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“”Insilco Evaluation of *Psoralea corylifolia* for Novel Anti-Tubercular Compounds: Virtual Screening, Molecular Docking, and Dynamics Simulation Analysis”

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Abstract

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), remains a significant global health concern, causing millions of infections and deaths each year. The emergence of drug-resistant strains of *M. tuberculosis* has further complicated the treatment of this infectious disease. Therefore, there is an urgent need to discover new and effective therapeutic strategies to combat TB. *P. corylifolia* was reported to possess antibacterial, anti-inflammatory, antifungal, antioxidant, estrogenic, antitumor, and immunomodulatory activities. The present study aimed to conduct an insilico evaluation of phytochemicals from the plant *Psorlea corylifolia* that are retrieved from databases and literature against potential targets of Mycobacterium tuberculosis. Around 69 compounds belonging to the plant was collected from previous literatures and they were subjected to SwissADME screening with various filters like Lipinski, Ghose, etc. Seven compounds have crossed these filters and ADMET prediction was done for these compounds using online tools like PreADME, ADMETlab2.0, etc. Additionally, molecular docking was performed for all seven compounds against three potential targets: 6B2Q, 6R9W, and 5W25 (protein structures related to Mycobacterium tuberculosis). Among the tested compounds, 2, 4-Di-tert-butylphenol exhibited interactions with all three targets, demonstrating favourable binding energy. The best docked complex was selected for a 100ns molecular dynamics simulation, which revealed stability between 70-90ns, indicating its potential interaction within a virtual biological environment. Further this compound requires *invitro* testing to know its ability to act as an anti-tuberculosis agent. These findings contribute to the identification of promising lead compounds for future drug discovery efforts against Mycobacterium tuberculosis.

Keywords: Insilico; *Mycobacterium tuberculosis; Psorlea corylifolia*, 2,4-Di-tert-butylphenol, Molecular Docking; Molecular Simulation; Anti-tubercular agent

Introduction

Mycobacterium tuberculosis, which causes tuberculosis (TB), continues to be one of the most prevalent causes of fatalities from a single infectious agent. Only three medications have been licensed and placed into the pharmaceutical market over the past 50 years, despite several attempts to find novel anti-TB treatments). Due to the rapid emergence of drug resistance and the lack of knowledge surrounding the cellular transition to dormancy that precedes it, many of the currently available anti-TB medications are ineffective. Additionally, nonrepli-

cating M. tuberculosis-fighting medications are currently scarce (1).

Psorlea corylifolia, a medicinal plant widely used in traditional medicine, has gained attention for its potential therapeutic properties. It contains a variety of bioactive compounds that have shown promising antimicrobial activities against several pathogens. In recent years, researchers have turned to *in silico* methods to explore the potential of Psorlea corylifolia and its bioactive compounds in combating *M. tuberculosis* (2).

In silico target validation is an essential step in drug discovery, enabling researchers to identify and validate potential protein targets that can be modulated by the bioactive compounds present in *Psorlea corylifolia.* By utilizing computational tools and databases, researchers can assess the drug-ability and functional significance of these targets, providing a foundation for subsequent analyses (3).

The crystal structure of the essential protein kinases A and B in Mycobacterium tuberculosis, represented by PDB ID 6B2Q (4), was chosen as one of the targets for the study. Targeting these protein kinases can potentially disrupt crucial cellular processes and inhibit the growth of MTB. Another target selected for investigation was the enoyl-ACP reductase, InhA, which is a validated drug target in MTB. InhA is involved in the synthesis of mycolic acids, essential components of the mycobacterial cell wall. The crystal structure of InhA in complex with the AP-124 inhibitor, represented by PDB ID 6R9W (5), was chosen to explore the binding interactions and potential inhibitory activity of *Psorlea corylifolia* against this target.

Additionally, the crystal structure of Aspartyl-t RNA synthetase (AspRS) from Mycobacterium tuberculosis, complexed with L-Aspartic Acid, represented by PDB ID 5W25, was selected as another target. AspRS plays a crucial role in protein synthesis by attaching aspartic acid to its cognate tRNA. Inhibition of AspRS can disrupt protein synthesis and potentially impair the survival of MTB (6). Through in silico target validation, molecular docking, and dynamics studies, we aim to investigate the binding affinity, potential inhibitory activity, and stability of *Psorlea corylifolia* compounds against the selected targets. These computational approaches will provide valuable insights into the interactions between *Psorlea corylifolia* and the target proteins, aiding in the identification of potential lead compounds for further experimental validation and development as novel anti-TB agents.

Materials and methods

Compound library preparation

The mass spectrometric data containing 69 compounds identified from the plant *Psorlea corylifolia* was retrieved from the literature (7,8). A compound library was prepared using these compounds along with canonical SMILES.

Physiochemical and ADME screening

The Canonical SMILES from the Library of Compounds were fed into the Swiss ADME prediction tool to obtain the physiochemical and ADME data. The screening was done using series of filters like Lipinski rule of 5, Ghose, Rotatable bonds, PAINS and Brenk (9).

Pharmacokinetics prediction

ADME prediction plays an important role in drug discovery and development as it helps assess the pharmacokinetic and pharmacodynamics properties of compounds. The ADME was predicted using the online tools Pre-ADME (10)and ADMETLAb2.0 (11). Pre ADME utilizes computational models and algorithms based on available databases and established methods to generate predictions for parameters like Lipophilicity, permeability, absorption, metabolism, etc., whereas ADMETLab2.0 gives all the ADME data along with clearance and half-life period of the compound.

