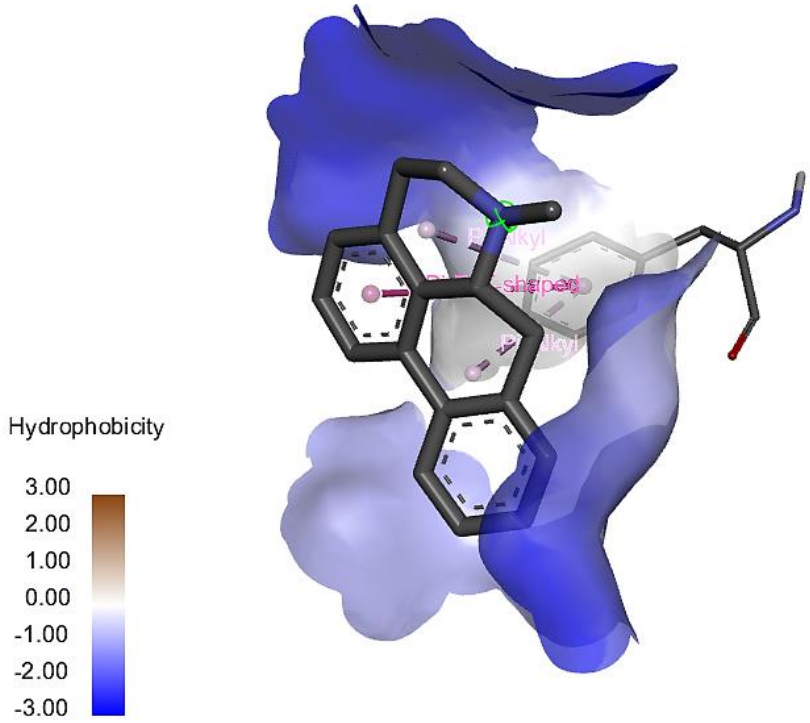

Figure 5. 2D and 3D diagram of interactions of Aporphine with ML2640.

Glutathione

Glutathione forms 2Dand 3D interaction with ML2640. It involves in conventional hydrogen bonding with Glu 251. It is also participated in an unfavourable donor- donor bonding with Gln 250 residue as shown in the Figure 6.