Toxicity prediction

Toxicity prediction is an important step

in drug discovery and development to evaluate the potential adverse effects of chemical compounds on biological systems. Several online tools are available to assist in toxicity prediction, including Preadmet, Protox II, and GUSAR.

Acute rat toxicity model

The GUSAR (General Unrestricted Structure-Activity Relationships) computer program is designed to create models of quantitative correlations between an organic molecule's structure and other attributes. Quantitative Neighborhoods of Atoms (QNA) descriptors, Prediction of Activity Spectra for Substances (PASS), and Self-Consistent Regression predictions are used in the QSAR approach to model acute toxicity for rats. With four methods of pharmacological substance administration (oral, intraperitoneal, intravenous, and subcutaneous) for rats, QSAR models of relationships "structure - acute toxicity" were developed through the GUSAR software (12).

Ames test and carcinogenicity predictions

Protox II is an online tool designed specifically for predicting the potential toxicity of small organic molecules towards various toxicological endpoints. It utilizes a structure-activity relationship (SAR) approach and provides predictions for toxicity endpoints such as mutagenicity, carcinogenicity, hepatotoxicity, and developmental toxicity. Protox II combines chemical similarity searching and QSAR (Quantitative Structure-Activity Relationship) models to estimate toxicity risks based on the molecular structure of the compound (13).

Ligand and protein preparation

The Ligand preparation was done by retrieving the SDF format of the compounds from Pubchem. The Mol format was generated using Pymol and the energy minimization of the compound structure was achieved using the Avogadro tool. Similarly, based on the literature the target protein PDB file for Dual Inhibition of the Essential Protein Kinases A and B in Mycobacterium tuberculosis(6B2Q), Crystal structure of InhA in complex with AP-124 inhibitor (6R9W) and Crystal structure of Aspartyl-tRNA synthetase from Mycobacterium tuberculosis complexed with L-Aspartic Acid (5W25) was obtained from Protein Data bank (14). Polar hydrogens were added during preparation to avoid undesired interactions during docking, while water molecules and hetero atoms were removed from the protein crystal structure. Using Auto dock, Kollman and Gasteiger charges were added to the ligands and protein during preparation. Additionally, conversion of protein and ligand structures from the PDB format into the PDBQT (Protein Data Bank, Partial Charge (Q), & Atom Type (T)) formats was made with the help of Auto dock (15).

Protein structure validation

Protein structure model validation was done by using ERRAT ("https://servicesn.mbi.ucla.edu/ERRAT/), (16) Verify3D ("http://servicesn.mbi.ucla.edu/Verify3D/) and PROCHECK (17) ("https://www.ebi.ac.uk/thornton-srv/software/PROCHECK).

Molecular docking

Auto Dock Vina is a molecular docking tool used in computational drug discovery to predict how small molecules (ligands) bind to a target protein. Configuration files were created for both the proteins by setting suitable Cartesian coordinates to generate Grid box. The protein's X, Y, and Z attributes were noted after choosing a specific ligand posture from among those present in the protein crystal structure to determine the binding affinity (18). The protein molecules were kept rigid during the docking process. For ligand conformation searching, the Lamarckian Genetic Algorithm (LGA), which is a local search algorithm, was employed.

Molecular simulation

Molecular dynamics simulation was carried out using Desmond module via Maestro of Schrodinger suite 2020-3 in the Linux platform.

Molecular simulation was performed to study the stability of docked complex with the ligand for around 50ns. The molecular system was solvated with crystallographic water (TIP3P) molecules under cubic periodic boundary conditions for a 20 Å buffer region. The system was neutralized with 0.1 M of Na+ and Cl- ions (19).

Results and Discussion

Psorlea corylifolia- compound library screening

The Compounds were screened using the validated rules used as filters in many pharmaceutical companies, as follows Lipinski rule of 5, Ghose, Rotatable bonds, PAINS and Brenk. From 69 compounds about 7 were able to cross all the filters and they were depicted in the figure 1. These compounds were further considered for the study against the proteins involved in the cell wall biosynthesis of the targeted pathway. In a similar study, *Sravika et al., 2021* used these filters to estimate individual ADME parameters of the phytoconstituents from *Bauhinia acuminata* for screening of compounds (20)*conventional medicinal plants analysis have constantly increased multinationally because plants allow them to complement modern pharmacological approaches. As computer mechanics approach, i.e in silico screening and pharmacokinetic screening can augment active compounds among the candidates and indicate mechanism of action of medicinal plants. The plant is well known for its precautionary action in tuberculosis. It has been established to possess some pharmacological activities such as Cytotoxic [1] , antibacterial [2, 3] , anti-nociceptive [4] , thrombolytic activity [5] , antioxidant [6] , anthelmintic [7] , anti-diarrheal [8] , Hepatoprotective [9]. The present focus on the use of in silico ADME tool called Swiss ADME for pharmacological and pharmacognostic profiling of Bauhinia acuminata. The results of these studies can be further carried forward by researcher to investigate the in vitro and in vivo studies to reveal the pharmacological basis of traditional medicinal plants. Introduction The prehistoric people have great consciousness of the tradition of medicinal plants as herbal medicines. In the world, more than 80% of the living in minor developed countries reveal on customary medicine and humans are dependent on herbs for their basic requirements such as food stuffs, clothing, flavor, shelter, fragrance, and medicines (Divya and Mini, 2011 & Manoj Kumar Mishra, 2016, Gurib-Fakim, 2006 and Brijesh & Madhusudan, 2015*.

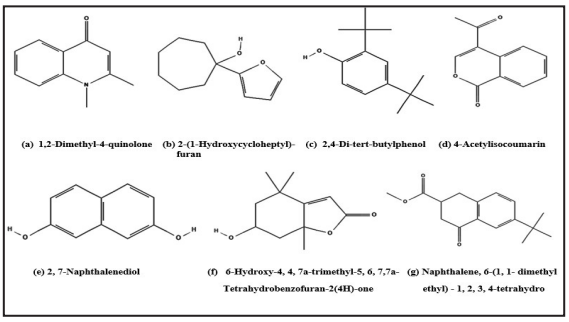

Figure 1: 2D structure of screened ligands

Physiochemical properties and drug likeness

The Physiochemical properties of the screened bioactive compounds were retrieved from swiss-ADME (Table 1). All the seven compounds were found to satisfy the Lipinski rule of five namely Molecular weight <500 g/mol, No of H-bond donors should be less than 5 and not more than 10 hydrogen bond acceptors (Table 2). The topological polar surface area was found to be less than 140 Å² describing the defined sum of polar atoms in the molecule. Moreover, the bioavailability scores of the compounds were computed based on their molecular weight, cLogP, number of hydrogen bond acceptors and hydrogen bond donor. In a previous Insilico validation study of 500 compounds focusing on different biological activity like antimicrobial, Antifungal, it was found that the predicted range of physiochemical properties exceeded the existing parameters even after applying all filters (21). The Log P (Octanol-Water partition coefficient) value remained within 5 indicating the water-soluble nature of compounds. According

to *Knoll et al., 2022* high lipophilicity indicates good permeation of compound through Mycobacterial cell wall (22). In the present study 2, 4-Di-tert-butylphenol exhibits moderate solubility with Log P value around 3.08 highlighting that it could penetrate through the cell wall of Mycobacterium and could exhibit potential activity at the target site.

Table 1: Physiochemical properties of predicted ligands

Ligands	Solubility and Lipophilicity							
	Molecular Formula	Molecular mass g/mol	No of H-bond acceptors	No of H-bond donors	Total polar surface Area Å²	Solubility (mg/mL)	Class	Log P
1,2-Dimethyl-4-quinolone	C11H11NO	173.21	1	0	22.00	2.83e-01	Soluble	2.07
2-(1-Hydroxycycloheptyl)-furan	C11H16O2	180.24	2	1	33.37	4.40e-01	Soluble	2.19
2,4-Di-tert-butylphenol	C14H22O	206.32	1	1	20.23	5.78e-03	Moderately soluble	3.08
2,7-Naphthalenediol	C10H8O2	160.17	2	2	40.46	3.61e-01	Soluble	1.31
4-Acetylisocoumarin	C11H8O3	188.18	3	0	47.28	9.55e-01	Soluble	1.75
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	C11H16O3	196.24	3	1	46.53	4.04e+00	Very soluble	1.88
Naphthalene, 6-(1,1-dimethyl ethyl)-1,2,3,4-tetrahydro	C16H20O3	260.33	3	0	43.37	8.12e-02	Soluble	2.88

Table 2: Drug likeness

Ligands	Lipinski rule of 5	Ghose	Bioavailability score
1,2-Dimethyl-4-quinolone	Yes; 0 violation	Yes ; No violation	0.55
2-(1-Hydroxycycloheptyl)-furan	Yes; 0 violation	Yes ; No violation	0.55
2,4-Di-tert-butylphenol	Yes; 0 violation	Yes ; No violation	0.55
2,7-Naphthalenediol	Yes; 0 violation	Yes ; No violation	0.55
4-Acetylisocoumarin	Yes; 0 violation	Yes ; No violation	0.55
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	Yes; 0 violation	Yes ; No violation	0.55
Naphthalene, 6-(1,1- dimethyl ethyl)- 1,2,3,4-tetrahydro	Yes; 0 violation	Yes ; No violation	0.55

ADMET prediction

The gastrointestinal absorption (GIA) of the identified compounds was evaluated using PreADME and the results are presented in Table 3A. 2, 7-Naphthalenediol was estimated to have low GIA, while 2, 4-Di-tert-butylphenol and 1, 2-Dimethyl-4-quinolone was predicted to have high potential to be absorbed in the gastrointestinal tract. High GIA is considered an advantage during oral administration. Thus, both 2, 4-Di-tert-butylphenol and 1, 2-Dimethyl-4-quinolone has good probability of being absorbed in the intestinal cells and exerts