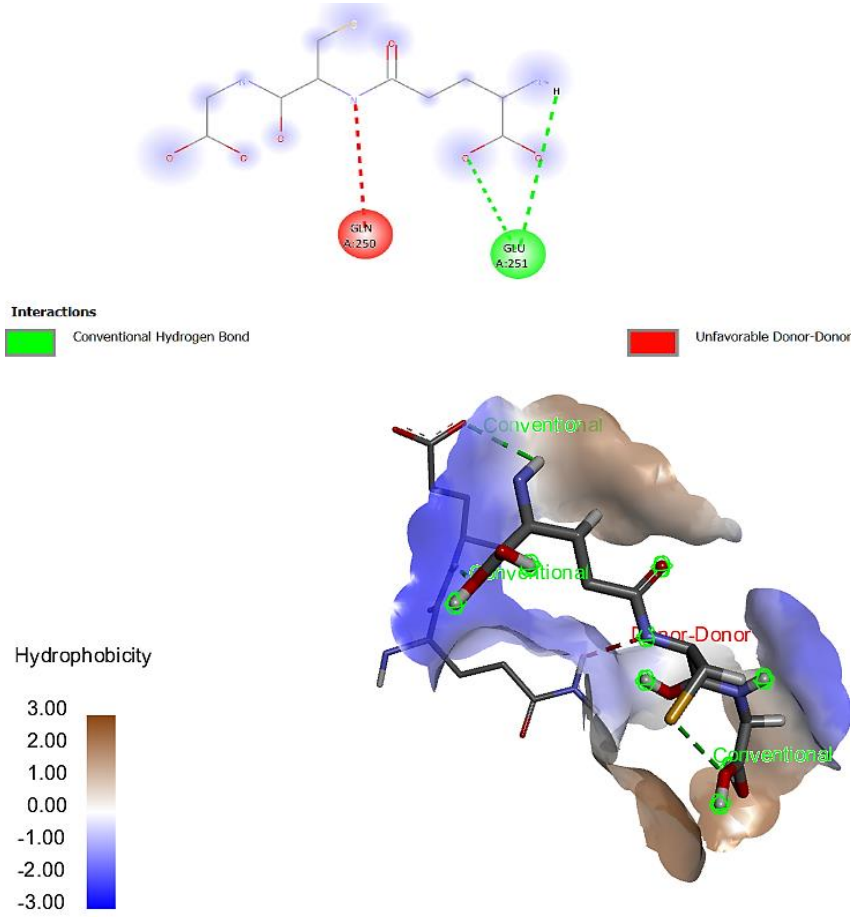

Figure 6. 2D and 3D diagram of interactions of Glutathione with ML2640.

Berberrubine Chloride

Berberrubine chloride only showing 3D interaction with ML2640. It is also involved in conventional hydrogen bonding and π- alkyl interaction with some of the residues of the target protein as shown in Figure 7.

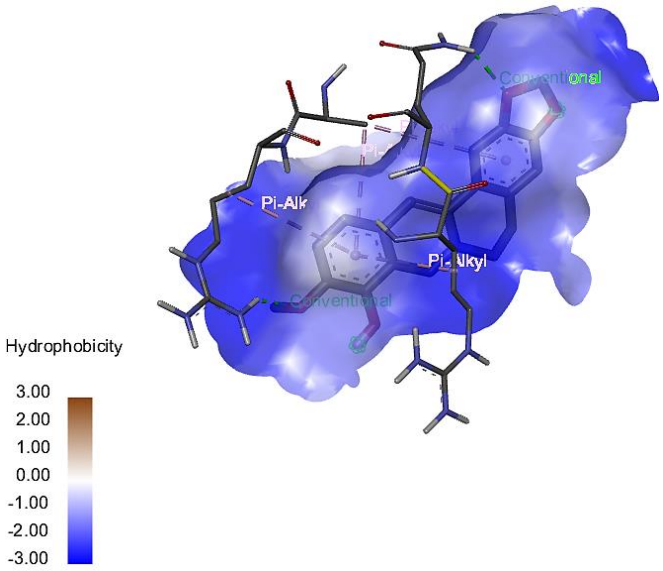

Figure 7. 3D diagram of interactions of Berberrubine chloride with ML2640.

Berberine

Berberine forms 2Dand 3D interaction with ML2640. It is forming carbon hydrogen bonding with Trp8, Asn 47 and Glu 146, π-π T shaped bonding with Tyr 139, alkyl and π- alkyl interaction with Ala 138 and Lys 135 residues as sown in the Figure 8.

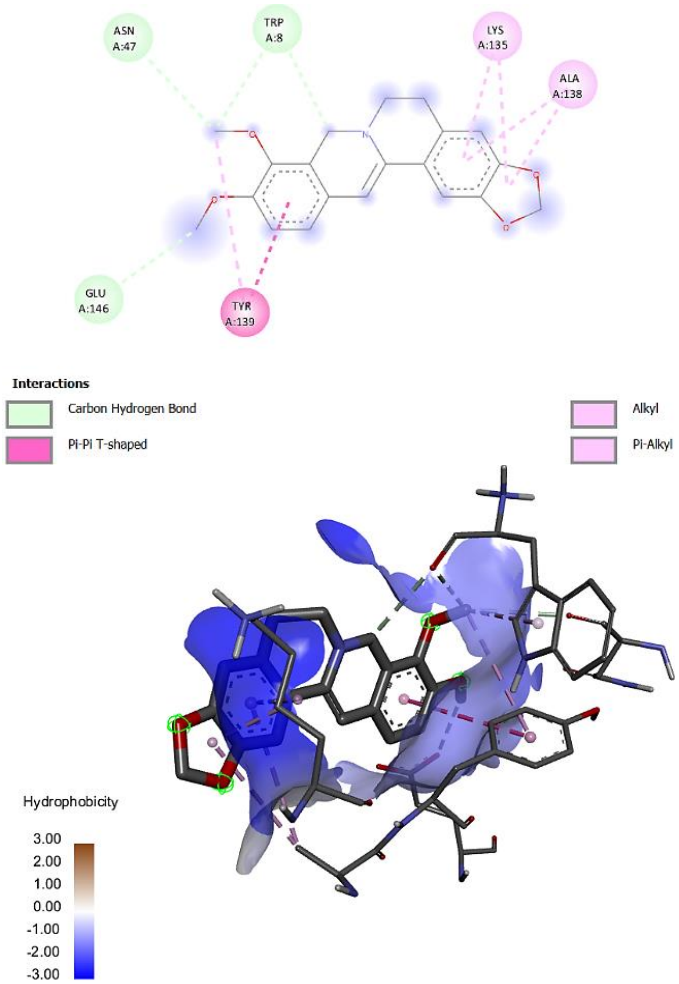

Figure 8. 2D and 3D diagram of interactions of Berberine with ML2640.