its biological activity. The Caco2 permeability study can aid in the selection of appropriate excipients to enhance the absorption of poorly permeable compounds. Values predicted using PreADME suggest that the Caco2 permeability of the compounds are within the moderate range. The skin permeation (LogKp) suggests that 2,4-Di-tert-butylphenol compound possess the less negative value of – 3.87 cm/s indicating its good skin permeability whereas 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one was predicted to contain moderate to high negative value of -6.79 cm/s depicting relatively low skin permeability. P-gp inhibitors are molecules that may block P-gp from excreting pharmaceuticals into cells, boosting the intracellular concentration and bioavailability of co-administered medications. In the current study it was found that compounds 1,2-Dimethyl-4-quinolone, 2,4-Di-tert-butylphenol and Naphthalene, 6-(1,1- dimethyl ethyl)- 1,2,3,4-tetrahydro are inhibitors of P-Glycoprotein indicating that these substances can overcome drug resistance and improve the therapeutic efficacy of drug by suppressing P-gp. Similarly, all the 7 compounds were found to be BBB permeable. The % plasma binding protein was found to be 100 for 2-(1-Hydroxycycloheptyl)-furan and 2, 4-Di-tert-butylphenol and least % of 23.88 for 6-Hydroxy-4, 4, 7a-trimethyl-5, 6, 7, 7a-tetrahydrobenzofuran-2(4H)-one once again highlighting that 2, 4-Di-tert-butylphenol has better systemic circulation. In an insilico study performed by *Khamouli et al., 2019* overall ADME predictions of amino-pyrimidine derivatives showed GIA of 90.926%, Caco2 around 15-18% and skin permeability -2.704 to -3. 721 cm/s respectively. Similarly, the plasma binding protein was found to be around 87% with moderate BBB penetration and some compounds are P-glycoprotein inhibitors (23). These predictions reveals that the GIA, Plasma binding protein, Caco2 permeability and BBB were found to be comparatively similar or more in the current study highlighting better Absorption and distribution property of the compound.

Table 3A: ADME Prediction of Screened compounds

COMPOUND NAME	ABSORPTION			DISTRIBUTION		
	Human intestinal Absorption	Caco-2 permeability	Log Kp (skin permeation) (cm/s)	P-glycoprotein inhibitor	BBB penetration	Plasma Protein Binding
1,2-Dimethyl-4-quinolone	100.000000	54.5989	-5.89	Yes	Yes	58.502030
2-(1-Hydroxycycloheptyl)-furan	95.451419	43.0929	-5.78	No	Yes	100.000000
2,4-Di-tert-butylphenol	100.000000	44.8684	-3.87	Yes	Yes	100.000000
2,7-Naphthalenediol	91.618383	20.3624	-5.93	No	Yes	95.587064
4-Acetylisocoumarin	98.036259	21.3184	-6.52	No	Yes	49.326538
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	93.378046	21.5562	-6.79	No	Yes	23.881949
Naphthalene, 6-(1,1-dimethyl ethyl)-1,2,3,4-tetrahydro	98.213233	22.3895	-5.62	Yes	Yes	92.984636

Compound metabolism impacts a substance's distribution, excretion, and absorption. A class of enzymes known as cytochrome P450 (CYP) monooxygenases affects how drugs are metabolized and eliminated from the body. The predicted metabolism of the identified compounds against six isomers of CYP is displayed in the table 3B. ADME prediction study done by *Aguinaldo et al., 2022,* revealed that caffeic acid extracted from *Cocos nucifera* caffeic acid was non-inhibitor of CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. Results also demonstrate that caffeic acid is a non-substrate of CYP1A2, CYP2C19, CYP2D6, and CYP3A4 (24) whereas in the present study, it was found that except 1, 2-Dimethyl-4-quinolone all other compounds are inhibitors of CYP2C9 and CYP3A4. Also, other than compounds 1, 2-Dimethyl-4-quinolone, 2, 4-Di-tert-butylphenol and 6-Hydroxy-4, 4, 7a-trimethyl-5, 6, 7, 7a-tetrahydrobenzofuran-2(4H)-one remaining were found to be the inhibitors of CYP2C19. On the other hand, none of the compounds showed inhibition against CYP2D6. While focussing on CYP substrates, 2, 4-Di-tert-butylphenol and Naphthalene, 6-(1, 1- dimethyl ethyl) - 1, 2, 3, 4-tetrahydro were found act as CYP3A4 substrates whereas no compound acts as a CYP2D6 substrates. From these data it has been predicted that among seven compounds most of them were found to be inhibitors of CYP2C19, CYP2C9 and CYP3A4 highlighting the metabolism of the compounds studied.

Table 3B: ADME Prediction of Screened compounds

COMPOUND NAME	METABOLISM						EXCRETION	
	CYP3A4 substrate	CYP3A4 inhibition	CYP2C19 inhibition	CYP2D6 substrate	CYP2D6 inhibition	CYP2C9 inhibition	$T_{1/2}$ (half-life period)	Clearance rate (ml/min/kg)
1,2-Dimethyl-4-quinolone	Weakly	No	No	No	No	No	0.345	5.226
2-(1-Hydroxycycloheptyl)-furan	No	Yes	Yes	No	No	Yes	0.255	8.533
2,4-Di-tert-butylphenol	Yes	Yes	No	No	No	Yes	0.324	6.300
2,7-Naphthalenediol	No	Yes	Yes	No	No	Yes	0.866	16.596
4-Acetylisocoumarin	No	Yes	Yes	No	No	Yes	0.618	3.049
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	Weakly	Yes	No	No	No	Yes	0.720	8.814
Naphthalene, 6-(1,1-dimethyl ethyl)-1,2,3,4-tetrahydro	Yes	Yes	Yes	No	No	Yes	0.193	7.212

A key pharmacokinetic factor that is related to drug bioavailability is drug clearance (CL). It is also a crucial factor in determining how frequently to administer drugs to reach steady-state concentrations. Suthar et al explains the half Life ($T_{1/2}$) of the compound explains the Volume of distribution and clearance of Drug. It falls under two category $T_{1/2}$> 3 (Category 0-

$T_{1/2}$ -) and $T_{1/2}$ ≤3 (Category 1- $T_{1/2}$+) The Output value is the probability of being $T_{1/2}$+ within the range 0 to 1. The values between 0-0.3 remarks excellent half-life of the compound whereas 0.3-0.7 gives us moderate half-life (25). In the present study, the compound Naphthalene,6-(1, 1- dimethyl ethyl) - 1, 2, 3, 4-tetrahydro had good half-life value of 0.193 whereas 2, 7-Naphthalenediol had poor half-life of 0.866. Also, the clearance rate was found to be relatively low for 4-Acetylisocoumarin with 3.049 ml/min/kg whereas 2, 7-Naphthalenediol exhibited higher clearance rate indicating that it can be rapidly excreted from the circulation. This implies that the compounds exhibited comparatively moderate half-life and clearance rate.

Toxicity panel

Rat toxicity model

The Acute toxicity prediction in Rat model was done using GUSAR Online and the results are tabulated Table 4. GUSAR gave predictions on rat model with Different routes of administration like Intraperitoneal, Intravenous, Oral and Subcutaneous whose LD50 are expressed in mg/kg. The globally standardized system of Classification and Labelling of Chemicals (GHS) defines the following hazardous classes as mentioned in (26): Class 1: if ingested, fatal (LD_{50} 5≤ mg/kg); Class 2 substances are deadly if consumed (5<LD_{50}≤50 mg/kg), hazardous if ingested (50<LD_{50}≤ 300 mg/kg), toxic if ingested (300<LD_{50}≤ 2000 mg/kg), potentially harmful if ingested (2000<LD50≤5000 mg/kg), and non-toxic (LD_{50}>5000 mg) substances. Previous research conducted by *Halder et al., 2019* confirms that *Psoralea corylifolia* was found to contain Neobavaisoflavone, a phytochemical which is predicted to possess an LD50 of 2500 mg/kg, and hence better than Dapsone, which is chemically produced (27). In contrast to the previous study, where the LD50 ranged between 2000 and 5000, the current investigation revealed that the LD 50 values of substances in all drug delivery routes, including IP, IV, SC, and oral, were expected to be larger than 5000mg/kg and thus found to be harmless. From these predictions, it was clear that the current study contains compounds with LD50 values that fall within the range indicating that they are non-toxic.

Table 4: Results of LD 50 value of the compound estimated with rat toxicity model

Ligands	Rat IP LD50 (mg/kg)	Rat IV LD50 (mg/kg)	Rat Oral LD50 (mg/kg)	Rat SC LD50 (mg/kg)
1,2-Dimethyl-4-quinolone	471,000	59,100	1327,000	732,200
2-(1-Hydroxycycloheptyl)-furan	326,700	37,890	858,600	486,800
2,4-Di-tert-butylphenol	797,100	72,050	1743,000	492,900
2,7-Naphthalenediol	483,900	98,270	1011,000	642,100
4-Acetylisocoumarin	380,600	39,010	2676,000	1275,000
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	479,300	20,150	3038,000	215,300
Naphthalene, 6-(1,1- dimethyl ethyl)-1,2,3,4-tetrahydro	612,100	36,540	1418,000	423,700

IP - Intraperitoneal route of administration Oral - Oral route of administration

IV - Intravenous route of administration SC - Subcutaneous route of administration

Ames test and carcinogenicity

AMES toxicity which is the test for mutagenicity. To prevent the creation of hazardous mutagenic and potentially carcinogenic medications, it is necessary to estimate the AMES toxicity since mutagenicity is a key end point of toxicity and has a strong connection to carcinogenicity. Except 2, 4-Di-tert-butylphenol rest of the compounds are reported to be mutagen. Other toxicity predictions like Hepatotoxicity, Carcinogenicity, Immunotoxicity, mutagenicity and Cytotoxicity has been done using protox2 which revealed that the compounds 2,4-Di-tert-butylphenol, 2,7-Naphthalenediol and Naphthalene, 6-(1,1- dimethyl ethyl)- 1,2,3,4-tetrahydro exhibited inactive remarks in all the above-mentioned toxicity parameters including Carcinogenicity Mouse and Rat model (Table 5). *Castro et al., 2021* conducted an evaluation of toxicity for compounds Isoeleutherin and Eleutherin isolated from a herb where insilico predictions revealed that both the compounds are positive towards rat carcinogenicity model and negative in mouse carcinogenicity model with medium risk in cytotoxicity (28). Whereas in the present study the compound 2,4-Di-tert-butylphenol was found to be a non-mutagen and negative or inactive in terms of all toxicity studies highlighting that this compound can be considered for further study.