ADME and Toxicity Analysis

After molecular docking, the phytochemicals exhibiting the highest binding scores, interactions, and binding affinities were selected for evaluating their ADME properties. The assessment was performed using the SwissADME online tool. Lipinski's rule of five was employed to predict the drug-likeness of the best docked compounds that is depicted in the Table 2.

Table 2. ADME analysis of best docked compounds: lipinski's rule evaluation.

S.N.	Ligand name	Molecular weight (g/mol)	H-bond donor	H-bond acceptor	Log-P-value	Molar refractivity
1	1-Benzylisoquinoline	219.28	0	1	2.55	71.20
2	Curcumin(synthetic)	368.38	2	6	3.27	102.80
3	Berberrubine	322.33	1	4	-0.04	90.41
4	Aporphine	235.32	0	1	2.79	78.97
5	Glutathione	307.32	5	7	0.73	70.37
6	Berberrubine chloride	357.79	1	4	-1.93	96.26
7	Berberine	336.36	0	4	-0.00	94.87

All the molecules in Table 2 having molecular weight are <500 Da, number of hydrogen bond donors and acceptor is <10 and <5, molar refractivity is between 40-130 and Log P value of 6 compounds ranges between -0.4 - 5, but Berberrubine chloride having least log p value depicts its hydrophilic nature.

In addition, Boiled egg analysis was also recorded (Figure 9). It indicates 1-Benzylisoquinoline, Berberrubine, Aporphine and Berberine shows higher probability for brain penetration, curcumin and Berberrubine chloride shows highest probability for gastrointestinal track passive absorption. Glutathione is not involving in any of the two permeations because is present outside the boiled egg plot.

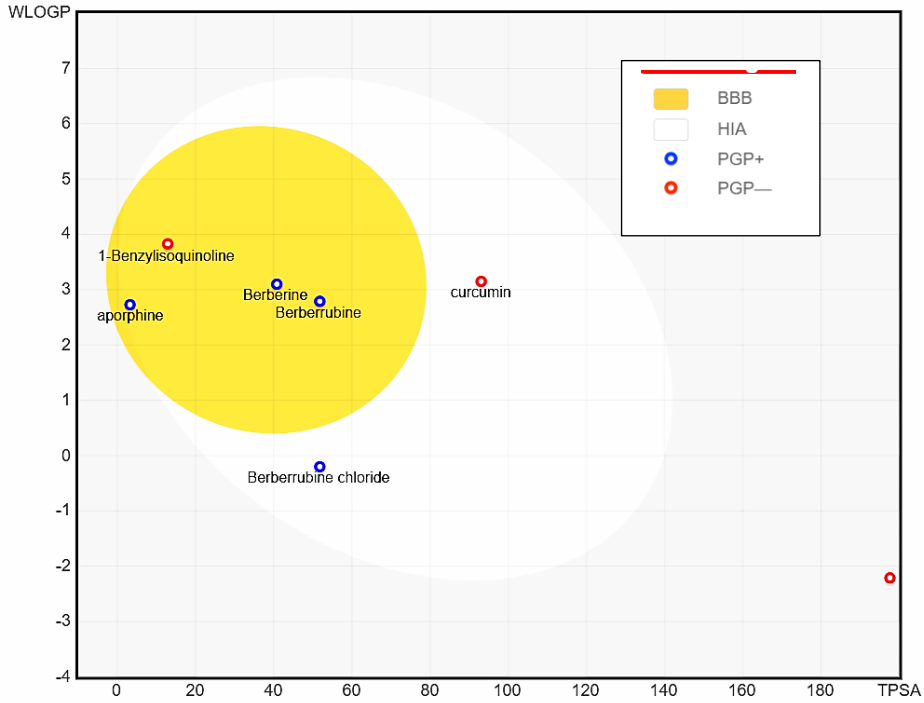

Figure 9. Boiled egg analysis of top 7 phytocompounds.