Table 5: Toxicity panel

Ligand	Ames test	Carcinogenicity Mouse model	Carcinogenicity Rat model	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
1,2-Dimethyl-4-quinolone	mutagen	Negative	Positive	Inactive	Inactive	Inactive	Active	Inactive
2-(1-Hydroxycycloheptyl)-furan	mutagen	Positive	Negative	Inactive	Inactive	Inactive	Inactive	Inactive
2,4-Di-tert-butylphenol	non-mutagen	Negative	Negative	Inactive	Inactive	Inactive	Inactive	Inactive
2,7-Naphthalenediol	mutagen	Negative	Negative	Inactive	Inactive	Inactive	Active	Inactive
4-Acetylisocoumarin	mutagen	Negative	Positive	Inactive	Active	Inactive	Inactive	Inactive
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	mutagen	Negative	Negative	Inactive	Active	Inactive	Inactive	Inactive
Naphthalene, 6-(1,1-dimethyl ethyl)-1,2,3,4-tetrahydro	mutagen	negative	negative	Inactive	Inactive	Inactive	Inactive	Inactive

Protein model validation

PROCHECK analysis and Ramachandran plot validation for the target proteins has been done following (29) protocol and has been illustrated in Figure 2, 3 and 4. In Target protein 6B2Q with more than 90.2% of the residues were found to be in favoured and altogether 9.6 % residues were found in additional and generously allowed regions, which validate the quality of homology models. Similarly, other proteins namely 6R9W and 5W25 shows around 91.6 and 88.2% of the residues in favoured region indicating better quality of protein model. The overall G-factor for 6B2Q, 6R9W and 5W25 were 0.12, 0.10 and 0.15. The modelled structures were also validated by other structure verification servers such as Verify 3D and Errat. Verify 3D assigned a 3D-1D score of >=0.1 for all the modelled proteins.

Table 6: Evaluation of Protein Model Quality

Protein Name	ERRAT Quality Score	Procheck	Verify 3D
6B2Q	92.7007	Out of 8 evaluations, Errors: 3 Warning: 3 Pass: 2	86.95 % of the residues have average 3D-1D score of >= 0.1
6R9W	96.1792	Out of 8 evaluations, Errors: 4 Warning: 2 Pass: 2	68.49 % of the residues have average 3D-1D score of >= 0.1
5W25	88.2459	Of 9 evaluations, Errors: 2 Warning: 3 Pass: 4	84.05% of the residues have averaged 3D-1D score >= 0.1

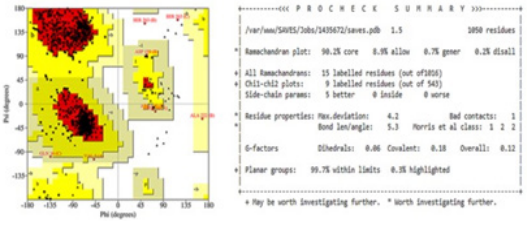

Figure 2: Ramachandran plot of protein 6B2Q obtained from procheck analysis

This implies that the models are compatible with their sequence. ERRAT showed overall quality factor 92.7007, 96.1792 and 88.2459 for 6B2Q, 6R9W and 5W25 respectively (Table 6). *Hamid et al., 2021* performed computational analysis of 3D structure of two catalytic proteins MOCS1A and MOCS1B where procheck analysis have been done and The Ramachandran plot statistics for model proteins MOCS1A and MOCS 1B revealed that for MOCS1A about 80% residues are in the allowed region and 3.6% residues (only 12 residues) are in disallowed region. For MOCS1B, more than 90% residues are in the allowed region and 1.8% residues (only 10 residues) are in disallowed region (30). Contrasting the current and previous study it can be inferred from Ramachandran plots that most data appear in the favoured region suggesting that the modelled structure is acceptable.

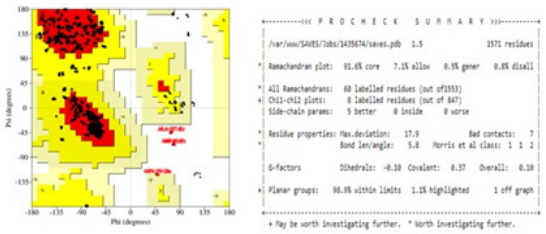

Figure 3: Ramachandran plot of protein 6R9W obtained from procheck analysis

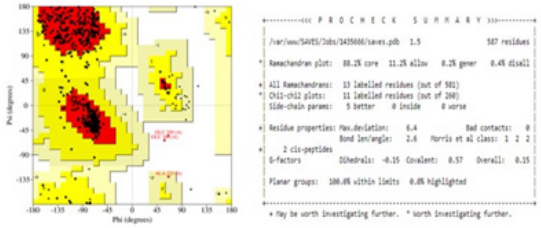

Figure 4: Ramachandran plot of protein 5W25 obtained from procheck analysis

Molecular docking

Molecular docking was done using Auto dock vina and their interactions are visualized using UCSF Chimera tool as per (31)many methods of molecular modeling have been em-

ployed to study complex biological and chemical systems. Experimental strategies are integrated with computational approaches for the identification, characterization, and development of novel drugs and compounds. In modern drug designing, molecular docking is an approach that explores the confirmation of a ligand within the binding site of a macromolecule. To date, many software and tools for docking have been employed. AutoDock Vina (in UCSF [University of California, San Francisco] Chimera. Docking was carried out with the seven screened compounds against the targets 6B2Q, 6R9W and 5W25. Among the docked compounds 2, 4-Di-tert-butylphenol exhibited strong binding energy of -8.8, -10.0 and -7.1 kcal/mol against all the three targets 6B2Q, 6R9W and 5W25 respectively (Table 7). They formed H-bonds with the amino acid residues Val98 A, Gly 96 A and Gly 563 A of the aforementioned corresponding targets (Refer Figure 5, 6 and 7). Similarly in terms of interactions, 2, 7-Naphthalenediol showed four H-bond interactions against 5W25 with the binding energy of -5.8 kcal/mol. Furthermore, 2-(1-Hydroxycycloheptyl)-furan and 4-Acetylisocoumarin displayed moderate binding energies of -5.6 and -5.5 kcal/mol against 6B2Q and 5W25. In a docking study conducted by *Wlodarchak et al., 2108* against 6B2Q, best binding affinity was found to be -8.87 kcal/mol for imidazopyridine aminofurazans (32) In another study nearly 25 bioactive pyrimidine derivatives have been docked against 6R9W where one of the derivatives showed better score of -11.59kcal/mol indicating its best interaction against the target (33). According to these two pioneering findings, the binding energy for 2, 4-Di-tert-butylphenol in the current research was discovered to be very similar with binding energy ranging between -8.8 and -10.0 kcal/mol.

Table 7: Docking scores of 6B2Q, 6R9W and 5W25 with the Compounds along with Bond Length

S.NO	Ligands	6B2Q				6R9W				5W25			
		Binding energy (Kcal/mol)	H-donor	H-acceptor	Bond length(Å)	Binding energy (Kcal/mol)	H-donor	H-acceptor	Bond length(Å)	Binding energy (Kcal/mol)	H-donor	H-acceptor	Bond length(Å)
1	1,2-Dimethyl-4-quinolone	-6.3	Asp 159 C	Ligand	5.558	-7.5	Gly 96A	Ligand	5.563	-5.6	Glu 230 A	Ligand	4.793
2	2-(1-Hydroxycycloheptyl)-furan	-5.6	Ligand	Gly 145 A	4.746	-6.5	Ligand	Ser 152 D	4.063	-5.8	Ligand	Arg 132 A	3.131
							Ligand	Ser 166 C	5.418				
3	2,4-Di-tert-butylphenol	-8.8	Ligand	Val 98 A	2.355	-10.0	Ligand	Gly 96 A	4.387	-7.1	Ligand	Gly 563 A	4.510
			Val 98 A	Ligand	2.823								
4	2,7-Naphthalenediol	-6.8	Val 98 A	Ligand	2.840	-7.9	Ligand	Gly 96 D	3.674	-5.8	Ligand	Ala 535 A	3.148
							Val 65 D	Ligand	2.361		Ligand	Gly 488 A	2.699
											Ligand	Asp 701 A	2.702
							Ligand	Leu 63 D	2.347		Ligand	Glu 485 A	2.942
5	4-Acetylisocoumarin	-6.8	Val 98 A	Ligand	3.755	-7.9	Gly 96 D	Ligand	3.405	-5.5	Gly 563 A	Ligand	5.064

6	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	-6.0	Leu 97 A	Ligand	2.912	-8.0	Ligand	Ser 166 D	3.324	-5.9	Ligand	Arg 492 A	3.250
7	Naphthalene, 6-(1,1-dimethyl ethyl)-1,2,3,4-tetrahydro	-7.6	Thr 21 D	Ligand	4.831	-7.6	Met 155B	Ligand	2.861	-5.8	Val 564 A	Ligand	4.292

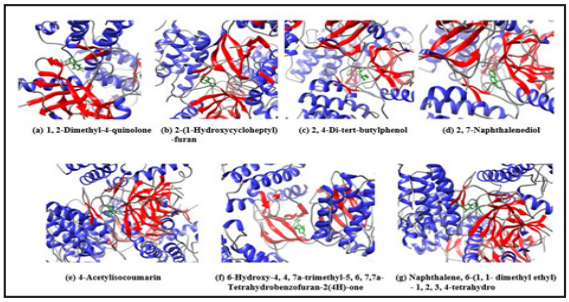

Figure 5: Visualization of interaction of Ligands with the target protein 6B2Q

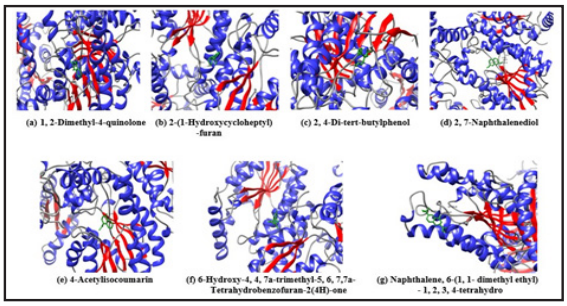

Figure 6: Visualization of interaction of Ligands with the target protein 6R9W

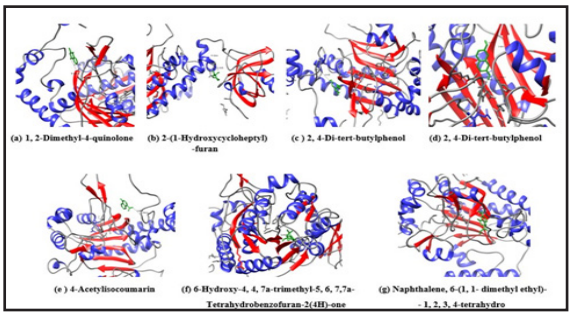

Figure 7: Visualization of interaction of Ligands with the target protein 5W25

Overall, the results indicate that the tested ligands have the potential to interact with the target proteins of Mycobacterium tuberculosis, suggesting their possible therapeutic relevance. The observed binding energies and hydrogen bonding interactions provide insights into the strength and specificity of ligand-protein interactions (1). Also, the current docking study highlights that compound 2, 4-Di-tert-butylphenol shows promising binding affinity against all the targets and hence the docked complex with better binding efficiency namely 6R9W_2, 4-Di-tert-butylphenol was further considered for Molecular dynamic simulation.