The best-docked compounds were assessed for various properties on a standard scale, including water solubility (LogS), human intestinal absorption (HIA), Blood-Brain Barrier (BBB) permeability, Permeability Glycoprotein substrate (Pgp-sub), carcinogenic effects, and validation of Lipinski's rule using Admetlab 2.0 is depicted in Table 3.

Table 3. ADME analysis using Admetlab 2.0.

S.N.	Ligand Name	LogS	HIA	Pgp-sub	BBB	Carcinogenicity	Lipinski's Rule
1	1-Benzylisoquinoline	-4.32	0.007	0.207	0.84	0.629	0 violations
2	Curcumin(synthetic)	-3.94	0.06	0.014	0.103	0.706	0 violations
3	Berberrubine	-4.34	0.004	0.999	0.614	0.923	0 violations
4	Aporphine	-3.69	0.003	0.09	0.986	0.216	0 violations
5	Glutathione	1.82	0.117	0.03	0.051	0.047	0 violations
6	Berberrubine chloride	-5.04	0.004	0.999	0.42	0.927	0 violations
7	Berberine	-4.55	0.003	1.0	0.914	0.906	0 violations

DISCUSSION

In the context of treating chronic diseases like leprosy caused by *Mycobacterium leprae*, the emergence of multiple drug resistance among pathogens has become a major concern. However,

recent studies have highlighted the potential of active compounds derived from medicinal plants in combating these drug-resistant pathogens [10]. Among these plants, *Coscinium fenestratum* stands out as a highly valuable medicinal plant known for its remarkable therapeutic efficacy across a wide range of diseases [12]. In the current era, In*silco* analysis has gained significant influence over invitro analysis due to its ability to reduce the number of bioassays required, saving time and costs associated with the drug discovery process [16]. Molecular docking and ADME analysis have proven to be particularly beneficial in further research, allowing for the analysis of binding affinity, interactions, and stability of ligands with the target. This predictive approach aids in identifying potential drug targets and assessing the drug-likeness properties of selected phytocompounds against specific microorganisms that causing chronic diseases.

In this study, the potential of phytocompounds derived from *Coscinium fenestratum*, known for their diverse medicinal properties, was investigated by docking them against the ML2640 protein of *Mycobacterium leprae*. Using the PyRx docking tool, the binding affinity of all 80 phytocompounds was analysed against the ML2640 protein (PDB ID: 6LU7) through in *silico* studies. Among these compounds, seven phytocompounds from *Coscinium fenestratum*, namely 1-Benzylisoquinoline, Curcumin (synthetic), Berberrubine, Aporphine, Glutathione, Berberrubine chloride, and Berberine, exhibited significant binding affinity. Following the molecular docking study, these seven compounds underwent further ADME analysis to assess their drug likeness and toxicity. Importantly, all of these phytocompounds complied with Lipinski's rule without any violations. All these compounds can be used as a protentional drug for reducing the host infection rate based on their significant binding affinity, Drug-likeness properties and ADMET prediction.

CONCLUSION

In this experiment, a total of eighty phytochemicals derived from *Coscinium fenestratum* were utilized to investigate their potential as drugs for treating *Mycobacterium leprae* infection. Among these seven compounds (namely 1-Benzylisoquinoline, Curcumin (synthetic), Berberrubine, Aporphine, Glutathione, Berberrubine chloride, and Berberine) were identified as the most effective for the target protein ML2640. By targeting the ML2640 protein, it disrupts bacterial survival and reduces its virulence. This study revealed the top 7 phytocompounds from *Coscinium fenestratum* demonstrated excellent binding affinity and were identified as the best-docked compounds with desirable drug-like properties. These compounds exhibited a safe ADMET profile, making them promising candidates for further optimization and development in the quest for effective treatments, there by inhibiting the host infection rate of Mycobacterium leprae.

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Authors Contribution

The contribution of all the authors to the manuscript has to be clearly stated

Conflict of Interest

The authors declare no conflict of interest.

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