Molecular simulation

Numerous dynamical, structural, and energetic details about the simulated system are revealed by MD simulations. For the purpose of running the simulations, the top-ranked docked pose 6R9W_2, 4-Di-tert-butylphenol based on free binding energy scores was selected as the structural model. The built trajectories of all the simulated systems were analysed in terms of its RMSD and Protein ligand Histogram. Complex stability was checked by analysis of the interaction map and the RMSD (root mean square deviation) plot of the ligand and protein. RMSD of the protein gives insights to the structural conformation throughout the simulation.The changes of the order of 1-3 Å is acceptable for small molecules.Here it was found to be around 2.4-3.3 Å with slight fluctuations from the beginning and it attained equilibration between 70- 90 ns (Figure 8). *Alzain et al., 2023* conducted an insil-

ico multitarget approach where 18 cytosporone E analogues were screened against 6R9W by docking and simulation using desmond schrodinger exhibited RMSD value below 3Å which reflected the stability of the compounds with few fluctuations (34). On comparing the present and previous study the RMSD and the stability was found to be more or less similar displaying that the compound, 2, 4-Di-tert-butylphenol can be considered as a potential anti-tubercular agent which requires further *invitro* evaluations to be marketed as drug molecule.

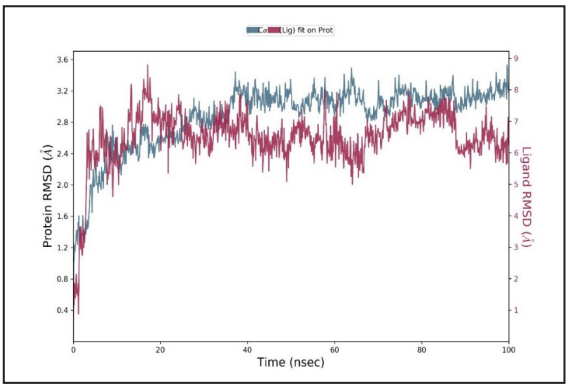

Figure 8: RMSD plot for the simulated 6R9W_2, 4-Di-tert-butylphenol complex

The protein Ligand contacts histogram depicts different types of interactions namely H-bond, Hydrophobic , Ionic and Water bridges. The stacked bar charts are normalized over the course of trajectory. In the Figure 9, H-bond interaction was seen with the aminoacid residues Met 98, Gly 96, ser 94, Arg 43 and Lys 165 indicating that there was a specific interaction maintained for about 30-50% of the simulation time of 100ns. Other than H-bond interactions, few hydrophobic contacts was observed at residue Met98, Ile 16, Phe41, Met 161, Ala 164 and Met 199 displaying the interaction of aromatic or aliphatic group on the ligand (Figure 9). According to *Alzain et al., 2023* water bridges are commonly found in the histogram since it mediates the hydrogen-bonded protein-ligand interactions. In their study, the average Hydrogen and hydrophobic bonds formed between the derivatives and the target was 5 that comprises of aminoacid residues like Phe149, Tyr 158, Trp222, Thr196 and Glu219 (34). This indicates that the current study had better H-bond interactions throughout the simulation time equally to that of the prior study.

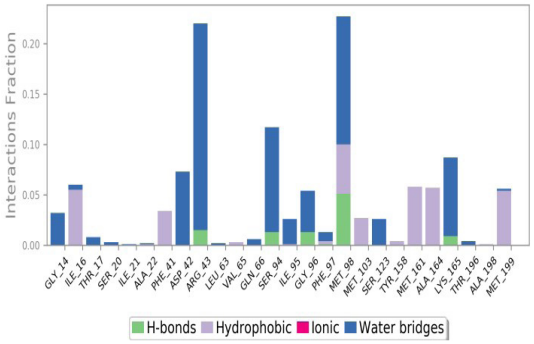

Figure 9: Protein Ligand Contacts Histogram

Conclusion

Overall, the study focused on in-silico evaluation of phytochemicals from *Psorlea corylifolia* against potential targets of Mycobacterium tuberculosis. Sixty-nine compounds from the plant were screened using SwissADME with various filters, resulting in the identification of seven compounds that passed these filters. ADME prediction using tools like PreADME and ADMETlab2.0 provided insights into the pharmacokinetic properties of these compounds. Furthermore, molecular docking against three potential targets related to Mycobacterium tuberculosis revealed better interaction between the compound 2, 4-Di-tert-butylphenol and the targets. Molecular dynamics simulation of the best docked complex demonstrated stability within a virtual biological environment. These findings highlight the potential of 2, 4-Di-tert-butylphenol as a lead compound for further investigation as an anti-tuberculosis agent. However, in vitro testing is necessary to validate its efficacy and determine its ability to act against Mycobacterium tuberculosis. The identified lead compound from this study contribute to ongoing drug discovery efforts against Tuberculosis.

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